

Polymer conjugates as anticancer nanomedicines

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Abstract | The transfer of polymer–protein conjugates into routine clinical use, and the clinical development of polymer–anticancer–drug conjugates, both as single agents and as components of combination therapy, is establishing polymer therapeutics as one of the first classes of anticancer nanomedicines. There is growing optimism that ever more sophisticated polymer-based vectors will be a significant addition to the armoury currently used for cancer therapy.

Controlled release

Controlled release dosage forms maintain a constant plasma concentration of drug over a prolonged period of time.

Nanomedicine

The newly emerging discipline called ‘nanomedicine’ describes the application of nanotechnology (usually viewed as 1–1,000 nm) to: the design of systems and devices that can be used to facilitate a better understanding of disease pathophysiology and therefore enable new target identification for therapeutic intervention; nanoimaging at the cellular and patient level; and the design of nanomedicines and nanodiagnosics. Underpinning fields are nano-related materials, nano-related engineering and nano-related toxicology.

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As mortality due to cancer continues to rise, two approaches are bringing hope of improved therapies. On one hand, genomics and proteomics research is identifying new tumour-specific molecular targets¹, and on the other, innovative drug-delivery systems^{2,3} are being designed to guide drugs more precisely to tumour cells and away from sites of toxicity, and/or to maintain drugs at a therapeutic concentration over long periods of time. The objective of ‘perfectly specific’ low-molecular-weight drug molecules able to prevent tumour cell growth without causing non-specific side effects has been difficult to realize in practice, particularly for the common solid tumours such as **breast**, **prostate**, **lung** and gastrointestinal cancers. This has been attributed to poorly predictive preclinical models⁴, a lack of drug specificity in the clinical setting and the problem of acquired drug resistance. Although sometimes overlooked⁵, drug-delivery systems, nano-sized vectors for tumour targeting and synthetic macromolecular therapeutics have begun to make an important contribution to cancer therapy over the last decade.

Biodegradable polymers containing entrapped drug can be placed in the body, and are used for localized drug delivery and/or the controlled release of a drug over a period of months. For example, small polymer rods (goserelin (Zoladex)) and polymer microparticles (leuprolide (Leupron Depot)) made from polylactide-co-glycolide-entrapping leutinizing hormone releasing hormone (LHRH) analogues are common treatments for prostate cancer. As the polymer slowly degrades, therapeutic levels of the anti-tumour peptide are maintained for up to 3 months, making the therapy very convenient for patient use^{6,7}. Another biodegradable polymeric implant, carmustine (Gliadel), is used to treat **brain cancer** (glioblastoma multiforme)^{8,9}. In this case, a biodegradable polyanhydride polymer is made

into small polymer discs containing the alkylating agent *bis*(2-chloroethyl)nitrosourea (BCNU). These discs are placed into the brain following the surgical removal of the tumour, and thereafter they slowly degrade to deliver the drug locally, therefore preventing tumour re-growth. Although such polymer-based drug-delivery systems have been important advances, the development of nano-sized vectors enables tumours to be targeted more precisely — the vectors can move around in the body and selectively localize a therapeutic drug payload to metastatic tumours.

The medical application of nanotechnology (that is, ‘nanomedicine’) has enormous potential to improve healthcare, particularly in cancer^{10,11,12}. On one hand, miniaturization is creating devices for use as diagnostics, biosensors and imaging agents, and on the other, ever more sophisticated synthetic chemistry is producing nanovectors for drug delivery. The terminology used is often contentious and can be confusing. Ferrari recently coined a useful definition of cancer nanotechnology¹² as “a vast and diverse array of devices derived from engineering, biology, physics and chemistry, including nanovectors for the targeted delivery of anticancer drugs and imaging contrast agents, and those detection systems such as nanowires and nanocantilever arrays under development for the early detection of precancerous and malignant lesions from biological fluids.” Nanovectors have also been called ‘nanopharmaceuticals or nanomedicines’. To distinguish them from biotech products, such as proteins and antibodies (which are also inherently 2–15 nm in size), the European Science Foundation’s Forward Look on Nanomedicine defined nanomedicines as “nanometer size scale complex systems, consisting of at least two components, one of which being the active ingredient”¹¹.

Nanopharmaceuticals or nanomedicines

The umbrella term used to describe nano-sized (1–1,000 nm) drugs and drug-delivery systems used as medicines that consist of more than one component.

Liposomes

Lipid-based vesicles used to entrap a drug payload and promote disease-specific targeting.

Nanoparticle

Tiny particles, usually of 20–500 nm dimensions, and formed from natural or synthetic polymers that are used to entrap drugs for improved drug targeting and controlled release.

Therapeutic index

The comparison of the drug dose that produces toxicity to the therapeutic dose.

Topical administration

The application of drugs to the skin.

Parenteral administration

The administration of drugs directly into the body by injection or infusion.

At a glance

- Water-soluble polymers conjugated to proteins and anticancer drugs are in routine clinical use and clinical development as both single agents and components of combination therapy. This is establishing polymer therapeutics as one of the first classes of anticancer nanomedicine. There is growing optimism about the use of ever more sophisticated polymer-based vectors for cancer therapy.
- The covalent conjugation of synthetic polymers, particularly poly(ethyleneglycol) (PEG), to protein drugs increases their plasma residence, reduces protein immunogenicity and can increase their therapeutic index. Several PEGylated enzymes (such as L-asparaginase) and cytokines (including interferon- α and granulocyte colony-stimulating factor) have now entered routine clinical use.
- Polymer conjugation alters the biodistribution of low-molecular-weight drugs, enabling tumour-specific targeting with reduced access to sites of toxicity. More than ten polymer-anti-tumour conjugates have been transferred into clinical development. They have been designed for lysosomotropic delivery following passive tumour targeting by the enhanced permeability and retention effect (EPR effect) or, in one case, for receptor-mediated targeting by the introduction of a cell-specific ligand. Polyglutamic acid-paclitaxel is showing particular promise in phase III trials in women with non-small-cell lung cancer.
- New strategies are making polymer conjugates active against new molecular targets (for example, anti-angiogenics), and the combination of polymer conjugates with low-molecular-weight drugs (which are routinely used in chemotherapy), radiotherapy or tailor-made prodrugs is showing promise. Moreover, the polymer platform provides an ideal opportunity to deliver a drug combination from a single carrier, and combined endocrine therapy and chemotherapy is showing preclinical potential as a breast cancer therapy.
- The polymers that have been used clinically so far have a linear polymer architecture. The principles for the design of polymer therapeutics are now being applied to new hyperbranched dendrimers and dendritic polymer architectures. Before clinical evaluation it is essential to establish the safety of new polymers, particularly in respect of general toxicity, immunogenicity and metabolic fate.

The notion of drug targeting is not new. A century ago Paul Ehrlich foresaw its potential and coined the phrase ‘magic bullet’ to describe a ‘perfect’ targeted therapy; the modern era has also popularized terms such as ‘guided missiles’ and ‘stealth delivery systems’. The basic principles of drug targeting and the array of technologies that are currently used to deliver anticancer therapy have been recently reviewed in detail elsewhere^{13–20}. After considerable effort, the last two decades has seen an increasing number of nanovectors progress from the laboratory to routine clinical use as anticancer therapies. These include antibody conjugates (for example, gemtuzumab (Mylotarg), Tositumomab and ibritumomab tiuxetan (Zevalin))^{14–16}, liposomes (for example, daunorubicin

(DaunoXome)), doxorubicin (Doxil/Caelyx)^{17,18}, and those polymer conjugates described below. Most recently (2005) the first therapeutic nanoparticle (albumin-entrapped paclitaxel (Abraxane))^{19,20} was approved as a treatment for metastatic breast cancer.

There has been a growing realization that the ideal anticancer nanovector requires multiple components. Polymers feature widely, and they have been used to prepare polymer-coated liposomes, polymer-antibody conjugates, polymer-protein (with or without drug) conjugates and nanoparticles coated with polymers and/or targeting ligands¹³. To achieve the optimal therapeutic index, careful assembly on the basis of sound biological rationale is required. The pathophysiology of the targeted disease (for example, tumour location, degree of vascularization and molecular biology) and the nature of the drug payload to be delivered must be considered¹³. It is equally essential that all components (not least synthetic and natural polymers) are inherently safe, amenable to reproducible manufacture on an industrial scale and suitable for transformation into a cost-effective pharmaceutical formulation providing a medicine that is practical to use clinically.

Polymer therapeutics as anticancer agents

Natural and synthetic polymers are used widely as components of new medical devices, for example, as rate-controlling coatings, as hydrogels or matrices for the topical administration of drugs, in tablets and capsules for oral administration and controlled-release systems for drugs, peptides and proteins, and as constructs for tissue engineering. However, it has only been during the last decade that the first polymer-based therapeutics emerged as clinically accepted medicines for parenteral administration. Although this article will focus

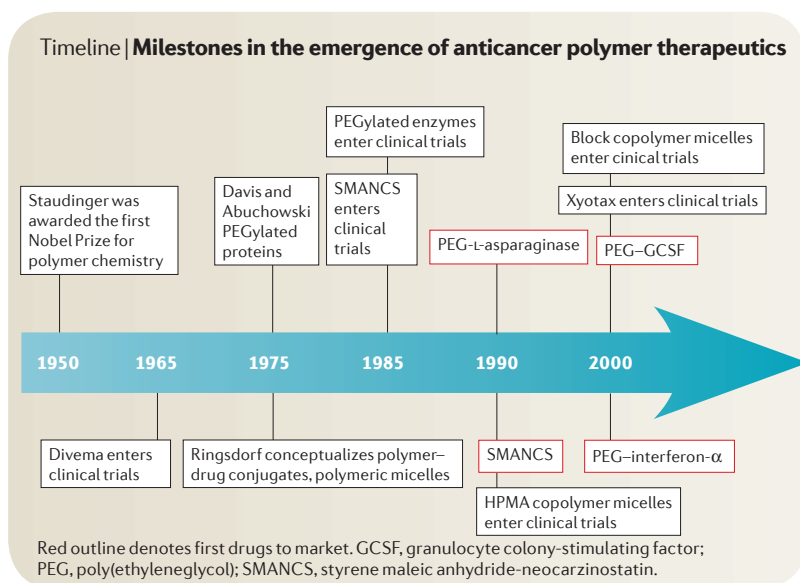


Table 1 | Polymer–protein conjugates in clinical use or development

Compound	Name	Status	Indication	Refs
SMANCS	Zinostatin Stimalmer	Market	Hepatocellular carcinoma	38,39
PEG–L-asparaginase	Oncaspar	Market	Acute lymphoblastic leukaemia	47
PEG–GCSF	Neulasta	Market	Prevention of neutropaenia associated with cancer chemotherapy	58
PEG–IFN α 2a	PEG–asys	Market	Hepatitis B and C	61
		Phase I/II	Melanoma, chronic myeloid leukaemia and renal-cell carcinoma	
PEG–IFN α 2b	PEG–Intron	Market	Hepatitis C	62
		Phase I/II	Melanoma, multiple myeloma and renal-cell carcinoma	
PEG–arginine deiminase	ADI-PEG20	Phase I	Hepatocellular carcinoma	52
PEG–glutaminase combined with a glutamine anti-metabolite 6-diazo-5-oxo-L-norleucine (DON)	PEG–PGA and DON	Phase I/II	Various cancers	137
PEG–D-amino acid oxidase (DAO) combined with the substrate DAO, D-proline	PEG–DAO and DAO, D-proline	Preclinical		138

GCSF, granulocyte colony-stimulating factor; IFN α , interferon- α ; PEG, polyethyleneglycol; SMANCS, styrene maleic anhydride-neocarzinostatin.

on polymer–protein and polymer–drug conjugates as anticancer agents, their wider application (for example, as antiviral agents, immunomodulators and for enzyme replacement) should be noted, and has been reviewed elsewhere²¹.

The term ‘polymer therapeutics’^{21,22} was coined to describe the biologically active polymeric drugs^{23–25}, polymer–drug conjugates^{26–28}, polymer–protein conjugates^{29,30}, polymeric micelles to which a drug is covalently bound³¹ and multi-component polyplexes (containing covalent linkers) being developed as non-viral vectors for gene and protein delivery^{32,33}. From the industrial standpoint, these are new chemical entities rather than conventional drug-delivery systems or formulations that simply entrap, solubilize or control drug release without resorting to chemical conjugation. The distinction is between a covalently bound biologically active system, and one that is non-covalently complexed or simply entrapped.

This article focuses on those polymer–protein and polymer–drug conjugates that have progressed into clinical testing. As macromolecular drugs are not bioavailable orally, they must be administered to patients parenterally. Polymer–protein conjugates are typically given subcutaneously or intramuscularly, whereas polymer–drug conjugates are administered intravenously. So far only linear polymers have been explored clinically, such as *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, polyglutamic acid (PGA), polyethyleneglycol (PEG), polysaccharides (for example, dextran) and those block copolymers that are used to assemble micelles²⁷. Although very different from low-molecular-weight pharmaceuticals and biologicals, hybrid polymer-based constructs have already shown that they can satisfy the stringent requirements of industrial development and regulatory authority approval. This

demands a cost-effective and profitable medicine or diagnostic on the one hand, and on the other, a safe and efficacious profile that justifies administration to patients. Key milestones in the history of polymer–anticancer conjugates are summarized in the **TIMELINE**. It is noteworthy that rapid advances in polymer chemistry are now creating many new polymers, such as intricate polymeric architectures including dendrimers and dendronized polymers (reviewed in **REFS** 21, 34, 35). Although it is hoped that they will have an important role in the next generation of nanovectors, they have not yet progressed to clinical trials, so they are not reviewed here in depth.

Polymer–protein conjugates

The first practical use of polymer therapeutics as anticancer agents was in the form of polymer–protein conjugates. The introduction of SMANCS and PEGylated proteins into clinical use in the early 1990s heralded the birth of a new class of anticancer agents (**TABLE 1**). Today, polymer–protein conjugates are used routinely as anticancer therapeutics, as an adjunct to chemotherapy and are also being developed as components of combination therapies.

Intra-hepatic artery delivery of SMANCS. Maeda and colleagues synthesized SMANCS by covalently linking the anti-tumour protein neocarzinostatin (NCS) to two styrene maleic anhydride (SMA) polymer chains³⁶. As the non-specific toxicity of NCS precludes its clinical development, the aim was to generate a polymeric derivative that was sufficiently hydrophobic to enable dispersion in the phase-contrast agent Lipiodol^{37,38}. This enabled local administration through the hepatic artery to patients with **liver cancer**, and with the aid of X-ray, the precise localization of SMANCS to tumour

Micelle

A self-assembling colloidal aggregate of amphipathic molecules, in this case polymeric block copolymers, to give a polymeric micelle, which occurs when the concentration reaches the critical micelle concentration.

Dendrimer

A macromolecule that contains symmetrically arranged branches arising from a multifunctional core. Repeated reaction sequences add a precise number of terminal groups at each step or generation.

Box 1 | Rationale for design of PEG–protein conjugates

Recombinant DNA and monoclonal antibody technology has created a growing number of peptide, protein and antibody-based drugs. The conjugation of poly(ethyleneglycol) (PEG) to proteins (PEGylation) is proving a useful tool to:

- Increase protein solubility and stability, and also to reduce protein immunogenicity^{29,30}.
- Prevent the rapid renal clearance of small proteins and receptor-mediated protein uptake by cells of the reticuloendothelial (RES) system.
- Prolong plasma half-life — leading to the need for less frequent dosing, which is of great patient benefit.

Although several water-soluble polymers have been successfully used for protein conjugation, PEG is particularly attractive because:

- PEG is used as a pharmaceutical excipient and is known to be non-toxic and non-immunogenic.
- PEG has a flexible, highly water-soluble chain that extends to give a hydrodynamic radius some 5–10 times greater than that of a globular protein of equivalent molecular weight. Its high degree of hydration means the polymer chain effectively has a 'water shell', and this helps to mask the protein to which it is bound.
- PEG can be prepared with a single reactive group at one terminal end, and this aids site-specific conjugation to a protein and avoids protein crosslinking during conjugation.

Although first generation protein conjugates were synthesized using linear monomethoxyPEGs (molecular weight (Mw) of ~5,000 g mol⁻¹), with many polymer chains randomly attached to each protein molecule, various sophisticated conjugation chemistries have now emerged that use linear or branched PEGs of Mw ~5,000–40,000 g mol⁻¹. Several techniques, most recently including phage display, enable site-specific peptide and protein modification. The specific linking chemistries and synthetic strategies being used are described in more detail elsewhere^{29,30,44,45}.

tissue. Preclinical studies showed a tumour–blood ratio of >2500 (REF. 37) — much higher than ever reported for other targeting approaches. Maeda defined this passive targeting phenomenon the “enhanced permeability and retention effect” (EPR effect)³⁹, and attributed it to the ‘leaky’ discontinuous endothelium of angiogenic tumour vasculature causing hyperpermeability coupled with the lack of effective tumour lymphatic drainage. It has recently been shown that Lipiodol is essential for the anti-tumour activity of SMANCS⁴⁰. The first clinical evaluation of SMANCS involved 44 patients given 3–4 mg of SMANCS every 3–4 weeks. X-ray imaging confirmed selective tumour retention. Remarkable activity was reported: 86% of patients showed decreased concentrations of α -fetoprotein (a biomarker for liver cancer), and 95% showed a decrease in tumour size³⁶. A subsequent multicentre phase II study involving 400 patients with primary hepatoma also reported a relatively high response rate (36–40%)^{41,42}. As the prognosis for primary liver cancer is so poor, and few agents are effective in treating this disease, SMANCS was approved in Japan in 1994 for the treatment of advanced and recurrent hepatocellular carcinoma⁴³. The most successful use of SMANCS has been seen when it is administered as a ‘patient-individualized treatment’ — that is, dose per tumour size (area) — and follow-up treatments are given on a need basis (if the tumour is not regressing). In this sense, SMANCS therapy was well ahead of its time, and only now is the possibility of individualized cancer therapy using nanomedicines being proposed as feasible.

PEGylated enzymes. In contrast to SMANCS, which is administered locally, PEG–protein conjugates⁴⁴ are administered parenterally, and are therefore useful for treating widely disseminated disease. Davis and Abuchowski proposed this approach, now called PEGylation (reviewed in REFS 29,30,44,45), in the 1970s. It has since been applied to various proteins, including enzymes, cytokines and monoclonal antibody Fab fragments. The biological rationale for the development of PEG–protein conjugates is described in BOX 1. Since the first PEGylated enzyme, PEG adenosine deaminase (Adagen, used to treat severe combined immunodeficiency syndrome), came to market in 1990, a steady stream of anticancer PEG–protein conjugates have progressed to market (TABLE 1).

PEG-L-asparaginase (Oncaspar) was the first to achieve US Food and Drug Administration (FDA) approval in 1994. It is used to treat acute lymphoblastic leukaemia (ALL)^{46,47}. Although L-asparaginase depletes asparagine (an amino acid that is essential for tumour growth) and is active against ALL and lymphoma, its administration can induce anaphylactic shock and other hypersensitivity reactions. Host antibody production can also lead to premature asparaginase clearance from the circulation. Phase I studies with PEG–L-asparaginase showed an increased plasma half-life (357 h) compared with the native enzyme (20 h), and the conjugate produced fewer hypersensitivity reactions⁴⁸. A subsequent phase II trial that involved patients with refractory **non-Hodgkin lymphoma**⁴⁹ being treated intramuscularly with PEG–L-asparaginase at a dose of 2,000 units per m² every 2 weeks led to partial response in some patients, and the absence of haematological toxicity. Most importantly, PEGylation consistently decreased hypersensitivity reactions^{49,50}.

PEG–recombinant arginine deiminase (PEG–rhArg) is currently being assessed as a treatment for hepatocellular carcinoma, both as a single agent that depletes arginine (which, again, is needed for tumour growth) and also in combination with 5-fluorouracil (5-FU)^{51,52}. In a phase I/II study involving 35 patients, PEG–rhArg was administered by weekly intramuscular injection. One patient showed tumour shrinkage, two had stable disease and all patients achieved arginine concentrations of <2 μ M. A PEG conjugate of recombinant methioninase (rMETase) has also been described. The ability of this conjugate to induce long-term methionine depletion *in vivo* has been shown, and PEGylation also reduces enzyme antigenicity⁵³.

PEGylated cytokines. PEG can also be linked to biological response modifiers such as interleukin 2 (IL2)^{54–56}, recombinant granulocyte colony-stimulating factor (GCSF)^{57,58} and interferon- α (IFN α). IL2 and IFN α can switch on the body’s own anti-tumour immune response, and GCSF promotes the regeneration of white blood cells, the levels of which can fall dramatically after chemotherapy. PEG modification prolonged the plasma half-life and decreased the immunogenicity of these proteins. PEGylated recombinant methionyl GCSF (Neulasta) has been developed for the prevention of

Table 2 | Polymer–drug conjugates

Compound	Name	Status	Indication	Refs
Polyglutamate–paclitaxel	CT-2103; Xyotax	Phase III	Various cancers, particularly non-small-cell lung cancer; ovarian cancer as a single agent or in combination therapy	107–111
Polyglutamate–camptothecin	CT-2106	Phase I	Various cancers	113
HPMA copolymer–doxorubicin	PK1; FCE28068	Phase II	Various cancers, particularly lung and breast cancer	88
HPMA copolymer–doxorubicin–galactosamine	PK2; FCE28069	Phase I/II	Particularly hepatocellular carcinoma	91
HPMA copolymer–paclitaxel	PNU166945	Phase I	Various cancers	93
HPMA copolymer–camptothecin	MAG-CPT	Phase I	Various cancers	95–97
HPMA copolymer–carboplatin platinite	AP5280	Phase I/II	Various cancers	99
HPMA copolymer–DACH-platinite	AP5346; ProLindac	Phase I/II	Various cancers	100
Dextran–doxorubicin	AD-70, DOX-OXD	Phase I	Various cancers	73
Modified dextran–camptothecin	DE-310	Phase I	Various cancers	74,75
PEG–camptothecin	Prothecan	Phase II	Various cancers	77

DACH, diaminocyclohexane; HPMA, N-(2-hydroxypropyl)methacrylamide; PEG, poly(ethyleneglycol).

severe cancer chemotherapy-induced neutropaenia^{59–61}. It needs less frequent administration, and is given by a single subcutaneous injection on day 2 of each chemotherapy cycle; in comparison with native GCSF, which must be given daily for 2 weeks to achieve the same protection.

Two PEGylated IFN α conjugates, PEG-asys (for 2a⁶²) and PEG-Intron (for 2b⁶³), have shown clinically superior antiviral activity compared with IFN α , and are approved treatments for hepatitis C. PEG–IFN α is now being studied as an anticancer therapy. The efficacy of IFN α in the treatment of melanoma and renal-cell carcinoma is well established, but protein administration presents problems including toxicity and the short plasma half-life ($t_{1/2} = 2.3$ h), which necessitates a 3-times-per-week administration schedule. In a phase I/II study PEGylated IFN α –2b was given once by subcutaneous injection every 12 weeks to patients with advanced solid tumours (primarily renal-cell carcinoma)⁶⁴. The maximum tolerated dose observed was 6.0 μ g per kg (body weight) a week, and a response rate of 14% was seen in 44 previously untreated patients with renal-cell carcinoma. PEGylated interferons are also currently undergoing clinical trials in other diseases (for example, glioma and metastatic melanoma) in combination with other agents known to have immunomodulatory properties and/or anti-angiogenic activity, but so far the results have not been particularly promising^{65,66}.

Polymer–drug conjugates

Ringsdorf had the idea of polymer–anticancer-drug conjugates in 1975 (REF. 67), and our subsequent research with Kopecek and colleagues beginning in the late 1970s designed the first synthetic polymer–drug conjugates to progress to clinical trial. Our biological rationale for the design of polymer–anticancer drug conjugates and

methods for their preclinical evaluation (reviewed in REFS 28,68) still hold true today. In all cases, the clinical aims of polymer–drug conjugation are to achieve improved drug targeting to the tumour, to reduce drug toxicity (by limiting access to the sites of toxicity) and to overcome the mechanisms of drug resistance. First generation conjugates sought to improve the therapeutic index of drugs already in routine clinical use (for example, doxorubicin, camptothecins, paclitaxel and platinates including carboplatin and diaminocyclohexane (DACH)-platinates). Conjugation to hydrophilic polymeric carriers can also improve the water solubility of hydrophobic drugs such as doxorubicin and paclitaxel, enabling easier formulation and patient administration. Ongoing clinical studies indicate the first polymer–drug conjugate product approval should occur within the next 2 years (TABLE 2). The rationale for polymer–drug conjugate design is described in BOX 2, and typical structures of polymer–drug conjugates are shown in FIG. 1. Almost all polymer–drug conjugates that have been clinically tested rely on increased tumour vascular permeability (that is, the EPR effect as described by Maeda³⁹) for tumour targeting. Many preclinical studies suggest opportunities for the tumour-specific targeting of polymer conjugates (reviewed in REFS 35,68) using antibodies, peptides (for example, melanocyte stimulating hormone⁶⁹) and other ligands (for example, folate⁷⁰); only one such targeted conjugate, HPMA copolymer-doxorubicin-galactosamine (see below), has so far been evaluated clinically.

Many polymers have been proposed as drug carriers⁷¹, but few have progressed to *in vivo* or clinical studies. Problems that are prohibiting further development include inherent polymer-related toxicity and/or polymer-related immunogenicity (polymers, as macromolecules, can cause an immune response (unlike PEG), so structures have to be chosen carefully⁷²); inadequate

Neutropaenia

A decrease in circulating neutrophils (white blood cells) in the peripheral blood.

Maximum-tolerated dose

The maximum dose of a drug that can be given to a patient without inducing severe or life-threatening side effects.

Box 2 | Rationale for the design of polymer–drug conjugates

Ringsdorf's vision of the idealized polymer chemistry for drug conjugation⁶⁷ and Trouet and De Duve's realization that the endocytic pathway might be useful for lysosomotropic drug delivery¹⁵⁶ led to the concept of targetable anticancer polymer–drug conjugates. Low-molecular-weight anticancer agents typically distribute randomly throughout the body, and this often leads to side effects. The attachment of drugs to polymeric carriers can:

- Limit cellular uptake to the endocytic route.
- Produce long-circulating conjugates. Most of the dose of low-molecular-weight drug typically leaves the circulation within minutes, whereas a polymer conjugate will ideally circulate for several hours to facilitate passive tumour targeting caused by the leakiness of angiogenic tumour blood vessels by the enhanced permeability and retention effect (EPR effect)³⁹. Conjugates have also been synthesized to contain targeting ligands (such as antibodies, peptides and sugars) with the aim of further promoting increased (building on the EPR effect) tumour targeting by receptor-mediated delivery^{26,28}.

Several features are needed for the effective design of polymer–drug conjugates:

- The polymer must be non-toxic and non-immunogenic. It must also be suitable for industrial-scale manufacture. Polymer molecular weight should be high enough to ensure long circulation, but for non-biodegradable polymeric carriers this molecular weight (Mw) must be less than 40,000 g mol⁻¹ to enable the renal elimination of the carrier following drug delivery. Therefore, the optimum (usually Mw 30,000–100,000 g mol⁻¹) must be tailored to suit the particular polymer being used.
- The polymer must be able to carry an adequate drug payload in relation to its potency.
- The polymer–drug linker must be stable during transport to the tumour, but able to release the drug at an optimum rate on arrival within tumour cells.
- If the drug exerts its effects through an intracellular pharmacological receptor, access to the correct intracellular compartment is essential. Peptidyl and ester polymer–drug linkers have been widely used. In particular, peptide sequences designed for cleavage by the lysosomal thiol-dependent protease cathepsin B^{81,82}, but pH-sensitive *cis*-aconityl, hydrazone and acetal linkages have also been used¹⁵⁷. They are hydrolysed within endosomal and lysosomal vesicles because of the local acidic pH (6.5–4.0). The ideal rate of release will vary according to the mechanism of action of the drug being delivered. Typically, conjugates containing doxorubicin linked by Gly-Phe-Leu-Gly release the drug payload over 24–48 h.
- The intracellular delivery and transfer of a drug out of the endosomal or lysosomal compartment is in many cases not only essential for therapeutic activity¹⁵⁸, it also provides the opportunity to bypass mechanisms of drug resistance that are reliant on membrane efflux pumps such as p-glycoprotein¹⁵⁹. The limitation of polymer Mw to <100,000 g mol⁻¹ ensures that the conjugate will be small enough to extravasate easily into the tumour, and will enable endocytic internalization by all types of tumour cell.

drug loading or inappropriate choice of drug (usually potency too low); and the use of unsuitable polymer–drug linkers — being either too stable (therefore preventing drug liberation and access to the pharmacological target), or degrading too quickly in aqueous solutions leading to premature drug release.

Many thought that natural polymers such as dextran would provide the safest platform. However, chain modification with pendant groups to enable drug attachment creates a non-biodegradable polymer, dextran can be immunogenic, and when a dextran–doxorubicin conjugate (AD-70) with a molecular weight (Mw) of ~70,000 g mol⁻¹ containing drug bound to oxidized dextran was tested in a phase I trial (administered every 21–28 days by a 30 minute infusion) the starting dose of 40 mg per m² (doxorubicin equivalent) showed unexpected toxicity. Dose reduction to 12.5 mg per m² showed thrombocytopenia, and severe hepatotoxicity was dose

limiting⁷³. This was probably due to dextran uptake by the reticuloendothelial system (RES). Recently, another dextran-based conjugate (DE-310) was explored clinically^{74,75}. It consists of carboxymethyl dextran polyalcohol (Mw = 340,000 g mol⁻¹) covalently bound by a peptidyl spacer to the camptothecin analogue DX-8951 — 6.6% of the final construct weight is drug (~6.6 wt% drug). In a phase I study DE-310 was given by 3 h infusion every 4 weeks up to 9 mg per m² (DX-8951 equivalent), and dose-limiting toxicities included thrombocytopenia and neutropenia, and reversible hepatotoxicity was observed⁷⁵. Although stable disease was seen in some patients, no evidence was found for EPR-mediated targeting.

Although PEG has also been used to synthesize drug conjugates⁷⁶, it contains only two terminal groups suitable for conjugation, which limits its drug-carrying capacity. A PEG–camptothecin conjugate (PEG–CPT; Prothecan), containing camptothecin (~1.7 wt%) linked to PEG at the C-20-OH position, therefore favouring the desired lactone ring configuration, has been tested clinically⁷⁷. In phase I studies it was administered every 3 weeks at doses of 600–4800 mg per m² (conjugate equivalent relating to 10–82 mg per m² camptothecin equivalent). A prolonged plasma half-life ($t_{1/2} > 72$ h) and activity were observed, with neutropenia and thrombocytopenia as dose-limiting toxicities⁷⁷. Instead, most clinical studies have involved HPMA-copolymer-based and PGA-based conjugates.

HPMA copolymer conjugates. Through the 1980s our systematic studies using HPMA copolymer–anticancer conjugates defined the principles for the design (BOX 2), preclinical evaluation, validated manufacture and formulation, and clinical development of these compounds^{27,28,68}. Based on early studies that evaluated the molecular-weight dependency of endocytosis⁷⁸ and biodistribution^{79,80}, an optimal HPMA copolymer Mw of ~30,000 g mol⁻¹ was chosen. PEG and HPMA copolymers are non-biodegradable in the main chain, so this size was chosen to ensure the elimination of the carrier from the body. A lysosomally degradable peptidyl linker (Gly-Phe-Leu-Gly) was favoured for drug conjugation, as it is stable in the circulation but is cleaved by lysosomal proteases (for example, cathepsin B) once it has been internalized by endocytosis^{81,82}. As high polymer–drug loading with hydrophobic or cationic moieties can promote substantial non-specific membrane binding^{83,84} (due to the net negative charge of the plasma membrane and the hydrophobic nature of the constituent lipids), a polymer–drug loading of ~10 wt% was chosen as optimal.

HPMA copolymer–drug conjugates can also contain targeting ligands (sugars, peptides and antibodies)⁶⁸, and the feasibility of receptor-mediated tumour targeting *in vivo* was shown for the first time with a multivalent galactose-containing conjugate⁸⁵. However, HPMA copolymer conjugates without a targeting ligand demonstrate EPR-mediated tumour targeting⁸⁶, and show improved anti-tumour activity (compared with free drug) *in vivo*⁸⁷. This justified their progression to clinical evaluation.

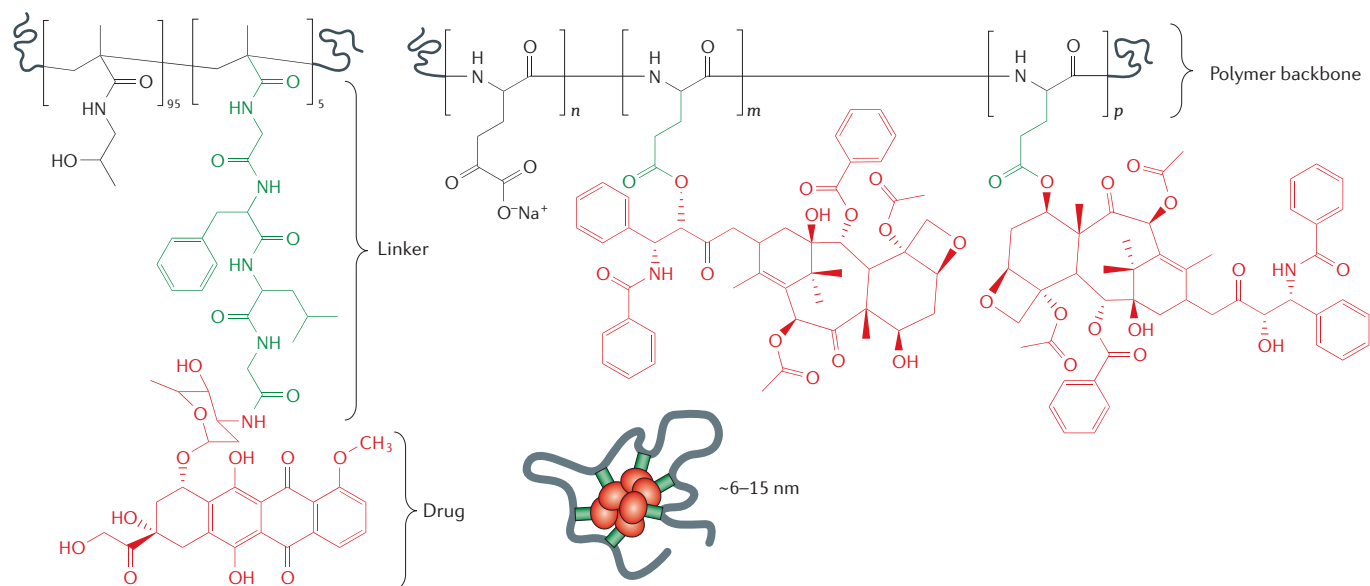
Thrombocytopenia

A decrease in platelets in the peripheral blood.

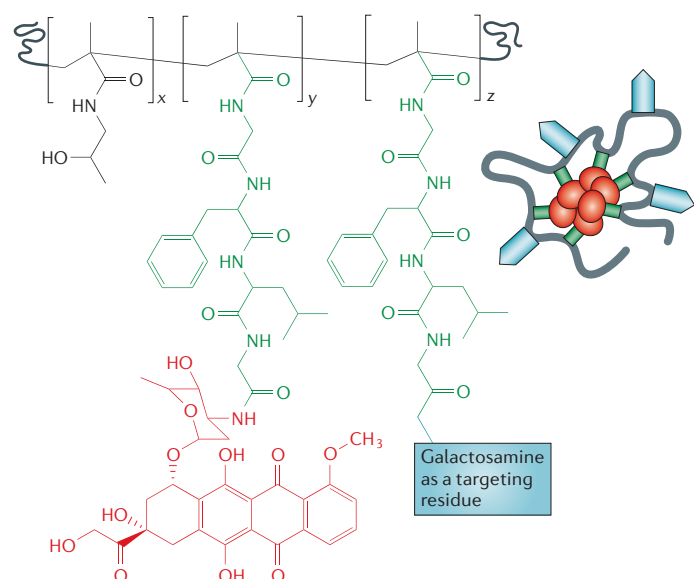
Dose-limiting toxicity

The particular type of toxicity responsible for the inability to raise a drug dose without the fear of severe or life-threatening side effects.

a Polymer–drug conjugate



b Targeted conjugate



c Polymeric combination therapy

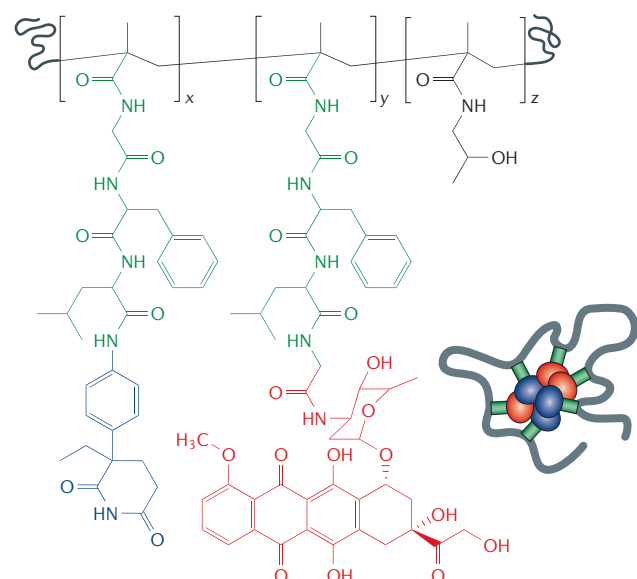


Figure 1 | Polymer–anticancer drug conjugates. Each panel shows both the detailed chemical structure and a cartoon of the general structure. The polymer backbone is shown in black, linker region in green, drug in red and additional components (for example, a targeting residue) in blue. **a** | Two examples of more ‘simple’ polymer–drug conjugates containing doxorubicin (left) and paclitaxel (right) that have progressed to clinical trial. **b** | A multivalent receptor-targeted conjugate containing galactosamine (light blue) to promote liver targeting. **c** | Polymeric combination therapy containing the aromatase inhibitor aminoglutethimide (red) and doxorubicin (blue).

Endocytosis

The internalization of a cell’s plasma membrane to form vesicles that capture macromolecules and particles present in the extracellular fluid and/or bound to membrane-associated receptors. These vesicles then undergo a complex series of fusion events directing the internalized vesicle to an appropriate intracellular compartment.

Phase I evaluation of HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin (PK1; FCE28068) (drug content ~ 8 wt%) began in 1994 (REF. 88). The conjugate was administered as a short intravenous infusion every 3 weeks at gradually increasing doses up to a maximum-tolerated dose of 320 mg per m^2 (doxorubicin equivalent). This is four–fivefold greater than the usual dose. The dose-limiting toxicities were typical of the anthracyclines (neutropenia and mucositis), and despite cumulative doses up to 1,680 mg per m^2

no cardiotoxicity was observed⁸⁸. Activity was also observed in chemotherapy-resistant patients; and doses as low as 80 mg per m^2 caused activity in patients with non-small-cell lung cancer (NSCLC), colorectal cancer and anthracycline-resistant breast cancer. Clinical pharmacokinetics showed prolonged plasma circulation ($t_{1/2\alpha} = 1.8$ h), an absence of liver accumulation and significant renal elimination (50–75% over the first 24 h)^{88,89}. Phase II trials showed no activity in patients with colorectal cancer, but partial responses were again

observed in patients with breast cancer and NSCLC⁹⁰. Most importantly, these early studies confirmed that high doses of this new polymeric carrier (>20 g per m²) could be administered without immunogenicity or polymer-related toxicity.

An HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin conjugate that also contained galactosamine (PK2; FCE28069) was designed to promote multivalent targeting of the hepatocyte asialoglycoprotein receptor (ASGR) to treat primary liver cancer^{68,91}. In a phase I/II trial this conjugate had a maximum-tolerated dose of 160 mg per m² doxorubicin equivalent. It is not clear why the galactosamine-containing conjugate is more toxic than that without galactosamine, as the dose-limiting toxicities were again typical of anthracyclines. Of the 23 patients treated who had primary hepatocellular carcinoma, two displayed partial responses, lasting >26 and >47 months, and a third showed a reduction in tumour volume. Eleven patients had stable disease⁹¹. Concentrations of drug in the liver were 15–20% of the administered dose after 24 h^{91,92}, and hepatoma-associated drug was 12–50-fold higher than would have been achieved through the administration of free doxorubicin.

However, phase I evaluations of HPMA copolymer conjugates containing paclitaxel (PNU166945)⁹³ and camptothecin (MAG-CPT)^{94–97} were disappointing. HPMA copolymer–camptothecin had no anti-tumour activity and severe cystitis as a dose-limiting toxicity, and HPMA copolymer–paclitaxel showed paclitaxel-like dose-limiting toxicity. These conjugates both contain an ester linkage (between the drug and the polymer) that can be cleaved during circulation in the bloodstream and renal elimination — unlike the peptide linkages mentioned earlier, which only release drug once the conjugate has entered the target cell. Moreover, in patients with colorectal cancer given a single dose of MAG-CPT before to surgery, the conjugate achieved similar levels in tumour and normal tissue at 24 h, but released drug levels were lower in tumour tissue than in normal tissue, indicating a lack of preferential accumulation by the EPR effect⁹⁷. These observations underline the need for careful conjugate design in relation to linker stability, drug loading and pharmacokinetics. Two HPMA copolymer platinates have had greater success: one that contains a malonate ligand (carboplatinum analogue) (AP5280)^{98,99} and a DACH platinatate (AP5346)¹⁰⁰. Both had reduced platinum-related toxicity, and the DACH platinatate also had anti-tumour activity.

Preliminary clinical experiments (not conducted to 'good clinical practice' (GCP) guidelines) have also been reported in six patients with refractory disease (including angiosarcoma and breast carcinoma) using HPMA copolymer–doxorubicin (or epirubicin) conjugates linked to human immunoglobulin (HuIg)¹⁰¹. The conjugates were synthesized on a case-by-case basis for patient treatment, and many biochemical and immunological parameters were assessed. Anti-tumour effects were observed in some patients (although this was not reported in sufficient detail to allow objective evaluation), and the conjugate did not seem to induce anti-Ig antibodies.

Polyglutamic acid conjugates. An exciting clinical programme is assessing a PGA–paclitaxel conjugate (CT-2103; Xyotax). In contrast to HPMA copolymer–paclitaxel, this conjugate contains a high drug loading (37 wt%). The drug is linked to PGA (Mw ~17,000 g mol⁻¹) through the 2' position to give a highly water-soluble product with an overall Mw of 49,000 g mol⁻¹ (REFS 102–105). Unlike HPMA copolymers, the PGA polymer chain is biodegradable. Paclitaxel is released to a small extent by slow hydrolytic release (up to 14% over 24 h), but is released to a greater extent following lysosomal cathepsin B degradation of the polymer backbone after endocytic uptake¹⁰⁶. Experiments in cathepsin-B-homozygous knockout mice confirmed the importance of enzymatic degradation. EPR-mediated tumour targeting and the greater efficacy of PGA–paclitaxel is observed in many preclinical tumour models, and it has an improved safety profile due to both decreased normal tissue exposure and improved drug solubility^{102–105}.

In phase I clinical trials, PGA–paclitaxel administered intravenously as a single agent over 30 minutes every 3 weeks had a maximum-tolerated dose of 233 mg per m² (paclitaxel equivalent)¹⁰⁷, and phase I/II studies showed a significant number of partial responses or stable disease, particularly in patients with **mesothelioma**, renal cell carcinoma, NSCLC and in paclitaxel-resistant **ovarian cancer**^{107,104}. Severe adverse events included neutropaenia and peripheral neuropathy. Both are classical paclitaxel-associated side effects, and neuropathy was cumulative and dose limiting. In one recent randomized phase III clinical trial, PGA–paclitaxel was compared with gemcitabine or vinorelbine as a first-line treatment for poor performance status (PS2) NSCLC patients^{108–110}. The conjugate showed significantly reduced severe side effects when compared with control patients, most of whom received gemcitabine. Although the conjugate failed to show an overall significantly improved survival in comparison with both compounds, there was a significant improvement in survival (40%) compared with vinorelbine. Interestingly, there was a greater increase in survival for women treated with PGA–paclitaxel (compared with men) that was most marked in pre-menopausal women¹¹¹. Activity might correlate with oestrogen levels, as oestrogen has been shown to increase the expression of cathepsin B¹¹². A pivotal trial is now ongoing to compare PGA–paclitaxel with paclitaxel (175 mg per m²) as a first-line therapy for women with NSCLC.

A PGA conjugate (CT-2106)¹¹³ of Mw ~50,000 g mol⁻¹ and containing a Gly linker to camptothecin (33–35 wt%) has also entered phase I/II trials.

Mechanism of action of drug conjugates

Increasing understanding of the mechanism of action of polymer–drug conjugates is helping to design second-generation conjugates and optimize clinical protocols for their evaluation. Many factors influence anti-tumour activity (FIG. 2). Conjugation of hydrophobic chemotherapy to hydrophilic polymers markedly improves solubility, and the synthesis of macromolecular prodrugs

Polymer–drug loading

The amount of drug carried by a polymer — this value is usually expressed as wt%.

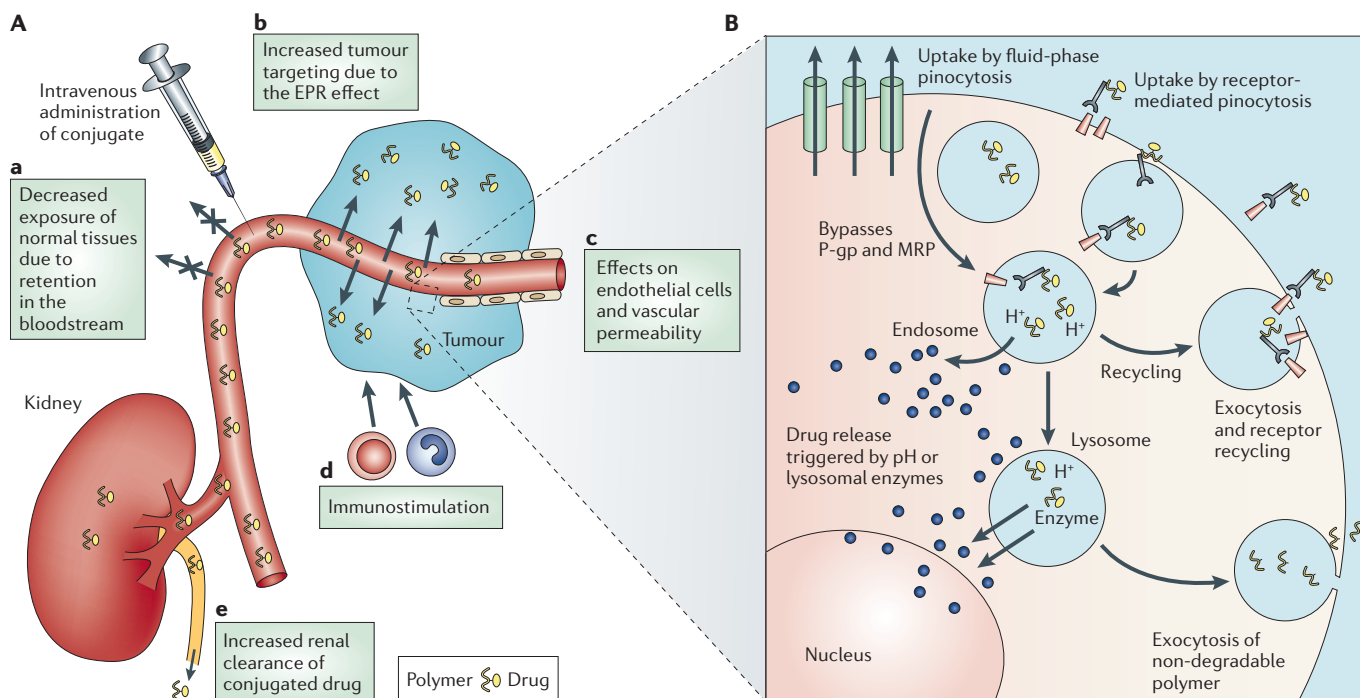


Figure 2 | Current understanding of the mechanism of action of polymer–drug conjugates. A | Hydrophilic polymer–drug conjugates administered intravenously can be designed to remain in the circulation — their clearance rate depends on conjugate molecular weight, which governs the rate of renal elimination. **a** | Drug that is covalently bound by a linker that is stable in the circulation is largely prevented from accessing normal tissues (including sites of potential toxicity), and biodistribution is initially limited to the blood pool. **b** | The blood concentration of drug conjugate drives tumour targeting due to the increased permeability of angiogenic tumour vasculature (compared with normal vessels), providing the opportunity for passive targeting due to the enhanced permeability and retention effect (EPR effect). **c** | Through the incorporation of cell-specific recognition ligands it is possible to bring about the added benefit of receptor-mediated targeting of tumour cells. **d** | It has also been suggested that circulating low levels of conjugate (slow drug release) might additionally lead to immunostimulation. **e** | If the polymer–drug linker is stable in the circulation, for example, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–Gly–Phe–Leu–Gly–doxorubicin, the relatively high level of renal elimination (whole body $t_{1/2}$ clearance >50% in 24 h) compared with free drug ($t_{1/2}$ clearance ~50% in 4 days) can increase the elimination rate. **B** | On arrival in the tumour interstitium, polymer-conjugated drug is internalized by tumour cells through either fluid-phase pinocytosis (in solution), receptor-mediated pinocytosis following non-specific membrane binding (due to hydrophobic or charge interactions) or ligand–receptor docking. Depending on the linkers used, the drug will usually be released intracellularly on exposure to lysosomal enzymes (for example, Gly–Phe–Leu–Gly and polyglutamic acid (PGA) are cleaved by cathepsin B) or lower pH (for example, a hydrazone linker degrades in endosomes and lysosomes (pH 6.5–<4.0)). The active or passive transport of drugs such as doxorubicin and paclitaxel out of these vesicular compartments ensures exposure to their pharmacological targets. Intracellular delivery can bypass mechanisms of resistance associated with membrane efflux pumps such as p-glycoprotein. If >10-fold, EPR-mediated targeting will also enable the circumvention of other mechanisms of drug resistance. Non-biodegradable polymeric platforms must eventually be eliminated from the cell by exocytosis. Rapid exocytic elimination of the conjugated drug before release would be detrimental and prevent access to the therapeutic target. In general, polymeric carriers do not access the cytosol. MRP, multidrug resistance protein.

dramatically alters drug biodistribution. Conjugate pharmacokinetics and the rate and location of drug liberation are the most important factors that define therapeutic index.

Rapid blood clearance after intravenous administration needs to be avoided to provide the high plasma concentration of the conjugate that is essential for EPR-mediated targeting. The failure of DE-310 to show EPR-mediated targeting in phase I trials might be explained by rapid initial plasma clearance caused by liver capture. If the polymer–drug linkage is stable in the bloodstream, the levels of free drug in plasma are low (>100–1,000 times less than for conjugated

drug)^{88,93,94,104}. Therefore, drug access to sites of toxicity (for example, the heart and bone marrow) can be reduced. This, together with the relatively rapid renal elimination of the conjugate, explains the reduction in HPMA copolymer–doxorubicin clinical toxicity^{88,91}. Conversely, if the linker is readily hydrolysed in the circulation then the dose-limiting toxicities of the parent drug are typically observed (for example, HPMA copolymer–paclitaxel⁹³) and no increase in therapeutic index results. Moreover, the hydrolysis of conjugates and drug liberation during renal elimination can bring unexpected toxicity, such as the dose-limiting cystitis seen for HPMA copolymer–camptothecin⁹⁴.

Conjugates can circulate much longer than free drug (typically $t_{1/2\alpha} > 1$ h for conjugate compared with < 5 minutes for free drug), which leads to significantly increased drug concentrations in tumours^{86,98,104}. Smaller tumours often show the highest uptake (up to 20% dose per g of tumour)¹¹⁵. However, it is not clear to what extent polymer–drug conjugates display such EPR-mediated targeting to clinical metastatic cancer. γ -Camera imaging showed EPR-mediated tumour localization, but the effect was modest and not evident in all patients⁸⁸. The fact that colon tumour targeting was not observed in the preliminary studies with HPMa copolymer–camptothecin is a cause for concern⁹⁷. More sensitive and quantitative clinical-imaging techniques are therefore needed. Knowledge of the efficiency of EPR-mediated targeting in individuals would enable the selection of those most suitable for treatment with polymer–drug conjugates.

Whether or not polymer–drug conjugates have an altered pharmacological mechanism of action compared with the parent drug is still a subject of debate. There are some indications that HPMa copolymer conjugates can circumvent multidrug resistance (MDR) clinically^{88,93}. This would be consistent with targeting to give a much higher local drug concentration and/or delivery by the lysosomotropic route. Even though conjugates frequently show superior *in vivo* anti-tumour activity^{87,102}, they are generally less cytotoxic *in vitro* than the parent drug. This is not surprising given the time that is needed for cellular uptake and drug release. However, this prevents the effective screening of conjugates using classical *in vitro* protocols. Preliminary experiments in rodent and xenograft tumour models indicated that variations in lysosomal thiol-protease activity might be more important in determining HPMa copolymer–doxorubicin activity than differences in the extent of EPR-mediated targeting¹¹⁵. The possibility that interactions with membrane-localized death receptors (Fas receptor pathway) might initiate cell killing has been discussed^{116,117}. However, the fact that HPMa copolymer conjugates containing non-degradable polymer–drug linkages have never shown activity *in vivo* does not support this hypothesis^{118–120}.

Several *in vitro* studies have suggested that HPMa copolymer–doxorubicin conjugates function through different molecular mechanisms (including stronger activation of apoptosis-signalling pathways)¹²¹ compared with free doxorubicin. By contrast, others indicate that cell death induced by the same conjugates occurs primarily by necrosis¹²². The kinetic complexities of *in vitro* experiments involving conjugates make the design and interpretation of such studies particularly challenging. However, there is growing evidence that HPMa copolymer–doxorubicin conjugates can exert an immunostimulatory effect. Accumulating evidence indicates that early anti-tumour activity *in vivo* occurs through cytotoxic or cytostatic drug action, but that the secondary immunostimulatory action of low levels of circulating conjugate augments this effect^{101,123,124}.

To the future

The molecular complexity of cancer means that drug combinations are most likely to improve long-term patient prognosis. Many interesting studies are examining for the first time the potential of polymer-based combinations.

Polymer–drug conjugate combinations. The clinical testing of PGA–paclitaxel combinations is furthest advanced. In a phase III trial, PS2 patients with advanced NSCLC were treated with either free or PGA-bound paclitaxel combined with carboplatin¹²⁵. Although the overall survival did not differ between the groups, gender differences were observed — there was an improvement in survival at 1 year and beyond for women who received PGA–paclitaxel. Women who received PGA–paclitaxel and had pre-menopausal oestradiol levels at the time of entry had a near doubling of their median (and 1 year) survival¹²⁶. Having evaluated PGA–paclitaxel as a single agent in ovarian cancer^{126,127}, an ongoing study is examining a combination of PGA–paclitaxel and carboplatin as a first-line therapy. Initial results showed tumour responses in 98% (80 out of 82) of patients¹²⁸, and this has led to a phase III trial to evaluate PGA–paclitaxel (135 mg per m² paclitaxel equivalent) as a monthly maintenance therapy in patients who have achieved a complete response following standard first-line chemotherapy. As PGA–paclitaxel combined with radiation treatment in preclinical models led to a marked improvement in EPR-mediated targeting and increased anti-tumour activity¹²⁹, phase I studies have also been initiated in which PGA–paclitaxel is given weekly in combination with radiation to patients with **oesophageal or gastric cancer**. The initial results are promising, with significant responses in 81% of patients¹³⁰.

The addition of polymer–drug conjugates to established low-molecular-weight chemotherapy regimens is a classical approach, but there is also the exciting possibility of single-polymer conjugates from which several drugs can be delivered simultaneously. HPMa copolymer conjugates containing both doxorubicin and the photoactivatable compound mesochlorin e6 showed better activity than HPMa copolymer–doxorubicin alone, and activity was increased with the addition of the OV-TL16 antibody to promote targeting^{131,132}. Recently, we developed a combination therapy for breast cancer — a conjugate that contains an HPMa copolymer, the aromatase inhibitor aminoglutethimide (an endocrine therapy) and doxorubicin (a chemotherapy)^{133,134}. The combination conjugate showed greater cytotoxicity against breast cancer cells *in vitro* than either of the individual conjugates alone or a simple mixture of them. Subsequent experiments confirmed the ability of HPMa copolymer–AGM to inhibit intracellular aromatase after drug liberation¹³⁵. Mechanistic studies indicate that the increased activity might be due to the kinetics of simultaneous drug liberation leading to increased apoptosis. This concept provides an interesting new opportunity for treating drug-resistant metastatic breast cancer.

Lysosomotropic

Molecules that are delivered to lysosomes and accumulate there. Applied in this context to polymeric constructs taken into the cell by endocytosis.

Polymer–protein combinations. Polymer–protein conjugates are also being studied in combination with chemotherapy regimens. PEG–GCSF is routinely used as an adjunct to chemotherapy. Recent phase II studies have examined a combination of PEG–IFN α -2b with thalidomide in patients with melanoma, although despite minimal side effects no clinical efficacy was seen¹³⁶. Several PEGylated enzymes are also being studied. A combination of PEGylated glutaminase (PEG–glut) and the glutamine anti-metabolite 6-diazo-5-oxo-L-norleucine (DON) is being evaluated in phase I trials¹³⁷. It is expected that DON will be more effective when glutamine levels are depleted. In a phase I study, patients were treated with 120 IU per m² of PEG–glut twice a week in combination with an increasing dose of DON 4-hours later. So far, five patients have shown stable disease and one patient with colorectal cancer showed a decline of the carcinoembryonic antigen marker. Experimentally, a PEG conjugate of D-amino acid oxidase (DAO) that targets tumour tissue by the EPR effect has been used to activate the DAO substrate, D-proline. This generates hydrogen peroxide intratumorally, which induces apoptosis, leading to marked *in vivo* anti-tumour activity¹³⁸.

An alternative two-step approach called polymer-directed enzyme prodrug therapy (PDEPT) can combine both polymer–drug and polymer–enzyme conjugates^{139,140}. This was developed because tumours might show new mechanisms of inherent or acquired resistance to polymer–drug conjugates if: endocytic internalization is too slow; intracellular trafficking does not efficiently direct the conjugate to lysosomes; and/or levels of activating enzyme (for example, cathepsin B) are too low. Therefore, PDEPT delivers the cytotoxic drug selectively and rapidly within the tumour interstitium using an externally administered trigger. To prove the concept, HPMA copolymer–Gly–Phe–Leu–Gly–doxorubicin was given intravenously, followed 5 hours later by HPMA copolymer–cathepsin B¹³⁹. This led to a rapid increase in doxorubicin release within tumour tissue but not normal tissue. Subsequently, a non-mammalian enzyme PDEPT combination, HPMA copolymer–Gly–Gly–cephalosporin–doxorubicin and HPMA copolymer–Gly–Gly– β -lactamase was shown to exhibit tumour targeting and significantly decrease tumour growth *in vitro*¹⁴⁰.

Using polymer conjugates to deliver anti-angiogenic therapy. Although fumagillol (TNP470) inhibits new blood vessel formation in tumours its clinical use has been limited by neurotoxicity. An HPMA copolymer conjugate that contains O-(chloroacetyl-carbamoyl) TNP470 was recently described as the first angiogenesis-inhibiting polymer conjugate¹⁴¹. It displays selective accumulation in tumour tissue through the EPR effect, and improved anti-angiogenic activity in both *in vivo* tumour models (A258 human melanoma and Lewis Lung carcinoma) and a hepatectomy model. Drug conjugation prevents TNP470 crossing the blood–brain barrier, so preventing the neurotoxicity of TNP470 itself¹⁴¹. Recent studies have shown that the combination

of HPMA copolymer–TNP470 and Avastin leads to synergistic effects in causing complete tumour regression (COLO-205 human colon carcinoma) *in vivo*¹⁴².

Conclusions, challenges and opportunities

Overshadowed by the larger investment in liposomal and antibody therapeutics, for two decades research in polymer therapeutics progressed almost unseen. The routine clinical use of PEGylated proteins in the 1990s, and the clinical development of polymer–drug conjugates and polymer combinations is rapidly changing this^{143,144}, and interest in ‘nanomedicines and nano-diagnostics’ is bringing the necessary investment to all classes of polymer therapeutics. Nevertheless, there are still challenges to ensure the safe and rapid translation of polymer therapeutics into routine clinical use. There is a need for the prudent design of new conjugates (especially bearing in mind the cellular and whole-organism pharmacokinetics of their macromolecular constructs), careful validated chemical characterization before clinical trial, and careful safety assessment of all new polymeric carriers.

Pharmacokinetically guided design. The unique pharmacokinetics of polymer–drug conjugates require new *in vitro* screening methodologies before clinical development. Cell-based assays for low-molecular-weight drugs rely on the rapid access of the drug to the intracellular target. However, polymer conjugates are taken up slowly by endocytosis, and thereafter drug is often released relatively slowly within endosomes or lysosomes. Clinical protocols require similar optimization.

Potential for new mechanisms of resistance. The mechanisms of resistance to conjugates and their dose-limiting toxicity might be quite distinct from those of existing chemotherapies. Although passive tumour targeting through the EPR effect can facilitate the tumour-selective delivery of macromolecules and nano-sized particles, there is still a need to better characterize clinical disease in respect of vascular permeability. The augmentation of EPR-mediated targeting by pulsatile infusion, co-administration of vasoactive agents or the combination of polymer therapeutics with radiotherapy might improve the tumour targeting of various polymer-based medicines.

New and related approaches. New polymeric carriers (including carriers of unique architecture)^{145–147}, innovative techniques for polymer–protein and peptide conjugation^{148–150} and the growing number of polymer–antibody¹⁵² and targeted polymer–drug conjugates¹⁵¹ should provide new candidates for clinical development. Another interesting related field is block copolymer micelles as carriers for chemotherapy^{152–155}. One system that shows particular promise simply mixes drug with a polymeric micelle¹⁵⁴, but drug can also be covalently bound to micelle carriers¹⁵⁵. The scene is set for significant advances in the application of anticancer polymer conjugates as nanomedicines.

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Competing interests statement

The author declares no competing financial interests.

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