THE DAWNING ERA OF POLYMER THERAPEUTICS

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As we enter the twenty-first century, research at the interface of polymer chemistry and the biomedical sciences has given rise to the first nano-sized (5–100 nm) polymer-based pharmaceuticals, the 'polymer therapeutics'. Polymer therapeutics include rationally designed macromolecular drugs, polymer–drug and polymer–protein conjugates, polymeric micelles containing covalently bound drug, and polyplexes for DNA delivery. The successful clinical application of polymer–protein conjugates, and promising clinical results arising from trials with polymer–anticancer-drug conjugates, bode well for the future design and development of the ever more sophisticated bio-nanotechnologies that are needed to realize the full potential of the post-genomic age.

MICELLES
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 crucial micelle concentration.

POLYPLEX
A polyelectrolyte complex. The term is usually used to describe the complex formed by a polycation and an anionic oligonucleotide or plasmid. The term interpolyelectrolyte complex (IPEC) is also used.

BIOACTIVE
A substance capable of eliciting a measurable biological response.

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The descriptor 'polymer therapeutics' is an umbrella term[1] used to describe polymeric drugs[2], polymer–drug conjugates[3], polymer–protein conjugates[4,5], polymeric MICELLES to which drug is covalently bound[6], and multi-component POLYPLEXES that are being developed as non-viral vectors[7] (FIG. 1). All subclasses use specific water-soluble polymers, either as the BIOACTIVE itself or as an inert functional part of a multifaceted construct for improved drug, protein or gene delivery. There is considerable hope that such bio-nanotechnologies, designed with an appreciation of the pathophysiology of normal and diseased tissue using advanced polymer chemistry and precision engineering at a molecular level, will help realize the full therapeutic potential of the post-genomic era. From the industrial standpoint, these nano-sized medicines are more like new chemical entities than conventional 'drug-delivery systems' or 'formulations' that simply entrap, solubilize or control drug release without resorting to chemical conjugation. Conceptually, polymer therapeutics share many features with other macromolecular drugs (proteins, antibodies, oligonucleotides) and macromolecular prodrugs including immunoconjugates. A bonus, however, is the versatility of synthetic chemistry, which allows tailoring of molecular weight, addition of BIOMIMETIC features to the man-made construct and even the possibility of including bioresponsive elements.

With the market approval of the first polymer–protein conjugates (polyethylene glycol (PEG)–adenosine deaminase, PEG–L-asparaginase and styrene maleic anhydride (SMANCS)) in the early 1990s (REF. 8), and promising results from clinical trials involving polymer–anticancer-drug conjugates[3], the field of polymer therapeutics is growing exponentially. Furthermore, it is evident that the lack of effective delivery systems for macromolecular medicines, including the peptides, proteins and oligonucleotides arising from genomics and proteomics research, is a bottleneck that must be overcome if we are to rapidly transform these new and exciting opportunities into practical-to-use therapies (BOX 1).

What contribution do polymer therapeutics make today? Until ten years ago they were regarded by many as a curiosity explored by those few who wished to work at the interface of polymer chemistry and biological sciences. Landmark historical events in this evolving field include the synthesis of *N*-vinylpyrrolidine conjugates of glycyl-L-leucine-mescaline as a drug depot formulation in 1955 (REF. 9); the first clinical testing of the synthetic polymeric anticancer agent divinylethermaleic anhydride (DIVEMA) in the 1960s (REF. 10); the elaboration of the concepts of polymer–drug conjugates[11], polymeric micelles[12] and PEGylated proteins[13] in the 1970s; and, most recently, the realization that non-viral

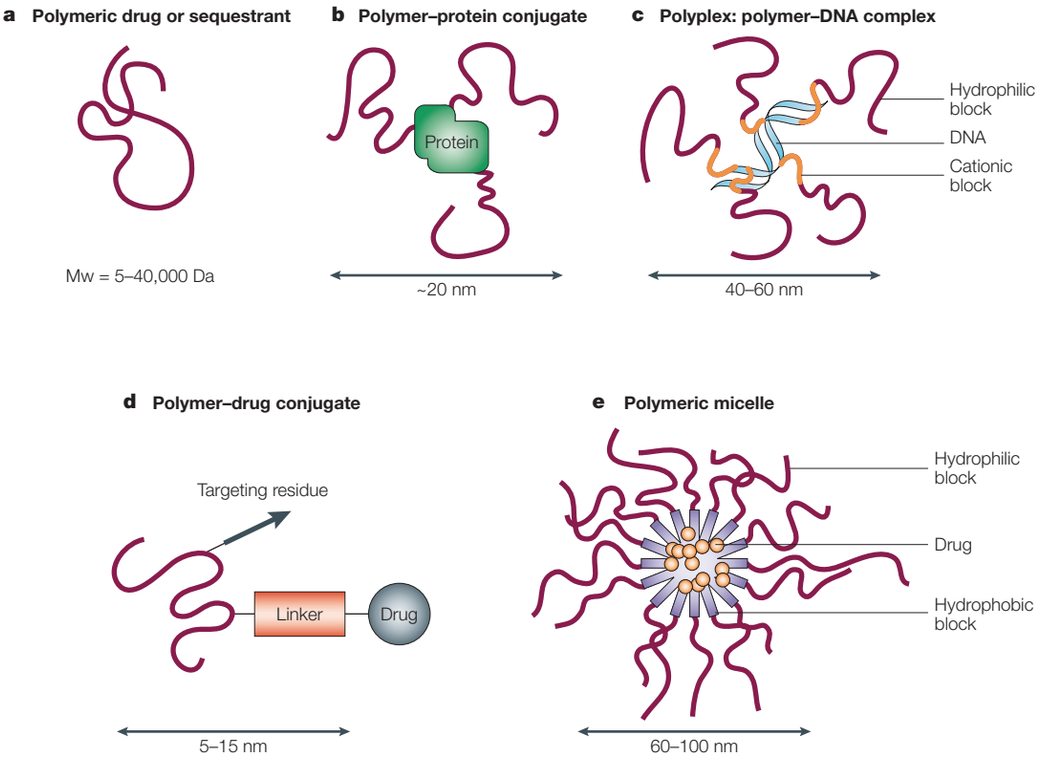

Figure 1 | **Schematic representation of polymer therapeutics now in, or progressing towards, clinical development.** The nano-sized and frequently multicomponent nature of these structures is visible. Mw, molecular weight.

vectors will be essential for gene therapy[14–16]. During the past two decades, an effective biological rationale has emerged for the design of each of the subclasses of polymer therapeutic. This, combined with increasingly innovative polymer and analytical chemistry and the realization that sophisticated polymer chemistry can be combined with biological macromolecules, such as proteins and oligonucleotides, to produce hybrid bionanotechnologies, has led to a pipeline of compounds that are suitable for both clinical development and routine clinical use (TABLES 1 and 2).

Which chemistry?

For systemic administration, the choice of an appropriate water-soluble polymer is crucial. The linear or branched polymer chain can function as a bioactive (a polymeric drug) or, alternatively, and most usually, as an inert structural component of a conjugate, a polymeric micelle or a non-viral vector. The polymer–drug and polymer–protein conjugates that have been clinically tested typically have a tripartite structure; the polymer, a linker and the bioactive[17]. However, much more elaborate multicomponent compositions now exist, with additional features for cell-specific targeting, to regulate intracellular trafficking and nuclear localization, and to allow the incorporation of drug combinations[18–20]. Modern polymer chemistry is producing increasingly intricate polymer structures, including multivalent polymers[21], branched polymers[22], graft polymers[23,24], DENDRIMERS[25–27], dendronized polymers[28], block copolymers[29], stars[30] and hybrid glyco-[31] and peptide derivatives[32] (FIG. 2). These will undoubtedly lead to the development of the polymer therapeutics of the future. Their potential advantages include a more defined chemical composition, tailored surface multivalency, and creation of defined three-dimensional architecture within either a synthetic water-soluble macromolecule (in the case of polymer therapeutics) or by the creation of new SUPRAMOLECULAR SYSTEMS such as polymeric nanotubes[33].

Polymers with a linear, random-coil structure have been used to synthesize the polymer therapeutics that have been transferred to the clinic. These include the synthetic polymers (PEG, *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, poly(vinylpyrrolidone) (PVP), poly(ethyleneimine) (PEI), linear polyamidoamines and DIVEMA); natural polymers (dextran (α-1,6 polyglucose), dextrin (α-1,4 polyglucose), hyaluronic acid, chitosans); and pseudosynthetic polymers, such as the man-made poly(amino acids) poly(L-lysine), poly(glutamic acid) (PGA), poly(malic acid) and poly(aspartamides)[17]. By their very nature, polymers present specific challenges for pharmaceutical development. A manufactured drug substance should be homogeneous and composed of a single, defined species. By contrast, all polymers are inherently heterogeneous and, as macromolecules, they can present special challenges for characterization. As it can affect

BIOMIMETIC
A term that describes a structure that is designed to mimic the properties of a natural macromolecule, for example, a synthetic multivalent ligand designed for receptor interaction.

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

SUPRAMOLECULAR SYSTEM
Self-assembled objects generated by intermolecular noncovalent interactions. They may be super molecules or polymolecular assemblies.

biological activity (for example, toxicity and efficacy), one particularly important issue is the fact that polymer samples contain individual molecules of different chain length. The average molecular weight is described by the terms 'weight average molecular weight' (Mw) and 'number average molecular weight' (Mn), and the ratio Mw/Mn gives a measure of the polydispersity[34]. Polysaccharides, chitosans and alginates extracted from natural sources are particularly polydisperse (Mw/Mn >2) and typically have high molecular weight (>200,000 g mol^{-1}) unless further processed to give lower-molecular-weight samples. However, depending on the mechanism of polymerization, some synthetic polymers have the advantage of very narrow polydispersity. For example, PEG has an Mw/Mn ~1.01. New synthetic methods and dendrimer chemistry are moving towards the production of synthetic macromolecules that, like proteins, are monodisperse.

Biologically active polymers: friend or foe?

The development of polymer therapeutics requires no more, or less, caution than that which is observed for any other drug or formulation. Like natural product and synthetic chemotherapy, protein-, peptide- and oligonucleotide-based therapeutics bring new, and specific, safety issues[35]. Past use of polymers as pharmaceutical excipients and as components of biomaterials provides considerable clinical experience to help guide the choice of polymer and polymer molecular weight for each application.

Polymeric drugs. Natural polymers extracted from plants, animals and seaweed — particularly the polyanions and polysulphates — have long been known to possess antiviral and antitumour activity[36]. DIVEMA (pyran copolymer) was the result of the first attempt to create a synthetic polyanionic modern medicine on the basis of this knowledge. Although known to induce APOPTOSIS and interferon release, and to activate macrophages to promote the killing of tumour cells, DIVEMA failed as an anticancer agent in early clinical trials because of its severe toxicity[37]. This was clearly related to polymer molecular weight and administration by the intravenous route[38]. Building on the lessons learnt in these early studies, modified polysaccharides, synthetic polypeptides and synthetic polymers have since been successfully transferred into the market as polymeric drugs. Sulphation of dextrin — a polysaccharide routinely used for peritoneal dialysis in patients with end-stage renal failure[39] — at the 2 (or 6) position produces a polymer that blocks infection of T-cell lines by laboratory-adapted strains of human immunodeficiency virus type 1 (HIV-1)[40]. Dextrin-2-sulphate (Mw = 25,000 g mol^{-1}) given to patients intraperitoneally daily for 28 days was well tolerated up to the maximal daily dose of 150 mg, and in Phase III clinical trials it reduced replication of HIV-1 in patients with AIDS[41]. Coincidentally, dextrin-2-sulphate also induced a gradual regression of Kaposi's sarcoma lesions in some patients. As dextrin-2 (and 6) -sulphate inhibits morphological differentiation of endothelial cells into tubes, this regression of lesions is probably the result of anti-ANGIOGENIC effect[42]. In gel form, dextrin-2-sulphate is now approved for use as an intravaginal virucide[43].

A steady stream of polymeric drugs are emerging. The synthetic polypeptide COPAXONE (Teva Pharmaceuticals) is a random copolymer of L-alanine, L-lysine,

Box 1 | The role of innovative drug-delivery systems in the realization of post-genomic medicines

Genomics and proteomics research is rapidly unveiling the molecular basis of many diseases[144]. With tools such as combinatorial chemistry, computer-assisted rational design, recombinant proteins and gene therapy, the progression towards better medicines should be accelerating. Although there are successes, including the tyrosine kinase inhibitor imatimib (Glivec/Gleevec; Novartis Pharmaceuticals) that is used to treat chronic myelogenous leukaemia[145], in general, progress has been disappointingly slow. There have been more than 600 gene therapy trials[146], but the first gene-therapy product is still awaited. Why is this? The answer, at least in one respect, is clear. The conversion of innovative therapeutics into medicines is frequently delayed by the lack of parallel investment in the enabling 'drug delivery' technologies[147] that are needed to guide the putative therapy into the correct intracellular compartment of the diseased cell, and, moreover, once there, to deliver it at an effective concentration for the appropriate duration of time. Modern approaches to parenteral drug targeting include liposomes, immunoconjugates, polymeric microparticles, and biodegradable polymeric implants that are designed for localized or sustained-controlled release[148,149]. Although most of these technologies have, in the past decade, led to marketed products, so far their benefit has been incremental. At one time, antibody conjugates, liposomes and polymer–conjugates were viewed as competing approaches. Naively, it was thought that one would emerge as a universal platform for delivery, but each has advantages and disadvantages. Antibodies provide selective targeting, but as proteins they can be immunogenic, their pharmacokinetics are governed by molecular weight, and they have a limited drug-carrying capacity. Liposomes have a high drug-carrying capacity, but stability can be an issue (either releasing drug too quickly or entrapping too strongly) and they are prone to reticulo-endothelial system capture. Polymer conjugates can be synthesized to specific molecular weight and composition, but their drug-carrying capacity is relatively low, and they can present challenges for characterization. The past decade has seen the realization that the ideal platform for drug delivery will marry the benefits of these three approaches into hybrid nanotechnologies for each application. A PEGylated liposome DOXIL (containing doxorubicin) (Sequus Pharmaceuticals) is a succesful anticancer treatment, the PEGylated anti-tumour necrosis factor humanized Fab fragment (CD870; Celltech)[69,70] is progressing well through clinical development as a treatment for arthritis, and many polymer conjugates look to antibodies to mediate cell specificity[94].

APOPTOSIS
A mechanism of programmed cell death, which occurs when a cell receives mixed internal signals for growth or when stimulated by an external trigger. Apoptosis can be initiated when a cell is no longer needed, or when a cell becomes a threat to the organism's health.

ANGIOGENESIS
The process by which small new blood vessels are formed by budding from existing vessels in both normal and diseased (for example, tumour) tissue.

Table 1 | **Polymer–protein conjugates on the market or in clinical development**

Compound	Name	Status (year to market)	Indication	References
PEG–adenosine deaminase	Adagen	1990	SCID syndrome	74
SMANCS	Zinostatin, Stimalmer	1993 (Japan)	Hepatocellular carcinoma	152
PEG–L-asparaginase	Oncaspar	1994	Acute lymphoblastic leukaemia	75
PEG–α-interferon 2b	PEG–INTRON™	2000	Hepatitis C	78
PEG–α-interferon 2b	PEG–INTRON™	Various clinical trials	Cancer, multiple sclerosis, HIV/AIDS	78,79
PEG–α-interferon 2a	PEGASYS	2002	Hepatitis C	77
PEG–HGR	Pegvisomant	2002 (approved EU)	Acromegaly	154
PEG–G-CSF	PEG–filgrastim, Neulasta™	2002	Prevention of neutropaenia associated with cancer chemotherapy	76
PEG–anti-TNF Fab	CD870	Phase II	Rheumatoid arthritis	70

EU, European Union; G-CSF, granulocyte colony-stimulating factor; HGR, human growth hormone; HIV, human immunodeficiency virus; PEG, polyethylene glycol; SCID, severe combined immunodeficiency; SMANCS, styrene maleic anhydride; TNF, tumour necrosis factor.

L-glutamic acid and L-tyrosine (Mw = 5–11,000 g mol^{-1}) (REF. 44) and when given subcutaneously it reduces both the frequency of relapse and disease progression in multiple sclerosis patients[45]. COPAXONE® mimics myelin basic protein, and although its precise mechanism of action remains unknown, it seems to minimize the autoimmune response to myelin that is seen in multiple sclerosis. Poly(allylamine)s have been developed clinically as polymeric sequestrants for oral administration. These polymers can be designed to bind phosphate, and thereby lower serum phosphorus and parathyroid hormone when administered orally to end-stage renal failure patients, or complex bile acids with consequent control of cholesterol absorption[46,47]. Experimentally, multivalent polymers are being investigated as alternative long-circulating polymers for protein conjugation, and as competitive inhibitors of viruses and toxins. These include poly(sialic acids), poly(acrylamide)–sialic acid and dendrimer–sialic acid[48–50].

Toxicology of polymer therapeutics. For polymeric drugs, inherent biological activity is a necessity, whereas inert, non-toxic polymers that are suitable for repeated administration are often required as components for other polymer-therapeutic applications. The general cytotoxicity, haematotoxicity, complement activation, carcinogenicity, teratogenicity, and cellular and humoral immunogenicity of many candidate polymers have now been defined[51,52]. Polycations are generally cytotoxic, HAEMOLYTIC and can activate complement, whereas polyanions are less cytotoxic, but can cause anticoagulant activity and can also stimulate cytokine release. As macromolecules, many polymers (including specific poly(aminoacids) and poly(saccharides)) elicit an immunoglobulin G (IgG) and/or an IgM response. All biological properties are molecular-weight-dependent and can change once the respective conjugates are prepared. Therefore, careful characterization of the potential toxicity of both the polymer — this will be a primary metabolite — and the final construct is required before clinical development. It must be remembered that pharmacokinetics at the whole body and cellular levels are also molecular-weight-dependent[53,54], and experience with PVP showed that accumulation of a non-biodegradable polymer occurred when it was administered parenterally using a molecular weight greater than the renal threshold[55]. An increased understanding of the potential deleterious properties of polymers and the molecular-weight dependence of these effects is continuing to shape the design of new, safer polymer chemistries.

The clinical development of HPMA copolymer–anticancer-drug conjugates was a milestone. Not only did these studies bring a new concept to clinical trial, this novel, non-biodegradable polymer had never before been administered to patients. Important lessons can be learnt from the preclinical programme. First, HPMA copolymer molecular weight (Mw ~ 30,000 g mol^{-1}) was optimized to ensure ultimate renal elimination at the same time as allowing tumour targeting[53,56]. Then, painstaking research probed the potential toxicity of the HPMA copolymer itself and each individual anticancer conjugate that was subsequently derived from it[57–61].

Biological rationale for polymer conjugate design

Polymer–protein and polymer–drug conjugates share many common features, but the biological rationale for their design is very different.

Polymer–protein conjugates. Recombinant DNA and monoclonal antibody technology have created a biotech revolution that is providing a growing number of peptide-, protein- and antibody-based drugs[62,63]. Their limitations often include a short plasma half-life, poor stability and, for proteins, immunogenicity. So, there has been a continuing search for improved protein-derived alternatives. In the 1970s, pioneering research by Davis, Abuchowski and colleagues foresaw the potential of the conjugation of PEG to proteins[13].

HAEMOLYTIC
Materials that cause breakage of the red blood cell membrane, and the release of haemoglobin.

This technique is now well established and is called PEGylation[4]. PEGylation is designed to increase protein solubility and stability, and to reduce protein immunogenicity[64–67]. Moreover, by preventing rapid renal clearance of small proteins and receptor-mediated protein uptake by cells of the reticuloendothelial system, PEGylation can be used to prolong plasma half-life. The resultant need for less frequent dosing is of great benefit to the patient and encourages compliance. PEG is a particularly attractive polymer for conjugation. It is widely used as a pharmaceutical excipient, and the flexible, highly water-soluble polymer chain extends to give a hydrodynamic radius that is some 5–10 times greater than that of a globular protein of equivalent molecular weight.

There are three requirements for optimized synthesis of a polymer–protein conjugate: a semi-telechelic polymer, that is, one with a single reactive group at one terminal end to avoid protein crosslinking during conjugation; the ability to introduce a linker that will not generate toxic or immunogenic by-products and that will provide appropriate stability characteristics (dependent on the protein being bound); and an approach that will provide reproducible site-specific protein modification. Linear and branched PEGs of Mw 5,000–40,000 g mol^{-1} have been used to create protein conjugates, sometimes with multiple PEGs attached per protein, or alternatively in a 1:1 ratio. First-generation protein conjugates used linear monomethoxyPEGs and a variety of conjugation chemistries[68], but these early strategies had significant disadvantages, including protein crosslinking (due to contaminating PEG-diol), modification of protein charge due to consumption of protein -NH_2 or -COOH groups during conjugation, unstable PEG–protein linkages, and, sometimes, the need for reaction conditions that led to protein denaturation. More recently, improved conjugation techniques have been developed, including site-specific modification following protein mutagenesis[69,70], the use of the enzyme transglutaminase to PEGylate selectively at glutamine in the protein[71], and the design of degradable PEG–protein linkages to maximize the return of protein bioactivity[71]. With increasingly sophisticated conjugate design, many of the early challenges for the clinical development of polymer–protein conjugates are being met[72,73].

The clinical value of PEGylation is now well established. PEG–adenosine deaminase (ADAGEN; Enzon) was the first PEGylated protein to enter the market, in 1990 (REF. 74). It is used to treat X-linked severe combined immunogenicity syndrome, as an alternative to bone marrow transplantation and enzyme replacement by gene therapy. Since the introduction of ADAGEN, a large number of PEGylated-protein and -peptide pharmaceuticals have followed (TABLE 1). PEG-L-asparaginase (ONCASPAR; Enzon) is used as a treatment for acute lymphoblastic leukaemia. Compared with the native enzyme, PEG-L-asparaginase has the advantages of reduced hypersensitivity, a longer plasma half-life and slower total clearance[75]. Consequently, PEG-L-asparaginase can be administered every two weeks, instead of the 2–3 times per week required for the native enzyme. Most importantly, PEGylation of L-asparaginase decreases hypersensitivity reactions (only 8% of patients show hypersensitivity reactions after administration of the conjugate) and the conjugate can be used to treat patients that are hypersensitive to the native enzyme. PEGylated-recombinant methionyl human granulocyte colony-stimulating factor (G-CSF) is used to prevent severe cancer chemotherapy-induced neutropaenia[76]. Again PEG–G-CSF (Neulasta; Amgen) has the benefit of less frequent administration, being given by a single subcutaneous injection on day 2 of each chemotherapy cycle. The native G-CSF must be given daily for two weeks to achieve the same protection.

Table 2 | **Polymer–drug conjugates and polymeric micelles in clinical trials as anticancer agents**

Compound	Name	Company	Linker	Status of development	References
HPMA copolymer–doxorubicin	PK1; FCE28068	CRC/Pharmacia	Amide	Phase II	81,95
HPMA copolymer– doxorubicin-galactosamine	PK2; FCE28069	CRC/Pharmacia	Amide	Phase I/II	96, 98
HPMA copolymer–paclitaxel	PNU166945	Pharmacia	Ester	Phase I	101
HPMA copolymer–camptothecin	MAG-CPT, PNU166148	Pharmacia	Ester	Phase I	102
HPMA copolymer–platinate	AP5280	Access Pharmaceuticals	Malonate	Phase I	112
Polyglutamate–paclitaxel	CT-2103, XYOTAX	Cell Therapeutics	Ester	Phase II/III	103–107
Polyglutamate–camptothecin	CT-2106	Cell Therapeutics	Ester	Phase I	114
PEG–camptothecin	PROTHECAN	Enzon	Ester	Phase II	108
PEG–aspartic acid-doxorubicin micelle	NK911	National Cancer Institute Japan	Amide/free drug	Phase I	117,118

CRC, UK Cancer Research Campaign; HPMA, *N*-(2-hydroxypropyl)methacrylamide; PEG, polyethylene glycol.

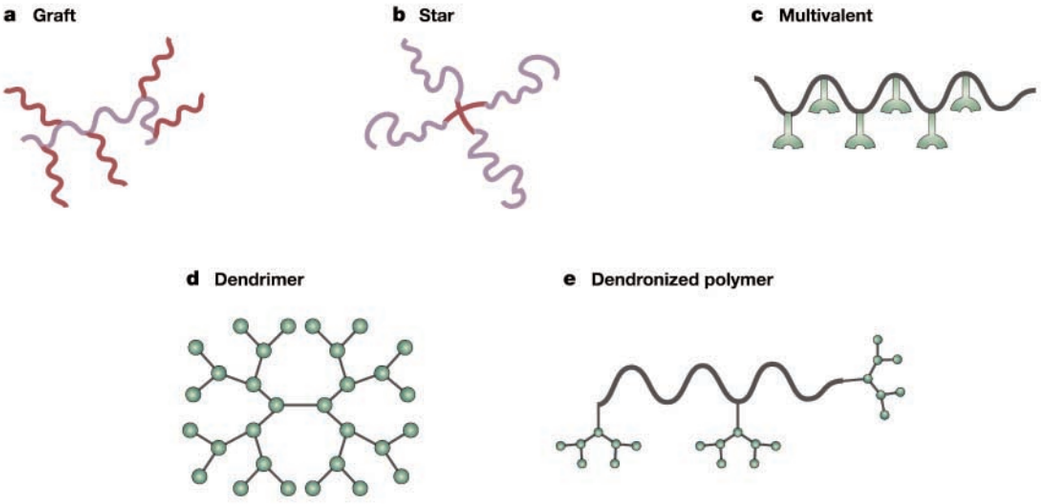

Figure 2 | **Novel polymeric architectures now being explored as the polymer therapeutics of the future.** The trend at present is towards more complex star-shaped and graft block-copolymer systems (**a** and **b**), tailored multivalent polymers providing improved opportunities for biorecognition (**c**) and three-dimensional biomimetic architectures, including dendrimers and dendronized polymers (**d** and **e**).

Two PEG–interferon-α conjugates (IFN-α–2a and IFN-α–2b), PEGASYS (Roche)[77] and PEG– INTRON (Schering)[78], have been approved as treatments for hepatitis C (REF. 4). IFN-α–2a and IFN-α–2b display similar biological activity and only differ in respect of a single amino acid, but the molecular weight of PEG used for conjugation and the linker employed is very different in each product. Consequently, PEGASYS has a higher specific activity *in vitro* and a longer plasma half-life than PEG–INTRON. Both conjugates have shown clinically superior antiviral activity compared to IFN-α, but without direct clinical comparison it is impossible to know whether either conjugate has a superior therapeutic index. PEG–IFN-α is also under clinical evaluation in other indications, including cancer, multiple sclerosis and HIV/AIDS. Efficacy of IFN-α in the treatment of melanoma and renal cell carcinoma is well established, but there are problems, including toxic side effects and a short plasma half-life ($t_{1/2}$ = 2.3 h) that necessitate administration three times per week. In a Phase I/II study, PEGylated IFN-α–2b was given by subcutaneous injection once per week for twelve weeks to patients with advanced solid tumours (primarily, renal cell carcinoma). PEGylated IFN-α–2b had a maximum tolerated dose of 6.0 μg kg^{-1} $week^{-1}$, and produced an objective response rate of 14% in 44 previously untreated renal-cell carcinoma patients[79].

Polymer–drug conjugates. Also in the 1970s, the combination of De Duve's realization that the ENDOCYTIC pathway might be useful for 'LYSOSOMOTROPIC drug delivery'[80] (FIGS 3 and 4) and Ringsdorf's vision of the idealized polymer chemistry for drug conjugation[11] produced the concept of targetable polymer–drug conjugates (FIG. 1). Whereas protein PEGylation was born from the desire to improve the properties of protein pharmaceuticals, polymer–drug conjugation was seen as a means of improving the cell specificity of low-molecular-weight drugs. Supplementary features are needed to design an effective polymer–drug conjugate. These include: a bioresponsive polymer–drug linker that is stable during conjugate transport and able to release drug at an optimum rate on arrival at the target site; adequate drug-carrying capacity in relation to the potency of the drug being carried; and the ability to target the diseased cell or tissue by an active (receptor–ligand) or a passive (pathophysiological) mechanism. As the drugs carried often exert their effects via an intracellular pharmacological receptor, it is essential that they eventually access the correct intracellular compartment[1,11,80].

A number of polymer–anticancer-drug conjugates are being tested clinically (TABLE 2). Routinely used cytotoxic chemotherapy distributes randomly in the body, and this feature, which is frequently combined with poor tumour selectivity in the mechanism of action, results in a relatively low therapeutic index. Those common solid tumours (breast, prostate, lung and colon cancer) that are the major cause of cancer mortality are particularly difficult to treat, hence the global quest for improved tumour targeting. Many researchers are trying to design improved low-molecular-weight prodrugs[81]. But covalent attachment of chemotherapy to a polymeric carrier is particularly attractive, as the increased molecular weight produces a radical change in the pharmacokinetics at both the whole body and cellular levels[82] (FIGS 3 and 4). Initially, it was believed that receptor-mediated targeting would be a prerequisite for tumour selectivity, and conjugates have been synthesized to contain a plethora of ligands, including antitumour antibodies and peptides. So far, no tumour-specific conjugate has progressed into clinical development. The realization that the prolonged plasma circulation of polymer-conjugated drug itself led to significant passive tumour targeting[83–85] by the 'enhanced permeability and

ENDOCYTOSIS
Internalization of the 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 substances to an appropriate intracellular compartment.

LYSOSOMOTROPIC
A term that describes molecules that are delivered to lysosomes and accumulate there. In this context, it is applied to polymeric constructs that are taken into the cell by endocytosis.

retention' (EPR) effect (BOX 2 and FIG. 3)[83] did, however, pave the way for the continued clinical development of simpler polymer conjugates that contain only covalently bound drug but no targeting ligand (TABLE 2).

Careful tailoring of polymer–drug linkers is essential to the creation of a polymeric prodrug that is inert during transport but allows drug liberation at an appropriate rate intratumorally. Peptidyl polymer–drug linkers were popularized by the successful design of HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin conjugates. This tetrapeptide linker is stable in the circulation[86], but is cleaved by the lysosomal thiol-dependent protease cathepsin B[87] following endocytic uptake of conjugate from the tumour interstitium (FIG. 4a). pH sensitive *cis*-aconityl, hydrazone and acetal linkages[17] have also been fashionable as an alternative for drug conjugation. The proton pump present in the endosomal and lysosomal membranes creates an acidic intravesicular environment — typically pH 6.5–4.0 — so drug liberation is triggered following internalization of the conjugate. An HPMA copolymer conjugate containing doxorubicin bound via hydrazone linkages has recently shown significantly improved antitumour activity against lymphoma *in vivo* compared with the tetrapeptide conjugate[88].

Most of the anticancer-drug conjugates that have been tested clinically have used HPMA copolymers as the carrier (TABLE 2). HPMA homopolymer was originally developed by Kopecek and colleagues as a plasma expander[89,90]. Collaborative research with Duncan and colleagues in the early 1980s produced two HPMA copolymer–doxorubicin conjugates[91–94] that subsequently progressed into Phase I/II evaluation under the co-sponsorship of the UK Cancer Research Campaign and Farmitalia Carlo Erba[95,96]. HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin (PK1; FCE28068) has a Mw ~ 30,000 g mol^{-1} and a doxorubicin content of ~ 8.5 wt% (FIG. 5a). In Phase I trials it was administered as a short infusion every three weeks, and the maximum tolerated dose was 320 mg m^{-2} (doxorubicin-equivalent)[95]. This is a four- to fivefold increase compared with the normal safe dose of free drug. The dose-limiting toxicities seen were typical of the anthracyclines, and included febrile neutropaenia and mucositis. Despite cumulative doses up to 1680 mg m^{-2} (doxorubicin-equivalent) no cardiotoxicity — a side effect that is typical of anthracyclines — was observed. Antitumour activity seen in patients considered to be chemotherapy resistant/refractory and at lower doxorubicin doses (80–180 mg m^{-2}) was consistent with EPR-mediated targeting, although GAMMA CAMERA imaging conducted as part of this study had poor resolution and failed to show clear evidence of selective tumour localization in all patients[95]. Clinical pharmacokinetics assessed by HPLC and gamma camera imaging confirmed prolonged plasma half-life when doxorubicin was administered in conjugate form ($t_{1/2\alpha}$ = 1.8 h), absence of liver accumulation and rapid renal elimination (50–75% over 24 h)[95]. Polymer-bound doxorubicin detected in plasma was always higher (>1,000) than levels of free doxorubicin. Neither dose-dependency in pharmacokinetics, nor contributory clinical factors causing changes in clearance of polymer-bound or free drug, were observed[95,97]. Most importantly, this pivotal clinical study confirmed that cumulative doses of HPMA copolymer >20 g m^{-2} could be administered without signs of immunogenicity or polymer-related toxicity. The fact that the rodent models established to document the preclinical pharmacokinetics and toxicology of PK1[61,84,93] correlated well with the subsequent clinical observations[95] validated the approach as a useful preclinical predictive tool.

HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin containing galactosamine (PK2; FCE28069)[96,98] is the only polymer–drug conjugate bearing a targeting ligand to be tested clinically. It was designed as an asialoglycoprotein (ASGP) biomimetic with the aim of targeting the hepatocyte asialoglycoprotein receptor[99] for the treatment of liver cancer. It should be emphasized that both normal hepatocytes and hepatoma bear the ASGP receptor. PK2 has an Mw ~ 25,000 g mol^{-1}, a doxorubicin content of ~ 7.5 wt%, and a galactosamine content of 1.5–2.5 mol% (FIG. 5b). Phase I/II trials were conducted in patients with primary hepatocellular carcinoma and the compound was given by intravenous infusion every three weeks. The maximum tolerated dose was 160 mg m^{-2} and, again, the dose-limiting toxicity was typical of anthracyclines[96]. Of the 23 patients entered, two had a measurable partial response and there were 11 others with stable disease[96]. Gamma camera imaging confirmed galactose-mediated liver targeting to 15–20% dose at 24 h[96,100]. Although most of the conjugate was localized to normal liver hepatocytes, it was estimated that the doxorubicin concentration in hepatoma tissue would still be 12–50-fold higher than could be achieved by administration of free drug.

Drug-delivery systems designed in the 1980s invariably used anthracyclines, as this was the most widely used chemotherapy at that time. Since then, other drugs have come to the fore, and the second-generation polymer conjugates now entering clinical trial all contain paclitaxel or camptothecins. Their poor water solubility, coupled with the hypersensitivity reactions associated with paclitaxel administration, made both paclitaxel and camptothecins attractive candidates for polymer conjugation. HPMA copolymer conjugates of paclitaxel (PNU166945)[101] and camptothecin (MAG–CPT)[102] — PGA–paclitaxel (CT-2103, XYOTAX; Cell Therapeutics)[103–107] and PEG–camptothecin (PROTHECAN; Enzon)[108] — have entered clinical evaluation. The PGA–paclitaxel conjugate (FIG. 5c) is proving to be the most interesting. It contains 37 wt% paclitaxel linked through the 2′ position — that is, via an ester bond — to the γ-carboxylic acid of PGA (Mw ~ 40,000 g mol^{-1}). PGA is the first biodegradable polymer to be used for conjugate synthesis; the polymer backbone is cleaved by cathepsin B to liberate diglutamyl-paclitaxel[108]. When administered intravenously as a single agent for 30 min every three weeks, the maximum tolerated dose of CT-2103 was 266 mg m^{-2}. In these early trials, a significant number of patients have shown partial responses or stable disease (mesothelioma, renal cell carcinoma,

GAMMA CAMERA
A device incorporating very sensitive radiation detectors, which produces images of the distribution of radioactivity in the body in patients who have been injected with small amounts of radioactive materials. These images can be used to detect and locate disease (such as cancer) if it is present. The device itself does not emit radiation.

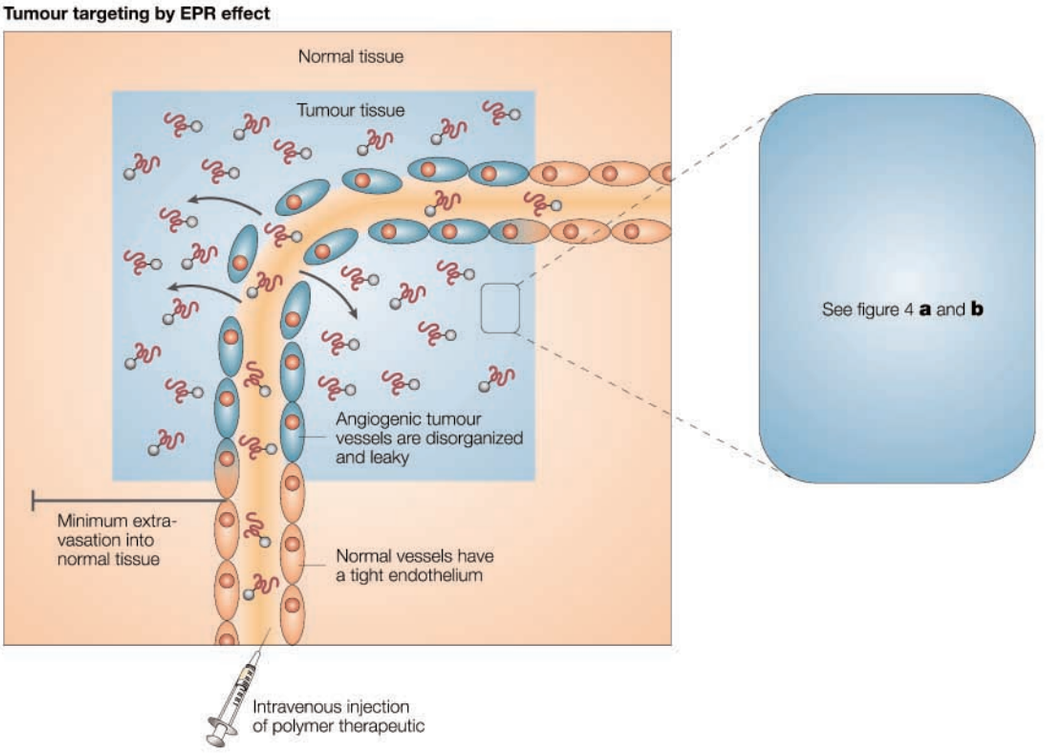

Figure 3 | **Biological rationale for the design of polymeric anticancer therapeutics (part 1).** Tumour targeting of long-circulating polymer therapeutics occurs passively by the 'enhanced permeability and retention' (EPR) effect. Hyperpermeable angiogenic tumour vasculature allows preferential extravasation of circulating macromolecules and polymeric micelles. Once present in the tumour interstitium, polymer therapeutics act either after endocytic internalization or extracellularly (FIG. 4).

non-small cell lung cancer and a paclitaxel-resistant ovarian cancer patient) and the extensive Phase II and Phase III programme now also includes combinations of CT-2103 with cisplatin and carboplatin[103–107]. By contrast, Phase I results with HPMA copolymer–paclitaxel and HPMA copolymer–camptothecin were disappointing, and underline the need for careful optimization of the polymer–drug linker to ensure stability during transit. Both conjugates contain relatively low drug loading (<10 wt% compared to 37 wt% paclitaxel in CT-2103). Rapid hydrolysis of the polymer–drug ester linkage could explain why MAG–CPT displayed dose-limiting cumulative bladder toxicity — probably due to drug liberation during renal elimination — and HPMA copolymer–paclitaxel displayed neurotoxicity, which is typical for free paclitaxel[101].

Following its successful application to protein conjugation, PEG has also been used to create drug conjugates[109,110]. The PEG–camptothecin conjugate PROTHECAN uses PEG of Mw 40,000 g mol^{-1}. Drug is bound via the C-20-OH position, and so favours the desired lactone ring configuration (FIG. 5d). The ratio of PEG–CPT to active drug is reported to be 60:1, indicating a drug content of 1.7 wt%. This is a rather low loading and illustrates the limitation of PEG as a drug carrier, namely, that drug can only be bound via the two reactive termini. In a Phase I study, PEG–CPT was administered every three weeks at doses of up to 4,800 mg m^{-2} (conjugate) (estimated to represent 82 mg m^{-2} camptothecin-equivalent); it had a maximum tolerated dose of 200 mg m^{-2} (camptothecin-equivalent)[108]. A Phase II programme with PEG–CPT is ongoing. Other conjugates in Phase I/II clinical trials include HPMA copolymer–platinates[85,111,112], a polysaccharide–camptothecin[113] and a PGA–camptothecin[114], and the results are eagerly awaited.

Polymeric micelles

Self-assembling block copolymer micelles have long been explored as drug carriers. A pluronic block copolymer micelle incorporating doxorubicin and able to circumvent p-glycoprotein-mediated resistance has recently shown promising results in Phase II clinical evaluation[115,116]. Like other more traditional micellar formulations, drug was non-covalently entrapped in this case. By contrast, Kataoka and colleagues have designed self-assembling polymeric micelles (NK911; 42 nm in diameter) using block copolymers of PEG (Mw ~ 5,000 g mol^{-1})–poly(aspartic acid) that also include a fraction of doxorubicin that is covalently bound to the polymer (~ 45%), as well as free drug[117,118]. This is, therefore, truly a polymer therapeutic as defined above. NK911 accumulates preferentially in tumour tissue by the EPR effect, leading to a three- to fourfold improvement in targeting[118]. But in this case, the covalently bound drug is inactive, and it is the free

drug slowly escaping over 8–24 h that destroys tumour cells. As the first such system to enter Phase I evaluation, clinical results will be very important.

To the future

What are the remaining long-term challenges and opportunities in this rapidly evolving field? Protein PEGylation is now a recognized tool. The first polymer–anticancer-drug conjugate should reach the market within the next three years, and non-viral vectors that are now being optimized will soon progress into clinical development. The combination of a sound biological rationale and advanced synthetic chemistry is creating ever-increasing opportunities for the design of novel

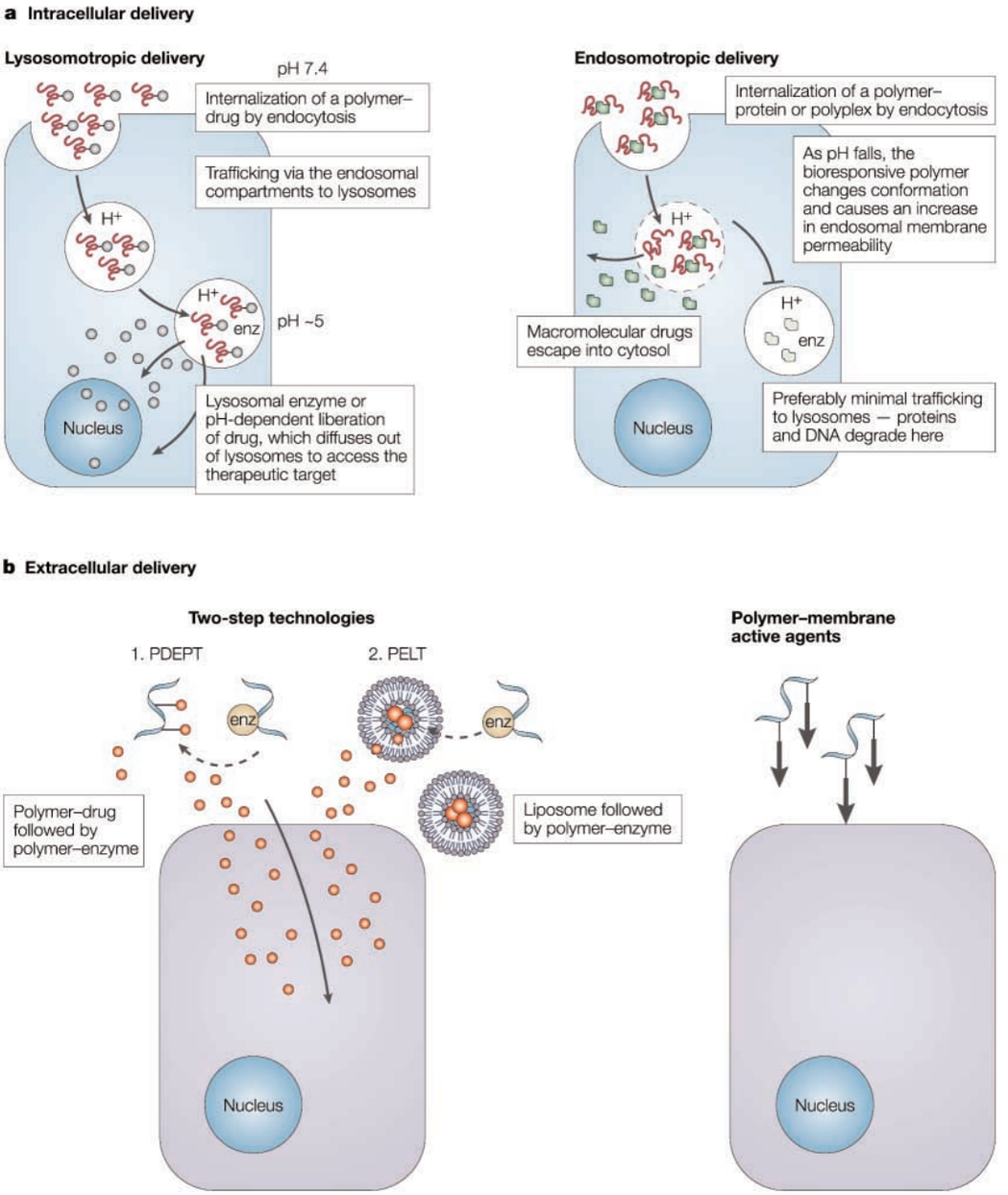

Figure 4 | **Biological rationale for the design of polymeric anticancer therapeutics (part 2). a** | Polymer–drug conjugates designed for lysosomotropic delivery of small-molecule drugs. Also shown is the use of bioresponsive, endosomolytic polymers to facilitate cytosolic access of genes and proteins from the endosome. **b** | Use of polymer-based systems to deliver drug within the tumour interstitium, or to destroy tumour cells following interaction with the cell membrane. Polymer-directed enzyme prodrug therapy (PDEPT) is a two-step approach that relies on activation of a polymer–drug conjugate by a complementary polymer–enzyme conjugate. Polymer–enzyme liposome therapy (PELT) relies on the liberation of drug from liposomes by the action of a polymer–phospholipase conjugate. Polymers that are conjugated to membrane active peptides or drugs that are known to activate the apoptosis pathway also have the potential to act at the level of the plasma membrane. enz, enzyme.

Box 2 | **Targeting tumours by the EPR effect**

The polymer–protein conjugate styrene maleic anhydride (SMA)–neocarzinostatin (NCS) called SMANCS (molecular weight = 16,000 g mol^{-1}) was originally synthesized by Hiroshi Maeda and colleagues, with the aim of hydrophobizing the antitumour protein NCS to allow dispersion in Lipiodol (a phase-contrast agent used for patient imaging) for administration locally via the hepatic artery[150]. Each protein molecule has two polymer chains bound to it via lysine 20 and the N-terminal amino group. While studying the pharmacokinetics of SMANCS using an *in vivo* tumour model, a liver tumour:blood ratio for SMANCS of >2,500 was observed[151]. This was much higher than that reported for any other targeting system, and SMANCS was subsequently approved in Japan as a treatment for hepatocellular carcinoma[152]. Maeda called the passive targeting phenomenon he observed the 'enhanced permeability and retention effect' (EPR effect)[83], and attributed it to two factors: the disorganized pathology of angiogenic tumour vasculature with its discontinuous endothelium, leading to hyperpermeability to circulating macromolecules, and the lack of effective tumour lymphatic drainage, which leads to subsequent macromolecular accumulation. It is now well established that long-circulating macromolecules — including albumin and polymer conjugates, polymeric micelles and liposomes — accumulate passively in solid tumour tissue by the EPR effect, and intravenously-administered drug-delivery systems can increase the tumour concentration of antitumour drugs up to 70-fold. Tumour biopsy following the administration of the liposomal anthracylines DOXIL and DaunoXome (NeXstar Pharmaceuticals) confirmed EPR-mediated tumour targeting in the clinical setting. The vasculature permeability on which the EPR effect relies varies during tumour progression. The greatest extravasation occurs in smaller tumours. Intertumoural hydrostatic pressure increases as the tumour grows, and angiogenic vessels are only present in the periphery. These features, together with irregular tumour blood flow, can lead to heterogeneity of the distribution of macromolecular medicines in tumour tissue[153]. However, a window of opportunity still exists to deliver high concentrations of antitumour agents to tumour tissue.

and improved polymer therapeutics. Of major importance is continued concerted effort at the interface of chemistry, biology and medicine, to define a sound biological rationale for construct design and to choose chemistries that are suitable for *in vivo* application and are amenable to industrial-scale development, not just laboratory-scale use.

In the context of cancer therapy, growing confidence that the EPR effect leads to tumour-selective delivery (FIG. 3 and BOX 2), the observation that smaller tumours exhibit the greatest vascular permeability[119], and the augmentation of targeting by pulsatile infusion[120], co-administration of vasoactive agents[121], or combination of polymer therapeutics with radiotherapy[122], indicate that this gateway will provide the opportunity for the delivery of a wide variety of polymer-based medicines (FIG. 4a). Long-circulating antitumour gene therapy and antitumour proteins could be selectively delivered to tumour micrometastases by exploiting EPR-mediated targeting. Although polymeric non-viral vectors still display a very poor transfection efficiency compared with viruses, improving the design of pH-responsive endosomolytic polymers gives hope that a synthetic viral mimetic will eventually become reality. The fundamental design requirements are identical to those described for the synthesis of drug conjugates, but once inside the cell the polyplex must also promote endosomal escape, ideally inhibit transfer to lysosomes (to minimize degradation of the gene or protein to be delivered), and, for gene therapy, ensure nuclear localization. Of the many polycations initially explored as non-viral vectors, the PEI-based polyplexes of Behr and colleagues have been most widely studied. PEI promotes endosomal escape via the proton-sponge mechanism[123,124,125]; linear polymers of Mw 22,000 g mol^{-1} are best able to overcome the nuclear barrier[126] and also yield the highest transfection rates[127]. Recent promising results obtained using PEI-polyplexes *in vivo*[128–130] have resulted in their development towards clinical evaluation in AIDS and cancer. PEI has the limitation of relatively high toxicity, and this could prove problematic for repeated systemic, rather than local, administration. Other emerging endosomolytic polymer libraries, including amphoteric polyamidoamines[131], polyacrylic acids[132] and poly-lysine-imidazoles[133], could provide interesting alternatives.

A prerequisite for pharmacological activity of constructs delivered via the lysosomotropic or ENDOSOMOTROPIC routes (FIG. 4a) is cellular internalization. Slow cellular uptake or transient cessation of endocytosis could, in theory, render cells resistant. To thwart this potential problem, polymer-based approaches containing cancer chemotherapy are now being designed for extracellular drug delivery (FIG. 4b). Polymer-directed enzyme prodrug therapy combines a polymeric prodrug and polymer–enzyme conjugate, thereby generating cytotoxic drug in the tumour interstitium. HPMA copolymer–cathepsin B combined with HPMA copolymer–Gly-Phe-Leu-Gly-doxorubicin, and an HPMA copolymer–β-lactamase conjugate (HPMA-Gly-Gly-β-L)/HPMA copolymer–Gly-Gly-cephalosporin-doxorubicin combination, have shown *in vivo* proof of concept[20,134]. Similarly, HPMA copolymer–phospholipase C conjugates can accelerate drug release from liposomes; this 'polymer-enzyme liposome therapy'[135] could present another opportunity for combination chemotherapy. The use of HPMA copolymer conjugates of catalytic antibodies for prodrug activation is an imaginative step further along this road[136]. An exciting alternative to cytotoxic chemotherapy is the destruction of angiogenic vasculature itself. Although a large number of anti-angiogenic agents are already in clinical development[137], the first polymeric anti-angiogenic conjugate — HPMA copolymer–TNP-470 — has recently been described and seems to hold significant promise[138].

ENDOSOMOTROPIC
A term that describes molecules that are delivered to the endosomal compartment of the cell and accumulate there. In this context, it is applied to polymer constructs that are designed as viral mimetics with the aim of breaching the endosomal membrane, and delivering proteins and oligonucleotides into the cytoplasm of the cell.

It is hoped that polymer therapeutics will become useful as treatments in a widening range of applications, perhaps as therapies for chronic, infectious and parasitological diseases, for diseases of the central nervous system, for the promotion of tissue regeneration, and even for improved vaccine delivery. The search is on for new biodegradable polymers that are more suited to repeated parenteral administration[139] and more elaborate three-dimensional biomimetic architectures[140]. Dendrimers and dendronized polymers provide multivalent surfaces for the immobilization of drugs, imaging agents and peptidyl epitopes[141], and they offer an opportunity to exploit different pathways of intracellular and transcellular trafficking[142]. Engineering shape-memory into polymers has recently been described for polymer materials[143]. The transfer of

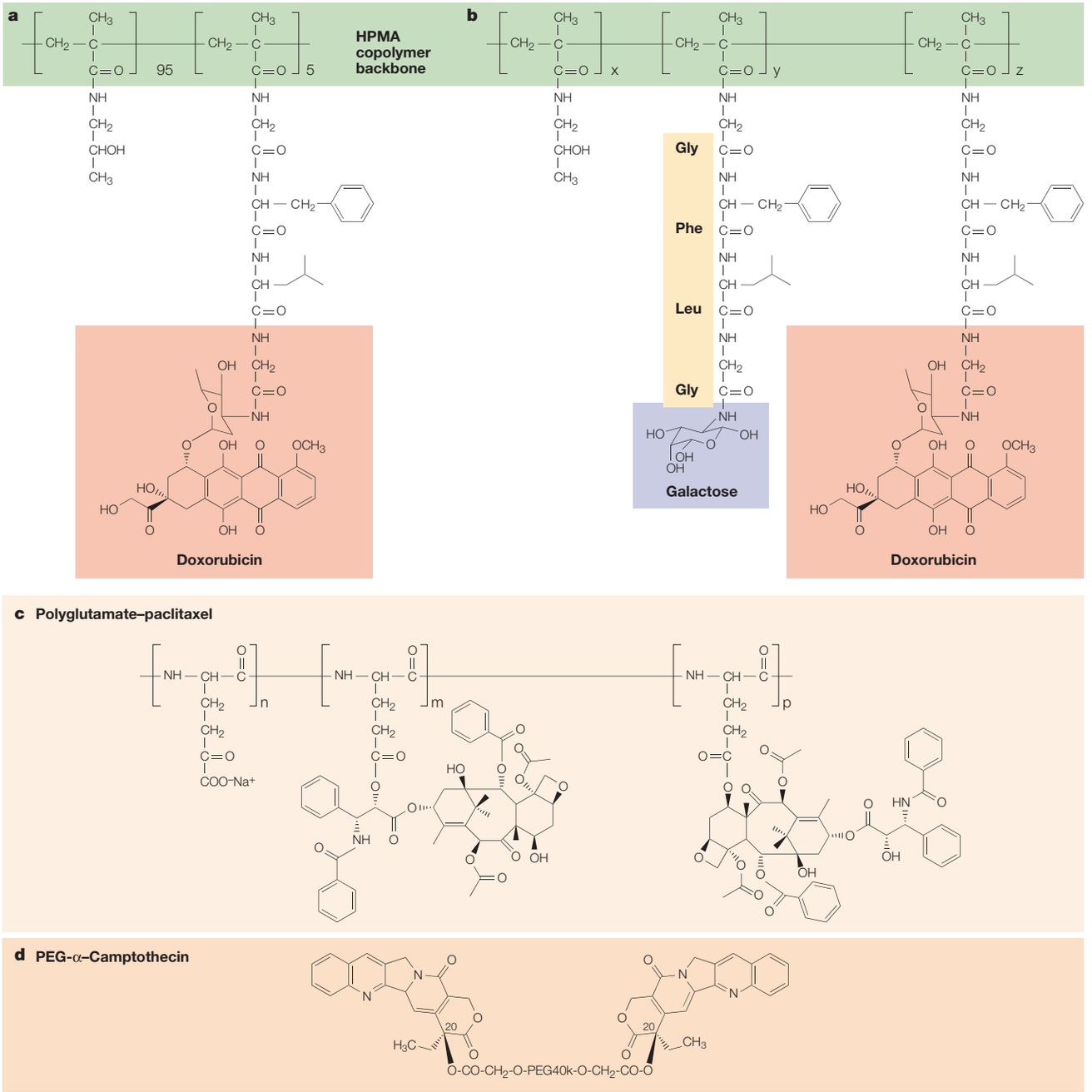

Figure 5 | **Examples of polymer–drug conjugates that are being evaluated as anticancer agents. a** | *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–doxorubicin (PK1; FCE28068). **b** | HPMA copolymer–doxorubicin containing galactosamine (PK2; FCE 28069) to promote liver targeting via the asialoglycoprotein receptor. These conjugates are composed of an HPMA copolymer backbone (green), a tetrapeptide Gly-Phe-Leu-Gly linker (yellow) and doxorubicin (red). **c** | Polyglutamate–paclitaxel. **d** | Polyethylene glycol (PEG)–camptothecin.

these concepts into water-soluble polymer architecture, perhaps using molecular imprinting techniques, remains an exciting challenge for the future.

Conclusions

The entry of hybrid conjugates that combine synthetic polymers with proteins or drugs, and polymer micelles that incorporate covalently bound drug, into clinical development has established polymer therapeutics as a credible option for medicine development. Although polymer chemistry can produce an almost infinite number of structures, the key to their successful medical application has been optimization of structure in light of a defined biological rationale, taking into account the pathophysiology of the disease and the proposed clinical use (route and frequency of administration). Growing expertise in this mutli-disciplinary field is helping to design ever more sophisticated second-generation technologies with bioresponsive elements, targeting ligands and three-dimensional architecture. There is real hope that, once optimized, these biomimetic nano-sized constructs will provide the improved treatments that are so urgently sought for life-threatening and debilitating diseases.

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