Journal of Controlled Release 329 (2021) 676–695

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Actively targeted nanomedicines for precision cancer therapy: Concept, construction, challenges and clinical translation

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ARTICLE INFO

Keywords:
Nanomedicines
Targeted delivery
Biopharmaceuticals
Active targeting
Clinical translation
Cancer therapy

ABSTRACT

The development of targeted nanomedicines for cancer therapy has been an utmost focus of research across different fields including materials science, nanotechnology, biotechnology, pharmaceutics, and clinical medicine. Vehicle-mediated, enhanced and tumor-selective delivery is deemed as a powerful tool to boost the efficacy and meanwhile minimize the off-target effect of potent chemo drugs, and to potentiate biopharmaceuticals such as nucleic acids (DNA, siRNA, miRNA, mRNA, CRISPR/Cas9, etc.), proteins and peptides that poorly penetrate the cell membrane on their own while having explicit effects intracellularly. The targeted nanomedicines may further provide imminent treatments for intractable brain tumors by transporting drugs across the blood-brain barriers, multi-drug resistant (MDR) tumors by evading the MDR pathways, metastatic tumors by inhibiting migratory tumor cells, and relapsed tumors by eliminating the cancer stem cells. The preclinical and clinical investigations demonstrate the clear benefits of targeted nanomedicines in treating advanced solid and hematological malignancies. In this review, we highlight the design and construction of conceptually interesting and clinically viable actively targeted cancer nanomedicines containing small molecular drugs, nucleic acid drugs, or protein/peptide drugs, discuss their pros and cons, and give perspectives on the future developments and clinical translation. We are convinced that with collaborative research and development across the disciplines, actively targeted cancer nanomedicines will make a breakthrough and become an indispensable platform for precision cancer therapy.

Abbreviation: ACUPA, 2-(3-(5-amino-1-carboxypentyl)-ureido) pentanedioic acid; AD, Adamantane; ADCs, Antibody-drug conjugates; AML, Acute myeloid leukemia; AuNP, Gold nanoparticles; B-ALL, Pre-B cell acute lymphoblastic leukemia; BBB, Blood-brain barriers; CDP, Cyclodextrin-based polycation; CED, Convection-enhanced delivery; CL2A, An acid-cleavable linker; CPs, Chimeric polymersomes; CRM107, Diphtheria toxin with a point mutation; CSCs, Cancer stem cells; CTCs, Circulating tumor cells; DAR, Drug-antibody ratio; DCs, Dendritic cells; DOX, Doxorubicin; DOX·HCl, Doxorubicin hydrochloride; DTX, Docetaxel; EGFR, Epidermal growth factor receptor; EPR, Enhanced permeability and retention; GBM, Glioblastoma multiforme; GO, Gemtuzumab ozogamicin; GrB, Granzyme B; GSDMB, Anti-gasdermin B; GSH, Glutathione; HA, Hyaluronic acid; HER2, Human epidermal growth factor receptor 2; HIF-1α, Hypoxia-inducible factor-1α; ILs, Immunoliposomes; IMMU-132, Sacituzumab govitecan; InO, Inotuzumab ozogamicin; LRP-1, Low-density lipoprotein receptor-related protein-1; mCRPC, Metastatic castration-resistant prostate cancer; MDR, Multi-drug resistant; MM, Multiple myeloma; MMAE, Monomethyl auristatin E; MMP-9, Matrix metalloproteinase-9; MTD, Maximum-tolerated dose; NHL, Non-Hodgkin lymphoma; NIR, Near-infrared; NSCLC, Non-small cell lung cancers; ORR, Overall response rate; PDCs, Polymer-drug conjugates; PECAM-1, Vascular cell adhesion molecule-1; P(TMC-DTC), Poly(trimethylene carbonate-co-dithiolane trimethylene carbonate); PEI, Polyethylenimine; PEM, Pemetrexed disodium; PFS, Progression-free survival; PHPMA, Poly(N-(2-hydroxypropyl)methacrylamide); PIC, Polyion complex; PK2, Galactosamine; PLA, Poly(lactic acid); PLGA, Poly(lactic-co-glycolic acid); PLL, Poly(L-lysine); PMSA, Prostate-specific membrane antigen; PS-DOX, Polymersomal DOX·HCl; PTX, Paclitaxel; RNAi, RNA interfere; RRM2, Ribonucleotide reductase; Sap, Saporin; scFv, Single-chain antibody Fv; SCLC, Small cell lung cancers; siPLK1, siRNA against polo-like kinase 1; SN-38, A cytotoxic topoisomerase I inhibitor; TAMs, Tumor associated macrophages; T-DM1, Ado-trastuzumab emtansine; Tf, Transferrin; TfR, Transferrin receptor; TMZ, Temozolomide; TNBC, Triple-negative breast cancer; Trop-2, Trophoblast cell-surface antigen 2; VEGF, Vascular endothelial growth factor.

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https://doi.org/10.1016/j.jconrel.2020.10.003
Received 9 July 2020; Received in revised form 13 September 2020; Accepted 1 October 2020
Available online 3 October 2020

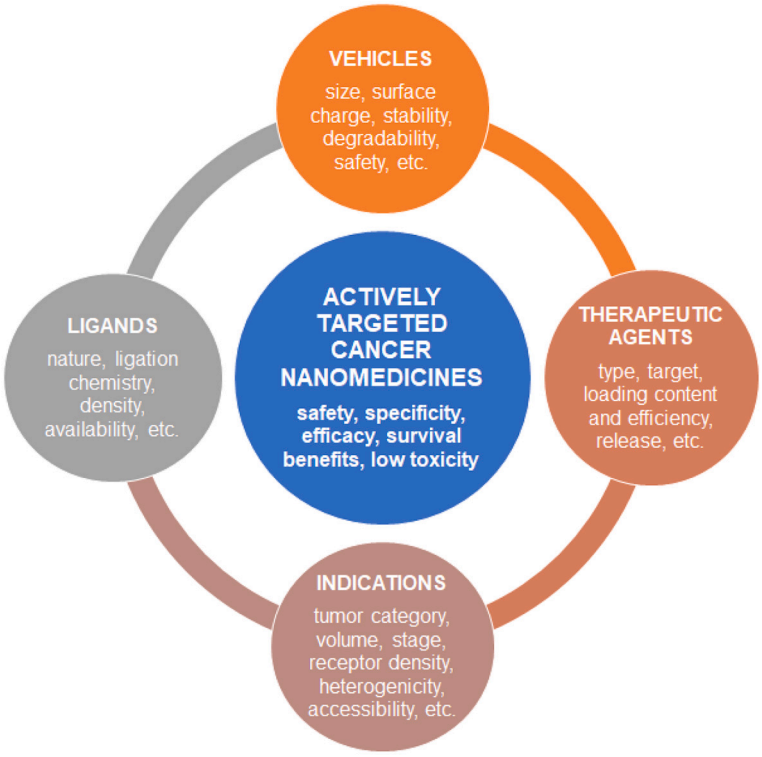
1. Introduction

The concept of targeted nanomedicines for cancer therapy, inspired by missiles that accurately hit and destroy the aimed objectives, has attracted overwhelming interest from the academia, to healthcare industry and the public [1,2]. It was hoped that vehicles would carry and specifically deliver anticancer agents into the tumor cells, just like transferrin transporting iron into the needed cells in human being [3]. This vehicle-mediated, enhanced and tumor-selective delivery was anticipated to not only boost the therapeutic efficacy but also minimize the off-target effects, which are often dose-limiting, of highly potent chemo drugs [4,5]. In contrast to chemo drugs, a majority of biopharmaceuticals such as nucleic acids (siRNA, miRNA, mRNA, DNA, CRISPR/Cas9, etc.), proteins and peptides that take explicit effects intracellularly possess poor cell membrane permeability and are subject to degradation *in vivo*, refraining them from direct application [6,7]. The clinical translation of these biopharmaceuticals relies genuinely on the advancement of safe and targeted vehicles that are capable of protecting them from degradation and selectively releasing them into tumor cells [8]. The development of targeted nanomedicines has been an utmost focus of research across different fields including materials science, nanotechnology, biotechnology, pharmaceutics, and clinical medicine [9,10].

The immense interest in targeted nanomedicines also stems from the fact that they possibly provide imminent treatments for intractable brain tumors, multi-drug resistant (MDR) tumors, metastatic tumors and relapsed tumors, which constitute the most formidable clinical challenges, by transporting therapeutic agents across the blood-brain barriers (BBB), evading the MDR pathways, inhibiting migratory tumor cells, and eliminating the cancer stem cells, respectively [11–13]. The past decade has witnessed exciting proof-of-concept results using targeted systems in different tumor models [14,15]. Interestingly, quite a few antibody-drug conjugates (ADCs) such as ado-trastuzumab emtansine (T-DM1), sacituzumab govitecan (IMMU-132), gemtuzumab ozogamicin (GO) and inotuzumab ozogamicin (InO) are used in the clinics for targeted treatment of patients with refractory/metastatic breast tumors and leukemia [16,17]. Several targeted nanomedicines such as BIND-014 (prostate-specific membrane antigen-targeted docetaxel nanoparticle) [18,19], CALAA-01 (transferrin-decorated siRNA nanotherapeutics) [20], and SGT-53 (anti-transferrin receptor single-chain antibody Fv (scFv) fragment-functionalized cationic liposome complexes of wild-type p53 plasmid) [21] were or are under phase I-II clinical investigations for treating various advanced solid tumors. These early clinical data have demonstrated the potentials of targeted nanomedicines in treating advanced solid and hematological malignancies.

It has to be noted that the performance of targeted cancer nanomedicines is critically dependent on the vehicles' properties (size, surface charge, stability, degradability, safety, etc.), ligands (nature, ligation chemistry, density, availability, etc.), therapeutic agents (type, target, loading content and efficiency, release, etc.) and indications (tumor category, volume, stage, receptor density, heterogenicity, accessibility, etc.) that interplay intimately with one another (Scheme 1) [4,22–24]. However, most of the preclinical and clinical studies did not give a full consideration of all these important factors, which partly explains why the reported targeted cancer nanomedicines have not yet met the clinical expectations. For instance, considering their good safety, poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been one of the most preferred vehicles for lipophilic anticancer drugs. However, PLA and PLGA nanoformulations are associated with inadequate stability, drug leakage, and fractional drug release, which would greatly reduce their targetability and efficacy [25–27]. The unmet endpoints of BIND-014 in its first clinical assessments might further be ascribed to the wrong choice of indications and lack of patient screening [19]. It is a common practice to screen suitable patients prior to administration of molecularly targeted drugs and antibody-conjugated formulations.

We are aware that there has been serious doubt about cancer nanomedicines [28,29] and polymeric cancer therapeutics [30–32].This open debate about cancer nanomedicines is very important and helpful to the field. It is, however, worthwhile to clarify that a majority of



Scheme 1. The clinical performance of actively targeted cancer nanomedicines is intimately dependent on the interplay between vehicles, ligands, therapeutic agents and indications.

nanomedicines reported to date are in fact not strict "targeted nanomedicines", which from their initial definition and purposes shall be safe, stable, long-circulating, tumor cell selective, and have specific receptor-mediated cell uptake and rapid release of therapeutic agents into the target cells. The so-called passively targeted nanomedicines relying on enhanced permeability and retention (EPR) effect in solid tumors cannot discriminate the cancer cells from the healthy cells [33], departing from the notion of targeted therapy. Let alone, there have been also questions regarding whether there is EPR effect and to what extent in the tumors of patients [34].

In this review, we highlight the cutting-edge design and construction of conceptually interesting and clinically viable actively targeted cancer nanomedicines. It should be stressed that in a strict sense, many of them also do not fully comply with the standards of "targeted nanomedicines". For example, in many cases, reported systems though equipped with a tumor specific ligand reveal poor stability, drug leakage in circulation, or do not readily release anticancer agents after internalization into cancer cells. In many other cases, the ligands have only moderate specificity and tumor cell affinity. Herein, the pros and cons of different actively targeted cancer nanomedicines containing lipophilic or hydrophilic small molecular drugs, nucleic acid drugs, as well as protein and peptide drugs, will be discussed. In the end, we give the conclusion and personal perspectives on future developments and clinical translation of actively targeted cancer nanomedicines.

2. Actively targeted lipophilic chemotherapeutic nanomedicines

The major chemical drugs used in the clinical settings e.g. paclitaxel (PTX) and docetaxel (DTX) are poorly water soluble and require formulating with solubilizers such as cremophor EL/ethanol mixture and tween 80, which give rise to moderate efficacy with pronounced adverse effects [35]. The past decade has witnessed rapid development of actively targeted nanomedicines containing diverse lipophilic small molecular drugs for treating different tumor models [2,16]. Today, the simplest and most successful targeted formulation is ADCs in which monoclonal antibody acts as both vehicle and targeting ligand [16,17]. The representative actively targeted pharmaceutic nanomedicines used in the clinics or in the clinical trials were summarized in Table 1.

Ado-trastuzumab emtansine (T-DM1), an anti-human epidermal growth factor receptor 2 (HER2) antibody-maytansinoid conjugate, was approved for refractory HER2-positive metastatic breast cancer patients in 2013 (Table 1) [36]. T-DM1 demonstrated also positive results in a phase II study in HER2-mutant lung cancer patients [37]. The phase II/III investigations in previously treated HER2-positive advanced gastric cancer patients concluded, however, no better than taxane in efficacy and toxicity profiles [38]. DM1 is a lipophilic, extremely cytotoxic microtubule-disrupting agent that is not applicable in itself. DM1 was conjugated to ado-trastuzumab via a stable thiosuccinimide bond using heterobifunctional succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate as a linker [39]. HER2-targeted delivery of DM1 imposed by T-DM1 evidently increased its tumor specificity, anti-tumor efficacy, and tolerability. Despite a reduced systemic toxicity over free DM1, T-DM1 holds a very narrow therapeutic window and is clinically applied at its maximum-tolerated dose (MTD) of 3.6 mg/kg [40]. T-DM1 has an average drug-antibody ratio (DAR) of 3.5, which corresponds to a low DM1 content of about 1.75 wt.% [41]. The analyses of T-DM1 further revealed a broad distribution of DAR from 0 to 8. T-DM1 with DAR of 0-2 would present low anti-tumor potency, while high DAR of 6-8 might form aggregates as a result of the highly lipophilic nature of DM1, reduce their HER2 affinity and accelerate clearance from circulation [42]. The HER2-targetability, efficacy and tolerability of ado-trastuzumab-maytansinoid conjugate might be greatly improved by site specific conjugation and precise control of DAR.

Sacituzumab govitecan (IMMU-132), an anti-trophoblast cell-surface antigen 2 (Trop-2) monoclonal antibody-SN-38 conjugate incorporating a hydrophilic, acid-cleavable CL2A linker, was granted a "breakthrough therapy" for previously treated metastatic triple-negative breast cancer (TNBC) patients in 2016 (Table 1) [43]. Trop-2 overexpresses in many epithelial cancers including a majority of TNBC tumors. SN-38, a moderately cytotoxic topoisomerase I inhibitor, was conjugated to mildly reduced anti-Trop-2 antibody (containing 8 thiol groups per antibody molecule) with a controlled and high DAR of 7.6 (Fig. 1) [52–54]. This site-specific conjugation and hydrophilic nature of linker afforded IMMU-132 with good aqueous solubility and serum stability and maintained Trop-2 binding ability. The DAR of IMMU-132 played a decisive role in treating subcutaneous NCI-N87 gastric carcinoma. Of note, SN-38 was not only released in the tumor cells following Trop-2-mediated uptake but also gradually released in the acidic tumor extracellular milieu thereby effectively eliminating both Trop-2-positive tumor cells and adjacent bystander cells [55]. The clinical investigations evidenced that IMMU-132 conferred significantly improved therapeutic index, less adverse effects (e.g. diarrhea) than irinotecan (a clinically used prodrug form of SN-38), and benefited patients with epithelial cancers including previously treated metastatic TNBC, small cell lung cancers (SCLC) [56] and non-small cell lung cancers (NSCLC) [57]. The recently updated clinical results demonstrated a response rate of 33% for heavily pretreated TNBC patients [58]. It should be noted that given the moderate potency, IMMU-132 was typically administered at a comparably high dose (10 mg/kg) and frequency (day 1 and 8 of 21-day cycles) [59]. The multi-step conjugation chemistry of IMMU-132 also poses significant issues on manufacturing and quality controls.

Gemtuzumab ozogamicin (GO, Mylotarg), an anti-CD33 antibody-calicheamicin conjugate, was the first approved ADC in 2000 for relapsed CD33-positive acute myeloid leukemia (AML) patients with an age of 60 and older [60]. CD33 overexpresses in the AML cells of majority patients but not on pluripotent stem cells. Calicheamicin is an extremely cytotoxic DNA-binding antibiotic with a half-maximal inhibitory concentration (IC_{50}) in ng/mL range. GO was obtained with an acid-cleavable hydrazone linkage and a stabilized disulfide bond by conjugating semi-synthetic calicheamicin to the lysine amine groups in gemtuzumab. GO would hydrolyze in the acidic endo/lysosomal compartments to release calicheamicin prodrug and further reduce to native

Table 1
A list of representative actively targeted pharmaceutic nanomedicines used in the clinics or in the clinical trials.

Name	Drug	Ligand/Vehicle	Target	Indication	Clinical phase	Ref.
T-DM1	Maytansinoid	Ado-trastuzumab	HER2	Metastatic breast cancer	Approved/2013	[36]
IMMU-132	SN-38	Sacituzumab	Trop-2	Metastatic TNBC	Approved/2016	[43]
GO	Calicheamicin	Gemtuzumab	CD33	AML	Approved/2001 Reapproved/2017	[44]
InO	Calicheamicin	Inotuzumab	CD22	Pre-B cell ALL	Approved/2017	[45]
DTXL-TNP	Docetaxel	ACUPA/PEG-PLA and PLGA	PMSA	Metastatic prostate cancer	Phase II	[46]
MM-302	DOX•HCl	Anti-ErbB2 (F5)-scFv antibody/ liposomes	HER2	Advanced breast tumor	Phase III	[47]
MCC-465	DOX•HCl	Anti-GAH antibody F(ab')$_2$ fragment /liposomes	GAH	Metastatic stomach tumor	Phase I	[48]
Anti-EGFR ILs-DOX	DOX•HCl	Cetuximab Fab′ fragment/liposomes	EGFR	EGFR-overexpressed advanced solid tumors	Phase I	[49]
2B3-101	DOX•HCl	GSH/liposomes	GSH	Metastatic brain tumor	Phase II	[50]
MBP-426	Oxaliplatin	Tf/liposomes	TfR	Advanced/metastatic solid tumors	Phase I	[51]

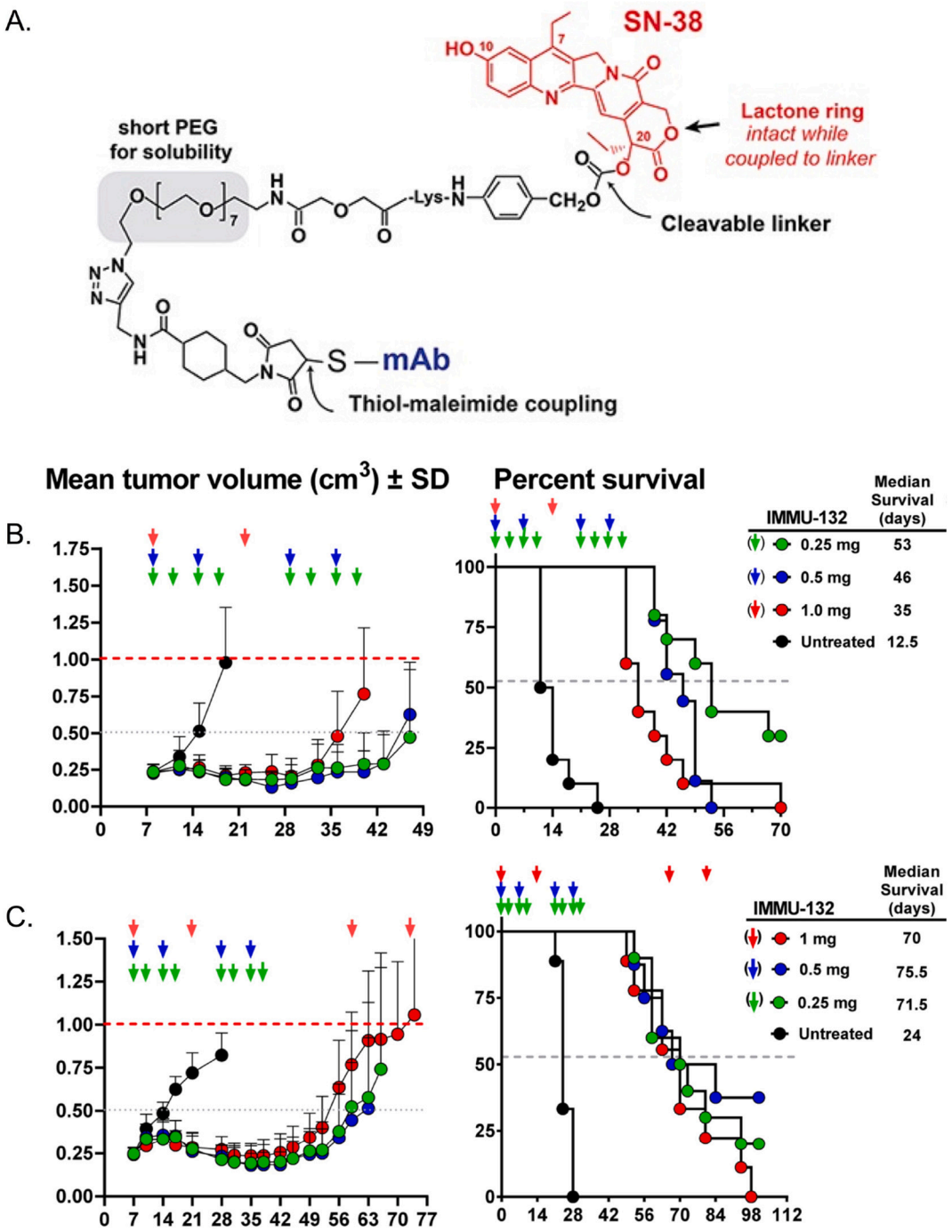


Fig. 1. A) Linker and linking chemistry of IMMU-132. B-C) Therapeutic efficacy of IMMU-132 with in subcutaneous BxPC-3 pancreatic or NCI-N87 gastric carcinoma-bearing mice. Reprinted with permission from ref. [54].

calicheamicin in the cytosols of tumor cells [61]. GO induced selective and potent cytotoxicity to HL-60 leukemic cells with IC_{50} in low to sub-pg/mL (calicheamicin equivalent) range. The confirmatory trials indicated, however, no clinical benefits and increased mortality rate of GO therapy over standard chemotherapy, leading to withdrawing of GO from the market in 2010. The retrospective analyses indicated that patient AML cells might differ greatly in their sensitivity to calicheamicin (over 100,000 times), and perhaps only patients with certain genotype could benefit from GO treatment. The clinical failure of GO is likely related to its broad DAR (ca. 50% antibody conjugated with 4-6 calicheamicin), high toxicity and narrow therapeutic window. The dosing regimen at a high dose of 9 mg/m^2 on days 1 and 14 gave significant toxicity to patients. Interestingly, obvious benefits were achieved for GO in combination with standard chemotherapy and as a monotherapy for newly diagnosed CD33-positive AML patients leading to renewed approval of GO in 2017 (Table 1) [44]. In this new regimen, GO was dosed at 3 mg/m^2 with multiple repetitions which reduced toxic side effects. This exemplifies that the clinical benefits of targeted formulations are critically dependent on the clinical design, selection of indications, dose, dosing scheme and patient screening.

In a similar way, anti-CD22 monoclonal antibody-targeted calicheamicin (inotuzumab ozogamicin, InO, DAR = 6) was developed and approved as a monotherapy for CD22-positive relapsed or refractory pre-B cell acute lymphoblastic leukemia (B-ALL) patients in 2017

(Table 1) [62]. CD22 overexpresses in most B-cell cancers such as B-ALL and B-cell non-Hodgkin lymphoma (NHL). The leukemia cells in more than 90% of ALL patients were CD22-positive [62]. InO revealed a low MTD of 1.8 mg/m^2 with thrombocytopenia as a dose-limiting toxic effect [63]. InO seemed also promising for relapsed/refractory B-cell NHL [45].

In spite of their evident tumor targeting effect, ADCs armed with highly toxic payloads would cause severe side effects beyond a certain dose. It is often the case that ADCs have a very narrow therapeutic window. The antibody-near-infrared (NIR) dye conjugates that are toxic only when bound to the target cells and irradiated with NIR light have appeared to be a more safe and efficient modality for targeted tumor therapy [64–66]. Unlike traditional ADCs, the dyes do not require to be released from the antibody to take effect. The anti-EGFR antibody-IR700-dye conjugate was reported to be most potent when anchored onto EGFR-overexpressing tumor cells while inducing little phototoxicity to non-bound cells. This photo-immunotherapy has been moved to FDA designated fast-track global Phase III clinical trial for inoperable head and neck cancer patients.

Water soluble polymer-drug conjugates (PDCs), which are analogous to ADCs, have been widely investigated for tumor therapy [67]. Compared to ADCs, PDCs have the advantages of easy preparation and increased drug content. Poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA) is a water soluble and biocompatible non-ionic polymer. PHPMA conjugated with 7.5 wt.% doxorubicin (DOX) via a lysosomally cleavable Gly-Phe-Leu-Gly tetrapeptide linkage and about 2.0 wt.% galactosamine (PK2) was developed for actively targeted liver tumor therapy. PK2 had a molecular weight (M_w) of 27.1 kg/mol and a polydispersity of 1.38. The preclinical toxicity studies revealed that PK2 had 2-3 times less acute toxicity and 5 times less cardiotoxicity than free DOX [68]. The initial clinical biodistribution investigations demonstrated about 30% drug accumulation in the hepatic region and a tumor-normal liver ratio of 1/3 in a multifocal hepatoma patient at 24 h post-injection [69]. Further studies in 31 liver tumor patients indicated that PK2 had an MTD of 160 mg DOX equiv./m^2, and delivered 16.9% ± 3.9% and 3.3% ± 5.6% of injected dose of DOX to the liver and tumor, respectively [70]. Three patients showed partial response. These data concluded that PK2 enabled liver-targeted delivery, but the healthy liver tissues had about 5 times higher DOX level than the tumor, likely due to the fact that the asialoglycoprotein receptors are highly expressed in the healthy hepatocytes. Galactose is not an optimal choice for hepatoma-targeted chemotherapy. PK2 did not continue for further clinical trials.

Prostate-specific membrane antigen (PSMA)-targeted docetaxel nanotherapeutics (DTXL-TNP, BIND-014) was developed through systematic optimization of size, surface, ligand density, drug encapsulation and release profiles from 2-(3-(5-amino-1-carboxypentyl)-ureido) pentanedioic acid (ACUPA)-functionalized PEG-PLA, PEG-PLA and PLGA copolymers (Fig. 2) [46,71]. PLA and PLGA are classical biodegradable polymers used widely in various biomedical fields. PSMA is a specific cell membrane glycoprotein overexpressed in a majority of prostate cancers as well as the neovasculatures of many advanced solid tumors such as breast, renal and lung carcinomas. The optimal DTXL-TNP had a size of 100 nm, DTX loading of 10 wt.%, and targeting density of 200 ACUPA molecules per nanoparticle. The preclinical studies revealed that DTXL-TNP afforded markedly prolonged circulation time with drug plasma levels kept 100-fold higher than DTX control over 24 hours, and significantly higher tumor accumulation and inhibition (Table 1) [46]. The first clinical results were very encouraging as efficacy was seen at

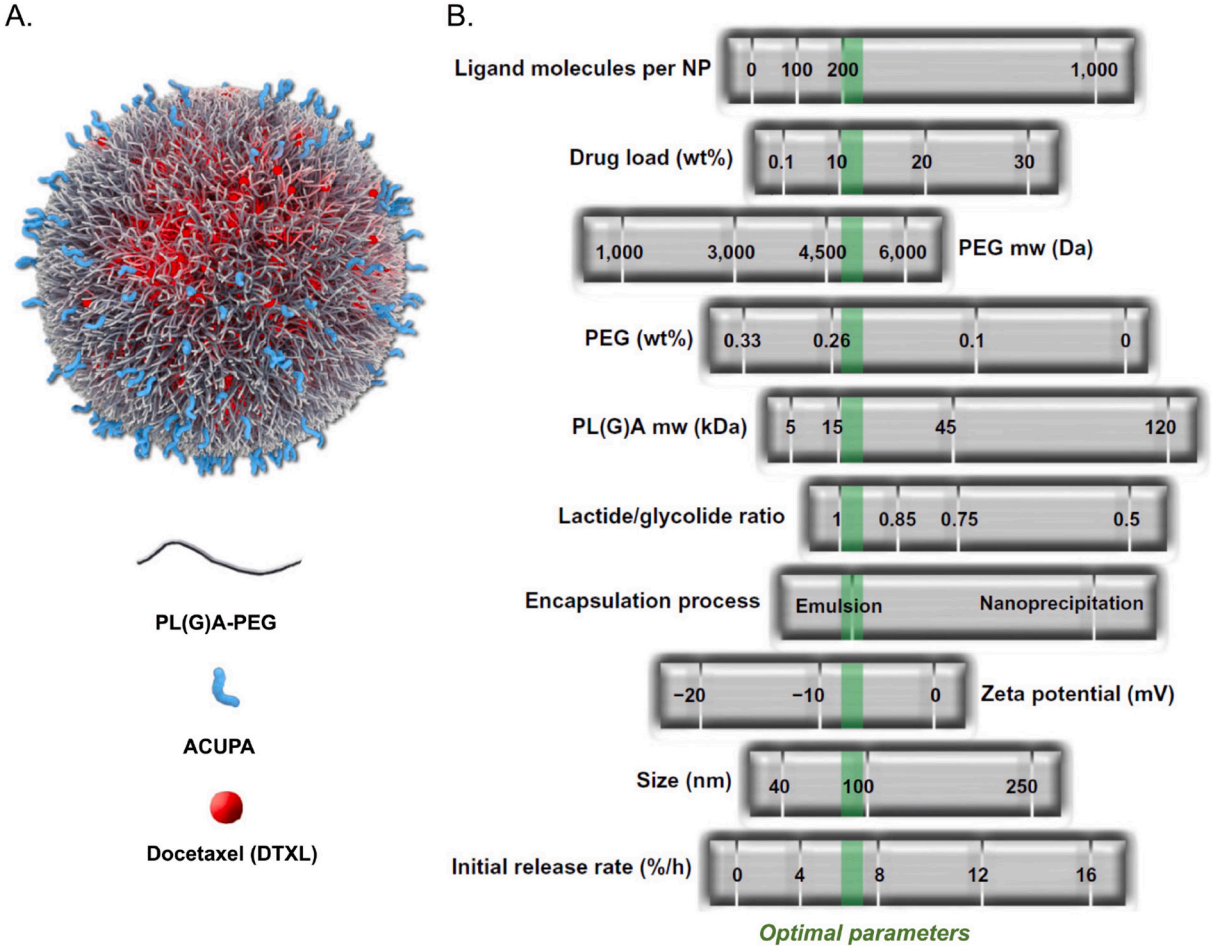


Fig. 2. A) Schematic illustration of BIND-014. B) BIND-014 with optimized parameters as indicated by the green dotted line. Adapted with permission from ref. [71].

lower doses compared to regular DTX injections [19]. The following efficacy studies in patients with advanced solid tumors including cervical, lung and head-and-neck cancers, however, did not show clinical benefits, which led to the bankruptcy of BIND Therapeutics [72]. The unmet endpoints of BIND-014 are at least partly due to wrong choice of indications and lack of patient screening. In fact, the clinically used molecularly targeted drugs and antibody-targeted formulations are prescribed only for a subset of tumor patients with particular mutations and high expression of specific antigens, respectively. It is impractical and deviates from the concept of precision therapy to apply targeted formulations to a broad range of indications. Even patients with the same type of tumor, screening is needed to increase response rate. In addition, tumor neovasculatures are likely not the best target as drugs have to be delivered to the tumor cells.

Having learned the lessons from the previous studies, the company has adjusted its clinical strategy of BIND-014 by focusing on metastatic castration-resistant prostate cancer (mCRPC) [18]. The phase I clinical evaluation recommended a regimen of 60 mg/m^2 dose and 3-week cycle [19]. The efficacy studies in 42 chemotherapy-naive mCRPC patients revealed a prostate-specific antigen response rate of 30%, preferential reduction of PSMA-positive circulating tumor cells (CTCs), and a median progression-free survival (PFS) of 9.9 months which exceeded that ($\geq$6 months) prespecified for the trial [18]. The major adverse effects were fatigue, nausea and neuropathy, indicating that BIND-014 does induce off-target toxicity. The findings that BIND-014 selectively and effectively reduced PSMA-positive CTCs validates PSMA as a target for the management of mCRPC patients. It was noted, nevertheless, that not all mCRPC express PSMA. The analyses of clinical samples revealed a low or negative PSMA expression in a portion of mCRPC patients and coexistence of both PSMA-positive and negative CTCs in another portion of patients [18]. The clinical benefits of BIND-014 might be amplified if only patients with a high and homogenous PSMA expression were selected. It should further be noted that the unmet clinical outcomes (efficacy and toxicity) of BIND-014 are perhaps also related to its inadequate stability, drug leakage, and fractional drug release in the tumor cells. Despite vast work done in optimization, BIND-014 released a significant amount of drug in hours, while insufficient release inside tumor cells due to the lack of mechanisms to facilitate drug release.

To realize high targetability and minimize toxicity, actively targeted nanomedicines ought to be steady with no or low drug leakage while immediately liberate drugs in the tumor cells. This has spurred the development of several novel strategies and targeted multifunctional systems such as acid or glutathione-activatable hyaluronic acid (HA)-drug conjugates [73], crosslinked HA nanoparticles [74], HA-PTX prodrug micelles [75], ligand-directed core-crosslinked micelles [76], and actively targeted prodrug nanoparticles [77,78]. Lipoic acid-crosslinked HA nanoparticles displayed robust DOX loading as a result of dense disulfide-crosslinking, speedy cytosolic DOX release due to glutathione-triggered cleavage of disulfide crosslinks, and evident CD44 affinity owing to presence of HA at the surface [79]. HA is a natural polysaccharide that possesses a specific binding affinity to CD44. A range of solid and hematological malignancies as well as cancer stem cells were reported to hold a high CD44 expression [80,81]. Interestingly, DOX-carrying HA nanotherapeutics exhibited a remarkable tumor accumulation of 12.71% ID/g and complete tumor repression with little adverse effects in CD44-overexpressing drug-resistant MCF-7 (MCF-7/ADR) human breast tumor model [79]. In contrast, free DOX could not inhibit MCF-7/ADR tumor growth and caused significant body weight loss. Though promising, the clinical translation of HA nanotherapeutics is challenged by the production and quality control. In another example, core-disulfide-crosslinked micelles based on biodegradable poly (ethylene glycol)-*b*-poly(ε-caprolactone) were surface-engineered with 20 mol% glioma-targeting Angiopep-2 peptide and 10 mol% cell-penetrating TAT peptide, via long and short PEG, respectively, to simultaneously enhance blood circulation, blood-brain barriers (BBB) penetration, glioblastoma cell specificity and uptake, and cytosolic release of DTX [82]. Notably, this dual-ligand core-disulfide-crosslinked micellar DTX displayed efficient glioblastoma accumulation, penetration and suppression, bringing about significantly increased survival of orthotopic U87MG human glioma-bearing nude mice. These glioma-targeting nanomedicines are potentially interesting for effective chemotherapy for glioblastoma. It should be noted, however, that all these newly designed actively targeted lipophilic pharmaceutic nanomedicines are still limited to the proof-of-concept studies. Their efficacy and in particular safety need to be systemically investigated prior to moving forward to clinical trials.

3. Actively targeted hydrophilic chemotherapeutic nanomedicines

Unlike lipophilic drugs that require a solubilizer or vehicle in clinical applications, hydrophilic agents are directly druggable. Hydrophilic drugs, however, usually display quick excretion, inferior efficacy and toxicity profiles. One typical example is doxorubicin hydrochloride (DOX·HCl) which has severe cardiotoxicity. Interestingly, DOX·HCl loading in the interior of liposomes and particularly PEGylated liposomes exhibited prolonged circulation time, enhanced tolerability, reduced cardiotoxicity and increased therapeutic index, which has led to the approval of first nanomedicines (Myocet, Doxil, Caelyx) for tumor therapy [83]. Liposomal DOX·HCl induced, however, only moderate improvement in therapeutic efficacy partly due to the lack of tumor cell selectivity [84]. In the past two decades, enormous endeavor has been directed to development of actively targeted liposomal DOX·HCl by decorating its surface with varying specific ligands such as antibodies, antibody fragments, small biomolecules, and peptides [85]. DOX·HCl could be efficiently loaded into liposomes via ammonium sulfate or pH gradient technique [86]. Importantly, several actively targeted PEGylated liposomal DOX·HCl formulations such as MM-302, MCC-465, Anti-epidermal growth factor receptor (EGFR) immunoliposomes (ILs)-DOX, and 2B3-101 have made progress to human clinical trials.

MM-302 is a HER2-targeted liposomal DOX·HCl designed for HER2-positive advanced breast tumor patients [47,87]. MM-302 containing 45 anti-ErbB2 (F5)-scFv antibodies per liposome was obtained by inserting anti-ErbB2-scFv-PEG-DSPE conjugate into PEGylated liposomal DOX·HCl (Table 1) [88]. Interestingly, MM-302 had little or no interference with cardiomyocyte cell function and HER2 signaling. The preclinical and clinical studies in HER2-positive breast and gastric tumor models showed that combination of MM-302 and trastuzumab enhanced tumor deposition and DNA damage leading to synergistic antitumor effect (Fig. 3) [87,89]. MM-302 and trastuzumab bound to HER2 extracellular subdomain I and IV, respectively and were found colocalized in BT474-M3 human breast xenografts in NCR/nu mice. The clinical trial in advanced HER2-positive breast cancer patients concluded that MM-302 at a dose of 30 mg/m^2 and higher, alone or in combination with 6 mg/kg trastuzumab, at a 3-week cycle caused little cardiotoxicity and afforded favorable safety (MTD $>$ 50 mg/m^2) and clinical activity (overall response rate (ORR) = 13%, median PFS = 7.4 months). ORR of 28.0% and median PFS of 10.9 months were achieved for cases of anthracycline-naïve patients [87]. The tumor tissue analysis confirmed the HER2-targeting effect. Positron emission tomography-computed tomography imaging using ^{64}Cu-labeled MM-302 indicated substantial deposition in liver, spleen and tumor (including bone and brain metastases) uptake of 0.52-18.5 %ID/kg at 24-48 h post-injection. Interestingly, tumor uptake of ^{64}Cu-MM-302 correlated favorably with treatment efficacy [90]. These results signified that MM-302 has a potential for treating metastatic HER2-positive breast cancer patients with bone or brain metastases.

MCC-465 is an anti-GAH antibody F(ab')$_2$ fragment-functionalized PEGylated liposomal DOX·HCl developed for metastatic stomach tumors (Table 1) [48]. GAH is a human monoclonal antibody that positively reacts to more than 90% of gastric cancer tissues. MCC-465 was fabricated with an average diameter of 143 nm and an anti-GAH-F

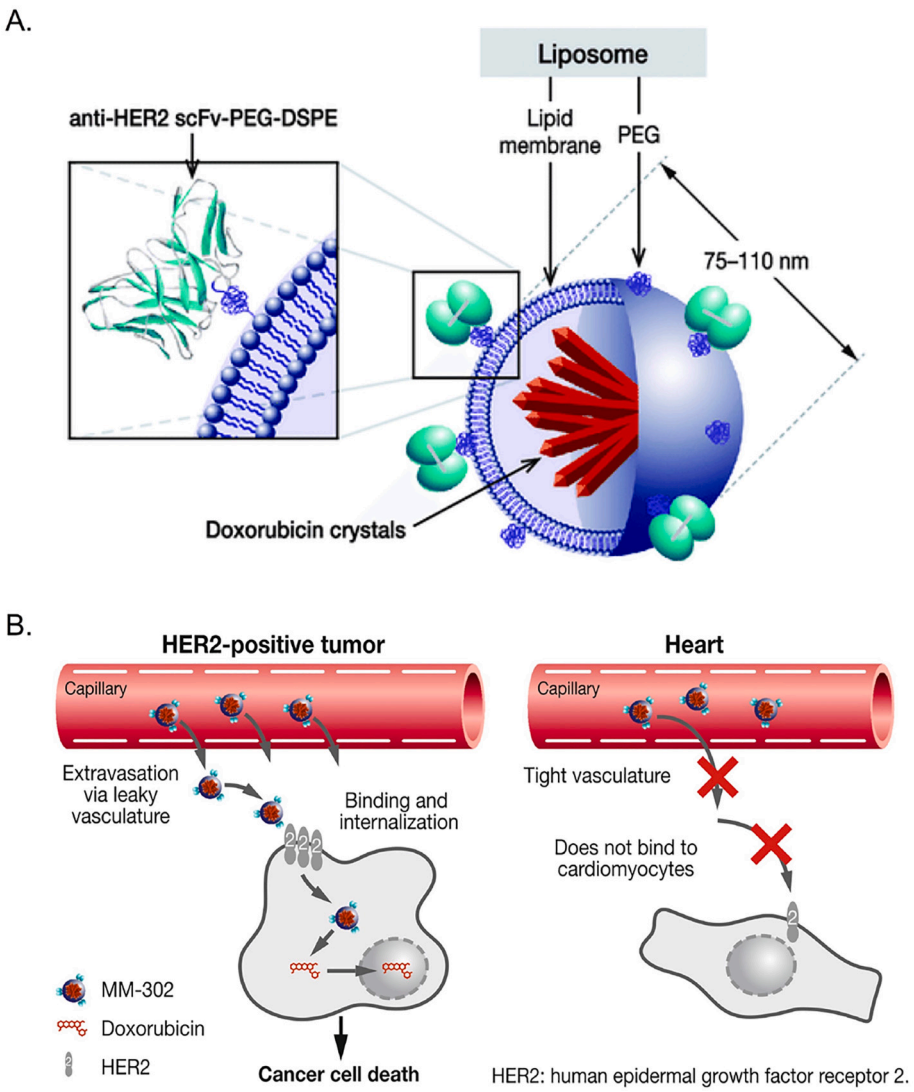


Fig. 3. A) Schematic illustration of MM-302. B) MM-302 remains in circulation for long periods of time, providing an opportunity to accumulate in tumors via leaky vasculature. Reprinted with permission from ref. [89].

$(ab')_2$/PEG(5 kDa) weight ratio of 1/4 by conjugating thiolated anti-GAH-F$(ab')_2$ and PEG to liposomes via thioether linkage [91]. MCC-465 displayed evident specificity and notably enhanced antitumor potency in GAH-positive B37 gastric cancer cells *in vitro* and *in vivo* compared with regular PEGylated liposomal DOX·HCl. The trials of MCC-465 in metastatic/recurrent gastric tumor patients demonstrated a similar pharmacokinetics to Doxil, absence of skin- and cardio-toxicity, an MTD of 45.5 mg/m^2 with myelosuppression and appetite loss as the dose-limiting toxicities, and stable disease in 10 out of 18 evaluated patients. MCC-465 was concluded with favorable safety and a dosing scheme of 32.5 mg/m^2 every 3 weeks was recommended for further efficacy studies.

Anti-EGFR ILs-DOX was manufactured by linking the Fab′ fragments of cetuximab to commercial PEGylated liposomal DOX·HCl (Caelyx) for treatment of EGFR-overexpressing advanced solid tumors (Table 1) [49]. In spite of similar tumor deposition, anti-EGFR ILs-DOX was far more efficiently internalized in EGFR-overexpressing tumor cells than regular liposomal DOX·HCl (92% *versus* <5%), significantly enhancing the antitumor potency [92,93]. Interestingly, anti-EGFR ILs-DOX greatly enhanced intracellular DOX delivery to EGFR-overexpressing multidrug-resistant tumor cells, producing one-to-two magnitudes higher antitumor activity than free DOX *in vitro*, and superior treatment of multidrug-resistant MDA-MB-231 Vb100 tumor xenografts *in vivo* [94]. The human trials of anti-EGFR ILs-DOX observed an MTD of 50 mg/m^2 (DOX equivalent), no cardiotoxicity or cumulative toxicity, and obvious clinical activity (out of 26 patients, 1 complete and 1 partial response, 10 stable disease) in EGFR-overexpressing advanced solid tumor patients that were not amenable to standard therapy [49]. Anti-EGFR ILs-DOX was recommended for phase II trials at 50 mg DOX·HCl per m^2.

2B3-101 is a glutathione (GSH)-tagged PEGylated liposomal DOX·HCl designated for enhanced chemotherapy of primary or metastatic brain tumors (Table 1) [50]. GSH is an endogenous tripeptide that is able to cross the BBB via the GSH transporters. 2B3-101 was prepared with a mean diameter of 95 nm through introducing GSH-PEG-DSPE (5 wt.% of total lipids) to liposomes followed by loading DOX·HCl via ammonium sulfate gradient method. The measurement of DOX concentrations in the brain at 5 h post-injection showed that 2B3-101 had 4.8 times higher brain-to-blood ratio of DOX than regular PEGylated liposomal DOX·HCl [95]. The preclinical studies in U87 MG-Luc human glioblastoma model in athymic FVB mice demonstrated good toleration and significantly better tumor repression of 2B3-101 than regular PEGylated liposomal DOX·HCl, with either once or twice-weekly dosing of 5 mg DOX·HCl equiv./kg [50]. 2B3-101 led to 38.5% and 16.1% increase of median survival time over saline and regular PEGylated liposomal DOX·HCl, respectively. The clinical studies in patients with metastatic brain tumors and recurrent gliomas certified its good safety

up to 70 mg/m^2 and antitumor activity at 40 mg/m^2 or higher doses, in which 12 out of 16 patients showed stable diseases. Phase II clinical trials were being carried out at 50 mg/m^2 3-week cycle and 60 mg/m^2 4-week cycle for patients with brain metastases and recurrent malignant glioma, respectively [96]. 2B3-101 is one of the very few nano-formulations advanced to clinical trials for brain tumor therapy. 2B3-101 is unique in that it exploits a small endogenous biomolecule to enhance drug delivery to brain. It should be noted, however, that 2B3-101 is not glioma-specific and might cause brain toxicity.

MBP-426 is a human transferrin (Tf)-conjugated oxaliplatin liposome formulation developed for Tf receptor (TfR)-overexpressing advanced/metastatic solid tumors (Table 1) [51]. Tf is a natural protein that transports iron to mammalian cells. TfR highly expresses in various cancer cells and Tf functionalization was shown to greatly enhance cancer cell uptake of different drugs [97]. Tf-tagged oxaliplatin liposome formulation revealed prolonged circulation time and decreased partitioning to erythrocytes compared with free oxaliplatin, and kept a high tumor drug level for over 72 h post-injection, resulting in more effective tumor inhibition than non-targeted liposomal formulation and free oxaliplatin at a dose of 5 mg/kg [98]. The clinical investigations in advanced or metastatic refractory solid tumor patients revealed an advantageous pharmacokinetics over oxaliplatin, favorable safety profile with thrombocytopenia as a main dose-limiting toxicity, and 15 patients with stable disease after two treatments [51]. A dose of 226 mg/m^2 was recommended for phase II trials.

MM-302, MCC-465, and anti-EGFR ILs-DOX have employed scFv antibody or antibody fragments to decorate the surface of liposomes to achieve tumor-selective delivery of DOX·HCl (Table 1). The scFv antibody and antibody fragments are big and often encounter manufacture hurdles. Another potential issue with scFv antibody and antibody fragments is their comparably low stability relative to monoclonal antibody. Increasing attention has been paid to the development of peptide-targeted nanomedicines in that peptides are small, stable, easy to fabricate, and amenable to different conjugation chemistry [99]. Various peptide-functionalized liposomal DOX·HCl formulations were designed and explored by different groups for the targeted treatment of diverse malignancies including metastatic, drug-resistant, and inaccessible tumors. For instance, glioma-targeted chemotherapy was enabled by introducing peptides like cyclic RGD, angiopep-2, T7, and Lyp-1 peptides, which could not only increase BBB penetration but also specifically bind to glioma cells [13]. The anti-glioma effect could further be enhanced by co-functionalizing liposomal DOX·HCl with two peptides [100,101]. In spite of their significant advantages over antibody- and antibody fragment-targeted formulations, peptide-guided liposomal DOX·HCl has not advanced to the clinical evaluation. This is at least partly due to the moderate tumor specificity and affinity of peptides and several intrinsic problems of liposomal formulations such as intricate fabrication procedure, insufficient stability, and slow drug release in the tumor cells. To increase their drug release at the target sites and thereby antitumor efficacy, stimuli (e.g. thermo and pH)-sensitive liposomes were developed at the cost of further complicating their preparation.

Polymersomes self-assembled from amphiphilic copolymers with enhanced stability, facile fabrication, and tailorable surface/membrane functions have recently appeared as an alternative platform to liposomes for targeted drug delivery [102–104]. For example, tumor-targeting disulfide-crosslinked polymersomal DOX·HCl (PS-DOX) formulations were constructed with mean sizes of 50 to 100 nm from co-self-assembly of poly(ethylene glycol)-*b*-poly(trimethylene carbonate-co-dithiolane trimethylene carbonate) (PEG-P(TMC-DTC)) and peptide-functionalized PEG-P(TMC-DTC) copolymers followed by loading DOX·HCl via a pH gradient approach [105]. PS-DOX exhibited extraordinary stability due to crosslinking of the membrane and efficient cytosolic drug release owing to reduction-triggered decrosslinking. Interestingly, PS-DOX displayed over 5-fold higher MTD than liposomal DOX·HCl. PS-DOX surface modified with ATN-161, cNGQ, cRGD, and GE11 peptides did cause little or low toxic effects while markedly enhance the treatment of diverse solid malignancies including melanoma, lung, liver, and ovarian tumors compared with liposomal DOX·HCl and non-targeted PS-DOX [106–109]. In a recent study, A6 peptide (KPSSPPEE)-decorated polymersomal epirubicin hydrochloride with 11 wt.% drug and small size of 55 nm was shown to specifically bind to and kill CD44-overexpressing LP-1 multiple myeloma (MM) cells *in vitro* and in orthotopic MM model, markedly enhancing mice survival rate and depleting bone destruction [110]. Unlike peptide-targeted ones, Tf and trastuzumab-targeted polymersomal formulations were obtained by post-modification method via thiol-ether linkage for the treatment of hepatocellular carcinoma and HER2-positive breast tumor, respectively [111,112]. In spite of observed specificity, the thiol-maleimide conjugation is not optimal due to the extensive and nonspecific thiolation of ligand and gradual hydrolysis of maleimide groups [113]. There is a need to develop more robust and specific post-modification methods. Intriguingly, incubating CGGGHKYLRW (Tf-binding peptide)-decorated PS-DOX with Tf could generate Tf-bound PS-DOX that displayed marked selectivity in TfR-overexpressing HCT-116 cells and significantly better treatment efficacy in HCT-116 tumor-bearing mice than the non-targeted control [114]. This employment of Tf-binding peptide is a novel strategy to circumvent post-modification. In addition to polycarbonate copolymers, functional polypeptide and polyester copolymers have been designed and prepared to construct actively targeted polymersomal DOX·HCl for treating various forms of malignancies [115].

As compared with liposomes, polymersomes have also the advantage to separately engineer the inner and outer surfaces of the membrane (coined as chimeric polymersomes, CPs) thereby uniquely achieving stable and efficient loading of different hydrophilic and amphipathic small molecular drugs such as pemetrexed disodium (PEM), methotrexate disodium and rigosertib, which were not possible with regular systems [116–118]. For instance, lung cancer specific polymersomal PEM, which was constructed with 14.2 wt.% PEM and a size of about 60 nm from co-assembly of PEG-P(TMC-DTC) block polyethyleneimine (PEG-P(TMC-DTC)-PEI) and CSNIDARAC peptide-functionalized PEG-P(TMC-DTC) in PEM-containing aqueous solution, showed 22 and 9.1 times increase in circulation time and tumor deposition over generic PEM formulation, and potent tumor suppression and significant survival benefits in H460 tumor-bearing mice at 12.5 mg/kg (PEM equivalent) [116].

Polymersomes, closely mimicking liposomes, are of particular interest to drug delivery. The plethora of preclinical and clinical data reported for liposomal systems are valuable and form a sound basis for designing new functional polymersomes as next generation vehicles for precision cancer therapy. The recently developed actively targeted polymersomal formulations demonstrated clear advantages over liposomal counterparts in terms of fabrication, stability, targetability, toxicity, safety, drug release, and efficacy, which warrant further investigation. These functional polymersomes are based on new polymers and will require rigorous safety and toxicity examinations prior to clinical translation.

4. Actively targeted nucleic acid nanomedicines

Nucleic acid-based biopharmaceuticals such as siRNA, miRNA, mRNA, DNA and CRISPR/Cas9 are an emerging and highly specific type of anticancer drugs that take explicit effects in the cytosol or nucleus of target cells [119,120]. In contrast to chemo drugs, nucleic acid drugs possess poor cell membrane permeability and are subject to degradation *in vivo*, refraining them from direct application [121,122]. The clinical translation of nucleic acids relies genuinely on the advancement of safe and targeted vehicles that are capable of protecting them from degradation and selectively releasing them into tumor cells [123]. Viruses though are able to mediate efficient delivery of certain nucleic acids are questioned by their safety, lack of cell selectivity, and potential immune response. Non-viral vehicles with better safety, facile engineering with

specific ligands, and ease of scale-up are regarded more viable for future cancer gene therapy [124,125]. Representative actively targeted biopharmaceutic nanomedicines in clinical trials were listed in Table 2.

SGT-53 is an anti-TfR single-chain antibody Fv fragment (TfRscFv)-functionalized cationic liposome complexes of wild-type p53 plasmid developed for targeted treatment of TfR-overexpressing advanced solid tumors by restoring p53 function (Table 2) [21,129]. The p53 gene has a tumor suppressing function. Loss of p53 functionality, either due to p53 mutation or dysfunction of p53 pathway, is found in a number of human tumors including breast cancers and glioblastoma multiforme (GBM). SGT-53 was prepared through decorating maleimide-functionalized cationic liposomes with TfRscFv-SH followed by complexing with wild-type 53 plasmid. The highest expression of p53 in the tumor was found at a pDNA/lipid ratio of 1 μg/13–14 nmol [130]. Interestingly, SGT-53 could penetrate the BBB and efficiently target to GBM cells and cancer stem cells (CSCs), leading to down-modulation of O6-methylguanine-DNA methyltransferase and apoptosis of intracranial GBM xenografts as a result of wild-type 53 expression. The co-administration of SGT-53 effectively sensitized both GBM cells and CSCs to temozolomide (TMZ) therapy, resulting in improved treatment and survival of TMZ-resistant GBM-bearing mice [131]. Moreover, combination of SGT-53 and anti-PD-1 antibody greatly augmented immune therapy for mouse syngeneic GL261 tumors, leading to GBM inhibition, T-cell infiltration and substantial survival benefits. The phase I clinical trials in advanced solid tumor patients displayed that SGT-53 induced minimum adverse effects at doses of 0.2–3.6 mg pDNA and most patients had stable disease, with evident p53 expression in metastatic lesions and one patient changing the status to resectable after one treatment, supporting favorable safety and antitumor activity of SGT-53 (Fig. 4) [21]. In another clinical trial, the combination of SGT-53 at 3.6 mg pDNA and 75 mg/m^2 DTX revealed good toleration and clear anticancer activity, in which 3 out of 12 metastatic/refractory cancer patients showed partial responses, 2 displayed stable disease with substantial tumor shrinkage, and 6 out of 9 patients failed previous taxane treatment became stable [129]. The combination of SGT-53 and DTX evidently reduced the metastatic lesions at 1 month after first round of treatment [132]. The preclinical and clinical results signified that these TfR-targeted p53 nanocomplexes have tremendous potential in treating GBM as well as sensitizing immune therapy and chemotherapy for 'cold' and refractory tumors, respectively. The same TfRscFv-functionalized cationic liposome was applied for targeted delivery of plasmid RB94 (a truncated RB gene), giving a new gene nanoformulation called SGT-94 [133]. RB94 is a gene that induces strong toxic effect to cancer cells but not healthy ones following transfection. The clinical studies in metastatic genitourinary cancer patients revealed low adverse effects and evident clinical activity at 2.4 mg pDNA with one complete remission and one partial remission. The expression of RB94 was detected in the lung metastases but not in the healthy lung of one patient, confirming its tumor specificity.

CALAA-01 is a Tf-decorated siRNA nanotherapeutics developed for targeted RNA interfere (RNAi) therapy for TfR-overexpressing solid tumors (Fig. 5) [126,134]. CALAA-01 was assembled with a mean diameter of ca. 70 nm in a modular approach from linear cyclodextrin-based polycation (CDP), adamantane (AD)-terminated PEG (AD-PEG), adamantane-functionalized Tf (AD-PEG-Tf), and siRNA against the M2 subunit of ribonucleotide reductase (RRM2) [135]. The silencing of RRM2 with siRNA was shown to induce pronounced antiproliferative effect in cancer cells [136]. The clinical trials of CALAA-01 performed in patients with refractory/metastatic solid tumors at 18, 24 and 30 mg/m^2 siRNA dosing on days 1, 3, 8 and 10 of a 21-day cycle revealed that the amount of intracellular nanocomplexes in the melanoma biopsies correlated with injected dose, both RRM2 mRNA and protein expression were inhibited by CALAA-01, and mRNA fragment was produced by explicit cleavage of RRM2 mRNA. These results certify that targeted siRNA nanoformulation can exert specific gene suppression via the mechanism of RNA interference (Table 2) [126,134]. The comparison of results from mice, rats, monkeys, and humans revealed that CALAA-01 was quickly eliminated in all species. The maximum blood concentration post-injection correlated positively with the body weight of different species, and the safety profile of CALAA-01 was similar in all species, except no kidney toxicity in humans, which likely resulted from predosing hydration protocol in the human clinical trials. These observations concluded that animals are good models to estimate the performance of CALAA-01 in humans [20].

The polyion complex (PIC) micelles that are assembled from block catiomers like PEG-*b*-poly(L-lysine) (PEG-PLL) and nucleic acids are an appealing nanosystem for actively targeted gene therapy. However, approaches such as disulfide-crosslinking, hydrophobic modification, and anchoring onto gold nanoparticles (AuNP) were required to prevent their fast disassembly in the circulation [137,138]. For example, cRGD-decorated PIC micelles with a mean diameter of less than 50 nm and stabilization via disulfide-crosslinking and cholesterol were shown to significantly enhance the siRNA deposition and gene knockdown activity compared with the non-stabilized controls in the subcutaneous cervical tumors after systemic injection [139]. cRGD-surfaced AuNP-cored PIC micelles (diameter $<$ 50 nm) demonstrated obvious active tumor-targetability and significant inhibition of subcutaneous HeLa tumor when loading with therapeutic siRNA against papilloma virus-derived E6 oncogene (Fig. 6) [140]. Interestingly, glucose-decorated AuNP-cored PIC micelles displayed selective and enhanced uptake by glucose transporter 1-overexpressing CSCs in the MDA-MB-231 breast cancer spheroids and significant suppression of CSC-rich orthotopic MDA-MB-231 tumor xenografts in mice when loading with siRNA against polo-like kinase 1 (siPLK1) [141]. Aiming to conquer different barriers in systemic siRNA delivery, increasingly more sophisticated constructs such as GE11 peptide-installed pH/redox dual-sensitive cationic unimolecular nanoparticle [142], folate-functionalized siRNA lipo-oligocation polyplexes [143], tumor-targeting aptamer- or folate-displaying exosomes [144,145], and anisamide-functionalized cationic liposomes wrapping polymetformin/HA-siRNA nanocomplex [146], were prepared and explored for targeted cancer RNAi therapy.

The actively targeted polymersomes with features closely mimicking viruses have recently been developed as a more safe, robust and efficient non-viral vehicles for systemic delivery of nucleic acids [147–149]. The short PEI or spermine as inner shell of CPs allowed complete and tight loading of siRNA at an N/P ratio of 1/1 or lower. Notably, cNGQ peptide-decorated CPs appeared to efficiently deliver siPLK1 to an orthotopic model of $\alpha_3\beta_1$-integrin-overexpressing A549 human lung tumor, leading to effective tumor retardation and significant survival benefits [150]. The angiopep-2 peptide-functionalized CPs further showed improved BBB penetration and targeted RNAi therapy for orthotropic U-87 MG GBM in mice through a low-density lipoprotein receptor-related protein-1 (LRP-1)-mediated pathway [151]. Lung cancer-selective cell penetrating peptide (CPP33)-installed CPs based on

Table 2
Representative actively targeted biopharmaceutic nanomedicines in clinical trials.

Name	Drug	Ligand/Vehicle	Target	Indication	Clinical phase	Ref.
SGT-53	Wild-type p53 DNA	TfRscFv/liposome complexes	TfR	Advanced solid tumors	Phase II	[21]
CALAA-01	siRNA against ribonucleotide reductase M2	Tf/polymeric NP	TfR	TfR-overexpressed solid tumors	Phase I	[126]
SGN-35	MMAE	cAC10 antibody	CD30	Relapsed/refractory Hodgkin's lymphoma	Approved/2011	[127]
Tf-CRM107	CRM107	Tf	TfR	Malignant brain tumor	Phase II	[128]

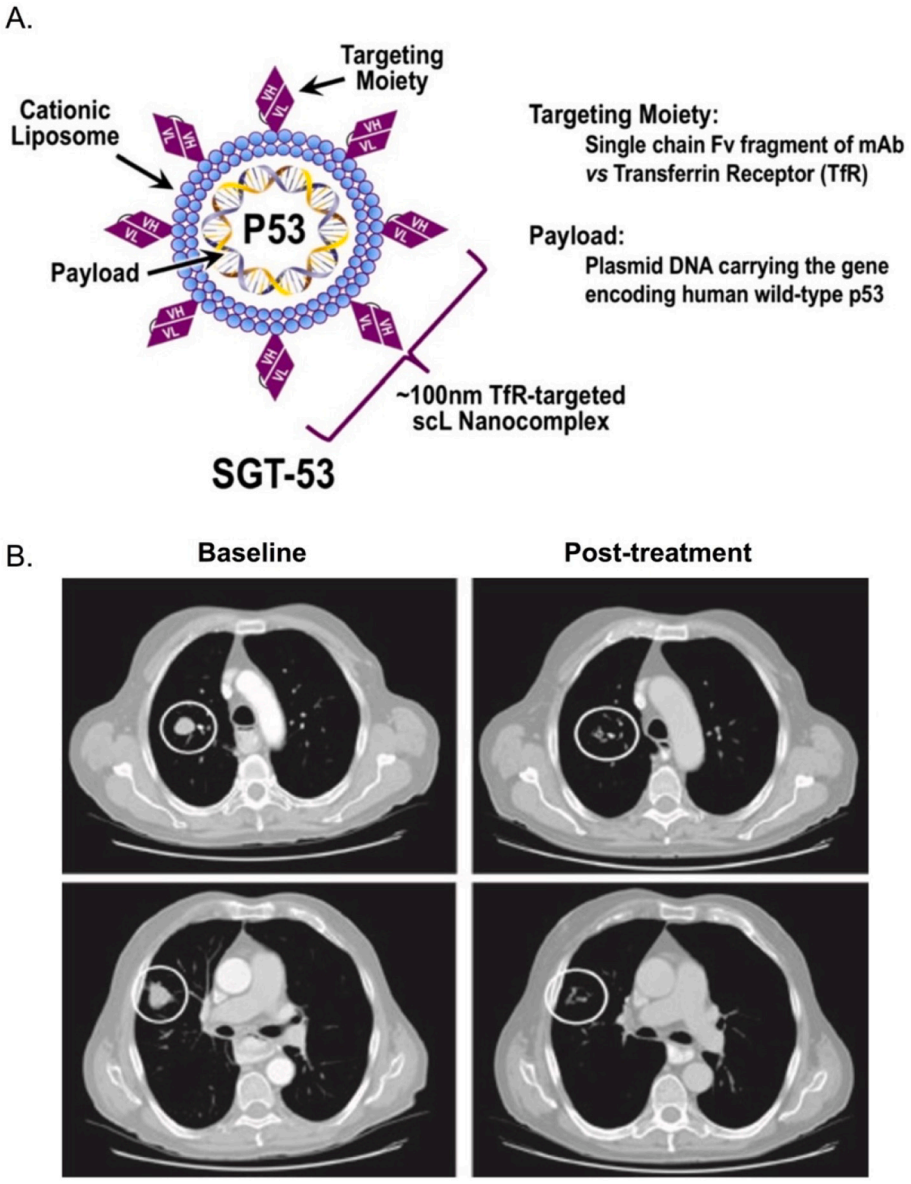


Fig. 4. A) Schematic illustration of SGT-53. B) The metastatic lesions (circled area) were evidently reduced at one month after first round of treatment with SGT-53 and DTX. Reprinted with permission from ref. [129, 132].

poly(ethylene glycol)-*b*-poly(α-aminopalmitic acid)-*b*-poly(L-lysine) triblock copolymer displayed efficient encapsulation and delivery of siPLK1 to the orthotopic A549 lung tumor xenografts in nude mice, leading to effective tumor repression and increased survival rate [152]. The CPs as envelope-type vehicle are robust and allow facile surface engineering with diverse ligands via either pre- or post-modification, which can be tailor-made for targeted gene therapy of diverse malignancies.

Unlike siRNA, miRNA though having a similar length plays multi-faceted roles in cancer cells via diverse mechanisms and possibly causes vastly different effects on cancer treatment depending on its cellular concentrations. The miRNA-based tumor therapy might be enforced by introducing miRNA to restore its function or anti-miRNA to suppress its overactivity in the target cells [153]. Oncogenic receptor tyrosine kinase Axl-specific aptamer-conjugated let-7g miRNA was reported to selectively silence let-7g genes and effectively retard the Axl-expressing lung adenocarcinoma models [154]. Interestingly, a simple conjugate of folate and miR-34a was shown to increase the delivery of miR-34a to folate receptor-overexpressing lung and breast tumors in mice, resulting in reduction of tumor volume [155]. The mucin1 aptamer-decorated hybrid nanoparticles augmented the release of miRNA-29b to target NSCLC cancer cells, leading to selective downmodulation of oncoprotein DNMT3B and suppression of lung tumor *in vivo* [156]. Galactose-functionalized pH and reduction dual-sensitive hybrid polypeptide nanoparticles and mannose-decorated lipid-coated calcium phosphonate nanovehicles wrapped by a pH-sensitive stealthy layer were developed for targeted delivery of miR155 into tumor associated macrophages (TAMs), which led to effective repolarizing of immunosuppressive TAMs to M1 macrophages and suppression of tumor growth [157,158]. CD133 specific aptamer-guided RNA nanoparticles carrying anti-miRNA21 were shown to be selectively internalized by breast CSCs and TNBC cells, which led to effective inhibition of miR21 expression and cell migration *in vitro* and repression of TNBC tumor progression *in vivo* [159].

Gene editing with CRISPR/Cas9 has recently emerged as a powerful tool for tumor therapy [160,161]. In contrast to siRNA and miRNA, CRISPR/Cas9 system is huge and needs to be transported and released into the nuclei of target cells, which sets immense barriers to reach its

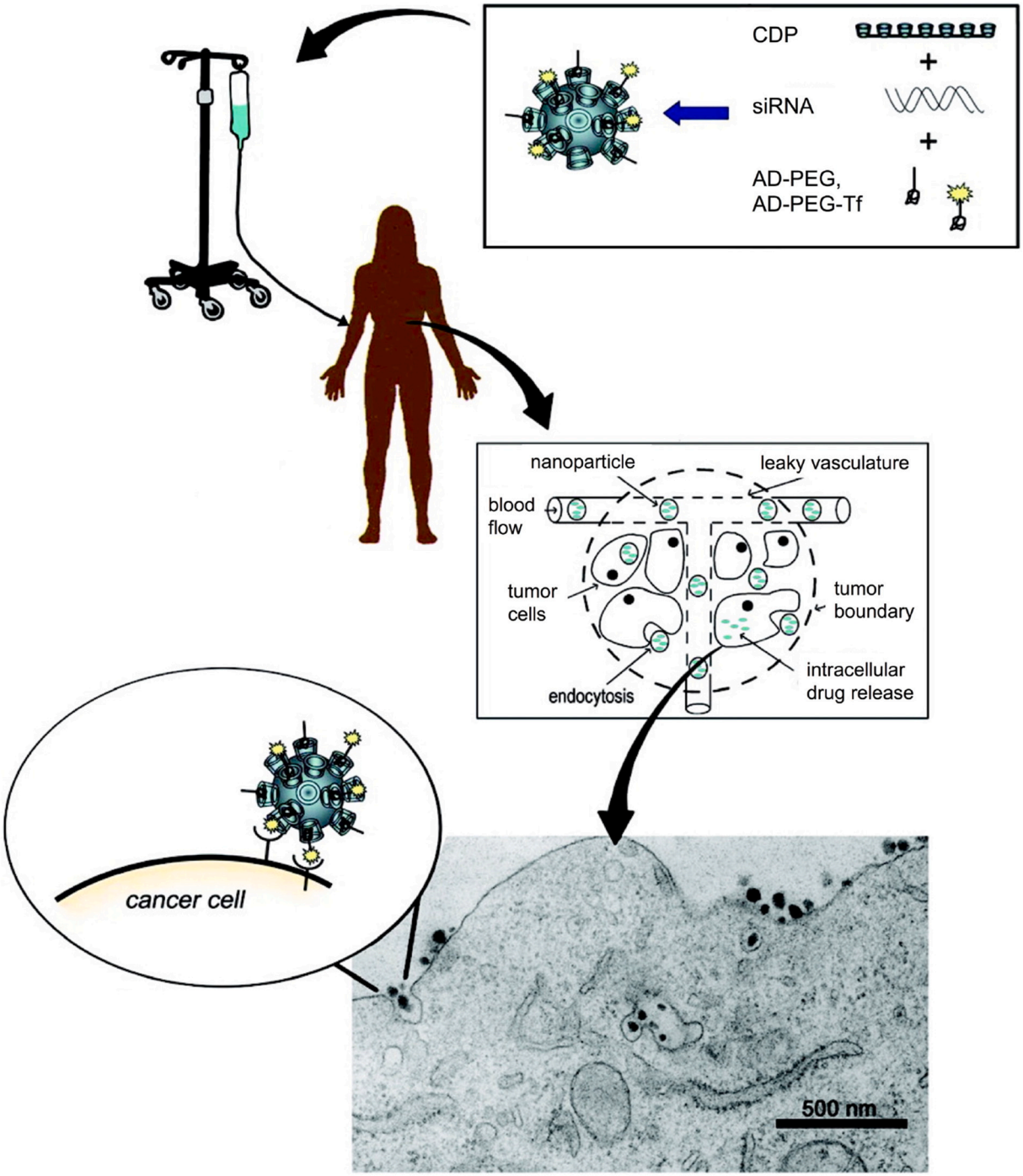


Fig. 5. Schematic of the preparation and function mechanism of CALAA-01. Adapted with permission from ref. [134].

therapeutic potential. The development of safe and highly selective nanovehicles for systemic delivery of CRISPR/Cas9 has become an immediate task, though several non-specific vehicles did show ability to deliver CRISPR/Cas9 to tumor xenografts *in vivo* [162,163]. The cancer-derived exosomes were reported to effectively deliver CRISPR/Cas9 to SKOV3 ovarian tumor xenografts in mice assumably via a cell tropism mechanism, leading to suppression of poly(ADP-ribose) polymerase-1, cell apoptosis, and cisplatin sensitization [164]. The MCF-7 cancer cell membrane-coated zeolitic imidazolate frameworks displayed cell-type-specific delivery of CRISPR/Cas9 affording enhanced inhibition of EGFP expression in MCF-7 cells [165]. AS1411 aptamer-targeted multifunctional CRISPR/Cas9 nanoformulation was constructed by decorating protamine sulfate/CRISPR/Cas9 plasmid (for CDK11 knockout) nanocomplexes with carboxymethyl chitosan conjugated with AS1411 aptamer and endosome-disrupting KALA peptide, and revealed notable repression of CDK11 expression, downregulation of matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) proteins, and upregulation of p53 protein in cancer cells [166]. The nanocomplexes of LC09 aptamer-functionalized PEG-PEI-cholesterol lipopolymer and CRISPR/Cas9 plasmids encoding VEGFA gRNA and Cas9 demonstrated selective and enhanced deposition of CRISPR/Cas9 in orthotopic osteosarcoma as well as lung metastasis, resulting in effective reduction of VEGFA expression, tumor angiogenesis and growth, lung metastasis, and bone lesion [167]. R8-dGR

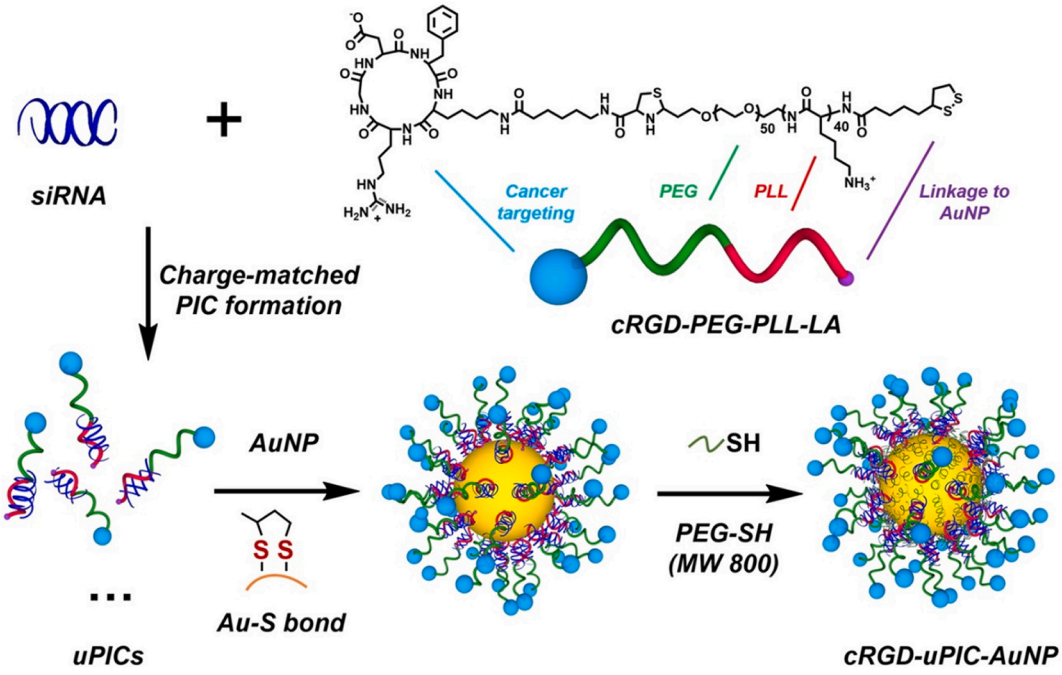


Fig. 6. Schematic illustration of cRGD-uPIC-AuNP preparation. Reprinted with permission from ref. [140].

peptide-functionalized cationic liposomes co-encapsulating hypoxia-inducible factor-1α (HIF-1α) sgRNA/Cas9 plasmids and PTX showed targeted delivery to pancreatic cancer cells, resulting in downregulation of HIF-1α expression as well as VEGF and MMP-9 proteins, which in turn increased the anticancer and anti-metastatic effects of PTX [168]. The results from the targeted CRISPR/Cas9 delivery have proven the concept that gene editing provides an effective treatment modality for tumors by silencing the oncogenes, modulating the tumor microenvironments, sensitizing chemotherapy, and/or boosting anticancer immunity. The clinical translation of CRISPR/Cas9, however, requires the development of more safe, specific and efficient delivery systems.

The non-viral delivery of mRNA especially mRNA vaccines is an increasingly focused area for tumor therapy [124]. Though direct injection of mRNA antigen was capable of eliciting specific cancer immunity and (pre)clinical activity, the efficacy was low [169]. The development of vehicles targeting to antigen-presenting cells, in particular dendritic cells (DCs), is of importance to mRNA vaccines because they would boost vaccine potency and specificity, reduce mRNA dose, and increase safety. The big size of mRNA (300–5000 kDa), however, poses greater challenge than siRNA and miRNA (~14 kDa). Given the fact that DCs overexpress mannose receptor [170], several mannose-functionalized vehicles were explored for targeted mRNA vaccine delivery [171,172]. Interestingly, mannose-decorated lysosomolytic lipopolyplexes showed four times higher transfection of splenic DCs than non-targeted control and enhanced cancer immunity when loading mRNA coding MART-1 antigen in B16F10 melanoma-bearing mice leading to better tumor inhibition and survival rate [173]. In a more recent study, lysosomolytic lipopolyplexes were functionalized with tri-antenna of α-D-mannopyranoside which induced abundant E7-specific T cells when loading mRNA coding E7 antigens as well as curative responses in murine TC1, EG7 and B16F0 tumor models when vaccinated with mRNA coding E7, OVA and MART-1 antigens, respectively, at 7 days after tumor implantation [174]. The mRNA-carrying sugar-capsules composed of polysaccharides particularly mannan from microbial cell wall displayed efficient drainage to lymph nodes, DC activation, mRNA transfection, and antigen presentation on DCs, eliciting specific T-cell responses and anticancer activity *in vivo* [175]. The membrane active GALA peptide-functionalized mRNA polyplexes with a mean diameter of 350 nm and negative charge of -7 mV were shown to enter DCs via sialic acid-mediated mechanism, afford 18-fold higher uptake than a liposome complexes control, and elicit improved immune responses when loading with mRNA coding OVA [176]. Moreover, mRNA encoding antitumor proteins was delivered to the cancer cells. In order to repress their hepatic uptake and enhance lung transfection, mRNA-lipid nanoparticles were decorated with an antibody against vascular cell adhesion molecule-1 (PECAM-1) via thioether linkage [177]. The transfection results showed that PECAM-1-targeted lipid formation augmented mRNA delivery and protein expression by about 200- and 25-fold, respectively, in the lungs following systemic injection compared with non-targeted control. cRGD-functionalized, core-disulfide-crosslinked and thermosensitive mRNA PIC micelles based on cRGD-PEG-PLys(thiol) and poly(*N*-isopropylacrylamide)-PLys(thiol) were shown to greatly improve tumor deposition and mRNA transfection *in vivo* [178].

There is no doubt that nucleic acid nanomedicines will play a key role in future tumor therapy [179,180]. The fast advance of biological engineering, cell and molecular biology, pathology, and so on has enabled identification of the genetic defects and mutations responsible for a distinct type or subtype of cancers, which facilitates the design of nuclei acid drugs to specifically eradicate the cancer cells. The nucleic acid drugs are also capable of boosting cancer immunity, in addition to reversing drug resistance, inhibiting tumor metastasis, and sensitizing cancer cells to chemotherapy [181,182]. In spite of great effort in developing novel and potent vehicles to enhance systemic nucleic acid delivery, there is lack of safe, clinically viable, specific, and efficient systems for targeted cancer gene therapy [183]. The innovation in actively targeted delivery approaches will certainly spur the clinical translation of nucleic acid drugs.

5. Actively targeted protein/peptide drug-based nanomedicines

The protein and peptide drugs that take effects intracellularly in a specific manner within cancer cells are a highly appealing class of biopharmaceuticals for tumor therapy [7,184]. Many proteins and peptides are extremely potent to cancer cells with IC_{50} values in the nano mole per litre (nM) range. For instance, granzyme B (GrB) is an endogenous

mammalian protein that plays a major role in the cytotoxic T lymphocytes and natural killer cells inducing the programmed death of mutated and infected cells in the human body. The assistance of perforin (pore-forming protein) is, however, critical for GrB to take effects, as GrB itself cannot enter cell cytosols. GrB had an IC_{50} of 1–20 nM depending on the delivery methods [185,186], however, free GrB revealed low cytotoxicity even at a concentration of 40 nM [187]. In addition to deficient cell entry, protein and peptide drugs are further associated with potential immunogenicity and rapid degradation *in vivo*. Moreover, the high potency of protein and peptide drugs would bring about acute cytotoxic effects if delivered to the healthy cells other than cancer cells. The development of efficient and cell-selective vehicles is, therefore, of paramount importance for protein and peptide drugs to be applied in the clinical settings [188].

Brentuximab Vedotin (SGN-35, Adcetris) is an anti-CD30 antibody-monomethyl auristatin E (MMAE) conjugate, a peptide based ADC, was approved in 2011 for the treatment of CD30-positive relapsed/refractory Hodgkin's lymphoma (Table 2) [127,189,190]. CD30 overexpresses in patients with malignant Hodgkin lymphoma and anaplastic large cell lymphoma. MMAE is a highly potent anti-tubulin peptide drug. MMAE was conjugated to CD30-specific cAC10 antibody via a protease-sensitive dipeptide linkage that is cleavable in the lysosomes. Upon the binding, the ADC-CD30 complex was internalized via antibody-dependent cellular phagocytosis, followed by the proteolytic cleavage of the linker and the release of MMAE. SGN-35 revealed a DAR of 4 with a distribution of 2 to 8 MMAE per antibody (Fig. 7). The human trials showed that SGN-35 given every 3 weeks had an MTD of 1.8 mg/kg [191]. Interestingly, SGN-35 generated high response rates of 75% and 87% in patients with Hodgkin lymphoma and anaplastic large cell lymphoma, respectively [190]. These notable response rates were ascribed to the enhanced cytotoxicity of MMAE to CD30-positive tumor cells and bystander effect to kill the non-targeted cells such as T-regulatory cells and supporting cells in the tumor microenvironment as a result of MMAE leakage from the malignant cells. The recent clinical trials showed efficacy of SGN-35 combining with chemotherapy for treating CD30-positive peripheral T-cell lymphoma and Hodgkin's lymphoma [192,193]. Although SGN-35 has demonstrated clear clinical benefits it suffers from a low therapeutic index.

Tf-CRM107 is a Tf conjugate of binding-inactivated genetic mutant of diphtheria toxin (CRM107) developed for regional treatment of malignant brain tumors (Table 2) [128]. CRM107 is a potent bacterial protein toxin that destroys cells intracellularly through impeding eukaryotic protein synthesis. CRM107 is a mutant lacking of native toxin binding. The conjugation of Tf to CRM107, via a thioester linkage, was intended to selectively deliver CRM107 to TfR-positive tumor cells like GBM cells. Tf-CRM107 showed high activity toward TfR-overexpressing mammalian cells at picomolar concentrations. The clinical studies revealed that administration of Tf-CRM107 by direct interstitial perfusion induced tumor shrinkage in 60% refractory malignant brain tumor patients without causing severe toxicity. The regional perfusion caused

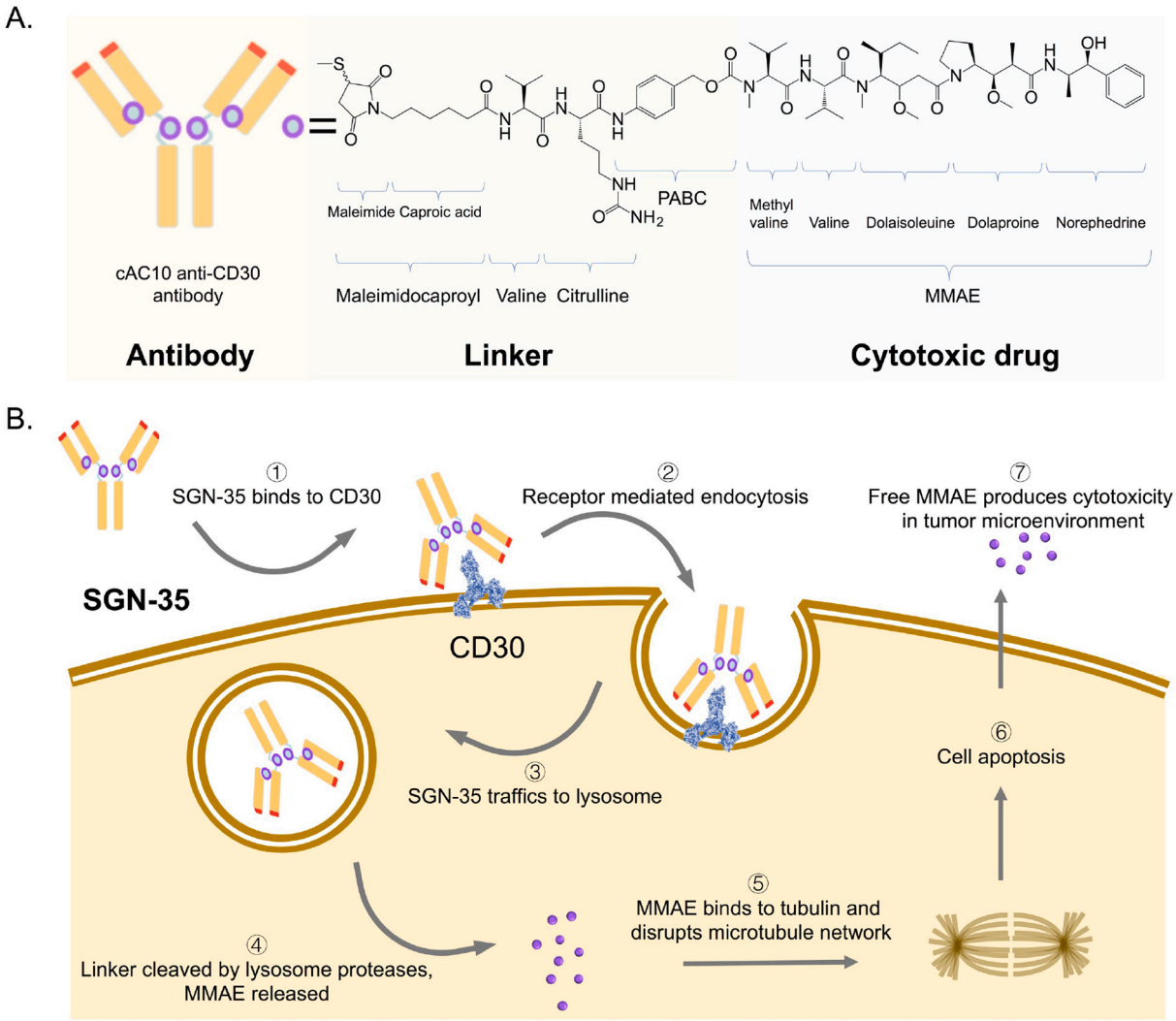


Fig. 7. A) Schematic illustration of the structure and B) mechanism action of SGN-35.

peritumoral toxicity at 1.0 μg/mL Tf-CRM107, which could be avoided by lowering Tf-CRM107 concentrations. The phase II studies revealed that 35% of the evaluable patients treated with Tf-CRM107 had complete or partial tumor response [194]. The convection-enhanced delivery (CED) of targeted toxin formulation might provide a novel and effective treatment for refractory or recurrent GBM patients [195]. The regional perfusion effectively circumvents the BBB issue, and targeted toxin formulation selectively binds and kills TfR-positive GBM cells and leaves non-targeted healthy cells intact, resulting in low neurotoxicity and systemic toxicity. However, CED is invasive and requires special training of clinicians.

Nanogels with an exceptional water content are one of the best nanovehicles to accomplish targeted intracellular protein delivery as they have not only excellent protein compatibility but also superior protein loading capacity [196,197]. The unique biocompatibility, biodegradability and CD44-targetability of HA renders it a fascinating substrate to create actively targeted nanogels for tumor therapy. Interestingly, reduction-sensitive fluorescent HA nanogels fabricated through nano-precipitation and "tetrazol-alkene" photo-click reaction could efficiently deliver GrB to CD44-overexpressing MCF-7 and A549 tumor cells, leading to effective repression of corresponding subcutaneous and orthotopic tumor models at 3.8–5.7 nmol GrB/kg [198]. Introducing GE11 peptide to photo-clicked HA nanogels further boosted their targetability and therapeutic efficacy of GrB toward CD44 and EGFR dual-positive SKOV-3 ovarian and MDA-MB-231 breast tumors. EGFR and CD44 dual-targeting multifunctional hyaluronic acid nanogels have appeared as a safe and efficacious platform for cancer protein therapy [199]. Saporin (Sap) as plant toxin is a single-stranded ribosome inactivating protein and shows high potency in a nM range. Sap-loaded dual-targeting HA nanogels revealed an IC_{50} of 5.36 nM Sap in CD44/EGFR-positive 4T1 metastatic breast cancer cells and effective repression of lung metastasis at 3.33 or 13.3 nmol Sap/kg *in vivo* [200]. The pH-sensitive HA nanogels obtained via nano-precipitation and coiled-coil peptide-crosslinking efficiently released Sap to MCF-7 cells yielding an IC_{50} of 12.2 nM [201]. The nanogels prepared by nano-precipitation, however, had somewhat big size (typically 140–180 nm) and broad distribution. The combination of microfluidics with photo-click chemistry could produce mono-dispersed HA nanogels with a small size of about 80 nm, which demonstrated improved internalization by 4T1 and MDA-MB-231 cells, and enhanced accumulation and penetration in MDA-MB-231 tumor models compared with 150 nm-sized counterparts [202].

HA nanogels were also obtained from co-self-assembly of HA-epigallocatechin gallate conjugate, linear PEI and GrB, and showed a high cytotoxic activity to CD44-positive HCT-116 colon cancer cells but not to CD44-negative cells [203]. HA nanogels were made from cholesteryl methacrylated HA and citraconic acid-modified HAase, and HAase was activated in the acidic tumor microenvironment and endosomal compartments of tumor cells, triggering the degradation of HA nanogels and intracellular release of deoxyribonuclease I, leading to enhanced antitumor activity [204]. In a recent study, anti-HER2 antibody-decorated HA nanocapsules were developed for targeted intracellular delivery of anti-gasdermin B (GSDMB) antibody into HER2-positive breast cancer cells [205]. GSDMB upregulates in ca. 60% of HER2-positive breast cancers, and attributes to cell migration, metastasis, and drug resistance. Interestingly, these HER2-targeted nanoformulations specifically and efficiently inhibited the activity of GSDMB, bringing about decreased migration, enhanced sensitivity to trastuzumab therapy, inhibited tumor growth, and diminished lung metastasis. In another study, aptamer-functionalized rectangular DNA origami nanosheets were developed for targeted intracellular delivery of ribonuclease (RNase) A [206].

Biodegradable polymersomes in particular CPs have recently emerged as a promising nanosystem for tumor-targeted intracellular protein delivery [102]. In contrast to nanogels, polymersomes load proteins and peptides in the watery compartment separated from the outside environment by a thick membrane, which would not only effectively inhibit protein/peptide leakage but also offer better protection of protein/peptide from degradation. The traditional polymersomes, however, displayed minimal encapsulation of protein with a loading efficiency of less than 7% [207]. CPs with a polyelectrolyte as inner shell could load notable amounts of proteins with different sizes at high efficiencies (up to 100%). PSMA and sigma receptor targeting, pH-sensitive degradable CPs efficiently delivered and released GrB to LNCaP prostate cancer cells (IC_{50} = 1.6 nM) and H460 lung cancer cells (IC_{50} = 6.25 nM), respectively [185,186]. GrB-loaded disulfide-crosslinked CPs decorated with anisamide and lung cell-selective penetrating peptide effectively suppressed the growth of subcutaneous H460 lung tumor at 1.56 nmol GrB/kg and orthotopic A549-Luc lung tumor at 2.88 nmol GrB/kg, respectively, without causing noticeable side effects [187,208]. In addition to solid tumors, GrB loaded in HA-functionalized CPs also exhibited high potency to CD44-positive LP1 human MM cells (IC_{50} = 8.1 nM) and strong suppression of both subcutaneous and orthotopic LP1 tumor in mice, significantly improving survival rates and alleviating bone loss [209]. Interestingly, angiopep-2 and ApoE peptide-functionalized disulfide-crosslinked CPs were shown to efficiently transport Sap across the BBB and accumulate at the orthotopic U-87 MG glioblastoma *in vivo*, leading to potent tumor suppression and great survival benefits [210]. ApoE peptide provides an ultrahigh-efficiency targeting strategy for GBM therapy [211]. cRGD peptide-installed Sap-loaded CPs based on poly(ethylene glycol)-b-poly(α-aminopalmitic acid)-b-poly(L-aspartic acid) triblock copolypeptide revealed an IC_{50} of 16.3 nM to A549 cancer cells and potent inhibition of orthotopic A549 lung tumors at 16.7 nmol Sap/kg [212]. The mannose-installed lipid-hybrid polymersomes co-loading ovalbumin antigen, TLR7/8 and TLR4 agonists revealed efficient internalization by DCs, improved passage to lymph nodes, and synergistic anticancer immune responses, effectively retarding tumor outgrowth [213]. There is no doubt that protein and peptide drugs with high specificity and potency will play an increasingly important role in treating malignant tumors. It is noted, however, that in spite of significant work on the development of nanosystems for intracellular protein and peptide drugs, a majority of nanovehicles are non-cell-selective and/or have toxicity and safety concerns.

6. Conclusions and future perspectives

The past decade has witnessed significant progress in the design, development and clinical translation of actively targeted nanomedicines for precision tumor therapy. Notably, several ADCs have been approved for treating patients with refractory or metastatic malignant tumors while a number of actively targeted liposome- and polymer-based formulations, with either classical small molecule anticancer drugs or emerging nucleic acid drugs, have made to human clinical trials for treating diverse advanced tumors. These novel actively targeted nanomedicines can be used either as a monotherapy or in combination with ongoing clinical treatments such as chemotherapy, molecular therapy, radiotherapy and immunotherapy. It should further be noted that a couple of actively targeted nanomedicines under clinical investigation have appeared promising for the treatment of patients with advanced glioblastoma that remains intractable in the clinics.

The field of actively targeted cancer nanomedicines is, however, at its infancy. Interestingly, up to date, non-actively targeted cancer nanomedicines still dominate the preclinical and especially human clinical studies [1,214,215], partly due to the fact that they are comparably simple to fabricate and/or already approved for clinical use (e.g. liposomes, lipid nanoparticles, biodegradable polymers, micelles, and PLGA nanoparticles). These early nanomedicines have shown to effectively alter the pharmacokinetics and biodistributions of various drugs, reducing drugs' adverse effects, and increasing drugs' therapeutic index and patient's quality of life. There is, nevertheless, modest improvement in the clinical efficacy and patient survival rates. The first-generation of actively targeted cancer nanomedicines have been

primarily constructed by (i) conjugating highly toxic chemical, peptide or protein drugs to specific proteins like monoclonal antibodies and transferrin, and (ii) functionalizing "safe" nanovehicles such as liposomes, lipid nanoparticles, and PLGA nanoparticles with selective ligands like antibody fragments, transferrin, or peptide. Among all, ADCs are the most successful and have shown to induce significant clinical responses and survival benefits by enhancing tumor deposition and antigen-mediated specific cell uptake, which certifies the feasibility of developing actively targeted nanomedicines for precision cancer therapy [216,217]. Of note, the real clinical success of ADCs was accomplished only in the early 2010s, more than 50 years after the pioneering work on ADCs [218,219]. The linker, linking chemistry and DAR are of great importance to the performance of ADCs [220,221]. The clinically used ADCs are all fabricated via non-specific conjugation which leads to a broad DAR distribution with 0-8 drugs per antibody. ADCs with both low and high DAR are not optimal and would contribute to either low anti-tumor potency or reduced specificity . The reasons lie in (i) ADCs with low DAR of 0–2 lead to not only low anticancer activity but also possibly blocked antigens on the tumor cells, and (ii) ADCs with high DAR of 6–8 tend to aggregate resulting in accelerated clearance and decreased tumor antigen affinity. In spite of increased tolerability compared with free drugs, all clinical ADCs bear a narrow therapeutic window, which partly is attributable to the heterogeneous conjugation and wide-ranging DAR. Undoubtedly, the clinical performance of ADCs can be further boosted by optimizing the function of linker, employing site-specific conjugation, and tailoring DAR [222]. We expect to see increasingly more ADCs with better selectivity and higher anticancer potency to stand out for precision cancer therapy in the coming decade. Differed from traditional ADCs containing highly toxic drug, antibody-NIR dye conjugates for photo-immunotherapy, which mitigates off-target side effects, appear to be a new approach for targeted tumor treatment.

It should be noted that ADCs are associated with several restrictions: (i) they have limited drug loading (typically less than 2 wt.%), which on one hand restrains them to the few extremely toxic drugs and on the other hand requires to use large amounts of antibodies; (ii) the highly toxic drugs are practically naked in the blood circulation, which might cause non-specific binding with proteins and cells, drug leakage, and/or drug degradation; and (iii) they are deficient in delivering most clinical drugs as well as emerging biopharmaceuticals like nuclei acid drugs. In this regard, actively targeted nanomedicines based on vehicles like liposomes, lipid nanoparticles and polymer nanoparticles are advantageous. The development of immunoliposomes for targeted tumor therapy started in the 1970s [223,224]. Interestingly, a great deal of work has centered on attaching specific antibody fragments onto PEGylated liposomal DOX•HCl. The human clinical trials began in the 2000s and did show tumor specificity, favorable safety and clinical activity in patients with refractory or metastatic tumors. Moreover, antibody fragment-functionalized cationic liposomes were shown promising for targeted tumor gene therapy. The clinical benefits are, however, far from meeting the expectation for actively targeted formulations. This unmet clinical performance of targeted liposomal formulations is possibly associated with: (i) their big sizes (often more than 100 nm), poor stability and drug leakage in circulation, and deficient drug release in the tumor cells, which are the fundamental problems for liposomal systems, (ii) low stability of antibody fragments, and (iii) non-optimal surface density and display of antibody fragments. It should further be noted that antibody-directed liposomal formulations involve typically delicate fabrication procedure and are difficult to scale up. The development of stabilized, stimuli-sensitive, and small-sized liposomes or lipid nanoparticles would probably improve the clinical activities of targeted formulations significantly. It is of interest to note that the first FDA approved RNAi therapeutics Onpattro (Patisiran) is a lipid nanoparticle formulation, which induces intrinsic liver targeting and effective reduction of transthyretin protein, offering a breakthrough therapy for polyneuropathy patients [225,226]. The liver-specific delivery of siRNA therapeutics renders lipid nanoparticles also interesting for the treatment of hepatocellular carcinoma and liver metastases.

The research on actively targeted nanoformulations based on biodegradable nanoparticles started only from the new millennium, which is more than 40 and 20 years later than ADCs and targeted liposomal formulations, respectively. In contrast to ADCs and targeted liposomal formulations, polymer nanoparticles are easy to produce, versatile, and facilely engineerable to suit for delivering different drugs ranging from small molecule drugs, proteins and peptides, to nucleic acids. The human clinical trials with the first actively targeted polymer nanomedicine (BIND-014), however, did not meet the endpoints. This clinical failure is not surprising given that patients with different solid tumors were enrolled, there was no patient screening, and tumor neovasculatures but not tumor cells were selected as the target. The clinical benefits could be achieved if only patients with PSMA-overexpressing prostate tumors were selected or different targeting ligands such as antibody and antibody fragments were used. Moreover, PLGA nanoparticles though safe are not the best vehicle. Despite systemic optimization, DTX nanoparticles were likely associated with instability and fast drug leakage (as shown by the *in vitro* drug release studies) and slow drug release in the target cells, both of which would significantly weaken the tumor targetability.

This first clinical trial taught us a lesson that vehicles, therapeutic agents, ligands, and indications are all vital to the success of actively targeted cancer nanomedicines. In the past years, a variety of novel, robust and environment-responsive biodegradable nanovehicles have been designed and developed to replace the classical essentially "inert" vehicles. The preclinical studies in diverse malignant tumor models have proven the concept that advanced actively targeted cancer nanomedicines based on multifunctional vehicles could boost both specificity and antitumor efficacy of various clinically used small molecule anticancer drugs as well as emerging biopharmaceuticals such as proteins, peptides, and nucleic acids. One issue with nanomedicines is that they accumulate also in the liver. The potential hepatotoxicity is a concern for actively targeted cancer nanomedicines though animal studies did not show obvious damage to the liver possibly owing to the inefficient uptake by healthy liver cells. In this regard, biopharmaceuticals like certain proteins and nucleic acid drugs are especially interesting because they, unlike toxic chemical drugs, are highly specific and will cause little or no toxicity to healthy cells. On the other hand, lacking safe and efficacious vehicles is the bottleneck for the clinical translation of biopharmaceuticals. We expect that increasingly more work will be dedicated to targeted delivery of biopharmaceuticals and breakthrough in tumor biotherapy will be achieved in the coming years. These actively targeted nanovehicles can further co-deliver chemotherapeutics and biotherapeutics leading to synergistic treatment of refractory, recurrent or metastatic tumors. Moreover, the selection of ligands and ligand density are among the decisive factors to the specificity of actively targeted cancer nanomedicines. Interestingly, several peptide-directed nanomedicines have shown to carry chemotherapeutics or biopharmaceutics across the BBB and target to malignant glioblastoma, significantly repressing tumor progression and enhancing mice survival rate [227]. The actively targeted nanomedicines might provide novel and effective treatments for glioblastoma. Of note, most previous studies employed peptides, aptamers or small molecules like folic acid as targeting ligands via pre-ligation approach because of easy fabrication, and typically ligand density was not systemically optimized. The moderate cancer cell specificity and affinity of peptides, aptamers and small molecule ligands coupled with un-optimized ligand density endow most nanomedicines with a mediocre tumor targeting ability *in vivo*. The monoclonal antibody and antibody fragments might offer exceptional selectivity and affinity; however, their use is hindered by their large size and lack of specific linking chemistry. The development of robust and small-sized nanovehicles that enable facile and controlled conjugation of monoclonal antibody and antibody fragments by post-modification might greatly enhance the tumor-targeting ability. The nanovehicles

could further be functionalized with dual ligands to enhance specificity, tumor cell uptake and/or tumor penetration [228]. Finally, patients have to be screened and only tumors highly expressing target receptors should be selected. The human tumors are very complicated and can vary to a great extent in receptor density, heterogenicity, and accessibility from different tumor types and subtypes, volumes, and stages. Hence, even for the same subtype of tumor, patients may respond differently to the same targeted nanomedicines. In this sense, it would be highly interesting to use the same targeted nanovehicles to load a diagnostic agent to screen the patients prior to administration of the actively targeted nanomedicines [23]. This personalized medicine approach might markedly increase the clinical response. It is recognized that one major bottleneck for the development of novel actively targeted cancer nanomedicines is their safety concern, which is in fact only partly true. The lag of clinical translation is more because current actively targeted cancer nanomedicines have not made breakthroughs in treatment efficacy and benefits as promised by “magic bullets” developed by the German Nobel laureate Paul Ehrlich in 1900 [229,230].

The field of actively targeted cancer nanomedicines, though facing numerous challenges, is booming. The unmet clinical requirements and huge market have stimulated broad interests and close cooperation between scientists from different fields, clinicians, entrepreneurs, pharmaceutical and biopharmaceutical industries, and investors. We are convinced that with collaborative research and development, actively targeted nanomedicines will become an indispensable precision treatment modality for diverse malignant tumors in the near future.

Credit Author Statement

Wenxing Gu and Fenghua Meng carried out literature survey and drafted the paper; Rainer Haag and Zhiyuan Zhong co-supervised the work, and wrote and revised the paper

Acknowledgement

This work was supported by research grants from the National Natural Science Foundation of China (NSFC 51633005, 51761135117, 51773146, 51861145310) and the Deutsche Forschungsgemeinschaft (DFG).

References

[1] D. Rosenblum, N. Joshi, W. Tao, J.M. Karp, D. Peer, Progress and challenges towards targeted delivery of cancer therapeutics, Nat. Commun. 9 (2018) 1410.
[2] P. Mi, H. Cabral, K. Kataoka, Ligand-installed nanocarriers toward precision therapy, Adv. Mater. 32 (2020) e1902604.
[3] K. Gkouvatsos, G. Papanikolaou, K. Pantopoulos, Regulation of iron transport and the role of transferrin, Biochim. Biophys. Acta. 1820 (2012) 188–202.
[4] A. Wicki, D. Witzigmann, V. Balasubramanian, J. Huwyler, Nanomedicine in cancer therapy: Challenges, opportunities, and clinical applications, J. Control. Release 200 (2015) 138–157.
[5] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25.
[6] C.F. Xu, S. Iqbal, S. Shen, Y.L. Luo, X. Yang, J. Wang, Development of "CLAN" nanomedicine for nucleic acid therapeutics, Small 15 (2019), e1900055.
[7] L. Cheng, L. Yang, F. Meng, Z. Zhong, Protein nanotherapeutics as an emerging modality for cancer therapy, Adv. Healthc. Mater. 7 (2018) 1800685.
[8] Y. Min, J.M. Caster, M.J. Eblan, A.Z. Wang, Clinical translation of nanomedicine, Chem. Rev. 115 (2015) 11147–11190.
[9] J.P.A. Ioannidis, B.Y.S. Kim, A. Trounson, How to design preclinical studies in nanomedicine and cell therapy to maximize the prospects of clinical translation, Nat. Biomed. Eng. 2 (2018) 797–809.
[10] J.I. Hare, T. Lammers, M.B. Ashford, S. Puri, G. Storm, S.T. Barry, Challenges and strategies in anti-cancer nanomedicine development: An industry perspective, Adv. Drug Deliv. Rev. 108 (2017) 25–38.
[11] J.L. Markman, A. Rekechenetskiy, E. Holler, J.Y. Ljubimova, Nanomedicine therapeutic approaches to overcome cancer drug resistance, Adv. Drug Deliv. Rev. 65 (2013) 1866–1879.
[12] S. Shen, J.-X. Xia, J. Wang, Nanomedicine-mediated cancer stem cell therapy, Biomaterials 74 (2016) 1–18.
[13] D. Furtado, M. Björnmalm, S. Ayton, A.I. Bush, K. Kempe, F. Caruso, Overcoming the blood–brain barrier: The role of nanomaterials in treating neurological diseases, Adv. Mater. 30 (2018) 1801362.
[14] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: Progress, challenges and opportunities, Nat. Rev. Cancer 17 (2017) 20–37.
[15] H. He, L. Liu, E.E. Morin, M. Liu, A. Schwendeman, Survey of clinical translation of cancer nanomedicines-lessons learned from successes and failures, Acc. Chem. Res. 52 (2019) 2445–2461.
[16] C.H. Chau, P.S. Steeg, W.D. Figg, Antibody–drug conjugates for cancer, The Lancet 394 (2019) 793–804.
[17] M.J. Birrer, K.N. Moore, I. Betella, R.C. Bates, Antibody-drug conjugate-based therapeutics: state of the science, J. Natl. Cancer Inst. 111 (2019) 538–549.
[18] K.A. Autio, R. Dreicer, J. Anderson, J.A. Garcia, A. Alva, L.L. Hart, M.I. Milowsky, E.M. Posadas, C.J. Ryan, R.P. Graf, R. Dittamore, N.A. Schreiber, J.M. Summa, H. Youssoufian, M.J. Morris, H.I. Scher, Safety and efficacy of BIND-014, a docetaxel nanoparticle targeting prostate-specific membrane antigen for patients with metastatic castration-resistant prostate cancer: A phase 2 clinical trial, JAMA Oncol. 4 (2018) 1344–1351.
[19] D.D. Von Hoff, M.M. Mita, R.K. Ramanathan, G.J. Weiss, A.C. Mita, P.M. LoRusso, H.A. Burris 3rd, L.L. Hart, S.C. Low, D.M. Parsons, S.E. Zale, J.M. Summa, H. Youssoufian, J.C. Sachdev, Phase I study of PSMA-targeted docetaxel-containing nanoparticle BIND-014 in patients with advanced solid tumors, Clin. Cancer Res. 22 (2016) 3157–3163.
[20] J.E. Zuckerman, I. Gritli, A. Tolcher, J.D. Heidel, D. Lim, R. Morgan, B. Chmielowski, A. Ribas, M.E. Davis, Y. Yen, Correlating animal and human phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA, Proc. Natl. Acad. Sci. U S A 111 (2014) 11449–11454.
[21] N. Senzer, J. Nemunaitis, D. Nemunaitis, C. Bedell, G. Edelman, M. Barve, R. Nunan, K.F. Pirollo, A. Rait, E.H. Chang, Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors, Mol. Ther. 21 (2013) 1096–1103.
[22] Q. Sun, Z. Zhou, N. Qiu, Y. Shen, Rational design of cancer nanomedicine: Nanoproperty integration and synchronization, Adv. Mater. 29 (2017) 1606628.
[23] R. van der Meel, E. Sulheim, Y. Shi, F. Kiessling, W.J.M. Mulder, T. Lammers, Smart cancer nanomedicine, Nat. Nanotechnol. 14 (2019) 1007–1017.
[24] I.A.B. Pijpers, L.K.E.A. Abdelmohsen, Y. Xia, S. Cao, D.S. Williams, F. Meng, J.C. M. van Hest, Z. Zhong, Adaptive polymersome and micelle morphologies in anticancer nanomedicine: From design rationale to fabrication and proof-of-concept studies, Adv. Ther. 1 (2018) 1800068.
[25] M. Mir, N. Ahmed, A.u. Rehman, Recent applications of PLGA based nanostructures in drug delivery, Colloids Surf. B 159 (2017) 217–231.
[26] E. Swider, O. Koshkina, J. Tel, L.J. Cruz, I.J.M. de Vries, M. Srinivas, Customizing poly(lactic-co-glycolic acid) particles for biomedical applications, Acta Biomater. 73 (2018) 38–51.
[27] K. Zhang, X. Tang, J. Zhang, W. Lu, X. Lin, Y. Zhang, B. Tian, H. Yang, H. He, PEG–PLGA copolymers: Their structure and structure-influenced drug delivery applications, J. Control. Release 183 (2014) 77–86.
[28] S. Wilhelm, A.J. Tavares, Q. Dai, S. Ohta, J. Audet, H.F. Dvorak, W.C.W. Chan, Analysis of nanoparticle delivery to tumours, Nat. Rev. Mater. 1 (2016) 1–12.
[29] D.L. Stirland, J.W. Nichols, S. Miura, Y.H. Bae, Mind the gap: A survey of how cancer drug carriers are susceptible to the gap between research and practice, J. Control. Release 172 (2013) 1045–1064.
[30] R. Haag, F. Kratz, Polymer therapeutics: Concepts and applications, Angew. Chem. Int. Ed. Engl. 45 (2006) 1198–1215.
[31] R. Duncan, The dawning era of polymer therapeutics, Nat. Rev. Drug Discov. 2 (2003) 347–360.
[32] M.J. Vicent, H. Ringsdorf, R. Duncan, Polymer therapeutics: Clinical applications and challenges for development, Adv. Drug Deliv. Rev. 61 (2009) 1117–1120.
[33] S.K. Golombek, J.N. May, B. Theek, L. Appold, N. Drude, F. Kiessling, T. Lammers, Tumor targeting via EPR: Strategies to enhance patient responses, Adv. Drug Deliv. Rev. 130 (2018) 17–38.
[34] F. Danhier, To exploit the tumor microenvironment: Since the EPR effect fails in the clinic, what is the future of nanomedicine? J. Control. Release 244 (2016) 108–121.
[35] M. Ashrafizadeh, Z. Ahmadi, N. Mohamadi, A. Zarrabi, S. Abasi, G. Dehghannoudeh, R.N. Tamaddondoust, H. Khanbabaei, R. Mohammadinejad, V.K. Thakur, Chitosan-based advanced materials for docetaxel and paclitaxel delivery: Recent advances and future directions in cancer theranostics, Int. J. Biol. Macromol. 145 (2020) 282–300.
[36] S. Verma, D. Miles, L. Gianni, I.E. Krop, M. Welslau, J. Baselga, M. Pegram, D. Y. Oh, V. Dieras, E. Guardino, L. Fang, M.W. Lu, S. Olsen, K. Blackwell, E.S. Group, Trastuzumab emtansine for HER2-positive advanced breast cancer, N. Engl. J. Med. 367 (2012) 1783–1791.
[37] B.T. Li, R. Shen, D. Buonocore, Z.T. Olah, A. Ni, M.F. Berger, J.e. Baselga, M. Scaltriti, M.E. Arcila, M.G. Kris, Ado-trastuzumab emtansine for patients with HER2 mutant lung cancers: Results from a phase II basket trial, J. Clin. Oncol. 36 (2018) 2532–2537.
[38] P.C. Thuss-Patience, M.A. Shah, A. Ohtsu, E. Van Cutsem, J.A. Ajani, H. Castro, W. Mansoor, H.C. Chung, G. Bodoky, K. Shitara, G.D.L. Phillips, T. van der Horst, M.-L. Harle-Yge, B.L. Althaus, Y.-K. Kang, Trastuzumab emtansine versus taxane use for previously treated HER2-positive locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma (GATSBY): An international randomised, open-label, adaptive, phase 2/3 study, Lancet Oncol. 18 (2017) 640–653.
[39] M. Barok, H. Joensuu, J. Isola, Trastuzumab emtansine: Mechanisms of action and drug resistance, Breast Cancer Res. 16 (2014) 209.

[40] H.A. Burris 3rd, H.S. Rugo, S.J. Vukelja, C.L. Vogel, R.A. Borson, S. Limentani, E. Tan-Chiu, I.E. Krop, R.A. Michaelson, S. Girish, L. Amler, M. Zheng, Y.W. Chu, B. Klencke, J.A. O'Shaughnessy, Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy, J. Clin. Oncol. 29 (2011) 398–405.
[41] I.E. Krop, M. Beeram, S. Modi, S.F. Jones, S.N. Holden, W. Yu, S. Girish, J. Tibbitts, J.H. Yi, M.X. Sliwkowski, F. Jacobson, S.G. Lutzker, H.A. Burris, Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer, J. Clin. Oncol. 28 (2010) 2698–2704.
[42] G.D. Lewis Phillips, G. Li, D.L. Dugger, L.M. Crocker, K.L. Parsons, E. Mai, W. A. Blattler, J.M. Lambert, R.V. Chari, R.J. Lutz, W.L. Wong, F.S. Jacobson, H. Koeppen, R.H. Schwall, S.R. Kenkare-Mitra, S.D. Spencer, M.X. Sliwkowski, Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate, Cancer Res. 68 (2008) 9280–9290.
[43] A. Bardia, I.A. Mayer, J.R. Diamond, R.L. Moroose, S.J. Isakoff, A.N. Starodub, N. C. Shah, J. O'Shaughnessy, K. Kalinsky, M. Guarino, V. Abramson, D. Juric, S. M. Tolaney, J. Berlin, W.A. Messersmith, A.J. Ocean, W.A. Wegener, P. Maliakal, R.M. Sharkey, S.V. Govindan, D.M. Goldenberg, L.T. Vahdat, Efficacy and safety of anti-trop-2 antibody drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer, J. Clin. Oncol. 35 (2017) 2141–2148.
[44] E.Y. Jen, C.W. Ko, J.E. Lee, P.L. Del Valle, A. Aydanian, C. Jewell, K. J. Norsworthy, D. Przepiorka, L. Nie, J. Liu, C.M. Sheth, M. Shapiro, A.T. Farrell, R. Pazdur, FDA approval: Gemtuzumab ozogamicin for the treatment of adults with newly diagnosed CD33-positive acute myeloid leukemia, Clin. Cancer Res. 24 (2018) 3242–3246.
[45] A. Advani, B. Coiffier, M.S. Czuczman, M. Dreyling, J. Foran, E. Gine, C. Gisselbrecht, N. Ketterer, S. Nasta, A. Rohatiner, I.G. Schmidt-Wolf, M. Schuler, J. Sierra, M.R. Smith, G. Verhoef, J.N. Winter, J. Boni, E. Vandendries, M. Shapiro, L. Fayad, Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: Results of a phase I study, J. Clin. Oncol. 28 (2010) 2085–2093.
[46] J. Hrkach, D. Von Hoff, M. Mukkaram Ali, E. Andrianova, J. Auer, T. Campbell, D. De Witt, M. Figa, M. Figueiredo, A. Horhota, S. Low, K. McDonnell, E. Peeke, B. Retnarajan, A. Sabnis, E. Schnipper, J.J. Song, Y.H. Song, J. Summa, D. Tompsett, G. Troiano, T. Van Geen Hoven, J. Wright, P. LoRusso, P.W. Kantoff, N.H. Bander, C. Sweeney, O.C. Farokhzad, R. Langer, S. Zale, Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile, Sci. Transl. Med. 4 (2012) 128–139.
[47] P. Munster, I.E. Krop, P. LoRusso, C. Ma, B.A. Siegel, A.F. Shields, I. Molnar, T. J. Wickham, J. Reynolds, K. Campbell, B.S. Hendriks, B.S. Adiwijaya, E. Geretti, V. Moyo, K.D. Miller, Safety and pharmacokinetics of MM-302, a HER2-targeted antibody-liposomal doxorubicin conjugate, in patients with advanced HER2-positive breast cancer: A phase 1 dose-escalation study, Br. J. Cancer 119 (2018) 1086–1093.
[48] Y. Matsumura, M. Gotoh, K. Muro, Y. Yamada, K. Shirao, Y. Shimada, M. Okuwa, S. Matsumoto, Y. Miyata, H. Ohkura, K. Chin, S. Baba, T. Yamao, A. Kannami, Y. Takamatsu, K. Ito, K. Takahashi, Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer, Ann. Oncol. 15 (2004) 517–525.
[49] C. Mamot, R. Ritschard, A. Wicki, G. Stehle, T. Dieterle, L. Bubendorf, C. Hilker, S. Deuster, R. Herrmann, C. Rochlitz, Tolerability, safety, pharmacokinetics, and efficacy of doxorubicin-loaded anti-EGFR immunoliposomes in advanced solid tumours: a phase 1 dose-escalation study, Lancet Oncol. 13 (2012) 1234–1241.
[50] P.J. Gaillard, C.C. Appeldoorn, R. Dorland, J. van Kregten, F. Manca, D.J. Vugts, B. Windhorst, G.A. van Dongen, H.E. de Vries, D. Maussang, O. van Tellingen, Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3-101), PLoS One 9 (2014), e82331.
[51] K.K. Sankhala, A.C. Mita, R. Adinin, L. Wood, M. Beeram, S. Bullock, N. Yamagata, K. Matsuno, T. Fujisawa, A. Phan, A phase I pharmacokinetic (PK) study of MBP-426, a novel liposome encapsulated oxaliplatin, J. Clin. Oncol. 27 (2009) 2535.
[52] D.M. Goldenberg, T.M. Cardillo, S.V. Govindan, E.A. Rossi, R.M. Sharkey, Trop-2 is a novel target for solid cancer therapy with sacituzumab govitecan (IMMU-132), an antibody-drug conjugate (ADC), Oncotarget 6 (2015) 22496–22512.
[53] A.N. Starodub, A.J. Ocean, M.A. Shah, M.J. Guarino, V.J. Picozzi Jr., L.T. Vahdat, S.S. Thomas, S.V. Govindan, P.P. Maliakal, W.A. Wegener, S.A. Hamburger, R. M. Sharkey, D.M. Goldenberg, First-in-human trial of a novel anti-trop-2 antibody-SN-38 conjugate, sacituzumab govitecan, for the treatment of diverse metastatic solid tumors, Clin. Cancer Res. 21 (2015) 3870–3878.
[54] T.M. Cardillo, S.V. Govindan, R.M. Sharkey, P. Trisal, R. Arrojo, D. Liu, E.A. Rossi, C.H. Chang, D.M. Goldenberg, Sacituzumab govitecan (IMMU-132), an anti-trop-2/SN-38 antibody-drug conjugate: Characterization and efficacy in pancreatic, gastric, and other cancers, Bioconjug. Chem. 26 (2015) 919–931.
[55] R.M. Sharkey, W.J. McBride, T.M. Cardillo, S.V. Govindan, Y. Wang, E.A. Rossi, C. H. Chang, D.M. Goldenberg, Enhanced delivery of SN-38 to human tumor xenografts with an anti-trop-2-SN-38 antibody conjugate (sacituzumab govitecan), Clin. Cancer Res. 21 (2015) 5131–5138.
[56] J.E. Gray, R.S. Heist, A.N. Starodub, D.R. Camidge, E. Kio, G. Masters, W. T. Purcell, M.J. Guarino, J. Misleh, C.J. Schneider, B.J. Schneider, A.J. Ocean, T. Johnson, L. Gandhi, K. Kalinsky, S.V. Govindan, P. Maliakal, B. Mudenda, W. A. Wegener, R.M. Sharkey, D.M. Goldenberg, Abstract CT155: Phase 2 study of sacituzumab govitecan (IMMU-132), an anti-Trop-2/SN-38 antibody-drug conjugate (ADC), in patients with pretreated metastatic small-cell lung cancer (mSCLC), Cancer Res. 77 (2017) CT155.
[57] R.S. Heist, M.J. Guarino, G. Masters, W.T. Purcell, A.N. Starodub, L. Horn, R. J. Scheff, A. Bardia, W.A. Messersmith, J. Berlin, A.J. Ocean, S.V. Govindan, P. Maliakal, B. Mudenda, W.A. Wegener, R.M. Sharkey, D.M. Goldenberg, D. R. Camidge, Therapy of advanced non–small-cell lung cancer with an SN-38-anti-trop-2 drug conjugate, sacituzumab govitecan, J. Clin. Oncol. 35 (2017) 2790–2797.
[58] A. Bardia, I.A. Mayer, L.T. Vahdat, S.M. Tolaney, S.J. Isakoff, J.R. Diamond, J. O'Shaughnessy, R.L. Moroose, A.D. Santin, V.G. Abramson, N.C. Shah, H. S. Rugo, D.M. Goldenberg, A.M. Sweidan, R. Iannone, S. Washkowitz, R. M. Sharkey, W.A. Wegener, K. Kalinsky, Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer, N. Engl. J. Med. 380 (2019) 741–751.
[59] A.J. Ocean, A.N. Starodub, A. Bardia, L.T. Vahdat, S.J. Isakoff, M. Guarino, W. A. Messersmith, V.J. Picozzi, I.A. Mayer, W.A. Wegener, P. Maliakal, S. V. Govindan, R.M. Sharkey, D.M. Goldenberg, Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate for the treatment of diverse epithelial cancers: Safety and pharmacokinetics, Cancer 123 (2017) 3843–3854.
[60] E.L. Sievers, M. Linenberger, Mylotarg: antibody-targeted chemotherapy comes of age, Curr. Opin. Oncol. 13 (2001) 522–527.
[61] P.R. Hamann, L.M. Hinman, I. Hollander, C.F. Beyer, D. Lindh, R. Holcomb, W. Hallett, H.-R. Tsou, J. Upeslacis, D. Shochat, A. Mountain, D.A. Flowers, I. Bernstein, Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody−calicheamicin conjugate for treatment of acute myeloid leukemia, Bioconjug. Chem. 13 (2002) 47–58.
[62] H.M. Kantarjian, D.J. DeAngelo, M. Stelljes, G. Martinelli, M. Liedtke, W. Stock, N. Gokbuget, S. O'Brien, K. Wang, T. Wang, M.L. Paccagnella, B. Sleight, E. Vandendries, A.S. Advani, Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia, N. Engl. J. Med. 375 (2016) 740–753.
[63] H. Kantarjian, D. Thomas, J. Jorgensen, E. Jabbour, P. Kebriaei, M. Rytting, S. York, F. Ravandi, M. Kwari, S. Faderl, M.B. Rios, J. Cortes, L. Fayad, R. Tarnai, S.A. Wang, R. Champlin, A. Advani, S. O'Brien, Inotuzumab ozogamicin, an anti-CD22–calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: A phase 2 study, Lancet Oncol. 13 (2012) 403–411.
[64] M. Mitsunaga, M. Ogawa, N. Kosaka, L.T. Rosenblum, P.L. Choyke, H. Kobayashi, Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules, Nat. Med. 17 (2011) 1685–1691.
[65] K. Sato, K. Ando, S. Okuyama, S. Moriguchi, T. Ogura, S. Totoki, H. Hanaoka, T. Nagaya, R. Kokawa, H. Takakura, M. Nishimura, Y. Hasegawa, P.L. Choyke, M. Ogawa, H. Kobayashi, Photoinduced ligand release from a silicon phthalocyanine dye conjugated with monoclonal antibodies: A mechanism of cancer cell cytotoxicity after near-infrared photoimmunotherapy, ACS Cent. Sci. 4 (2018) 1559–1569.
[66] H. Kobayashi, P.L. Choyke, Near-infrared photoimmunotherapy of cancer, Acc. Chem. Res. 52 (2019) 2332–2339.
[67] I. Ekladious, Y.L. Colson, M.W. Grinstaff, Polymer-drug conjugate therapeutics: Advances, insights and prospects, Nat. Rev. Drug Discov. 18 (2019) 273–294.
[68] J.W. Hopewel, R. Duncan, D. Wilding, K. Chakrabarti, Preclinical evaluation of the cardiotoxicity of PK2: A novel HPMA copolymer-doxorubicin-galactosamine conjugate antitumour agent, Hum. Exp. Toxicol. 20 (2001) 461–470.
[69] P.J. Julyan, L.W. Seymour, D.R. Ferry, S. Daryani, C.M. Boivin, J. Doran, M. David, D. Anderson, C. Christodoulou, A.M. Young, S. Hesslewood, D.J. Kerr, Preliminary clinical study of the distribution of HPMA copolymers bearing doxorubicin and galactosamine, J. Control. Release 57 (1999) 281–290.
[70] L.W. Seymour, D.R. Ferry, D. Anderson, S. Hesslewood, P.J. Julyan, R.P. Doran, A.M. Young, S. Burtles, D.J. Kerr, Hepatic drug targeting: Phase I evaluation of polymer-bound doxorubicin, J. Clin. Oncol. 20 (2002) 1668–1676.
[71] V. Sanna, N. Pala, M. Sechi, Targeted therapy using nanotechnology: Focus on cancer, Int. J. Nanomed. 9 (2014) 467–483.
[72] H. Ledford, Bankruptcy of nanomedicine firm worries drug developers, Nature 533 (2016) 304–305.
[73] Y. Liu, C. Zhou, W. Wang, J. Yang, H. Wang, W. Hong, Y. Huang, CD44 receptor targeting and endosomal pH-sensitive dual functional hyaluronic acid micelles for intracellular paclitaxel delivery, Mol. Pharm. 13 (2016) 4209–4221.
[74] H.Y. Seok, N. Sanoj Rejinold, K.M. Lekshmi, K. Cherukula, I.K. Park, Y.C. Kim, CD44 targeting biocompatible and biodegradable hyaluronic acid cross-linked zein nanogels for curcumin delivery to cancer cells: In vitro and in vivo evaluation, J. Control. Release 280 (2018) 20–30.
[75] Y. Zhong, K. Goltsche, L. Cheng, F. Xie, F. Meng, C. Deng, Z. Zhong, R. Haag, Hyaluronic acid-shelled acid-activatable paclitaxel prodrug micelles effectively target and treat CD44-overexpressing human breast tumor xenografts in vivo, Biomaterials 84 (2016) 250–261.
[76] M. Talelli, S. Oliveira, C.J. Rijcken, E.H. Pieters, T. Etrych, K. Ulbrich, R.C. van Nostrum, G. Storm, W.E. Hennink, T. Lammers, Intrinsically active nanobody-modified polymeric micelles for tumor-targeted combination therapy, Biomaterials 34 (2013) 1255–1260.
[77] S.A. Costa, D. Mozhdehi, M.J. Dzuricky, F.J. Isaacs, E.M. Brustad, A. Chilkoti, Active targeting of cancer cells by nanobody decorated polypeptide micelle with bio-orthogonally conjugated drug, Nano Lett. 19 (2019) 247–254.
[78] P. Zhong, H. Meng, J. Qiu, J. Zhang, H. Sun, R. Cheng, Z. Zhong, alphavbeta3 Integrin-targeted reduction-sensitive micellar mertansine prodrug: Superb drug loading, enhanced stability, and effective inhibition of melanoma growth in vivo, J. Control. Release 259 (2017) 176–186.

[79] Y. Zhong, J. Zhang, R. Cheng, C. Deng, F. Meng, F. Xie, Z. Zhong, Reversibly crosslinked hyaluronic acid nanoparticles for active targeting and intelligent delivery of doxorubicin to drug resistant CD44+ human breast tumor xenografts, J. Control. Release 205 (2015) 144–154.
[80] V. Orian-Rousseau, CD44, a therapeutic target for metastasising tumours, Eur. J. Cancer 46 (2010) 1271–1277.
[81] S. Misra, P. Heldin, V.C. Hascall, N.K. Karamanos, S.S. Skandalis, R.R. Markwald, S. Ghatak, Hyaluronan-CD44 interactions as potential targets for cancer therapy, FEBS J. 278 (2011) 1429–1443.
[82] Y. Zhu, Y. Jiang, F. Meng, C. Deng, R. Cheng, J. Zhang, J. Feijen, Z. Zhong, Highly efficacious and specific anti-glioma chemotherapy by tandem nanomicelles co-functionalized with brain tumor-targeting and cell-penetrating peptides, J. Control. Release 278 (2018) 1–8.
[83] Y. Barenholz, Doxil(R)–the first FDA-approved nano-drug: lessons learned, J. Control. Release 160 (2012) 117–134.
[84] S.T. Duggan, G.M. Keating, Pegylated liposomal doxorubicin, Drugs 71 (2011) 2531–2558.
[85] G.T. Noble, J.F. Stefanick, J.D. Ashley, T. Kiziltepe, B. Bilgicer, Ligand-targeted liposome design: challenges and fundamental considerations, Trends Biotechnol. 32 (2014) 32–45.
[86] J. Gubernator, Active methods of drug loading into liposomes: Recent strategies for stable drug entrapment and increased in vivo activity, Expert Opin. Drug Deliv. 8 (2011) 565–580.
[87] C.W. Espelin, S.C. Leonard, E. Geretti, T.J. Wickham, B.S. Hendriks, Dual HER2 targeting with trastuzumab and liposomal-encapsulated doxorubicin (MM-302) demonstrates synergistic antitumor activity in breast and gastric cancer, Cancer Res. 76 (2016) 1517–1527.
[88] J.G. Reynolds, E. Geretti, B.S. Hendriks, H. Lee, S.C. Leonard, S.G. Klinz, C. O. Noble, P.B. Lucker, P.W. Zandstra, D.C. Drummond, K.J. Olivier Jr., U. B. Nielsen, C. Niyikiza, S.V. Agresta, T.J. Wickham, HER2-targeted liposomal doxorubicin displays enhanced anti-tumorigenic effects without associated cardiotoxicity, Toxicol. Appl. Pharmacol. 262 (2012) 1–10.
[89] K. Miller, J. Cortes, S.A. Hurvitz, I.E. Krop, D. Tripathy, S. Verma, K. Riahi, J. G. Reynolds, T.J. Wickham, I. Molnar, D.A. Yardley, HERMIONE: A randomized phase 2 trial of MM-302 plus trastuzumab versus chemotherapy of physician's choice plus trastuzumab in patients with previously treated, anthracycline-naive, HER2-positive, locally advanced/metastatic breast cancer, BMC Cancer 16 (2016) 352.
[90] H. Lee, A.F. Shields, B.A. Siegel, K.D. Miller, I. Krop, C.X. Ma, P.M. LoRusso, P. N. Munster, K. Campbell, D.F. Gaddy, S.C. Leonard, E. Geretti, S.J. Blocker, D. B. Kirpotin, V. Moyo, T.J. Wickham, B.S. Hendriks, (64)Cu-MM-302 positron emission tomography quantifies variability of enhanced permeability and retention of nanoparticles in relation to treatment response in patients with metastatic breast cancer, Clin. Cancer Res. 23 (2017) 4190–4202.
[91] S. Hosokawa, T. Tagawa, H. Niki, Y. Hirakawa, K. Nohga, K. Nagaike, Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner, Br. J. Cancer 89 (2003) 1545–1551.
[92] C. Mamot, D.C. Drummond, C.O. Noble, V. Kallab, Z. Guo, K. Hong, D.B. Kirpotin, J.W. Park, Epidermal growth factor receptor–targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs in vivo, Cancer Res. 65 (2005) 11631–11638.
[93] Y. Zhou, D.C. Drummond, H. Zou, M.E. Hayes, G.P. Adams, D.B. Kirpotin, J. D. Marks, Impact of single-chain Fv antibody fragment affinity on nanoparticle targeting of epidermal growth factor receptor-expressing tumor cells, J. Mol. Biol. 371 (2007) 934–947.
[94] C. Mamot, R. Ritschard, A. Wicki, W. Küng, J. Schuller, R. Herrmann, C. Rochlitz, Immunoliposomal delivery of doxorubicin can overcome multidrug resistance mechanisms in EGFR-overexpressing tumor cells, J. Drug Target. 20 (2012) 422–432.
[95] T. Birngruber, R. Raml, W. Gladdines, C. Gatschelhofer, E. Gander, A. Ghosh, T. Kroath, P.J. Gaillard, T.R. Pieber, F. Sinner, Enhanced doxorubicin delivery to the brain administered through glutathione PEGylated liposomal doxorubicin (2B3-101) as compared with generic Caelyx,((R))/Doxil((R))–a cerebral open flow microperfusion pilot study, J. Pharm. Sci. 103 (2014) 1945–1948.
[96] P.J. Gaillard, B.M. Kerklaan, P. Aftimos, S. Altintas, A. Jager, W. Gladdines, F. Lonnqvist, P. Soetekouw, H. Verheul, A. Awada, J. Schellens, D. Brandsma, Abstract CT216: Phase I dose escalating study of 2B3-101, glutathione PEGylated liposomal doxorubicin, in patients with solid tumors and brain metastases or recurrent malignant glioma, Cancer Res. 74 (2014) CT216.
[97] T.R. Daniels, E. Bernabeu, J.A. Rodriguez, S. Patel, M. Kozman, D.A. Chiappetta, E. Holler, J.Y. Ljubimova, G. Helguera, M.L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, Biochim. Biophys. Acta. 1820 (2012) 291–317.
[98] R. Suzuki, T. Takizawa, Y. Kuwata, M. Mutoh, N. Ishiguro, N. Utoguchi, A. Shinohara, M. Eriguchi, H. Yanagie, K. Maruyama, Effective anti-tumor activity of oxaliplatin encapsulated in transferrin-PEG-liposome, Int. J. Pharm. 346 (2008) 143–150.
[99] H. Sun, Y. Dong, J. Feijen, Z. Zhong, Peptide-decorated polymeric nanomedicines for precision cancer therapy, J. Control. Release 290 (2018) 11–27.
[100] X. Wei, J. Gao, C. Zhan, C. Xie, Z. Chai, D. Ran, M. Ying, P. Zheng, W. Lu, Liposome-based glioma targeted drug delivery enabled by stable peptide ligands, J. Control. Release 218 (2015) 13–21.
[101] C. Chen, Z. Duan, Y. Yuan, R. Li, L. Pang, J. Liang, X. Xu, J. Wang, Peptide-22 and cyclic rgd functionalized liposomes for glioma targeting drug delivery overcoming BBB and BBTB, ACS Appl. Mater. Interfaces 9 (2017) 5864–5873.
[102] S. Iqbal, M. Blenner, A. Alexander-Bryant, J. Larsen, Polymersomes for therapeutic delivery of protein and nucleic acid macromolecules: From design to therapeutic applications, Biomacromolecules 21 (2020) 1327–1350.
[103] F. Wang, J. Xiao, S. Chen, H. Sun, B. Yang, J. Jiang, X. Zhou, J. Du, Polymer vesicles: Modular platforms for cancer theranostics, Adv. Mater. 30 (2018) 1705674.
[104] B. Discher, Y.Y. Won, D. Ege, J. Lee, F. Bates, D. Discher, D. Hammer, Polymersomes: Tough vesicles made from diblock copolymers, Science 284 (1999) 1143–1146.
[105] Y. Zou, F. Meng, C. Deng, Z. Zhong, Robust, tumor-homing and redox-sensitive polymersomal doxorubicin: A superior alternative to Doxil and Caelyx? J. Control. Release 239 (2016) 149–158.
[106] N. Zhang, Y. Xia, Y. Zou, W. Yang, J. Zhang, Z. Zhong, F. Meng, ATN-161 peptide functionalized reversibly cross-linked polymersomes mediate targeted doxorubicin delivery into melanoma-bearing C57BL/6 mice, Mol. Pharm. 14 (2017) 2538–2547.
[107] Y. Zou, J. Wei, Y. Xia, F. Meng, J. Yuan, Z. Zhong, Targeted chemotherapy for subcutaneous and orthotopic non-small cell lung tumors with cyclic RGD-functionalized and disulfide-crosslinked polymersomal doxorubicin, Signal Transduct. Target Ther. 3 (2018) 32.
[108] Y. Zou, Y. Xia, F. Meng, J. Zhang, Z. Zhong, GE11-directed functional polymersomal doxorubicin as an advanced alternative to clinical liposomal formulation for ovarian cancer treatment, Mol. Pharm. 15 (2018) 3664–3671.
[109] Y. Fang, W. Yang, L. Cheng, F. Meng, J. Zhang, Z. Zhong, EGFR-targeted multifunctional polymersomal doxorubicin induces selective and potent suppression of orthotopic human liver cancer in vivo, Acta Biomater. 64 (2017) 323–333.
[110] W. Gu, J. An, H. Meng, N. Yu, Y. Zhong, F. Meng, Y. Xu, J. Cornelissen, Z. Zhong, CD44-specific A6 short peptide boosts targetability and anticancer efficacy of polymersomal epirubicin to orthotopic human multiple myeloma, Adv. Mater. 31 (2019) 1904742.
[111] Y. Wei, X. Gu, L. Cheng, F. Meng, G. Storm, Z. Zhong, Low-toxicity transferrin-guided polymersomal doxorubicin for potent chemotherapy of orthotopic hepatocellular carcinoma in vivo, Acta Biomater. 92 (2019) 196–204.
[112] L. Ding, W. Gu, Y. Zhang, S. Yue, H. Sun, J. Cornelissen, Z. Zhong, HER2-specific reduction-sensitive immunopolymersomes with high loading of epirubicin for targeted treatment of ovarian tumor, Biomacromolecules 20 (2019) 3855–3863.
[113] L. Martinez-Jothar, S. Doulkeridou, R.M. Schiffelers, J. Sastre Torano, S. Oliveira, C.F. van Nostrum, W.E. Hennink, Insights into maleimide-thiol conjugation chemistry: Conditions for efficient surface functionalization of nanoparticles for receptor targeting, J. Control. Release 282 (2018) 101–109.
[114] Y. Wei, X. Gu, Y. Sun, F. Meng, G. Storm, Z. Zhong, Transferrin-binding peptide functionalized polymersomes mediate targeted doxorubicin delivery to colorectal cancer in vivo, J. Control. Release 319 (2020) 407–415.
[115] M. Qiu, H. Sun, F. Meng, R. Cheng, J. Zhang, C. Deng, Z. Zhong, Lipopepsomes: A novel and robust family of nano-vesicles capable of highly efficient encapsulation and tumor-targeted delivery of doxorubicin hydrochloride in vivo, J. Control. Release 272 (2018) 107–113.
[116] W. Yang, L. Yang, Y. Xia, L. Cheng, J. Zhang, F. Meng, J. Yuan, Z. Zhong, Lung cancer specific and reduction-responsive chimaeric polymersomes for highly efficient loading of pemetrexed and targeted suppression of lung tumor in vivo, Acta Biomater. 70 (2018) 177–185.
[117] W. Yang, Y. Xia, Y. Fang, F. Meng, J. Zhang, R. Cheng, C. Deng, Z. Zhong, Selective cell penetrating peptide-functionalized polymersomes mediate efficient and targeted delivery of methotrexate disodium to human lung cancer in vivo, Adv. Healthc. Mater. 7 (2018) 1701135.
[118] H. Qin, Y. Jiang, J. Zhang, C. Deng, Z. Zhong, Oncoprotein inhibitor rigosertib loaded in ApoE-targeted smart polymersomes reveals high safety and potency against human glioblastoma in mice, Mol. Pharm. 16 (2019) 3711–3719.
[119] D. Vercauteren, J. Rejman, T.F. Martens, J. Demeester, S.C. De Smedt, K. Braeckmans, On the cellular processing of non-viral nanomedicines for nucleic acid delivery: Mechanisms and methods, J. Control. Release 161 (2012) 566–581.
[120] U. Lachelt, E. Wagner, Nucleic acid therapeutics using polyplexes: A journey of 50 years (and beyond), Chem. Rev. 115 (2015) 11043–11078.
[121] H. Lee, A.K.R. Lytton-Jean, Y. Chen, K.T. Love, A.I. Park, E.D. Karagiannis, A. Sehgal, W. Querbes, C.S. Zurenko, M. Jayaraman, C.G. Peng, K. Charisse, A. Borodovsky, M. Manoharan, J.S. Donahoe, J. Truelove, M. Nahrendorf, R. Langer, D.G. Anderson, Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery, Nat. Nanotechnol. 7 (2012) 389–393.
[122] H.J. Kim, A. Kim, K. Miyata, K. Kataoka, Recent progress in development of siRNA delivery vehicles for cancer therapy, Adv. Drug Deliv. Rev. 104 (2016) 61–77.
[123] J.C. Kaczmarek, P.S. Kowalski, D.G. Anderson, Advances in the delivery of RNA therapeutics: From concept to clinical reality, Genome Med. 9 (2017) 60.
[124] K.A. Hajj, K.A. Whitehead, Tools for translation: Non-viral materials for therapeutic mRNA delivery, Nat. Rev. Mater. 2 (2017) 17056.
[125] L. Yin, Z. Song, K.H. Kim, N. Zheng, N.P. Gabrielson, J. Cheng, Non-viral gene delivery via membrane-penetrating, mannose-targeting supramolecular self-assembled nanocomplexes, Adv. Mater. 25 (2013) 3063–3070.
[126] M.E. Davis, J.E. Zuckerman, C.H. Choi, D. Seligson, A. Tolcher, C.A. Alabi, Y. Yen, J.D. Heidel, A. Ribas, Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles, Nature 464 (2010) 1067–1070.
[127] R.A. de Claro, K. McGinn, V. Kwitkowski, J. Bullock, A. Khandelwal, B. Habtemariam, Y. Ouyang, H. Saber, K. Lee, K. Koti, M. Rothmann, M. Shapiro, F. Borrego, K. Clouse, X.H. Chen, J. Brown, L. Akinsanya, R. Kane, E. Kaminskas, A. Farrell, R. Pazdur, U.S. Food and Drug Administration approval summary: Brentuximab vedotin for the treatment of relapsed Hodgkin lymphoma or

relapsed systemic anaplastic large-cell lymphoma, Clin. Cancer Res. 18 (2012) 5845–5849.
[128] D.W. Laske, R.J. Youle, E.H. Oldfield, Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors, Nat. Med. 3 (1997) 1362–1368.
[129] K.F. Pirollo, J. Nemunaitis, P.K. Leung, R. Nunan, J. Adams, E.H. Chang, Safety and efficacy in advanced solid tumors of a targeted nanocomplex carrying the p53 gene used in combination with docetaxel: A phase 1b study, Mol. Ther. 24 (2016) 1697–1706.
[130] L. Xu, C.-C. Huang, W. Huang, W.-H. Tang, A. Rait, Y.Z. Yin, I. Cruz, L.-M. Xiang, K.F. Pirollo, E.H. Chang, Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes, Mol. Cancer Ther. 1 (2002) 337–346.
[131] S.-S. Kim, A. Rait, E. Kim, K.F. Pirollo, M. Nishida, N. Farkas, J.A. Dagata, E. H. Chang, A nanoparticle carrying the p53 gene targets tumors including cancer stem cells, sensitizes glioblastoma to chemotherapy and improves survival, ACS Nano 8 (2014) 5494–5514.
[132] J. Harford, S.-S. Kim, K. Pirollo, A. Rait, M. Moghe, E. Chang, TP53 gene therapy emanating from the investigational agent SGT-53 is capable of augmenting cancer immunotherapy in multiple murine syngeneic tumor models, J. Immunother. Cancer 6 (2018) P457.
[133] A. Siefker-Radtke, X.Q. Zhang, C.C. Guo, Y. Shen, K.F. Pirollo, S. Sabir, C. Leung, C. Leong-Wu, C.M. Ling, E.H. Chang, R.E. Millikan, W.F. Benedict, A phase l study of a tumor-targeted systemic nanodelivery system, SGT-94, in genitourinary cancers, Mol. Ther. 24 (2016) 1484–1491.
[134] M.E. Davis, The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: From concept to clinic, Mol. Pharm. 6 (2009) 659–668.
[135] D.W. Bartlett, M.E. Davis, Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles, Bioconjug. Chem. 18 (2007) 456–468.
[136] J.D. Heidel, J.Y. Liu, Y. Yen, B. Zhou, B.S. Heale, J.J. Rossi, D.W. Bartlett, M. E. Davis, Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation in vitro and in vivo, Clin. Cancer Res. 13 (2007) 2207–2215.
[137] H.J. Kim, H. Takemoto, Y. Yi, M. Zheng, Y. Maeda, H. Chaya, K. Hayashi, P. Mi, F. Pittella, R.J. Christie, K. Toh, Y. Matsumoto, N. Nishiyama, K. Miyata, K. Kataoka, Precise engineering of siRNA delivery vehicles to tumors using polyion complexes and gold nanoparticles, ACS Nano 8 (2014) 8979–8991.
[138] S. Uchida, K. Kataoka, Design concepts of polyplex micelles for in vivo therapeutic delivery of plasmid DNA and messenger RNA, J. Biomed. Mater. Res. A 107 (2019) 978–990.
[139] Y. Oe, R.J. Christie, M. Naito, S.A. Low, S. Fukushima, K. Toh, Y. Miura, Y. Matsumoto, N. Nishiyama, K. Miyata, K. Kataoka, Actively-targeted polyion complex micelles stabilized by cholesterol and disulfide cross-linking for systemic delivery of siRNA to solid tumors, Biomaterials 35 (2014) 7887–7895.
[140] Y. Yi, H.J. Kim, P. Mi, M. Zheng, H. Takemoto, K. Toh, B.S. Kim, K. Hayashi, M. Naito, Y. Matsumoto, K. Miyata, K. Kataoka, Targeted systemic delivery of siRNA to cervical cancer model using cyclic RGD-installed unimer polyion complex-assembled gold nanoparticles, J. Control. Release 244 (2016) 247–256.
[141] Y. Yi, H.J. Kim, M. Zheng, P. Mi, M. Naito, B.S. Kim, H.S. Min, K. Hayashi, F. Perche, K. Toh, X. Liu, Y. Mochida, H. Kinoh, H. Cabral, K. Miyata, K. Kataoka, Glucose-linked sub-50-nm unimer polyion complex-assembled gold nanoparticles for targeted siRNA delivery to glucose transporter 1-overexpressing breast cancer stem-like cells, J. Control. Release 295 (2019) 268–277.
[142] G. Chen, Y. Wang, R. Xie, S. Gong, Tumor-targeted pH/redox dual-sensitive unimolecular nanoparticles for efficient siRNA delivery, J. Control. Release 259 (2017) 105–114.
[143] P.M. Klein, S. Kern, D.J. Lee, J. Schmaus, M. Hohn, J. Gorges, U. Kazmaier, E. Wagner, Folate receptor-directed orthogonal click-functionalization of siRNA lipopolyplexes for tumor cell killing in vivo, Biomaterials 178 (2018) 630–642.
[144] F. Pi, D.W. Binzel, T.J. Lee, Z. Li, M. Sun, P. Rychahou, H. Li, F. Haque, S. Wang, C.M. Croce, B. Guo, B.M. Evers, P. Guo, Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression, Nat. Nanotechnol. 13 (2017) 82–89.
[145] Z. Zheng, Z. Li, C. Xu, B. Guo, P. Guo, Folate-displaying exosome mediated cytosolic delivery of siRNA avoiding endosome trapping, J. Control. Release 311-312 (2019) 43–49.
[146] Y. Zhao, W. Wang, S. Guo, Y. Wang, L. Miao, Y. Xiong, L. Huang, PolyMetformin combines carrier and anticancer activities for in vivo siRNA delivery, Nat. Commun. 7 (2016) 11822.
[147] Y. Kim, M. Tewari, J.D. Pajerowski, S. Cai, S. Sen, J. Williams, S. Sirsi, G. Lutz, D. E. Discher, Polymersome delivery of siRNA and antisense oligonucleotides, J. Control. Release 134 (2009) 132–140.
[148] R. Cheng, F. Feng, F. Meng, C. Deng, J. Feijen, Z. Zhong, Glutathione-responsive nano-vehicles as a promising platform for targeted intracellular drug and gene delivery, J. Control. Release 152 (2011) 2–12.
[149] F. Wang, J. Gao, J. Xiao, J. Du, Dually gated polymersomes for gene delivery, Nano Lett. 18 (2018) 5562–5568.
[150] Y. Zou, M. Zheng, W. Yang, F. Meng, K. Miyata, H.J. Kim, K. Kataoka, Z. Zhong, Virus-mimicking chimaeric polymersomes boost targeted cancer siRNA therapy in vivo, Adv. Mater. 29 (2017) 1703285.
[151] Y. Shi, Y. Jiang, J. Cao, W. Yang, J. Zhang, F. Meng, Z. Zhong, Boosting RNAi therapy for orthotopic glioblastoma with nontoxic brain-targeting chimaeric polymersomes, J. Control. Release 292 (2018) 163–171.
[152] M. Qiu, J. Ouyang, Y. Wei, J. Zhang, Q. Lan, C. Deng, Z. Zhong, Selective cell penetrating peptide-functionalized envelope-type chimeric lipopepsomes boost systemic RNAi therapy for lung tumors, Adv. Healthc. Mater. 8 (2019) 1900500.
[153] A. Ganju, S. Khan, B.B. Hafeez, S.W. Behrman, M.M. Yallapu, S.C. Chauhan, M. Jaggi, miRNA nanotherapeutics for cancer, Drug Discov. Today 22 (2017) 424–432.
[154] C.L. Esposito, L. Cerchia, S. Catuogno, G. De Vita, J.P. Dassie, G. Santamaria, P. Swiderski, G. Condorelli, P.H. Giangrande, V. de Franciscis, Multifunctional aptamer-miRNA conjugates for targeted cancer therapy, Mol. Ther. 22 (2014) 1151–1163.
[155] E.A. Orellana, S. Tenneti, L. Rangasamy, L.T. Lyle, P.S. Low, A.L. Kasinski, FolamiRs: Ligand-targeted, vehicle-free delivery of microRNAs for the treatment of cancer, Sci. Transl. Med. 9 (2017) eaam9327.
[156] M. Perepelyuk, K. Sacko, K. Thangavel, S.A. Shoyele, Evaluation of MUC1-aptamer functionalized hybrid nanoparticles for targeted delivery of miRNA-29b to nonsmall cell lung cancer, Mol. Pharm. 15 (2018) 985–993.
[157] L. Liu, H. Yi, H. He, H. Pan, L. Cai, Y. Ma, Tumor associated macrophage-targeted microRNA delivery with dual-responsive polypeptide nanovectors for anti-cancer therapy, Biomaterials 134 (2017) 166–179.
[158] X. Zang, X. Zhang, X. Zhao, H. Hu, M. Qiao, Y. Deng, D. Chen, Targeted delivery of miRNA 155 to tumor associated macrophages for tumor immunotherapy, Mol. Pharm. 16 (2019) 1714–1722.
[159] H. Yin, G. Xiong, S. Guo, C. Xu, R. Xu, P. Guo, D. Shu, Delivery of anti-miRNA for triple-negative breast cancer therapy using RNA nanoparticles targeting stem cell marker CD133, Mol. Ther. 27 (2019) 1252–1261.
[160] T. Zhan, N. Rindtorff, J. Betge, M.P. Ebert, M. Boutros, CRISPR/Cas9 for cancer research and therapy, Semin. Cancer Biol. 55 (2019) 106–119.
[161] L. Zhang, P. Wang, Q. Feng, N. Wang, Z. Chen, Y. Huang, W. Zheng, X. Jiang, Lipid nanoparticle-mediated efficient delivery of CRISPR/Cas9 for tumor therapy, NPG Asia Mater. 9 (2017), e441.
[162] H.X. Wang, Z. Song, Y.H. Lao, X. Xu, J. Gong, D. Cheng, S. Chakraborty, J.S. Park, M. Li, D. Huang, L. Yin, J. Cheng, K.W. Leong, Nonviral gene editing via CRISPR/Cas9 delivery by membrane-disruptive and endosomolytic helical polypeptide, Proc. Natl. Acad. Sci. U S A 115 (2018) 4903–4908.
[163] Y.L. Luo, C.F. Xu, H.J. Li, Z.T. Cao, J. Liu, J.L. Wang, X.J. Du, X.Z. Yang, Z. Gu, J. Wang, Macrophage-specific in vivo gene editing using cationic lipid-assisted polymeric nanoparticles, ACS Nano 12 (2018) 994–1005.
[164] S.M. Kim, Y. Yang, S.J. Oh, Y. Hong, M. Seo, M. Jang, Cancer-derived exosomes as a delivery platform of CRISPR/Cas9 confer cancer cell tropism-dependent targeting, J. Control. Release 266 (2017) 8–16.
[165] M.Z. Alyami, S.K. Alsaiari, Y. Li, S.S. Qutub, F.A. Aleisa, R. Sougrat, J. S. Merzaban, N.M. Khashab, Cell-type-specific CRISPR/Cas9 delivery by biomimetic metal organic frameworks, J. Am. Chem. Soc. 142 (2020) 1715–1720.
[166] B.Y. Liu, X.Y. He, R.X. Zhuo, S.X. Cheng, Tumor targeted genome editing mediated by a multi-functional gene vector for regulating cell behaviors, J. Control. Release 291 (2018) 90–98.
[167] C. Liang, F. Li, L. Wang, Z.K. Zhang, C. Wang, B. He, J. Li, Z. Chen, A.B. Shaikh, J. Liu, X. Wu, S. Peng, L. Dang, B. Guo, X. He, D.W.T. Au, C. Lu, H. Zhu, B. T. Zhang, A. Lu, G. Zhang, Tumor cell-targeted delivery of CRISPR/Cas9 by aptamer-functionalized lipopolymer for therapeutic genome editing of VEGFA in osteosarcoma, Biomaterials 147 (2017) 68–85.
[168] M. Li, H. Xie, Y. Liu, C. Xia, X. Cun, Y. Long, X. Chen, M. Deng, R. Guo, Z. Zhang, Q. He, Knockdown of hypoxia-inducible factor-1 alpha by tumor targeted delivery of CRISPR/Cas9 system suppressed the metastasis of pancreatic cancer, J. Control. Release 304 (2019) 204–215.
[169] N. Pardi, M.J. Hogan, F.W. Porter, D. Weissman, mRNA vaccines - a new era in vaccinology, Nat. Rev. Drug Discov. 17 (2018) 261–279.
[170] L. Martinez-Pomares, The mannose receptor, J. Leukoc. Biol. 92 (2012) 1177–1186.
[171] Y. Wang, L. Zhang, Z. Xu, L. Miao, L. Huang, mRNA vaccine with antigen-specific checkpoint blockade induces an enhanced immune response against established melanoma, Mol. Ther. 26 (2018) 420–434.
[172] F. Perche, D. Gosset, M. Mevel, M.L. Miramon, J.J. Yaouanc, C. Pichon, T. Benvegnu, P.A. Jaffres, P. Midoux, Selective gene delivery in dendritic cells with mannosylated and histidylated lipopolyplexes, J. Drug Target. 19 (2011) 315–325.
[173] F. Perche, T. Benvegnu, M. Berchel, L. Lebegue, C. Pichon, P.A. Jaffres, P. Midoux, Enhancement of dendritic cells transfection in vivo and of vaccination against B16F10 melanoma with mannosylated histidylated lipopolyplexes loaded with tumor antigen messenger RNA, Nanomedicine 7 (2011) 445–453.
[174] A. Le Moignic, V. Malard, T. Benvegnu, L. Lemiegre, M. Berchel, P.A. Jaffres, C. Baillou, M. Delost, R. Macedo, J. Rochefort, G. Lescaille, C. Pichon, F. M. Lemoine, P. Midoux, V. Mateo, Preclinical evaluation of mRNA trimannosylated lipopolyplexes as therapeutic cancer vaccines targeting dendritic cells, J. Control. Release 278 (2018) 110–121.
[175] S. Son, J. Nam, I. Zenkov, L.J. Ochyl, Y. Xu, L. Scheetz, J. Shi, O.C. Farokhzad, J. J. Moon, Sugar-nanocapsules imprinted with microbial molecular patterns for mRNA vaccination, Nano Lett. 20 (2020) 1499–1509.
[176] B. Lou, S. De Koker, C.Y.J. Lau, W.E. Hennink, E. Mastrobattista, mRNA polyplexes with post-conjugated GALA peptides efficiently target, transfect, and activate antigen presenting cells, Bioconjug. Chem. 30 (2019) 461–475.
[177] H. Parhiz, V.V. Shuvaev, N. Pardi, M. Khoshnejad, R.Y. Kiseleva, J.S. Brenner, T. Uhler, S. Tuyishime, B.L. Mui, Y.K. Tam, T.D. Madden, M.J. Hope, D. Weissman, V.R. Muzykantov, PECAM-1 directed re-targeting of exogenous mRNA providing two orders of magnitude enhancement of vascular delivery and

expression in lungs independent of apolipoprotein E-mediated uptake, J. Control. Release 291 (2018) 106–115.
[178] Q. Chen, R. Qi, X. Chen, X. Yang, S. Wu, H. Xiao, W. Dong, A targeted and stable polymeric nanoformulation enhances systemic delivery of mRNA to tumors, Mol. Ther. 25 (2017) 92–101.
[179] A. Akinc, M.A. Maier, M. Manoharan, K. Fitzgerald, M. Jayaraman, S. Barros, S. Ansell, X. Du, M.J. Hope, T.D. Madden, B.L. Mui, S.C. Semple, Y.K. Tam, M. Ciufolini, D. Witzigmann, J.A. Kulkarni, R. van der Meel, P.R. Cullis, The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs, Nat. Nanotechnol. 14 (2019) 1084–1087.
[180] P. Barata, A.K. Sood, D.S. Hong, RNA-targeted therapeutics in cancer clinical trials: Current status and future directions, Cancer Treat Rev. 50 (2016) 35–47.
[181] A.K. Iyer, Z. Duan, M.M. Amiji, Nanodelivery systems for nucleic acid therapeutics in drug resistant tumors, Mol. Pharm. 11 (2014) 2511–2526.
[182] W. Huang, L. Chen, L. Kang, M. Jin, P. Sun, X. Xin, Z. Gao, Y.H. Bae, Nanomedicine-based combination anticancer therapy between nucleic acids and small-molecular drugs, Adv. Drug Deliv. Rev. 115 (2017) 82–97.
[183] C. Chen, Z. Yang, X. Tang, Chemical modifications of nucleic acid drugs and their delivery systems for gene-based therapy, Med. Res. Rev. 38 (2018) 829–869.
[184] S. Mitragotri, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: Formulation and delivery strategies, Nat. Rev. Drug Discov. 13 (2014) 655–672.
[185] X. Li, W. Yang, Y. Zou, F. Meng, C. Deng, Z. Zhong, Efficacious delivery of protein drugs to prostate cancer cells by PSMA-targeted pH-responsive chimaeric polymersomes, J. Control. Release 220 (2015) 704–714.
[186] L. Lu, Y. Zou, W. Yang, F. Meng, C. Deng, R. Cheng, Z. Zhong, Anisamide-decorated pH-sensitive degradable chimaeric polymersomes mediate potent and targeted protein delivery to lung cancer cells, Biomacromolecules 16 (2015) 1726–1735.
[187] W. Yang, Y. Wei, L. Yang, J. Zhang, Z. Zhong, G. Storm, F. Meng, Granzyme B-loaded, cell-selective penetrating and reduction-responsive polymersomes effectively inhibit progression of orthotopic human lung tumor in vivo, J. Control. Release 290 (2018) 141–149.
[188] A. Fu, R. Tang, J. Hardie, M.E. Farkas, V.M. Rotello, Promises and pitfalls of intracellular delivery of proteins, Bioconjug. Chem. 25 (2014) 1602–1608.
[189] P.D. Senter, E.L. Sievers, The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma, Nat. Biotechnol. 30 (2012) 631–637.
[190] J. Katz, J.E. Janik, A. Younes, Brentuximab vedotin (SGN-35), Clin. Cancer Res. 17 (2011) 6428–6436.
[191] A. Younes, N.L. Bartlett, J.P. Leonard, D.A. Kennedy, C.M. Lynch, E.L. Sievers, A. Forero-Torres, Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas, N. Engl. J. Med. 363 (2010) 1812–1821.
[192] S. Horwitz, O.A. O'Connor, B. Pro, T. Illidge, M. Fanale, Z. Hua, M. Little, S. Rao, J. Woolery, T. Manley, L. Trümper, Brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma (ECHELON-2): a global, double-blind, randomised, phase 3 trial, The Lancet 393 (2019) 229–240.
[193] J.M. Connors, W. Jurczak, D.J. Straus, S.M. Ansell, W.S. Kim, A. Gallamini, A. Younes, S. Alekseev, A. Illes, M. Picardi, E. Lech-Maranda, Y. Oki, T. Feldman, P. Smolewski, K.J. Savage, N.L. Bartlett, J. Walewski, R. Chen, R. Ramchandren, P.L. Zinzani, D. Cunningham, A. Rosta, N.C. Josephson, E. Song, J. Sachs, R. Liu, H.A. Jolin, D. Huebner, J. Radford, Brentuximab vedotin with chemotherapy for stage III or IV hodgkin's lymphoma, N. Engl. J. Med. 378 (2018) 331–344.
[194] M. Weaver, D. Laske, Transferrin receptor ligand-targeted toxin conjugate (Tf-CRM107) for therapy of malignant gliomas, J. Neurooncol. 65 (2003) 3–13.
[195] A. Jahangiri, A.T. Chin, P.M. Flanigan, R. Chen, K. Bankiewicz, M.K. Aghi, Convection-enhanced delivery in glioblastoma: A review of preclinical and clinical studies, J. Neurosurg. 126 (2017) 191–200.
[196] D. Li, C.F. van Nostrum, E. Mastrobattista, T. Vermonden, W.E. Hennink, Nanogels for intracellular delivery of biotherapeutics, J. Control. Release 259 (2017) 16–28.
[197] D. Huang, H. Qian, H. Qiao, W. Chen, J. Feijen, Z. Zhong, Bioresponsive functional nanogels as an emerging platform for cancer therapy, Expert Opin. Drug Deliv. 15 (2018) 703–716.
[198] J. Chen, Y. Zou, C. Deng, F. Meng, J. Zhang, Z. Zhong, Multifunctional click hyaluronic acid nanogels for targeted protein delivery and effective cancer treatment in vivo, Chem. Mater. 28 (2016) 8792–8799.
[199] J. Chen, J. Ouyang, Q. Chen, C. Deng, F. Meng, J. Zhang, R. Cheng, Q. Lan, Z. Zhong, EGFR and CD44 dual-targeted multifunctional hyaluronic acid nanogels boost protein delivery to ovarian and breast cancers in vitro and in vivo, ACS Appl. Mater. Interfaces 9 (2017) 24140–24147.
[200] J. Chen, H. He, C. Deng, L. Yin, Z. Zhong, Saporin-loaded CD44 and EGFR dual-targeted nanogels for potent inhibition of metastatic breast cancer in vivo, Int. J. Pharm. 560 (2019) 57–64.
[201] L. Ding, Y. Jiang, J. Zhang, H.A. Klok, Z. Zhong, pH-sensitive coiled-coil peptide-cross-linked hyaluronic acid nanogels: synthesis and targeted intracellular protein delivery to CD44 positive cancer cells, Biomacromolecules 19 (2018) 555–562.
[202] K. Huang, Y. He, Z. Zhu, J. Guo, G. Wang, C. Deng, Z. Zhong, Small, traceable, endosome-disrupting, and bioresponsive click nanogels fabricated via microfluidics for CD44-targeted cytoplasmic delivery of therapeutic proteins, ACS Appl. Mater. Interfaces 11 (2019) 22171–22180.
[203] K. Liang, S. Ng, F. Lee, J. Lim, J.E. Chung, S.S. Lee, M. Kurisawa, Targeted intracellular protein delivery based on hyaluronic acid-green tea catechin nanogels, Acta Biomater. 33 (2016) 142–152.
[204] Q. Zhu, X. Chen, X. Xu, Y. Zhang, C. Zhang, R. Mo, Tumor-specific self-degradable nanogels as potential carriers for systemic delivery of anticancer proteins, Adv. Funct. Mater. 28 (2018) 1707371.
[205] A. Molina-Crespo, A. Cadete, D. Sarrio, M. Gamez-Chiachio, L. Martinez, K. Chao, A. Olivera, A. Gonella, E. Diaz, J. Palacios, P.K. Dhal, M. Besev, M. Rodriguez-Serrano, M.L. Garcia Bermejo, J.C. Trivino, A. Cano, M. Garcia-Fuentes, O. Herzberg, D. Torres, M.J. Alonso, G. Moreno-Bueno, Intracellular delivery of an antibody targeting gasdermin-B reduces HER2 breast cancer aggressiveness, Clin. Cancer Res. 25 (2019) 4846–4858.
[206] S. Zhao, F. Duan, S. Liu, T. Wu, Y. Shang, R. Tian, J. Liu, Z.G. Wang, Q. Jiang, B. Ding, Efficient intracellular delivery of RNase A using DNA origami carriers, ACS Appl. Mater. Interfaces 11 (2019) 11112–11118.
[207] G. Liu, S. Ma, S. Li, R. Cheng, F. Meng, H. Liu, Z. Zhong, The highly efficient delivery of exogenous proteins into cells mediated by biodegradable chimaeric polymersomes, Biomaterials 31 (2010) 7575–7585.
[208] W. Yang, Y. Xia, Y. Zou, F. Meng, J. Zhang, Z. Zhong, Bioresponsive chimaeric nanopolymersomes enable targeted and efficacious protein therapy for human lung cancers in vivo, Chem. Mater. 29 (2017) 8757–8765.
[209] Y. Zhong, F. Meng, W. Zhang, B. Li, J.C.M. van Hest, Z. Zhong, CD44-targeted vesicles encapsulating granzyme B as artificial killer cells for potent inhibition of human multiple myeloma in mice, J. Control. Release 320 (2020) 421–430.
[210] Y. Jiang, W. Yang, J. Zhang, F. Meng, Z. Zhong, Protein toxin chaperoned by LRP-1-targeted virus-mimicking vesicles induces high-efficiency glioblastoma therapy in vivo, Adv. Mater. 30 (2018) 1800316.
[211] Y. Jiang, J. Zhang, F. Meng, Z. Zhong, Apolipoprotein E peptide-directed chimeric polymersomes mediate an ultrahigh-efficiency targeted protein therapy for glioblastoma, ACS Nano 12 (2018) 11070–11079.
[212] M. Qiu, Z. Zhang, Y. Wei, H. Sun, F. Meng, C. Deng, Z. Zhong, Small-sized and robust chimaeric lipopepsomes: A simple and functional platform with high protein loading for targeted intracellular delivery of protein toxin in vivo, Chem. Mater. 30 (2018) 6831–6838.
[213] D. Zhu, C. Hu, F. Fan, Y. Qin, C. Huang, Z. Zhang, L. Lu, H. Wang, H. Sun, X. Leng, C. Wang, D. Kong, L. Zhang, Co-delivery of antigen and dual agonists by programmed mannose-targeted cationic lipid-hybrid polymersomes for enhanced vaccination, Biomaterials 206 (2019) 25–40.
[214] L. Salvioni, M.A. Rizzuto, J.A. Bertolini, L. Pandolfi, M. Colombo, D. Prosperi, Thirty years of cancer nanomedicine: Success, frustration, and hope, Cancers 11 (2019) 1855.
[215] D. Bobo, K.J. Robinson, J. Islam, K.J. Thurecht, S.R. Corrie, Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to date, Pharm. Res. 33 (2016) 2373–2387.
[216] H. Shim, Bispecific antibodies and antibody-drug conjugates for cancer therapy: Technological considerations, Biomolecules 10 (2020) 360.
[217] A. Beck, L. Goetsch, C. Dumontet, N. Corvaia, Strategies and challenges for the next generation of antibody-drug conjugates, Nat. Rev. Drug Discov. 16 (2017) 315–337.
[218] R.D. Rubens, Antibodies as carriers of anticancer agents, The Lancet 303 (1974) 498–499.
[219] G. Gregoriadis, Targeting of drugs, Nature 265 (1977) 407–411.
[220] K. Tsuchikama, Z. An, Antibody-drug conjugates: Recent advances in conjugation and linker chemistries, Protein Cell 9 (2018) 33–46.
[221] C. Duerr, W. Friess, Antibody-drug conjugates- stability and formulation, Eur. J. Pharm. Biopharm. 139 (2019) 168–176.
[222] S. Coats, M. Williams, B. Kebble, R. Dixit, L. Tseng, N.S. Yao, D.A. Tice, J.C. Soria, Antibody-drug conjugates: Future directions in clinical and translational strategies to improve the therapeutic index, Clin. Cancer Res. 25 (2019) 5441–5448.
[223] T.D. Heath, R.T. Fraley, D. Papahdjopoulos, Antibody targeting of liposomes: Cell specificity obtained by conjugation of F(ab')2 to vesicle surface, Science 210 (1980) 539–541.
[224] T.M. Allen, E.H. Moase, Therapeutic opportunities for targeted liposomal drug delivery, Adv. Drug Deliv. Rev. 21 (1996) 117–133.
[225] D. Adams, A. Gonzalez-Duarte, W.D. O'Riordan, C.C. Yang, M. Ueda, A.V. Kristen, I. Tournev, H.H. Schmidt, T. Coelho, J.L. Berk, K.P. Lin, G. Vita, S. Attarian, V. Plante-Bordeneuve, M.M. Mezei, J.M. Campistol, J. Buades, T. H. Brannagan 3rd, B.J. Kim, J. Oh, Y. Parman, Y. Sekijima, P.N. Hawkins, S. D. Solomon, M. Polydefkis, P.J. Dyck, P.J. Gandhi, S. Goyal, J. Chen, A.L. Strahs, S.V. Nochur, M.T. Sweetser, P.P. Garg, A.K. Vaishnaw, J.A. Gollob, O.B. Suhr, Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis, N. Engl. J. Med. 379 (2018) 11–21.
[226] R.L. Setten, J.J. Rossi, S.-p. Han, The current state and future directions of RNAi-based therapeutics, Nat. Rev. Drug Discov. 18 (2019) 421–446.
[227] J. Xie, Z. Shen, Y. Anraku, K. Kataoka, X. Chen, Nanomaterial-based blood-brain-barrier (BBB) crossing strategies, Biomaterials 224 (2019) 119491.
[228] Y. Zhu, J. Feijen, Z. Zhong, Dual-targeted nanomedicines for enhanced tumor treatment, Nano Today 18 (2018) 65–85.
[229] T. Gura, Magic bullets hit the target, Nature 417 (2002) 584–586.
[230] S.Y. Tan, S. Grimes, Paul Ehrlich (1854-1915): Man with the magic bullet, Singapore Med. J. 51 (2010) 842–843.