Polymer–drug conjugate therapeutics: advances, insights and prospects

Iriny Ekladious[1], *Yolonda L. Colson*[2]* *and Mark W. Grinstaff*[1]*

Abstract | Polymer–drug conjugates have long been a mainstay of the drug delivery field, with several conjugates successfully translated into clinical practice. The conjugation of therapeutic agents to polymeric carriers, such as polyethylene glycol, offers several advantages, including improved drug solubilization, prolonged circulation, reduced immunogenicity, controlled release and enhanced safety. In this Review, we discuss the rational design, physicochemical characteristics and recent advances in the development of different classes of polymer–drug conjugates, including polymer–protein and polymer–small-molecule drug conjugates, dendrimers, polymer nanoparticles and multifunctional systems. Current obstacles hampering the clinical translation of polymer–drug conjugate therapeutics and future prospects are also presented.

Bioactives
Molecules that elicit a biological response.

Excipient
A non-active substance formulated with an active pharmaceutical ingredient to enhance physicochemical characteristics such as stability and solubility.

Polymer–drug conjugate therapeutics are pharmacologically active macromolecular constructs comprising one or more therapeutic agents — including small molecules, peptides, proteins and aptamers — covalently bound to a polymeric carrier. The conjugation of bioactives to polymers confers numerous benefits, including enhanced drug solubilization, controlled delivery, increased efficacy and improved pharmacokinetics. Although the first polymer–drug conjugate was reported in 1955 by Jatzkewitz (BOX 1), the field of polymer therapeutics was propelled by the seminal work of Ringsdorf, Kopecek and Duncan, among others, in the 1970s[1–3]. The tangible clinical utility of polymer conjugation was realized in 1990 with the first market approval of a polymer–protein conjugate, Adagen, an enzyme replacement therapy for adenosine deaminase deficiency in patients with severe combined immunodeficiency.

Since these pioneering efforts, the field of polymer–drug conjugates has exhibited remarkable growth. Advances in synthetic polymer chemistry and materials engineering are expanding the repertoire of available materials, architectures and functionalities. In particular, responsive and multifunctional carriers afford the exciting opportunity to use rational design and precise molecular engineering to control and optimize the delivery of therapeutic agents. Integrating the present versatility of polymer–drug conjugate design with our growing understanding of the complex pathophysiology of various indications will transform drug development efforts as well as treatment paradigms.

In this Review, we discuss recent advances in the preclinical and clinical development of different classes of polymer–drug conjugates, including polymer–protein and polymer–small-molecule drug conjugates, dendrimers and polymer nanoparticles (FIG. 1). For the purposes of this Review, antibody fragments are grouped with proteins, given their size and structural similarity. Although peptides are similar to proteins in their composition, they are grouped with small-molecule drugs owing to their size as well as their regulatory classification. We focus, in particular, on polyethylene glycol (PEG) for protein conjugation, given that it has exhibited the greatest clinical success thus far. Central design considerations, including choice of linker chemistry, implementation of responsive modalities and integration of targeting moieties, are also discussed. We additionally highlight the design and development of sophisticated multifunctional systems that enable the simultaneous implementation of multiple therapeutic and/or imaging modalities in a single macromolecular carrier. Despite the progress achieved in the field, several obstacles currently impede the clinical translation of polymer–drug conjugate therapeutics. We therefore provide an overview of present translational challenges and discuss future prospects.

Classes of polymer–drug conjugates

Polymer–protein conjugates

The launch of recombinant insulin in 1982 propelled a remarkable upsurge in the development of protein therapeutics[4]. However, despite their clinical value, these agents commonly suffer from poor stability, rapid clearance and immunogenicity[5–8]. Pioneering work by Abuchowski et al. in 1977 demonstrated the utility of conjugation with PEG in ameliorating protein immunogenicity, improving solubility and extending the plasma half-life[9,10]. PEG is a highly water-soluble, flexible, uncharged and biocompatible polymer that is widely used as an excipient in the pharmaceutical industry. When bound to a protein therapeutic, PEG shields

[1]*Departments of Biomedical Engineering, Chemistry, and Medicine, Boston University, Boston, MA, USA.*

[2]*Department of Surgery, Brigham and Women's Hospital, Boston, MA, USA.*

**e-mail: ycolson@bwh.harvard.edu; mgrin@bu.edu*

https://doi.org/10.1038/s41573-018-0005-0

Box 1 | **The first polymer–drug conjugate**

The first synthesis of a polymer–drug conjugate was reported in 1955 by Von Horst Jatzkewitz[84]. Jatzkewitz hypothesized that the conjugation of the psychedelic alkaloid, mescaline, to a copolymer of *N*-vinylpyrrolidone and acrylic acid, would provide a controlled release form of the bioactive. He reasoned that the non-physiological, high-molecular-mass polymer carrier would increase the in vivo residence time of mescaline. Meanwhile, incorporation of a physiological dipeptide linker of glycine and leucine would afford enzymatic cleavage and release of the active agent.

Jatzkewitz monitored the urinary excretion of mescaline in mice that received intraperitoneal or subcutaneous injections of mescaline hydrochloride, glycine-leucine-mescaline hydrochloride, a mixture of the polymer carrier with glycine-leucine-mescaline hydrochloride, polymer-conjugated glycine-leucine-mescaline or a polymer conjugate of mescaline without the dipeptide linker. Mescaline is rapidly cleared in animals receiving either intraperitoneal or subcutaneous injections of mescaline hydrochloride, glycine-leucine-mescaline hydrochloride or a mixture of the polymer vehicle with glycine-leucine-mescaline hydrochloride, with excretion products falling below the detection limit within 16 hours after administration. However, animals that receive either subcutaneous or intraperitoneal injections of polymer-conjugated glycine-leucine-mescaline exhibit detectable mescaline excretion for extended periods of up to 21 or 9 days, respectively. Interestingly, mescaline excretion is not detected in animals receiving polymer-bound mescaline without the cleavable dipeptide linker. Given that a simple mixture of dipeptide-mescaline and the polymer carrier does not extend residence time, Jatzkewitz importantly notes that the extended residence time of polymer-conjugated glycine-leucine-mescaline is due to the sustained cleavage and release of the bioactive, rather than a passive association with the polymer carrier. Accordingly, he proposes the use of polymer-bound mescaline as a chronic, long-acting form of the bioactive. This seminal work demonstrates the utility of macromolecular carriers in extending in vivo residence time and additionally provides early insight on the impact of linker chemistry.

antigenic epitopes via steric repulsion, thereby reducing the immunogenicity of the agent[5]. Additionally, steric repulsion prevents degradation by proteolytic enzymes, as well as opsonization and subsequent clearance by the mononuclear phagocyte system (MPS). The increase in molecular mass and hydrodynamic radius conferred by polymer conjugation further reduces plasma clearance by renal filtration. The subsequent improvements in protein stability, plasma half-life and immunogenicity reduce the frequency of dosing and improve the overall safety profile of the therapeutic agent.

The first marketed PEG–protein therapeutic, Adagen, received clinical approval in 1990 (REF.[11]). Adagen is a PEG–adenosine deaminase conjugate used for the treatment of severe combined immunodeficiency caused by an inherited deficiency of the adenosine deaminase enzyme (that is, ADA-SCID)[12]. The clinical utility and impact of PEGylation have since been firmly established, with several PEG–protein conjugates receiving approval as therapies for a variety of indications, including hepatitis C, acute lymphatic leukaemia and rheumatoid arthritis[13] (TABLE 1). Furthermore, PEGylation is not limited to proteins; nucleic acids can be PEGylated to reduce renal clearance and extend the circulation half-life of, for example, aptamer therapeutics, including the approved drug pegaptanib sodium (Macugen) for macular degeneration[14,15]. These successes have encouraged the growth of a substantial pipeline of PEGylated proteins, including enzymes, cytokines, growth factors and antibodies, entering into clinical evaluation[11] (TABLE 2).

Opsonization
The process by which foreign materials are coated with opsonin proteins to enhance their phagocytic uptake and clearance.

Applications. A popular application of PEGylation has been to improve on the characteristics of an established protein therapeutic. For example, pegaspargase (Oncaspar) is a PEG–L-asparaginase conjugate that was approved in 1994 for the treatment of acute lymphoblastic leukaemia[16]. Oncaspar exhibits an extended plasma half-life of 357 hours compared with 20 hours for the unmodified asparaginase enzyme, reducing the frequency of dosing from 2–3 times per week to a single biweekly dose. The immunogenicity of the native enzyme is also minimized, enabling the treatment of patients who exhibit hypersensitivity reactions to the native enzyme. Interestingly, despite the increased cost per dose, the PEGylated protein provides cost savings of up to 78%, on an inpatient basis, compared with unmodified L-asparaginase, demonstrating the potential for new therapeutic technologies to improve outcomes while reducing costs[17]. Another example of improvement on established drugs is provided by the PEG–epoetin beta conjugate, Mircera, which received approval for the treatment of renal anaemia in patients with chronic kidney disease (CKD) in 2007. Mircera exhibits an extended plasma half-life of 134 hours compared with other erythropoiesis-stimulating agents (ESAs), which have plasma half-lives of <25 hours[18], enabling extended dosing intervals and potentially reducing the costs associated with anaemia management in patients with CKD[19,20].

The improvements in pharmacokinetics afforded by protein PEGylation have similarly transformed the treatment of several other indications, including chronic hepatitis C and chemotherapy-induced neutropenia. In the treatment of chronic hepatitis C, peginterferon alfa-2b (PegIntron) and peginterferon alfa-2a (Pegasys) provide enhanced efficacy and increased rates of sustained virological response via a single weekly dose compared with three weekly doses of the unmodified interferon α2b and interferon α2a, respectively[21,22]. Pegfilgrastim (Neulasta), a PEGylated granulocyte colony-stimulating factor (G-CSF) indicated for the treatment of chemotherapy-induced neutropenia, exhibits equivalent efficacy as a single dose per chemotherapy cycle relative to 11 daily injections of unmodified G-CSF[23]. This can be attributed to a significant reduction in the renal clearance of the PEGylated protein, which in turn allows self-regulating, neutrophil-mediated clearance to dominate. That is, as Neulasta increases the production of neutrophils and neutrophil precursors, these cells clear the therapeutic from circulation.

In addition to therapeutic benefit, PEGylation can improve the design of existing protein therapeutics. For example, although the antigen-binding fragment (Fab′) of an antibody can be produced more easily and at lower cost relative to the full protein, the Fab′ generally exhibits shorter circulation times. PEGylation addresses this limitation as exemplified by certolizumab pegol (Cimzia), a PEGylated anti-tumour necrosis factor (TNF) Fab′ that exhibits a half-life of 14 days and is administered as a single biweekly dose[24]. Of note, antibody fragments are grouped here with proteins given their size and structural similarity.

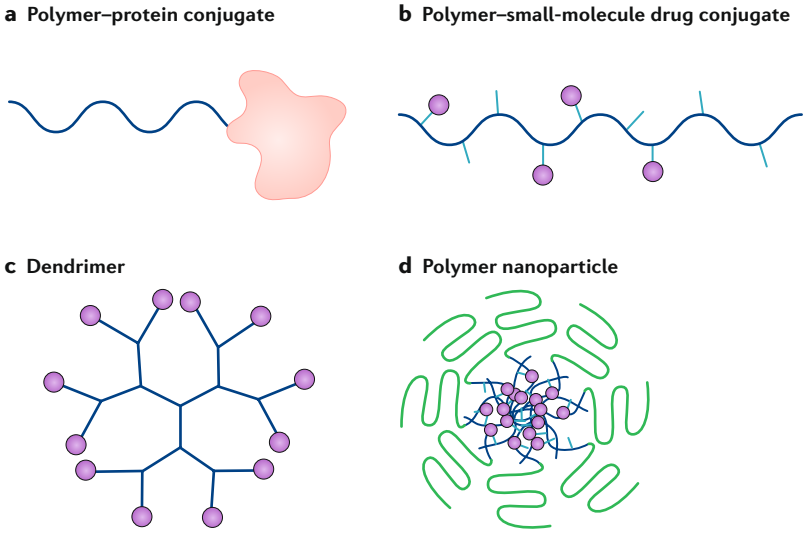

Fig. 1 | **Classes of polymer–drug conjugates on the market or in clinical development.** Water-soluble polymers are employed for the conjugation of protein therapeutics (for example, Mircera; part **a**), as well as small-molecule drugs (for example, paclitaxel poliglumex (Opaxio); part **b**). Dendrimers are highly branched, 3D polymeric architectures with high functionality for drug conjugation (part **c**). Currently, two dendrimer conjugates are in clinical development: DEP docetaxel and DEP cabazitaxel. Polymeric nanoparticles are colloidal carriers with dimensions on the nanoscale (part **d**). As exemplified by the depicted polymeric micelle, polymeric nanoparticles typically possess a core–shell architecture, with a hydrophobic core sequestered by a hydrophilic corona. CriPec docetaxel is an example of a polymer nanoparticle currently in clinical development.

PEGylation can also facilitate the translation of novel therapeutic agents that would otherwise be limited by poor pharmacokinetics. There is considerable interest in the clinical development of fibroblast growth factor 21 (FGF21) as a treatment for type 2 diabetes. However, FGF21 exhibits a plasma half-life of ≤2 hours[25,26], while a PEGylated FGF21, BMS-986036, exhibits an extended half-life of 19–24 hours. BMS-986036 improved insulin sensitivity in patients with type 2 diabetes and decreased the hepatic fat fraction in patients with nonalcoholic steatohepatitis[27–29]. In a subsequent phase I clinical trial, the PEG–FGF21 conjugate, BMS-986171, exhibited an increased half-life of 83–96 hours and greater plasma exposure than BMS-986036, enabling weekly dosing[30]. Similarly, the PEGylation of IL-10 extends the plasma half-life from <5 hours to 20 hours[31,32]. Interestingly, in preclinical evaluation, PEGylated IL-10, pegilodecakin, activates $CD8^+$ tumour-specific cytotoxic T cells and induces tumour rejection, whereas non-PEGylated IL-10 does not[32,33]. In a phase I study, daily subcutaneous administration of pegilodecakin resulted in stable serum concentrations for the duration of treatment and demonstrated sustained immune stimulation as well as evidence of antitumour activity[32,34].

In addition to extending the plasma half-life and reducing immunogenicity, PEGylation provides an opportunity to control the selectivity of protein binding. As an example, NKTR-214, a PEGylated IL-2 clinical candidate, displays reduced binding to the IL-2 receptor α-subunit (IL-2Rα) owing to site-specific PEGylation at the lysine residues of the IL-2–IL-2Rα interface[35,36]. Binding to the IL-2 receptor β-subunit (IL-2Rβ) is minimally impacted. Consequently, NKTR-214 affords increased proliferation of $CD8^+$ tumour-killing memory effector T cells, reduced proliferation of immunosuppressive regulatory T cells and enhanced antitumour efficacy relative to IL-2 in preclinical evaluation[35]. Conversely, NKTR-358, an alternative PEG–IL-2 conjugate designed for the treatment of autoimmune indications, exhibits reduced affinity for IL-2Rβ while maintaining its affinity for IL-2Rα, enabling preferential activation of regulatory T cells[37]. In preclinical evaluation, NKTR-358 suppresses antigen-induced inflammation and exhibits efficacy in a model of systemic lupus erythematosus.

Site-specific modification. Protein PEGylation is typically achieved via the post-polymerization modification of semi-telechelic monomethoxy-PEG to prevent protein crosslinking. Previously, the most common reactive groups targeted for coupling were the amino groups of the amino terminus and lysine residues[38]. However, owing to the prevalence of lysine, first-generation conjugation approaches lead to uncontrolled, multi-site PEGylation, resulting in heterogeneous mixtures of PEG–protein conjugates (that is, PEGmers). Meanwhile, uncontrolled PEGylation at or near binding or catalytic sites results in reduced and varied bioactivity among PEGmers[6]. First-generation conjugation approaches additionally suffer from protein crosslinking owing to the presence of contaminating PEG-diol[3,39]. Newer PEGylation approaches therefore rely on precise conjugation chemistries and methodologies (FIG. 2). Currently, chemical ligation strategies are available for efficient and site-specific conjugation at cysteine[40,41], tyrosine[42] and arginine[43] residues, as well as at histidine [44]. Recombinant gene technology can also be employed to achieve precise expression of specific residues for PEGylation. For example, an engineered cysteine in Cimzia is used to afford site-specific PEGylation, extending the half-life of the Fab′ without impairing its ability to bind TNF[15,45]. Methods for the selective modification of the amino terminus[46,47] and carboxyl terminus[48,49] have also been reported. Expansion of the ligation strategies available for protein PEGylation provides increased molecular control, facilitating the rational design of polymer–protein conjugates in which structure and bioactivity are retained.

Elegant techniques using enzyme-mediated ligation along with genetically engineered expression of unnatural amino acids are also utilized for site-specific polymer conjugation. For example, the use of transglutaminase enables the selective incorporation of PEG at glutamine residues, which are not otherwise chemically accessible[50,51]. Recently, sortase-specific motifs were expressed in therapeutic cytokines and subsequently utilized to achieve sortase-mediated, site-specific PEGylation at protein termini, yielding homogeneous conjugates with extended plasma half-lives and full biological activity[52]. A selective enzymatic approach has also been developed to convert surface cysteines to formylglycines, which bear an aldehyde functionality for subsequent conjugation (SMARTag)[53]. Site-specific modification of glycans, rather than the peptide sequence, can also be

Semi-telechelic
A polymer with one reactive end group.

Table 1 | **Marketed polymer–drug conjugates**

Name (company)	Polymer carrier	Drug	Indication(s)	Year approved[a]	Refs
Adagen (Enzon Pharmaceuticals)	PEG	ADA	ADA-SCID	1990	12
SMANCS (Astellas Pharma)	Poly(styrene-co-maleic acid)	Neocarzinostatin	Liver and renal cancer	1993 (Japan)	260
Oncaspar (Enzon Pharmaceuticals)	PEG	L-asparaginase	Acute lymphoblastic leukaemia	1994	16
PegIntron (Merck & Co.)	PEG	Interferon α2b	Hepatitis C	2001	261
Pegasys (Genentech)	PEG	Interferon α2a	Hepatitis B and hepatitis C	2002	262
Neulasta (Amgen)	PEG	G-CSF	Chemotherapy-induced neutropenia	2002	263
Somavert (Pfizer)	PEG	HGH receptor antagonist	Acromegaly	2003	264
Macugen (Bausch & Lomb)	PEG	Anti-VEGF aptamer	Neovascular age-related macular degeneration	2004	265
Mircera (Roche)	PEG	Epoetin beta	Anaemia associated with chronic kidney disease	2007	18
Cimzia (UCB Pharma)	PEG	Anti-TNF Fab′	Crohn's disease, rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis	2008	266
Krystexxa (Horizon Pharma)	PEG	Uricase	Chronic gout	2010	267
Plegridy (Biogen)	PEG	Interferon β1a	Relapsing multiple sclerosis	2014	268
Movantik (AstraZeneca)	PEG	Naloxone	Opioid-induced constipation	2014	94
Adynovate (Baxalta)	PEG	Factor VIII	Haemophilia A	2015	269
Palynziq (BioMarin)	PEG	Phenylalanine ammonia lyase	Phenylketonuria	2018	270
Jivi (Bayer)	PEG	Factor VIII	Haemophilia A	2018	271

ADA, adenosine deaminase; Fab′, antigen-binding fragment; G-CSF, granulocyte colony-stimulating factor; HGH, human growth hormone; PEG, polyethylene glycol; SCID, severe combined immunodeficiency; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. [a]Approved by the US Food and Drug Administration unless otherwise stated; biosimilar versions of approved drugs are omitted.

achieved via sialyltransferase-mediated PEGylation[54]. This approach has been recently employed in the development of turoctocog alfa pegol, a PEG–factor VIII conjugate that maintains full biological activity and exhibits prolonged therapeutic efficacy[55]. The site-specific expression of unnatural amino acids with orthogonal chemical reactivity provides a previously unrealized opportunity to create libraries of PEG–protein conjugates by modulating the precise position of the target non-native amino acid on the protein of interest[56,57] (FIG. 2a). This is particularly important for the screening and optimization of PEGylated protein therapeutics, as PEGylation at different sites gives rise to unpredicted and considerable variability in pharmacokinetics and pharmacodynamics[57,58].

An alternative approach to the post-polymerization modification of semi-telechelic polymers (that is, 'grafting to') is the chemoselective 'grafting from' approach, in which in situ polymerization proceeds from an initiating site incorporated into the target protein[7,59] (FIG. 2b). In addition to affording synthetic precision, this technique provides the advantage of minimized steric hindrance and therefore improved reaction efficiency. Moreover, the synthetic and purification steps are simplified, which is a considerable advantage for the large-scale production of polymer–protein therapeutics. Currently, more versatile approaches are being employed, including the direct incorporation of reversible addition fragmentation chain transfer agents into proteins[60,61] and the introduction of atom-transfer radical polymerization initiators via genetically encoded unnatural amino acids[62] and intein-mediated ligation[63].

PEG alternatives. Despite its widespread use, a considerable limitation of PEG and its subsequent utility in therapeutics is its non-biodegradability. At present, approved PEGylated protein therapeutics employ PEGs of ≤40 kDa molecular mass, close to the glomerular filtration threshold of approximately 50 kDa (REF.[15]). Although increased molecular mass generally affords extended circulation time, concerns regarding the accumulation of non-biodegradable PEG limit the optimization of polymer molecular mass and the resultant pharmacokinetics[64]. Consequently, chemical approaches are being developed to impart biodegradability to PEG, for example, via the incorporation of biocleavable units into the main chain[65,66] (FIG. 2c). Alternatively, natural biopolymers, such as polysaccharides, including dextran, polysialic acid and hyaluronic acid, as well as polypeptides, are being utilized for protein conjugation[67]. In fact, the first polymer–protein conjugate tested in humans was a dextran–streptokinase conjugate, streptodekase[67]. A promising emerging approach is the use of unstructured hydrophilic polypeptides, such as XTEN and PAS — amino acid repeats of Gly-Ala-Pro-Glu-Ser-Thr and Pro-Ala-Ser, respectively[68,69]. Notably, these polymer–protein conjugates can be encoded by a single genetic construct, eliminating the need for

Table 2 | **Polymer–protein and polymer–aptamer conjugates in clinical development**

Name (company)	Polymer carrier	Drug	Indication(s)[a]	Stage (ClinicalTrials.gov identifier)	Refs
Turoctocog alfa pegol (Novo Nordisk)	PEG	Factor VIII	Haemophilia A	Pre-registration (NCT01480180)	272
Calaspargase pegol (Shire)	PEG	Asparaginase	Acute lymphoblastic leukaemia and lymphoblastic lymphoma	Pre-registration (NCT01574274)	273
Elapegademase (Leadiant Biosciences)	PEG	ADA	ADA-SCID	Pre-registration (NCT01420627)	274
Pegvorhyaluronidase alfa (Halozyme Therapeutics)	PEG	Hyaluronidase	Pancreatic cancer	Phase III (NCT02715804)	275
TransCon Growth Hormone (Ascendis Pharma)	PEG	Human growth hormone	Growth hormone deficiency	Phase III (NCT03344458)	187
Pegilodecakin (ARMO BioSciences)	PEG	IL-10	Pancreatic cancer	Phase III (NCT02923921)	34
Pegargiminase (Polaris Pharmaceuticals)	PEG	Arginine deiminase	Mesothelioma	Phase II/III (NCT02709512)	276
BCT-100 (Bio-Cancer Treatment International)	PEG	Arginase 1	Acute myeloid leukaemia	Phase II (NCT02899286)	277
Pegsiticase (Selecta Biosciences)	PEG	Uricase	Chronic gout	Phase II (NCT02959918)	278
Sanguinate (Prolong Pharmaceuticals)	PEG	Carboxyhaemoglobin	Sickle cell disease	Phase II (NCT02411708)	279
Pegzilarginase (Aeglea BioTherapeutics)	PEG	Arginase 1	Arginase 1 deficiency	Phase II (NCT03378531)	280
BMS-986036 (Bristol-Myers Squibb)	PEG	FGF21	Nonalcoholic steatohepatitis	Phase II (NCT03486899)	27
Dapirolizumab pegol (UCB Pharma)	PEG	Anti-CD40L Fab′	Systemic lupus erythematosus	Phase II (NCT02804763)	281
Zimura (Ophthotech Corporation)	PEG	Aptamer complement C5 inhibitor	Neovascular age-related macular degeneration	Phase II (NCT03362190)	282
NKTR-214 (Nektar Therapeutics)	PEG	IL-2	Solid tumours	Phase I/II (NCT02869295)	35
BIVV001 (Bioverativ Therapeutics)	XTEN	Recombinant factor VIII Fc–von Willebrand factor	Haemophilia A	Phase I/II (NCT03205163)	283
Olaptesed pegol (NOXXON Pharma)	PEG	Anti-CXCL12 aptamer	Colorectal cancer and pancreatic cancer	Phase I/II (NCT03168139)	284
Fovista (Ophthotech Corporation)	PEG	Anti-PDGFB aptamer	Ocular von Hippel–Lindau syndrome	Phase I/II (NCT02859441)	285
BMS-986171 (Bristol-Myers Squibb)	PEG	FGF21	Nonalcoholic steatohepatitis	Phase I (NCT02538874)	30
NKTR-358 (Nektar Therapeutics)	PEG	IL-2	Systemic lupus erythematosus	Phase I (NCT03556007)	37

ADA, adenosine deaminase; CXCL12, CXC-chemokine ligand 12; Fab′, antigen-binding fragment; FGF21, fibroblast growth factor 21; IL, interleukin; PEG, polyethylene glycol; PDGFB, platelet-derived growth factor subunit B; SCID, severe combined immunodeficiency. [a]Some drugs are in different clinical trial stages for other indications; biosimilar versions of approved drugs are omitted.

chemical conjugation. New synthetic and biodegradable alternatives to PEG are also being employed to enhance protein pharmacokinetics and present an exciting opportunity in the development of the next generation of polymer–protein conjugates[63,70,71].

Conflicting reports on the immunogenicity and antigenicity of PEG have further prompted interest in the use and development of non-fouling and biocompatible PEG replacements, such as poly(2-oxazoline)s and polypeptoids[72–76]. Similar to PEG, hydrophilic poly(2-oxazoline)s, namely, poly(2-ethyl-2-oxazoline) and poly(2-methyl-2-oxazoline), are highly water-soluble, biocompatible and non-fouling. Accordingly, these polymers have been used for the modification of several proteins, providing conjugates with similar characteristics to PEGylated proteins[74]. Polypeptoids, which are synthetic polypeptide mimetics based on an N-substituted glycine backbone, likewise demonstrate promise as PEG alternatives. In a recent study, the modification of interferon α2b with polysarcosine, a polypeptoid composed of the naturally occurring amino acid sarcosine, provided an equivalent improvement in circulation half-life compared with the PEGylated protein, while significantly reducing the generation of anti-interferon antibodies and exhibiting more rapid receptor association as well as improved efficacy[76]. Novel zwitterionic and biomimetic alternatives have also been described[77–79]. Notably, a heparin-mimicking polymer affords stabilization of a heparin-binding protein — basic FGF — which is otherwise unstable, even after PEGylation[79]. In addition to the

Zwitterionic
An electrically neutral molecule with at least one positive and one negative functional group.

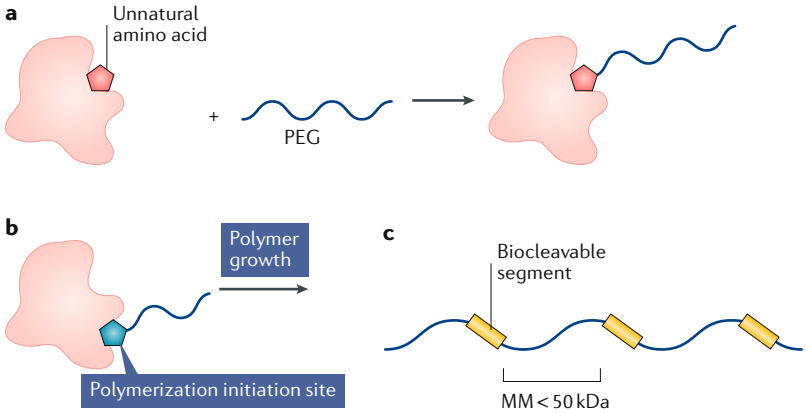

Fig. 2 | **Emerging approaches in polymer–protein conjugate development.** **a** | The site-specific expression of unnatural amino acids with orthogonal reactivities affords tuneable and precise control of the position of polyethylene glycol (PEG) conjugation. **b** | Incorporation of a polymerization initiation site in the target protein enables site-specific, in situ polymerization. **c** | Biodegradable PEGs with high molecular mass (MM) are synthesized via the incorporation of biocleavable segments in the main chain. Resulting PEG segments of <50 kDa are subsequently eliminated via renal clearance.

traditional advantages of polymer conjugation, the ligation of responsive polymers affords 'smart' conjugates in which protein activity and release can be modulated[80,81]. Additionally, amphiphilic polymer–protein conjugates can be incorporated into sophisticated superstructures to alter their pharmacokinetic profile and to increase functionality via the entrapment and co-delivery of additional active agents[82,83].

Polymer–small-molecule drug conjugates

The first polymer–small-molecule drug conjugate was reported over 60 years ago, in 1955, by Jatzkewitz, who demonstrated that the conjugation of mescaline, a psychedelic alkaloid, to a copolymer of *N*-vinylpyrrolidone and acrylic acid extends the residence time of the bioactive in mice[84] (BOX 1). In the 1970s, Ringsdorf proposed the concept of a pharmacologically active polymeric carrier, which concurrently affords drug solubilization and targeting[1]. Subsequently, work by Kopecek and colleagues in the late 1970s led to the development of the first synthetic polymer–small-molecule drug conjugate to advance to clinical trials, with many others following[2] (TABLE 3).

The ligation of small-molecule bioactives to polymeric carriers offers several advantages, including improved aqueous solubility, enhanced stability, extended plasma half-life, active intracellular delivery, altered biodistribution and the potential for targeted delivery via the incorporation of targeting moieties. Additionally, the choice of linker chemistry confers control over the release and subsequent activity of the molecule. These advantages are particularly pertinent to cytotoxic chemotherapeutics, which often exhibit poor solubility, rapid clearance and limited tumour exposure. Importantly, the efficacy of anticancer agents is severely limited by the off-target toxicity, prompting the development of approaches that afford control of biodistribution and activity. In the 1980s, Matsumura and Maeda reported that a polymeric conjugate of the anticancer protein neocarzinostatin, SMANCS, preferentially accumulates in tumour tissue following intravenous administration[85]. They proposed that the hyperpermeability of tumour vasculature, in combination with impaired lymphatic drainage, results in the passive accumulation of macromolecules in tumours and termed this phenomenon the enhanced permeability and retention (EPR) effect. Consequently, polymer–drug conjugates of small-molecule anticancer agents exhibit improved safety and efficacy in preclinical animal models, with many advancing to clinical evaluation (see below).

Applications. The first polymer–small-molecule drug conjugates to advance to clinical trials employed copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA), polyglutamic acid or PEG as carriers of cytotoxic chemotherapeutic agents. PK1 (FCE28068), the first clinically investigated water-soluble polymer–small-molecule drug conjugate, comprises doxorubicin bound to an HPMA copolymer via a lysosomally cleavable peptidyl linker[3]. Owing to the non-biodegradability of the HPMA copolymer, a size of 30 kDa was chosen to ensure renal elimination of the carrier. In preclinical animal models, PK1 had a 15-fold improvement in plasma half-life, an enhanced safety profile and greater antitumour efficacy than unconjugated doxorubicin[86]. Notably, PK1 showed 17–77-fold greater tumour accumulation of drug relative to free doxorubicin, and passive tumour targeting was also observed, with 45–250-fold higher drug concentrations in tumour tissues relative to healthy tissues[86]. However, when PK1 was tested in clinical trials, tumour accumulation of the conjugate was observed in only a small subset of patients, and efficacy was marginal, although it showed extended circulation time and an overall improved safety profile[87,88]. Interestingly, in phase II evaluation, the two patients who exhibited tumour accumulation did not respond to treatment, while patients who responded to treatment had not been among those imaged[88]. Taken together, these data suggest that tumour permeability is heterogeneous and cannot be relied on to achieve therapeutic efficacy. Additionally, given that tumour accumulation did not invariably result in a therapeutic response, it is possible that even among permeable tumours, PK1 exhibited an insufficient increase in half-life and, accordingly, suboptimal tumour accumulation. Consequently, these results discouraged additional investment, and the development of PK1 was abandoned[89].

More recently, a four-arm PEG conjugate of the cytotoxic chemotherapeutic irinotecan, Onzeald, progressed to phase III trials. Onzeald employs a cleavable ester linkage to bind one irinotecan molecule per PEG arm and has a nominal molecular mass of 20 kDa (REF.[90]). The ester linkages slowly hydrolyse to release irinotecan, which is subsequently metabolized into the active anticancer agent, SN-38. In preclinical animal models, Onzeald exhibits an extended circulation half-life, sustained concentrations in plasma and tumour and up to 400-fold increased plasma exposure (AUC) compared with conventional irinotecan. Of note, while tumour C_{max} is up to tenfold greater after administration of Onzeald compared with irinotecan, plasma C_{max} is reduced,

Biodistribution
The distribution of a molecule within the body.

AUC
The area under the plasma drug concentration versus time curve. Larger AUC values are indicative of increased drug exposure.

C_{max}
The maximal concentration of drug achieved after administration.

Table 3 | **Polymer–small-molecule drug conjugates, dendrimers and polymer nanoparticles in clinical development**

Name (company)	Polymer carrier	Drug	Indication(s)[a]	Stage (ClinicalTrials.gov identifier)	Refs
Onzeald (Nektar Therapeutics)	PEG	Irinotecan	Breast cancer	Pre-registration (NCT02915744)	92
NKTR-181 (Nektar Therapeutics)	PEG	μ-Opioid receptor agonist	Chronic low back pain and chronic non-cancer pain	Pre-registration (NCT02367820)	95
PEX168 (Jiangsu Hansoh Pharmaceutical)	PEG	Loxenatide	Type 2 diabetes	Phase III (NCT02477969)	286
NC-6004 (NanoCarrier)	PEG-b-poly(glutamic acid) micelle	Cisplatin	Pancreatic cancer	Phase III (NCT02043288)	287
Opaxio (CTI BioPharma)	Polyglutamic acid	Paclitaxel	Ovarian cancer, peritoneal cancer and fallopian tube cancer	Phase III (NCT00108745)	288
APL-2 (Apellis Pharmaceuticals)	PEG	Cyclic peptide complement C3 inhibitor	Paroxysmal nocturnal haemoglobinuria	Phase III (NCT03500549)	97
CRLX101 (BlueLink Pharmaceuticals)	Cyclodextrin-PEG that self-assembles into nanoparticles	Camptothecin	Ovarian cancer, peritoneal cancer and fallopian tube cancer	Phase II (NCT01652079)	289
NK012 (Nippon Kayaku)	PEG-b-poly(glutamic acid) micelle	SN-38	Breast cancer	Phase II (NCT00951054)	290
OsteoDex (DexTech Medical)	Dextran	Alendronate	Prostate cancer	Phase II (NCT02825628)	291
Somadex (DexTech Medical)	Dextran	Somatostatin	Neuroendocrine tumours and acromegaly	Phase II (NA)	292
BP-C1 (Meabco A/S)	Benzo-polycarbonic acid polymer	Pt(II)	Breast cancer	Phase II (NCT02783794)	293
Pegcantratinib (Sienna Biopharmaceuticals)	PEG	TrkA inhibitor	Pruritus	Phase II (NCT03322137)	98
DEP docetaxel (Starpharma)	PEG-polylysine dendrimer	Docetaxel	Solid tumours	Phase II (NA)	123
NC-6300 (NanoCarrier)	PEG-b-poly(aspartic acid) micelle	Epirubicin	Solid tumours and soft tissue sarcoma	Phase I/II (NCT03168061)	199
CRLX301 (BlueLink Pharmaceuticals)	Cyclodextrin-PEG that self-assembles into nanoparticles	Docetaxel	Solid tumours	Phase I/II (NCT02380677)	150
DEP cabazitaxel (Starpharma)	PEG-polylysine dendrimer	Cabazitaxel	Solid tumours	Phase I/II (NA)	294
NKTR-262 (Nektar Therapeutics)	PEG	TLR7/TLR8 agonist	Solid tumours	Phase I/II (NCT03435640)	295
CriPec docetaxel (Cristal Therapeutics)	Core-crosslinked PEG-polymer micelle	Docetaxel	Solid tumours	Phase I (NCT02442531)	158
NC-4016 (NanoCarrier)	PEG-b-poly(glutamic acid) micelle	Oxaliplatin	Solid tumours and lymphoma	Phase I (NCT03168035)	296
RadProtect (Original BioMedicals)	PEG-b-poly(glutamic acid) chelating complex micelle	Amifostine	Acute radiation syndrome	Phase I (NCT02587442)	297
SER-214 (Serina Therapeutics)	Poly(2-ethyl-2-oxazoline)	Rotigotine	Parkinson disease	Phase I (NCT02579473)	298
DFP-13318 (ProLynx)	PEG	SN-38	Solid tumours	Phase I (NCT02646852)	299

PEG, polyethylene glycol; TLR, Toll-like receptor; TrkA, tropomyosin receptor kinase A. [a]Some drugs are in different clinical trial stages for other indications.

potentially affording an improved therapeutic index. Consequently, administration of Onzeald results in sustained tumour suppression and regression for weeks after treatment in preclinical models. In phase I first-in-human studies, Onzeald demonstrated similarly improved pharmacokinetics, with SN-38 exhibiting an elimination half-life of 50 days compared with 12–47 hours following irinotecan administration[91]. However, in a phase III trial published in 2015, Onzeald did not confer an improvement in overall survival compared with a single-agent treatment of physician's choice in patients with advanced breast cancer[92]. Notably, the patients in this trial had been heavily pretreated and possessed locally recurrent or metastatic breast cancer, and it is possible that Onzeald would have provided a survival benefit in a patient population with less advanced

Therapeutic index
The ratio of the dose needed to elicit a toxic side effect relative to the dose needed to elicit a therapeutic effect. A larger therapeutic index is indicative of a safer drug.

disease. Furthermore, while the preclinical efficacy of Onzeald can be potentially attributed to increased and sustained tumour drug concentrations, there are presently no clinical data to confirm that Onzeald confers a similar benefit in human tumours. Nonetheless, administration of Onzeald demonstrated a survival benefit in a subset of patients with a history of brain or liver metastases[92,93], and a phase III trial involving such patients is ongoing (TABLE 3).

Polymer–small-molecule drug conjugates are also being developed for non-cancer indications. The only marketed polymer–small-molecule drug conjugate to date, naloxegol (Movantik), received clinical approval in 2014 for the treatment of opioid-induced constipation in patients with chronic pain[94]. Notably, Movantik is an orally administered compound, and its approval highlights the potential utility of polymeric carriers in the design of non-parenteral therapies. Movantik, a PEG oligomer conjugate of the opioid antagonist naloxone, exhibits reduced permeability and penetration into the central nervous system relative to the small-molecule drug. Consequently, Movantik significantly improves opioid-related constipation while reducing the ability of naloxone to counteract opioid-induced analgesia. Similarly, NKTR-181 employs a PEG side chain to reduce the transfer rate of the μ-opioid agonist pharmacophore across the blood–brain barrier[95]. Notably, the rate of drug transfer across the blood–brain barrier is a contributing factor in the addictive qualities and abuse potential of opioid analgesics. Consequently, NKTR-181 exhibited reduced abuse potential as well as significant analgesic efficacy in patients[95,96].

Peptide drugs are likewise amenable to polymer conjugation. Although peptides are similar to proteins in composition, they are grouped here with small-molecule drugs owing to their size and their regulatory classification. Peptides (≤40 amino acids) are not generally considered biological products by the US Food and Drug Administration (FDA) and are regulated as conventional small-molecule drugs. APL-2, a current clinical candidate, is a PEGylated cyclic peptide inhibitor of complement C3. In clinical evaluation, APL-2 was well tolerated and provided sustained haemolysis suppression in patients with paroxysmal nocturnal haemoglobinuria[97]. Interestingly, polymer conjugation may also be useful in the development of topical agents such as pegcantratinib. Pegcantratinib, a PEGylated small-molecule TrkA kinase inhibitor, demonstrated significant reductions in the pruritus of patients with psoriasis and is currently in phase II trials[98].

Advances. Owing to their non-biodegradability and subsequent constraints on molecular mass, first-generation conjugates of PEG and HPMA copolymers exhibit suboptimal pharmacokinetics. Therefore, in addition to well-established polypeptides[99], current research efforts focus on the evaluation of conjugates employing new biodegradable and backbone-degradable water-soluble polymers. For example, backbone-degradable HPMA polymers are synthesized via the incorporation of enzymatically degradable oligopeptide sequences in the main chain[100–102]. Accordingly, degradable high-molecular-mass HPMA polymer–small-molecule drug conjugates demonstrate improved pharmacokinetics and pharmacodynamics while exhibiting eventual renal clearance. New water-soluble, biocompatible and biodegradable polymers, such as polycarbonates, are likewise being synthesized and evaluated, expanding the repertoire of polymers available for drug conjugation[103,104]. Additionally, biologically active polymers, such as peptide molecular transporters, are being employed as drug carriers owing to the added biological functionality they afford[105–107]. While not readily biodegradable, polypeptoids and poly(2-oxazoline)s are also being investigated as potentially promising drug carriers[74,75,108].

Molecular architecture is emerging as an important modulator of the in vivo fate and subsequent therapeutic efficacy of polymer–small-molecule drug conjugates, with circular and branched structures (FIG. 3a) generally exhibiting extended plasma half-lives and greater efficacy than their linear counterparts[109,110]. Importantly, polymer branching affords an alternative approach to the synthesis of high-molecular-mass conjugates without compromising the drug loading efficiency of di-end-functional polymers, such as PEG. In addition to polymer molecular mass and architecture, linker chemistry can be modulated to achieve an optimized drug release profile and, subsequently, enhanced therapeutic efficacy. For example, slow-releasing and fast-releasing HPMA–dexamethasone polymeric constructs are designed via the incorporation of hydrazone and hydrazone benzyl ester linkers, respectively[111]. In an in vivo rat model of arthritis, the slow-releasing conjugate exhibits sustained anti-inflammatory activity, leading to an improved therapeutic outcome compared with the fast-releasing conjugate. The evaluation of polymer–small-molecule drug conjugate libraries in which molecular mass, architecture and linker chemistry are varied is a valuable approach for the identification of clinically promising therapeutic candidates and will greatly contribute to our knowledge of the respective macromolecular therapeutic design requirements for a variety of indications.

While first-generation polymer–small-molecule drug conjugates enhance the pharmacokinetics and pharmacodynamics of previously approved small-molecule therapeutics, the use of polymeric carriers to facilitate the translation and clinical utility of novel agents is a particularly exciting and promising avenue for the next generation of polymer therapeutics[112,113]. Presently, several conjugates of novel agents are in clinical development, including NKTR-181, APL-2 and pegcantratinib[95,97,98] (TABLE 3). The synthesis of biomimetic polymer therapeutics that incorporate the structural and chemical features of therapeutically active molecules also presents an innovative paradigm in the rational design of the next generation of 'drug-free' macromolecular therapies. Recently, Kopecek and colleagues developed drug-free bioactive conjugates of anti-CD20 Fab′[114–116]. In particular, owing to their multivalency, these constructs afford CD20 crosslinking and directly induce apoptosis in malignant B cells, eliminating the need for Fc-mediated crosslinking by immune effector cells. Importantly, apoptosis induction relies on the presence of the polymer component, as the Fab′ alone is not pharmacologically active.

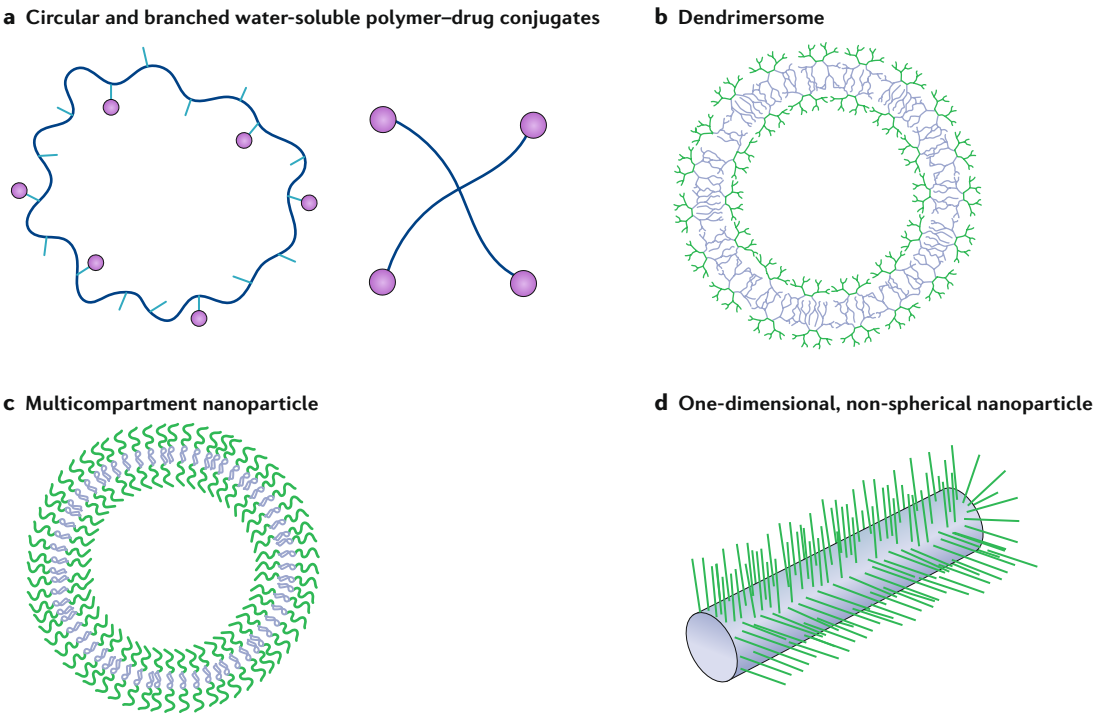

Fig. 3 | **Architectural diversity of polymer therapeutics.** Circular and branched polymer–drug conjugates exhibit increased circulation times relative to linear polymers of similar molecular mass owing to their increased hydrodynamic radii (part **a**). Onzeald is an example of a current clinical candidate with a branched polymeric architecture. Dendrimersomes are unilamellar vesicles formed via the self-assembly of amphiphilic dendrimers (part **b**). These structures maintain the high functionality of the dendrimer components while simultaneously enabling the physical entrapment of additional agents in their aqueous core. Amphiphilic linear polymers can self-assemble to afford multicompartment nanoparticles with a hydrophobic polymer compartment and an aqueous core (part **c**), as well as 1D and non-spherical nanoparticles (part **d**).

Dendrimers

Dendrimers are unimolecular, highly branched, 3D polymeric macromolecules, which were first described by Newkome and Tomalia in 1985 (REFS[117,118]). Repeat branching units, or generations, radiating from an initiating core are added stepwise, affording high synthetic precision and low dispersity. Importantly, dendrimers exhibit high surface functionality, with the number of surface groups increasing exponentially with the addition of each generation. Owing to their utility in generating synthetically precise libraries of monodisperse and tuneable nanostructures with high functionality, dendrimers have been extensively explored as drug delivery vehicles. Similar to linear polymer drug carriers, dendritic drug carriers afford improved aqueous solubility, as well as extended circulation time and altered biodistribution in preclinical animal models. However, owing to their relatively large hydrodynamic radii, dendrimers exhibit reduced renal clearance and greater plasma exposure than linear polymers of similar molecular mass[119]. Additionally, owing to their high functionality, drugs, imaging and targeting agents are incorporated at high density in dendritic carriers. Although dendrimers are most often conjugated to small-molecule drugs, they are also used as drug carriers for protein therapeutics[120,121].

Recently, the first dendrimer-based drug product, SPL7013 (VivaGel), received market approval in Australia and the European Union for the treatment of bacterial vaginosis. VivaGel is a poly(L-lysine) dendrimer-based topical microbicide, in which the macromolecular dendrimer itself is the active pharmaceutical agent[122]. A poly(L-lysine) dendrimer–docetaxel conjugate, DEP docetaxel, is also under development by Starpharma and has recently demonstrated enhanced safety, longer plasma half-life, lower peak blood concentrations and greater overall drug exposure than conventional docetaxel in a phase I clinical trial[123].

The most widely studied dendrimers to date are non-biodegradable, cationic amine-terminated polyamidoamine (PAMAM) dendrimers. However, in addition to being non-biodegradable, the clinical utility of PAMAM dendrimers is considerably limited by the nonspecific toxicity and haemolysis attributed to their cationic surface charge[124]. To improve their biocompatibility, the surface charge of cationic dendrimers is minimized via surface functionalization[125,126]. Additionally, biodegradable and biocompatible alternatives, including peptide-based, polyurea-based and polyester-based dendrimers, are currently being synthesized and evaluated for biomedical applications[127–132]. While therapeutic entities can be physically entrapped within a dendrimer, conjugation affords high drug loadings as well as controlled and sustained drug release kinetics[125,133–135]. For example, the conjugation of fluocinolone acetonide to a PAMAM dendrimer affords sustained drug release

over an extended period of 90 days[134]. Subsequently, one intravitreal injection of the carrier attenuated retinal degeneration for up to 30 days in a preclinical animal model[134]. Interestingly, the release of conjugated active agents buried within dendrimers can be modulated by varying the number of generations as well as the surface chemistry, providing an additional layer of control for the design of optimal drug carriers[135].

Dendrimers additionally afford modulation and optimization of cellular interactions via controlled surface modification. Notably, amine-terminated PAMAM dendrimers exhibit pH-dependent ligand exposure and, subsequently, tuneable cellular interactions[136]. The extent of cellular interactions of targeted, PEGylated dendritic structures also depends on the nanoscale surface features, including PEG corona length and targeting ligand density, rendering control and optimization of surface features essential for the rational design of clinically transformative drug carriers[137]. Furthermore, owing to their high surface functionality, the conjugation of many targeting ligands on a dendrimer enables increased avidity, enhancing the utility of targeting moieties that otherwise possess low binding affinities. For example, the enhanced avidity of multivalent targeted dendrimers can be employed to bind resistant bacteria as well as circulating tumour cells[138,139]. The conjugation of epitopes to dendritic carriers likewise affords inventive self-adjuvating vaccines[140].

Interestingly, non-targeted dendrimers exhibit transepithelial transport, which can be modulated by surface functionalization with PEG[141]. Therefore, dendritic carriers are emerging as useful vehicles for achieving and controlling drug delivery across biological barriers. For example, dendrimers traverse the blood–brain barrier and localize in injured neurons and microglia upon systemic administration[125,142]. Additionally, dendrimer drug carriers are amenable to oral and pulmonary drug administration, opening new paradigms in the delivery of therapeutic agents[128,143]. Dendritic carriers are of particular interest as cancer nanomedicines owing to their concomitant ability to exhibit passive tumour accumulation as well as deep tumour penetration[144,145]. Consequently, dendrimer–drug conjugates are incorporated into sophisticated nanoarchitectures to combine the long circulation time and increased tumour accumulation of large nanostructures (~100 nm) with the increased tumour penetration of smaller dendrimers (~5 nm)[145]. Similarly, dendrimersomes (FIG. 3b) combine the synthetic precision of dendrimers with the added functionality of unilamellar vesicles, which can, for example, carry hydrophilic agents in their aqueous core[146].

Polymer nanoparticles

In the late 1970s, Kopf et al. reported the first preparation of a polymeric nanoparticulate carrier[147]. Polymeric nanoparticles, which are colloidal carriers with dimensions on the nanoscale, have since been widely employed as drug delivery vehicles[148]. Like other macromolecular drug carriers, polymeric nanoparticles afford improved delivery of hydrophobic agents as well as extended circulation and altered biodistribution of encapsulated therapeutics. However, compared with water-soluble linear polymers and dendrimers, colloidal polymeric nanoassemblies exhibit greater architectural complexity and afford enhanced tunability via modular design of both core and surface components. Here, we focus on nanoparticles of polymer–drug conjugates.

Polymer nanoparticles include self-assembling polymer aggregates, such as the cyclodextrin-based polymeric conjugate nanoassemblies CRLX101 and CRLX301 (TABLE 3), which are produced by intermolecular and intramolecular inclusion complex formation between cyclodextrin and the conjugated lipophilic agents: camptothecin and docetaxel, respectively[149,150]. However, the most widely investigated polymeric nanoassemblies possess a core–shell architecture, in which the hydrophobic polymer core is sequestered and stabilized by a hydrophilic corona. Solid polymer nanoparticles are prepared via active processing, either precipitation or emulsification, in the presence of a surfactant. Alternatively, the self-assembly of amphiphilic block copolymers in aqueous media affords the facile preparation of polymeric micelles. Owing to their solid nature, polymeric nanoparticle drug carriers offer a unique set of advantages, including the sequestration of agents in the hydrophobic nanocarrier core, high drug loading capacity and controlled drug release via diffusion or controlled degradation of the polymer matrix. Although physicochemical interactions between a drug and its carrier can be employed to physically entrap active agents in polymeric nanoparticles, the loading capacity is often limited to ≤10 wt% (REFS[151–153]). Additionally, these systems suffer from significant burst release, with >50% of the drug being released within 24 hours. By contrast, conjugation of active agents to polymeric nanocarriers enables incorporation at high and predefined loadings, while choice of linker chemistry and modulation of core properties afford controlled release kinetics.

The first generation of polymeric nanocarriers to advance to clinical trials was polymeric micelles of PEG-poly(amino acid) amphiphilic block copolymers developed by Kataoka and colleagues in the late 1980s[154]. The first of these was NK911, a 40 nm micelle of PEG-b-poly(aspartic acid) copolymer conjugated to doxorubicin through amide bonds, which entered clinical evaluation in 2001. In preclinical studies, NK911 exhibited an extended plasma half-life, increased overall drug exposure, greater tumour accumulation and improved efficacy compared with free doxorubicin[154]. Similarly, in phase I clinical testing, NK911 was well tolerated and exhibited a longer half-life and a larger AUC than free doxorubicin[155]. Nonetheless, the recommended dosage of NK911 was ultimately similar to that of standard doxorubicin, and no recent developments have been reported.

Challenges and recent developments. Concerns regarding the stability of self-assembled nanostructures upon dilution in the bloodstream are motivating the development of core-crosslinked polymeric micelles, as well as solid polymer nanoparticles[156]. Recently, a biodegradable, core-crosslinked polymeric micelle with covalently entrapped docetaxel, CriPec docetaxel, entered clinical

Avidity
The cumulative strength of a binding interaction.

development and completed phase I testing in 2018. The CriPec nanoparticle platform is promising, as it affords tailored design via control of nanocarrier size, drug release kinetics, degradation profile and surface functionality[157]. In preclinical animal models, CriPec docetaxel exhibits improved efficacy, controlled and sustained plasma drug levels, enhanced tumour accumulation and improved safety at high doses relative to the clinical formulation of docetaxel[157,158]. Although several polymer–drug conjugate nanoparticles are in clinical development (TABLE 3), none have entered the market to date.

One of the greatest challenges in the clinical translation of polymeric nanocarriers is their physicochemical heterogeneity. The uncontrolled conjugation of therapeutic agents to polymeric carriers contributes to this heterogeneity, resulting in polydisperse polymer mixtures with varied drug loadings and sites of modification. New precise synthetic approaches are emerging to enable the synthesis of well-defined polymer–drug conjugate nanoassemblies. In particular, novel methodologies, such as drug-initiated, living in situ polymerization and living polymerization of prodrug monomers, are being developed and employed for the regioselective and chemoselective incorporation of bioactives into synthetically precise polymeric nanoparticles at nearly quantitative loading efficiencies[159–161]. Currently, the most widely used hydrophobic polymers in the fabrication of polymeric nanocarriers are biodegradable and biocompatible poly(amino acid)s and polyesters. Although widely employed in FDA-approved devices, hydrophobic polyesters such as polycaprolactone, poly(lactic acid), poly(glycolic acid) and poly(lactide-co-glycolide) exhibit limited functionality for drug conjugation. Consequently, polyester-based nanocarriers primarily rely on the physical entrapment of active agents. However, recent progress in polymer chemistry is enabling the synthesis of new biocompatible and biodegradable polymers with high functionality for conjugation[162–164]. Additionally, advances in genetic engineering afford the biosynthesis of polypeptide carriers with precisely defined compositions, molecular masses and polydispersities[165–167].

The synthesis of novel multifunctional polymer backbones, as well as the development of new precise synthetic approaches, is facilitating the synthesis of polymeric nanocarriers with high and controlled drug loadings[159,161,168]. For example, the atom-efficient incorporation of paclitaxel in a poly(glycerol carbonate) backbone affords high, controlled drug loadings of up to 74 wt%[163]. Additionally, harnessing the compatibility of free paclitaxel with the high-density paclitaxel-conjugate polymer matrix facilitates the additional physical entrapment of the free drug and affords previously unattainable ultrahigh drug loadings >100 wt%[169]. Importantly, concurrent maximization of the drug/material efficiency (that is, the drug loading) and the therapeutic efficacy is imperative to the successful clinical development of the next generation of polymeric nanocarriers, as it minimizes a patient's exposure to synthetic carrier material. The inventive development of responsive and multifunctional polymers in recent years is also facilitating the sophisticated molecular design of smart polymer nanoparticles with a wide range of added functionalities, including pH-responsive fluorescence, endosomal membrane destabilization, thermosensitive aggregation and solubilization, and triggered pulsatile drug release[170–173].

The rational design of core components, including choice of active agents, drug loading, polymer composition, polymer hierarchical structure and polymer chain length, affords fine-tuned control of nanocarrier properties such as release kinetics, mechanical stiffness, stability and core mobility[163,169,174–178]. For example, more compact polymer aggregation is achieved via increased drug loading or increased polymer molecular mass, resulting in reduced rates of drug release[163,175]. The additional physical entrapment of active agents in polymer–drug conjugate nanocarriers can provide added control. Interestingly, the physical entrapment of free paclitaxel in a high-density paclitaxel-conjugate nanocarrier modulates the mechanical stiffness and drug release kinetics, while maintaining sustained release over extended periods of 15–70 days[169]. The continuous and prolonged release of active agents from macromolecular drug carriers is especially promising because it affords long-term therapeutic efficacy in the treatment of pathologies that otherwise require frequent, repeated administrations of conventional drugs[163,179]. Control of spatial arrangement and hierarchical structure also enables the synthesis of nanoassemblies with increased functionality, for example, via the implementation of multiple compartments in a single nanocarrier platform[180,181] (FIG. 3c). Recently reported 1D, non-spherical micellar carriers (FIG. 3d) similarly expand the repertoire of architectures available for the design of novel polymeric nanomedicines[182].

The most commonly employed route of administration in the preclinical evaluation of polymeric nanoparticles is systemic, intravenous injection. Despite their utility and clinical prevalence, alternative routes of administration are seldom employed owing to challenges in achieving oral bioavailability and crossing mucosal barriers. Nonetheless, polymeric nanoparticles are currently being developed for the controlled delivery of therapeutic agents across biological membranes[179,183,184]. One such example is a low-molecular-weight chitosan (LMWC) nanoparticle developed for the oral delivery of exendin-4, a peptide used for the treatment of type 2 diabetes[184]. Presently, exendin-4 is administered via subcutaneous injection, which reduces patient compliance and presents a risk of infection at the injection site. Incorporation of exendin-4 in the nanocarrier provides proteolytic stability, while LMWC affords mucoadhesion and facilitates paracellular transport.

Stimuli-responsive systems

Stimuli-responsive, smart drug delivery systems are of widespread interest owing to their ability to undergo physical and/or chemical changes in response to endogenous biological or external triggers[185]. The triggered response of these carriers is most often employed to achieve control over the spatial and temporal release of bioactives but is also being utilized to access more

sophisticated functionalities, such as modulation of tissue penetration and cellular internalization. Linker chemistry primarily provides control of drug release, while responsive polymer backbones are being designed to modulate physicochemical parameters such as solubility, stability, conformation and hydrodynamic radius.

Spatiotemporal control of drug release

Choice of linker chemistry is important in the design of polymer–drug conjugate therapeutics, as it confers spatiotemporal control over the cleavage and subsequent release of active agents. Without sufficient linker stability, a conjugated drug can exhibit premature release, annulling the advantages of its macromolecular carrier. However, in the case of an inactive polymeric prodrug, insufficient drug release may result in sub-therapeutic drug levels and, consequently, suboptimal therapeutic efficacy. Therefore, a sustained drug release profile that affords prolonged therapeutic efficacy is highly desirable. For example, the long-acting TransCon Growth Hormone (GH) is an inactive prodrug of unmodified human GH bound to a PEG carrier through a proprietary linker designed to achieve similar exposure as daily GH administration in a more convenient single weekly dose via physiological pH-dependent and temperature-dependent hydrolysis[186,187]. Harnessing the chemical and biological distinguishing features of the target indication to trigger site-specific release is also a promising strategy for achieving high local concentrations of active drug and minimizing off-target toxicities. Consequently, many linker chemistries have been described and utilized for the sustained or triggered release of therapeutic agents (FIG. 4a).

Hydrolytic cleavage is frequently employed in sustained release systems and is primarily achieved using ester linkages, which afford sustained and continuous release for periods ranging from days to months[134,163,175,188,189]. Nonetheless, to achieve precise control of the drug release site, linkers exhibiting selective cleavage in the biochemical microenvironment of the disease site are being developed. These linkers combine innovation in chemical synthesis and materials design with the growing understanding of the basic biochemical characteristics of the target indication. For example, enzymatically cleavable peptide linkers are employed to achieve tumour-specific drug release in the presence of pathologically overexpressed enzymes such as matrix metalloproteinases and cathepsin B[190–193]. Acid-responsive linkers are also widely employed and represent a diverse set of chemical bonds, including hydrazone, aconityl and acetal linkages[194]. Consequently, drug release can be triggered following cellular internalization and subsequent trafficking to acidic endosomal and lysosomal compartments[195]. Importantly, these linkers are useful for the selective delivery of anticancer agents in the mildly acidic tumour microenvironment[128,151,167,174,196,197]. The utility of acid-labile linkers is evident in the design of NC-6300, a clinical candidate in which the anticancer agent epirubicin is incorporated in a PEG-b-poly(aspartic acid) micelle via a hydrazone linkage[198,199]. The micelles are stable in the bloodstream but release epirubicin upon exposure to the acidic environment of endosomal and lysosomal compartments. In a phase I study, NC-6300 exhibited an improved safety profile relative to standard epirubicin, allowing the administration of higher doses via the micellar therapeutic[199]. Specific intracellular drug delivery is also achieved using reduction-sensitive linkages, such as disulfide and thioether, which are cleaved in the reducing environment of the cell[105,125,200,201]. This capability is particularly advantageous for the treatment of indications in which high intracellular drug concentrations are required to overcome efflux-pump-mediated drug resistance[105,202].

Additionally, systems targeted for specific cell localization must be designed to retain their payload before cellular internalization in order to harness their targeting capability. Specific cellular localization, combined with triggered intracellular release, affords optimized drug delivery by concurrently enhancing therapeutic efficacy and minimizing systemic toxicity. Notably, the triggered intracellular delivery of valproic acid using PAMAM dendrimers, which selectively localize in activated microglia, results in improved efficacy, with only one-tenth of the dose necessary to elicit an equivalent therapeutic effect in a large animal model of brain injury[125]. The conjugate additionally prevents the adverse cardiovascular side effects associated with the free drug.

Linker chemistry has been shown to affect the solution conformation of a polyglutamic acid-based combination-drug conjugate[203] and, thus, the impact of linker chemistry on the physical properties of a polymer therapeutic must be thoroughly assessed and considered. Given the complexity of macromolecular drug carriers, it is also important to note that in addition to linker chemistry, many other physicochemical parameters, such as sterics, polymer molecular mass and nanocarrier composition, also impact the rate of drug cleavage and release. Therefore, careful evaluation and judicious choice of all system design parameters are necessary for the synthesis of well-defined and programmable macromolecular carriers.

Responsive polymers

Stimuli-responsive polymeric materials are of considerable interest, principally in the design of smart drug delivery systems, which exhibit physicochemical changes in response to endogenous or external stimuli (FIG. 4b). Consequently, these materials are utilized to access a wide range of added functionalities and to enhance carrier-mediated drug delivery. For example, while drug-linker chemistry affords control over drug cleavage and release, the incorporation of enzymatically cleavable or trigger-responsive domains in polymer backbones imparts concomitant control of polymer degradation[101,173]. Additionally, cellular internalization is controlled, for example, by modulating surface charge or ligand accessibility in response to a given stimulus[136,197].

Thermally responsive polymers, such as poly(*N*-isopropylacrylamide) and genetically engineered elastin-like polypeptides, exhibit temperature-dependent changes in solubility and aggregation. Consequently, these polymers are used to externally regulate the activity

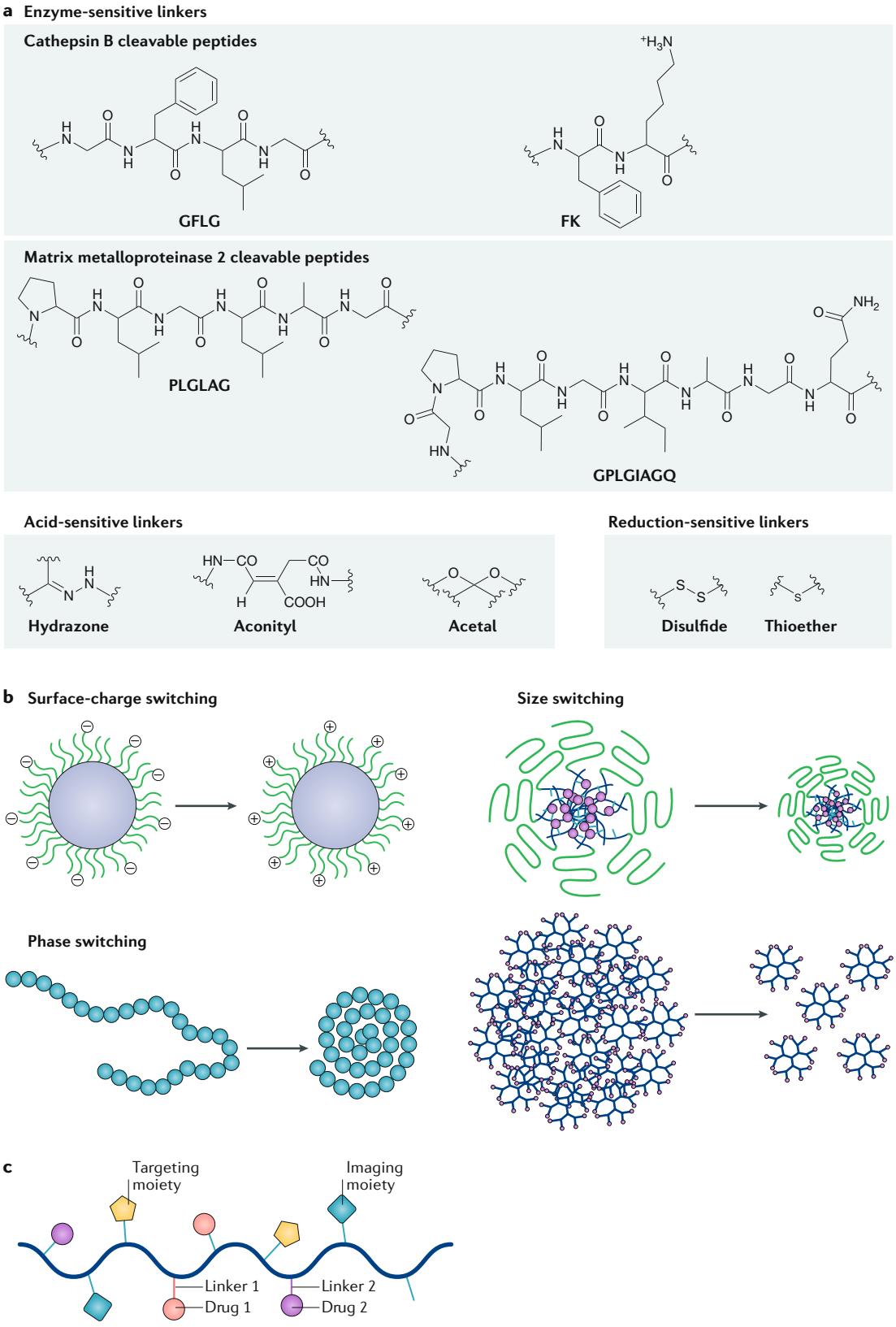

Fig. 4 | **Stimuli-responsive functionalities and schematic representation of a multifunctional polymer therapeutic.** **a** | Stimuli-sensitive linking chemistries afford spatiotemporal control of drug release. For example, the clinical candidate NC-6300 employs a hydrazone linkage to release epirubicin in the acidic environment of endosomal and lysosomal compartments. **b** | Responsive, smart polymer carriers exhibit physicochemical changes in response to a stimulus. **c** | Multifunctional polymeric carriers afford the simultaneous incorporation of multiple therapeutic and diagnostic functionalities, including combinations of active agents, targeting moieties and imaging modalities.

of a conjugated protein or the in vivo localization of the macromolecular therapeutic[80,171]. Size-switchable drug carriers, which exhibit reversible volume changes or irreversible fragmentation into smaller polymeric carriers, are also being developed to enable maximization of both plasma half-life and target tissue penetration[145,204,205]. In an important study illustrating the therapeutic potential of smart polymer therapeutics, size-switchable 80 nm nanoparticles were designed to dissociate into <10 nm dendrimer–platinum conjugates in response to tumour acidity[205]. Therefore, while the large nanocarriers extend blood circulation time, dissociation into small dendrimer carriers facilitates enhanced tumour penetration. Consequently, these nanoparticles exhibit prolonged half-life, significantly enhanced tumour drug accumulation and improved overall efficacy compared with non-responsive nanoparticle–platinum and dendrimer–platinum controls.

Targeted delivery

Polymer therapeutics confer many valuable benefits to conjugated active agents; nonetheless, these systems do not inherently provide control of specific interactions at the tissue or cellular level. Consequently, Ringsdorf's vision of an ideal, pharmacologically active polymer therapeutic included a homing device that would enable precise tissue or cellular localization[1]. The specific delivery of active agents to target tissues or cells could improve the success of a wide range of drug candidates for which off-target effects are a concern. Accordingly, this strategy is of particular interest in the delivery of cytotoxic anticancer agents, which are typically limited by considerable off-target toxicities. The recent clinical success of antibody–drug conjugates, which combine the potency of cytotoxic chemotherapeutics with the selectivity of recombinant monoclonal antibodies, highlights the potential of this approach[206].

Currently, PK2 (FCE28069), an HPMA copolymer–doxorubicin conjugate bearing galactosamine to target the hepatocyte asialoglycoprotein receptor, is the only targeted polymer–drug conjugate to advance to clinical trials[3,207]. In phase I clinical evaluation, PK2 demonstrated selective hepatic delivery in patients with liver cancer in contrast to the analogous non-targeted conjugate, PK1 (REF.[208]). Although the theoretical tumour drug concentrations were calculated to be 12–50-fold greater than doxorubicin concentrations in biopsied liver tumours after administration of the free drug, only 3 of 31 patients treated with PK2 exhibited evidence of tumour response. Additionally, tumour accumulation was fivefold lower than PK2 levels in healthy liver tissue, potentially owing to decreased asialoglycoprotein receptor expression in the tumour relative to normal liver. Solubility issues have been speculated to contribute to the observed lung localization at early time points[209]. Precipitated or aggregated polymer in the bloodstream would have been filtered out in the lungs, and this could have also potentially contributed to the observed liver accumulation, although low levels of spleen uptake were observed. The clinical development of PK2 was subsequently terminated during phase II evaluation in 2008 (REF.[210]).

Current active targeting approaches principally exploit the overexpression of surface receptors on cells of interest and rely on the specificity and binding affinity of ligands that bind these receptors. Alternatively, the unique composition of certain tissues affords targeting via molecules that possess high binding affinity for the distinctive biochemical features of the tissue. Available targeting moieties represent a diverse class of molecules, including antibodies, carbohydrates, small molecules, peptides and aptamers[211–213]. Notably, high-throughput combinatorial screening of libraries of peptides and aptamers is facilitating the identification of ligand–receptor pairs[212]. The field of macromolecular drug delivery has accordingly benefited from advances in target ligand identification, with preclinical polymer therapeutics employing a diverse array of targeting moieties to achieve localized drug delivery and enhanced therapeutic efficacy[166,177,188,191,214]. For example, the incorporation of a cyclic Arg-Gly-Asp (cRGD) targeting peptide in a PEG-polyglutamic acid polymeric micelle affords significantly increased tumour platinum accumulation as well as enhanced overall efficacy compared with oxaliplatin and control micelles bearing a non-targeting peptide[214]. The integration of various functional ligands into polymer–drug conjugate molecular constructs is further facilitated by new generalizable and facile techniques, such as chemo-enzymatic bioorthogonal conjugation and mussel-inspired nanocarrier surface functionalization[166,215–217]. Importantly, the incorporation of epithelium-specific targeting ligands opens new paradigms in the delivery of polymer–drug conjugate therapeutics by enabling their translocation across biological barriers[218–220]. For example, receptors on brain capillary endothelial cells, such as the nicotine acetylcholine receptor, recognize ligands in circulation and facilitate their transfer across the blood–brain barrier. Accordingly, PEGylated liposomes incorporating a peptide ligand for the nicotine acetylcholine receptor exhibit greater brain localization in a preclinical animal model[218]. Following their translocation, additional targeting ligands can facilitate the specific delivery of polymer therapeutics to a precise location within the target tissue[219].

The incorporation of targeting moieties in polymer therapeutic platforms also provides other advantages and functionalities. For example, the multiplicity of surface functional groups in polymeric carriers accommodates the integration of multiple targeting ligands and, consequently, multivalent binding[138,139]. Owing to their ability to simultaneously bind multiple surface receptors, multivalent molecules exhibit greater binding affinity than their monovalent counterparts, enabling the use of ligands that otherwise exhibit low binding affinity for their target. For example, vancomycin exhibits weak binding affinity for vancomycin-resistant bacterial cell wall. However, the multivalent incorporation of vancomycin in a dendritic carrier increases the binding avidity by four to five orders of magnitude, restoring the ability of vancomycin to bind the resistant cell surface[138]. Additionally, modulation of nanocarrier surface features, such as PEG corona length and target ligand density, provides fine-tuned control of avidity

and cellular interactions[137]. Similarly, the incorporation of targeting moieties in responsive polymeric carriers affords controlled ligand accessibility, an example being the triggered occlusion of ligands in collapsed PAMAM dendrimeric architectures[136]. Control of the subcellular localization of polymeric drug carriers is also achieved through the incorporation of ligands targeted to specific intracellular compartments, such as the nucleus or mitochondria[221,222].

Multifunctional polymeric carriers

One of the most exciting opportunities presented in the development of polymer therapeutics is the ability to engineer multiple therapeutic functionalities in a single drug delivery system. The multiplicity of functional groups available in polymeric carriers allows the concomitant incorporation of multiple, distinct active agents, as well as the combination of imaging and therapeutic modalities (FIG. 4c).

Combination therapy

Although most preclinical and clinical polymer therapeutics developed to date deliver a single active agent, the administration of drug combinations is a promising approach for treating diseases in which molecular complexity, heterogeneity and/or resistance impede the efficacy of single-agent therapy[223]. Combination therapy is particularly well established in cancer treatment but is also being explored for other indications, such as multiple sclerosis and diabetes[224,225]. Combinations of active agents can maximize therapeutic efficacy by targeting different molecular pathways or acting on different cell subpopulations. Additionally, certain drug combinations exhibit superadditive efficacy, or synergism[226]. Interestingly, in vitro studies demonstrate that for a given drug combination, certain agent ratios are synergistic, while others are antagonistic[226]. However, ensuring that the optimal ratio of drugs is maintained in vivo is challenging, as most therapeutic agents exhibit differing pharmacokinetics and biodistribution. Nonetheless, macromolecular therapeutics, which afford precise control of drug incorporation, are uniquely positioned to maintain and deliver predefined ratios of drug cocktails to target tissues or cells.

Owing to the broad range of chemical functionalities and architectures afforded, polymeric carriers can be synthesized with multiple conjugated and/or physically entrapped agents, enabling the facile and controlled incorporation of a wide range of therapeutics[174,181,227–229]. The combination of physical entrapment and chemical conjugation, in particular, is a facile means by which to deliver hydrophilic and hydrophobic agents in a single carrier and with differential release kinetics. For example, the self-assembly of amphiphilic camptothecin conjugates into nanocapsules allows the concurrent encapsulation of hydrophilic doxorubicin hydrochloride in the aqueous core[181]. Additionally, temporal drug release is achieved, with the physically entrapped doxorubicin exhibiting relatively accelerated release kinetics owing to its ability to diffuse from the polymeric carrier without the need for active cleavage. Similarly, differential release kinetics of a cocktail of conjugated agents can be realized via the implementation of multiple, distinct linking chemistries and provides an additional layer of control in combination therapy[229]. Importantly, the utility of single-carrier combination therapy is highlighted by the ability of these systems to exhibit increased synergism as well as enhanced efficacy relative to the analogous combinations of free drugs or single-agent carriers[174,227,228,230]. In addition to the delivery of drug combinations, polymeric carriers can also be employed to simultaneously implement multiple therapeutic modalities, such as chemotherapy, photodynamic therapy and photothermal therapy[192,231,232].

Theranostics

The concurrent integration of imaging and therapeutic modalities in theranostic polymer carriers allows patient-specific visualization of drug delivery. Consequently, these multifunctional therapies provide valuable information on in vivo pharmacokinetics and biodistribution, expediting the realization of next-generation, personalized medicines[233]. For example, the incorporation of a radionuclide in PK1 allowed quantification of organ and tumour uptake during clinical evaluation[87,88]. It was therefore possible to identify the subset of patients exhibiting preferential tumour accumulation of the conjugate. Importantly, the clinical implementation of such systems has the potential to enable personalized treatment monitoring and informed treatment selection.

Today, increasingly sophisticated and smart polymer theranostics are being developed and evaluated[192,200,231,232]. For example, the incorporation of a gadolinium complex in a hydrophobic micelle core prevents the exchange of water, reducing the magnetic resonance signal[200]. However, upon exposure to intracellular reducing conditions, release of disulfide-linked camptothecin affords a hydrophobic-to-hydrophilic transition of the nanocarrier polymer matrix. Consequently, a magnetic resonance signal is activated only following drug release. Furthermore, multiple imaging modalities, such as fluorescence, magnetic resonance imaging, photoacoustic imaging and positron emission tomography, can be simultaneously incorporated for increased clinical functionality[192,232]. New ultrasensitive and responsive polymeric nanoprobe technologies also present new opportunities in the development of effective polymer theranostics[127,172,234,235]. The additional incorporation of targeting moieties expands the applications of these nanoreporters to include molecular imaging and diagnosis[236–238]. Notably, the simultaneous integration of therapeutics, imaging agents and targeting ligands provides the clinically transformative capability to both 'see and treat' disease. For example, the rational design of a fluorescent, cRGD-linked polymeric nanocarrier affords targeted, image-guided drug delivery[231]. On-demand therapy is achieved via light irradiation, which generates reactive oxygen species for photodynamic therapy as well as doxorubicin cleavage. Interestingly, polymer–drug conjugates additionally demonstrate utility in the preparation of multifunctional 'super' nanoassemblies that combine the functionality of these carriers with inorganic particles[239].

Theranostic
A system that combines therapeutic and imaging modalities for both treatment and diagnosis.

Challenges

Polymer–protein conjugates

The established clinical success of multiple polymer–protein conjugates (TABLE 1) substantiates the continued preclinical and clinical development of the extensive pipeline of such conjugates (TABLE 2). However, several challenges remain. First, polymer conjugation often reduces the bioactivity of protein therapeutics. This limitation can be mitigated via site-specific PEGylation, as well as the implementation of approaches that restore the active protein, including the use of cleavable linkers[15,240]. As an example, turoctocog alfa pegol, a current clinical candidate, retains full biological activity owing to the selective PEGylation of an *O*-linked glycan in the factor VIII B domain[55]. The circulatory half-life of factor VIII is extended, while unmodified and fully active factor VIIIa is released following thrombin cleavage of the B domain. Another challenge is the potential immunogenicity of PEG. The presence of anti-PEG antibodies has been correlated with the rapid clearance and loss of efficacy of PEGylated therapeutics[73,241]. Furthermore, owing to its non-biodegradability, optimization of the molecular mass of PEG is limited by the renal clearance cut-off. PEG additionally presents a risk of intracellular accumulation, which is of particular concern for chronically administered conjugates[242]. Biodegradable and non-immunogenic PEG alternatives may therefore enable the development of the next generation of polymer–protein conjugates[39,72].

Polymeric conjugates of small-molecule drugs

By contrast, the translation of polymeric conjugates of small-molecule drugs to clinical practice has been limited, with only one such therapeutic, Movantik, successfully entering the market so far. Two issues are worth expanding on here. First, Movantik and several other late-stage candidates in the pipeline (TABLE 3) are for non-oncology indications, even though the preclinical and clinical development of conjugates of small-molecule bioactives has focused heavily on anticancer agents, as these have been considered to have the greatest opportunity and potential based on preclinical studies. This suggests that the expanding polymer therapeutics toolbox discussed above merits further attention in addressing challenges related to pharmacokinetics and off-target effects in small-molecule drug development outside of oncology.

The second related issue is why there has been such little clinical success with polymeric conjugates of small-molecule drugs in oncology despite the major focus on them and their preclinical promise. Although several polymer conjugate systems have demonstrated enhanced pharmacokinetics and reduced toxicity, improvements in anticancer efficacy have been marginal[86,207]. The preclinical efficacy of anticancer polymer therapeutics has been largely attributed to EPR-mediated tumour accumulation, but the passive accumulation of macromolecular therapies in heterogeneous human tumours is now a topic of intense debate[243,244]. For example, only 8 of 37 patients treated with PK1 and imaged in phase I and phase II clinical evaluations exhibited verifiable tumour uptake via radionuclide imaging[87,88]. The gap between preclinical findings and patient data is likely a result of the failure of murine models to accurately recapitulate human cancer. For example, the rapid growth of murine tumours leads to irregular blood vessel formation and, consequently, leaky vasculature[245]. However, not all blood vessels in human tumours are leaky. Similarly, a recent study in canines with spontaneous solid tumours demonstrated highly variable liposomal accumulation[246]. Interestingly, a recent meta-analysis of preclinical EPR-mediated tumour accumulation of drug carriers showed that, on average, only 0.7% of the intravenously injected dose reaches the tumour[247], suggesting that, even in preclinical models exhibiting passive accumulation of drug carriers, current systems achieve inadequate delivery of chemotherapeutics to tumours. However, an analysis of the tumour localization of the analogous small-molecule agents was not performed, confounding a direct comparison between drug carriers and their small-molecule counterparts. The limited translation of molecularly targeted polymeric small-molecule drug conjugates can be similarly attributed to an insufficient delivery advantage to the pathological target relative to healthy cells[211].

One promising approach to facilitate the translation of polymeric conjugates of small-molecule anticancer agents is to identify patients in whom the therapeutic does accumulate passively in tumours[248] and/or to develop approaches to increase the passive tumour accumulation of drug carriers[249]. Notably, a recent clinical study demonstrated that high tumour localization of radiolabelled liposomal doxorubicin is correlated with improved response rates, progression-free survival and overall survival in patients receiving a combination of liposomal doxorubicin and cisplatin[250]. Similarly, other reports demonstrate positive correlations between the tumour accumulations of clinically approved ferumoxytol iron nanoparticles, as well as radiolabelled HER2-targeted liposomal doxorubicin, and improved treatment outcomes in patients receiving liposomal chemotherapy[251,252]. These findings have further motivated the development of a drug-free radiolabelled liposomal companion diagnostic[253]. It is nonetheless important to bear in mind that increased tumour drug concentrations do not invariably translate to improved efficacy. For example, PEGylated liposomal doxorubicin (that is, Doxil) exhibits equivalent efficacy to free doxorubicin in metastatic breast cancer and multiple myeloma, despite demonstrating higher accumulation of drug in patient tumours[86,254,255]. However, Doxil successfully mitigates the cardiotoxicity of doxorubicin and enables extended dosing intervals. Therefore, target tissue accumulation, efficacy and enhanced safety are among many parameters contributing to the clinical success of drug carriers[256].

Another issue that potentially contributed to the insufficient improvements in pharmacokinetics and therapeutic outcomes for the first clinically evaluated water-soluble conjugates of non-biodegradable HPMA copolymers, such as PK1, is that they had molecular masses below the renal cut-off of 50 kDa to ensure their elimination[207]. However, since these early clinical trials, advances in polymer chemistry have enabled the

synthesis of high-molecular-mass biodegradable and biocompatible polymeric carriers. Given the proven clinical safety of HPMA-based conjugates, biodegradable high-molecular-mass HPMA copolymers, synthesized via the incorporation of biocleavable units in the polymer backbone, are a potentially promising alternative[100]. The complex biological obstacles a drug carrier encounters upon infusion may additionally limit its ability to exhibit enhanced pharmacokinetics and biodistribution. Most notably, cells of the MPS rapidly sequester and clear foreign materials from the circulation, reducing their half-life and bioavailability[257]. In addition to traditional PEGylation, emerging approaches to minimize MPS clearance include the use of 'self' peptides and red blood cell membrane surface coatings[257]. Furthermore, minimal evidence validates the extended stability of drug carriers in vivo[156]. In particular, self-assembled structures, such as polymeric micelles, are prone to dissociation upon dilution in the bloodstream or in the presence of plasma and tissue components that can bind the individual amphiphilic polymers. Although the macromolecular therapeutic may nonetheless provide a formulation benefit, immediate disassembly and drug release minimize the utility of the polymer carrier. Therefore, careful design implementations, such as core-crosslinking, must be considered to ensure that carrier stability is maintained in vivo and that the spatiotemporal release of the active agent is not compromised. Similarly, thorough preclinical evaluation of pharmacokinetics and biodistribution must be conducted to ensure sufficient in vivo stability of the chosen linking chemistry.

Development considerations

For all types of polymer–drug conjugate, the current development approaches are largely empirical. However, the diverse and complex challenges presented in the clinical translation of polymer therapeutics necessitate a holistic design approach, rigorous optimization and systematic, high-throughput evaluation of libraries of conjugates[258]. In particular, the development of clinically viable drug carriers requires a bottom-up design approach in which the active molecule, indication and even patient subset are defined at the outset. Similarly, robust and quantifiable design criteria are critical, especially given that preclinical models are poorly predictive of clinical outcomes. For example, for a given therapeutic, indication and patient population, what quantifiable modifications in pharmacokinetics and/or biodistribution are necessary to improve patient outcomes? While such questions are challenging to address, computational and theoretical modelling approaches can be employed to correlate outcomes with concrete design parameters.

It is also important that systems maintain their theoretical physicochemical characteristics and behaviour in vivo. The complexity of macromolecular therapeutics presents additional challenges in development and manufacture, such as reproducibility, scalability and cost[243]. In particular, clinical-scale manufacture necessitates that critical quality attributes are defined and that set standards for these attributes are reproducibly met[259]. This becomes increasingly challenging as complexity and, accordingly, the number of critical quality attributes are increased. At a minimum, chemical and physical stability, molecular mass, polydispersity, drug loading and sites of drug conjugation must be considered in the development of a polymer–drug conjugate. Therefore, the utility of each structural and/or functional component of a polymer–drug conjugate must be carefully evaluated in order to optimize function and efficacy while minimizing complexity.

Outlook and conclusions

The field of polymer–drug conjugates has matured substantially in the past two decades. In fact, today, generic polymer–drug conjugates are also entering the market[259]. Great strides have been made in the development and expansion of a robust toolbox for the rational and modular design of effective polymer therapeutics, including an extensive array of synthetic methods, compositions and architectures. Given the tailored design and advantages afforded by polymer conjugation, we anticipate that this strategy will continue to facilitate the translation of innovative treatments, even in the face of a changing therapeutic landscape. Accordingly, polymeric carriers are being developed to deliver a wide range of therapeutic modalities, including small molecules, peptides, aptamers and proteins. Furthermore, given the growing promise of cancer immunotherapy, it is not surprising that polymeric immunotherapies, including pegilodecakin and NKTR-214 (TABLE 2), are already in clinical development. Similarly, the advantages afforded by more sophisticated approaches, including molecular targeting, combination therapy and the concurrent integration of therapeutics and diagnostics in a single carrier, herald the forthcoming clinical development of polymeric drug conjugates that integrate these functionalities. Rational design and continued multidisciplinary collaboration will expedite the realization of clinically transformative polymer–drug conjugate therapeutics in the forthcoming years.

Published online 12 December 2018

1. Ringsdorf, H. Structure and properties of pharmacologically active polymers. *J. Polym. Sci. C* **51**, 135–153 (1975).
2. Kopecek, J. & Kopeckova, P. HPMA copolymers: origins, early developments, present, and future. *Adv. Drug Deliv. Rev.* **62**, 122–149 (2010).
3. Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.* **2**, 347–360 (2003).
4. Nagle, T., Berg, C., Nassr, R. & Pang, K. The further evolution of biotech. *Nat. Rev. Drug Discov.* **2**, 75–79 (2003).
5. Caliceti, P. & Veronese, F. M. Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. *Adv. Drug Deliv. Rev.* **55**, 1261–1277 (2003).
6. Tong, R. & Cheng, J. Anticancer polymeric nanomedicines. *Polym. Rev.* **47**, 345–381 (2007).
7. Canalle, L. A., Lowik, D. W. & van Hest, J. C. Polypeptide-polymer bioconjugates. *Chem. Soc. Rev.* **39**, 329–353 (2010).
8. Mitragotri, S., Burke, P. A. & Langer, R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat. Rev. Drug Discov.* **13**, 655–672 (2014).
9. Abuchowski, A., van Es, T., Palczuk, N. C. & Davis, F. F. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. *J. Biol. Chem.* **252**, 3578–3581 (1977).
10. Abuchowski, A., McCoy, J. R., Palczuk, N. C., van Es, T. & Davis, F. F. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *J. Biol. Chem.* **252**, 3582–3586 (1977).
11. Alconcel, S. N. S., Baas, A. S. & Maynard, H. D. FDA-approved poly(ethylene glycol)-protein conjugate drugs. *Polym. Chem.* **2**, 1442–1448 (2011).
12. Hershfield, M. S. et al. Treatment of adenosine deaminase deficiency with polyethylene glycol-

modified adenosine deaminase. *N. Engl. J. Med.* **316**, 589–596 (1987).
13. Ulbrich, K. et al. Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies. *Chem. Rev.* **116**, 5338–5431 (2016).
14. Keefe, A. D., Pai, S. & Ellington, A. Aptamers as therapeutics. *Nat. Rev. Drug Discov.* **9**, 537–550 (2010).
15. Turecek, P. L., Bossard, M. J., Schoetens, F. & Ivens, I. A. PEGylation of biopharmaceuticals: a review of chemistry and nonclinical safety information of approved drugs. *J. Pharm. Sci.* **105**, 460–475 (2016).
16. Graham, M. L. PEGaspargase: a review of clinical studies. *Adv. Drug Deliv. Rev.* **55**, 1293–1302 (2003).
17. Peters, B. G., Goeckner, B. J., Ponzillo, J. J., Velasquez, W. S. & Wilson, A. L. PEGaspargase versus asparaginase in adult all: a pharmacoeconomic assessment. *Formulary* **30**, 388–393 (1995).
18. Macdougall, I. C. et al. Pharmacokinetics and pharmacodynamics of intravenous and subcutaneous continuous erythropoietin receptor activator (C.E.R.A.) in patients with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* **1**, 1211–1215 (2006).
19. Curran, M. P. & McCormack, P. L. Methoxy polyethylene glycol-epoetin beta: a review of its use in the management of anaemia associated with chronic kidney disease. *Drugs* **68**, 1139–1156 (2008).
20. Bezditko, N., Iakovlieva, L., Mishchenko, O., Gerasymova, O. & Kyrychenko, O. Pharmacoeconomic aspects of use of erythropoietin drugs in patients on hemodialysis in ukraine. *Value Health* **15**, A459 (2012).
21. Wang, Y. S. et al. Structural and biological characterization of PEGylated recombinant interferon alpha-2b and its therapeutic implications. *Adv. Drug Deliv. Rev.* **54**, 547–570 (2002).
22. Rajender Reddy, K., Modi, M. W. & Pedder, S. Use of PEGinterferon alfa-2a (40 KD) (Pegasys) for the treatment of hepatitis C. *Adv. Drug Deliv. Rev.* **54**, 571–586 (2002).
23. Yang, B. B. & Kido, A. Pharmacokinetics and pharmacodynamics of PEGfilgrastim. *Clin. Pharmacokinet.* **50**, 295–306 (2011).
24. Deeks, E. D. Certolizumab pegol: a review of its use in the management of rheumatoid arthritis. *Drugs* **73**, 75–97 (2013).
25. Kharitonenkov, A. & Adams, A. C. Inventing new medicines: the FGF21 story. *Mol. Metab.* **3**, 221–229 (2014).
26. So, W. Y. & Leung, P. S. Fibroblast growth factor 21 as an emerging therapeutic target for type 2 diabetes mellitus. *Med. Res. Rev.* **36**, 672–704 (2016).
27. Sanyal, A. et al. BMS-986036 (PEGylated FGF21) in patients with non-alcoholic steatohepatitis: a phase 2 study. *J. Hepatol.* **66**, S89–S90 (2017).
28. Charles, E. D. et al. A phase 1 study of BMS-986036 (PEGylated FGF21) in healthy obese subjects. *Hepatology* **64**, 546A (2016).
29. Charles, E. D., Tetri, B. A., Luo, Y., Wu, C. K. & Christian, R. A phase 2 study of BMS-986036 (PEGylated FGF21) in obese adults with type 2 diabetes and a high prevalence of fatty liver. *Hepatology* **64**, 17A (2016).
30. Wu, C. K., Charles, E. D., Bui, A., Christian, R. & Abu Tarif, M. Phase 1 study of BMS-986171 (PEGylated FGF21) in healthy obese subjects. *Hepatology* **64**, 564A–565A (2016).
31. Huhn, R. D. et al. Pharmacodynamics of subcutaneous recombinant human interleukin-10 in healthy volunteers. *Clin. Pharmacol. Ther.* **62**, 171–180 (1997).
32. Naing, A. et al. CD8$^+$ T cell stimulation with PEGylated recombinant human IL-10 in the patient with advanced solid tumors — a phase I study. *J. Immunother. Cancer* **3**, P204 (2015).
33. Mumm, J. B. et al. Il-10 elicits IFNgamma-dependent tumor immune surveillance. *Cancer Cell* **20**, 781–796 (2011).
34. Naing, A. et al. Safety, antitumor activity, and immune activation of PEGylated recombinant human interleukin-10 (AM0010) in patients with advanced solid tumors. *J. Clin. Oncol.* **34**, 3562–3569 (2016).
35. Charych, D. H. et al. NKTR-214, an engineered cytokine with biased IL2 receptor binding, increased tumor exposure, and marked efficacy in mouse tumor models. *Clin. Cancer Res.* **22**, 680–690 (2016).
36. Charych, D. et al. Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a kinetically-controlled interleukin-2 (IL2) receptor agonist for cancer immunotherapy. *PLOS ONE* **12**, e0179431 (2017).
37. Langowski, J. et al. NKTR-358: a selective, first-in-class IL-2 pathway agonist which increases number and suppressive function of regulatory T cells for the treatment of immune inflammatory disorders. *Arthritis Rheumatol.* **69**, 2 (2017).
38. Roberts, M. J., Bentley, M. D. & Harris, J. M. Chemistry for peptide and protein PEGylation. *Adv. Drug Deliv. Rev.* **54**, 459–476 (2002).
39. Pelegri-O'Day, E. M., Lin, E. W. & Maynard, H. D. Therapeutic protein-polymer conjugates: advancing beyond PEGylation. *J. Am. Chem. Soc.* **136**, 14323–14332 (2014).
40. Smith, M. E. et al. Protein modification, bioconjugation, and disulfide bridging using bromomaleimides. *J. Am. Chem. Soc.* **132**, 1960–1965 (2010).
41. Jones, M. W. et al. Polymeric dibromomaleimides as extremely efficient disulfide bridging bioconjugation and PEGylation agents. *J. Am. Chem. Soc.* **134**, 1847–1852 (2012).
42. Jones, M. W. et al. Direct peptide bioconjugation/ PEGylation at tyrosine with linear and branched polymeric diazonium salts. *J. Am. Chem. Soc.* **134**, 7406–7413 (2012).
43. Gauthier, M. A. & Klok, H. A. Arginine-specific modification of proteins with polyethylene glycol. *Biomacromolecules* **12**, 482–493 (2011).
44. Cong, Y. et al. Site-specific PEGylation at histidine tags. *Bioconjug. Chem.* **23**, 248–263 (2012).
45. Nesbitt, A. M., Stephens, S. & Chartash, E. K. in *Pegylated Protein Drugs: Basic Science and* Clinical *Applications* (ed. Veronese, F. M.) 229–254 (Birkhäuser, 2009).
46. Gilmore, J. M., Scheck, R. A., Esser-Kahn, A. P., Joshi, N. S. & Francis, M. B. N-terminal protein modification through a biomimetic transamination reaction. *Angew. Chem. Int. Ed.* **45**, 5307–5311 (2006).
47. Obermeyer, A. C., Jarman, J. B. & Francis, M. B. N-terminal modification of proteins with *o*-Aminophenols. *J. Am. Chem. Soc.* **136**, 9572–9579 (2014).
48. Zhang, X., Li, F., Lu, X. W. & Liu, C. F. Protein C-terminal modification through thioacid/azide amidation. *Bioconjug. Chem.* **20**, 197–200 (2009).
49. Thom, J., Anderson, D., McGregor, J. & Cotton, G. Recombinant protein hydrazides: application to site-specific protein PEGylation. *Bioconjug. Chem.* **22**, 1017–1020 (2011).
50. Sato, H. Enzymatic procedure for site-specific PEGylation of proteins. *Adv. Drug Deliv. Rev.* **54**, 487–504 (2002).
51. Fontana, A., Spolaore, B., Mero, A. & Veronese, F. M. Site-specific modification and PEGylation of pharmaceutical proteins mediated by transglutaminase. *Adv. Drug Deliv. Rev.* **60**, 13–28 (2008).
52. Popp, M. W., Dougan, S. K., Chuang, T. Y., Spooner, E. & Ploegh, H. L. Sortase-catalyzed transformations that improve the properties of cytokines. *Proc. Natl Acad. Sci. USA* **108**, 3169–3174 (2011).
Sortase-mediated, site-specific PEGylation of cytokines extends plasma half-life without compromising biological activity.
53. Appel, M. J. & Bertozzi, C. R. Formylglycine, a post-translationally generated residue with unique catalytic capabilities and biotechnology applications. *ACS Chem. Biol.* **10**, 72–84 (2015).
54. DeFrees, S. et al. GlycoPEGylation of recombinant therapeutic proteins produced in Escherichia coli. *Glycobiology* **16**, 833–843 (2006).
55. Stennicke, H. R. et al. A novel B-domain O-glycoPEGylated FVIII (N8-GP) demonstrates full efficacy and prolonged effect in hemophilic mice models. *Blood* **121**, 2108–2116 (2013).
56. Wang, L., Brock, A., Herberich, B. & Schultz, P. G. Expanding the genetic code of Escherichia coli. *Science* **292**, 498–500 (2001).
57. Cho, H. et al. Optimized clinical performance of growth hormone with an expanded genetic code. *Proc. Natl Acad. Sci. USA* **108**, 9060–9065 (2011).
This is the first clinical study of a PEG–protein conjugate prepared via the incorporation of an unnatural amino acid.
58. Mu, J. et al. FGF21 analogs of sustained action enabled by orthogonal biosynthesis demonstrate enhanced antidiabetic pharmacology in rodents. *Diabetes* **61**, 505–512 (2012).
59. Bontempo, D. & Maynard, H. D. Streptavidin as a macroinitiator for polymerization: in situ protein-polymer conjugate formation. *J. Am. Chem. Soc.* **127**, 6508–6509 (2005).
60. Liu, J. et al. In situ formation of protein-polymer conjugates through reversible addition fragmentation chain transfer polymerization. *Angew. Chem. Int. Ed.* **46**, 3099–3103 (2007).
61. Boyer, C. et al. Well-defined protein-polymer conjugates via in situ raft polymerization. *J. Am. Chem. Soc.* **129**, 7145–7154 (2007).
62. Peeler, J. C. et al. Genetically encoded initiator for polymer growth from proteins. *J. Am. Chem. Soc.* **132**, 13575–13577 (2010).
63. Gao, W., Liu, W., Christensen, T., Zalutsky, M. R. & Chilkoti, A. In situ growth of a PEG-like polymer from the C terminus of an intein fusion protein improves pharmacokinetics and tumor accumulation. *Proc. Natl Acad. Sci. USA* **107**, 16432–16437 (2010).
64. Zhou, Y. & Kopecek, J. Biological rationale for the design of polymeric anti-cancer nanomedicines. *J. Drug Target.* **21**, 1–26 (2013).
65. Lee, Y. et al. Poly(ethylene oxide sulfide): new poly(ethylene glycol) derivatives degradable in reductive conditions. *Biomacromolecules* **6**, 24–26 (2005).
66. Lundberg, P. et al. Poly[(ethylene oxide)-co-(methylene ethylene oxide)]: a hydrolytically-degradable poly(ethylene oxide) platform. *ACS Macro Lett.* **1**, 1240–1243 (2012).
67. Pasut, G. Polymers for protein conjugation. *Polymers* **6**, 160–178 (2014).
68. Podust, V. N. et al. Extension of in vivo half-life of biologically active molecules by XTEN protein polymers. *J. Control. Release* **240**, 52–66 (2016).
69. Gebauer, M. & Skerra, A. Prospects of pasylation® for the design of protein and peptide therapeutics with extended half-life and enhanced action. *Bioorg. Med. Chem.* **26**, 2882–2887 (2018).
70. Urakami, H. & Guan, Z. Living ring-opening polymerization of a carbohydrate-derived lactone for the synthesis of protein-resistant biomaterials. *Biomacromolecules* **9**, 592–597 (2008).
71. Steinbach, T. & Wurm, F. R. Degradable polyphosphoester-protein conjugates: "PPEylation" of proteins. *Biomacromolecules* **17**, 3338–3346 (2016).
72. Knop, K., Hoogenboom, R., Fischer, D. & Schubert, U. S. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew. Chem. Int. Ed.* **49**, 6288–6308 (2010).
73. Garay, R. P., El-Gewely, R., Armstrong, J. K., Garratty, G. & Richette, P. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. *Expert Opin. Drug Deliv.* **9**, 1319–1323 (2012).
74. Luxenhofer, R. et al. Poly(2-oxazoline)s as polymer therapeutics. *Macromol. Rapid Commun.* **33**, 1613–1631 (2012).
75. Gangloff, N., Ulbricht, J., Lorson, T., Schlaad, H. & Luxenhofer, R. Peptoids and polypeptoids at the frontier of supra- and macromolecular engineering. *Chem. Rev.* **116**, 1753–1802 (2016).
76. Hu, Y., Hou, Y., Wang, H. & Lu, H. Polysarcosine as an alternative to PEG for therapeutic protein conjugation. *Bioconjug. Chem.* **29**, 2232–2238 (2018).
77. Hu, J., Wang, G., Zhao, W. & Gao, W. In situ growth of a C-terminal interferon-alpha conjugate of a phospholipid polymer that outperforms pegasys in cancer therapy. *J. Control. Release* **237**, 71–77 (2016).
78. Keefe, A. J. & Jiang, S. Poly(zwitterionic)protein conjugates offer increased stability without sacrificing binding affinity or bioactivity. *Nat. Chem.* **4**, 59–63 (2012).
79. Nguyen, T. H. et al. A heparin-mimicking polymer conjugate stabilizes basic fibroblast growth factor. *Nat. Chem.* **5**, 221–227 (2013).
The rational design of a heparin-mimicking polymer affords stabilization of basic FGF.
80. De, P., Li, M., Gondi, S. R. & Sumerlin, B. S. Temperature-regulated activity of responsive polymer-protein conjugates prepared by grafting-from via raft polymerization. *J. Am. Chem. Soc.* **130**, 11288–11289 (2008).
81. Hardwicke, J. T. et al. The effect of dextrin-rhEGF on the healing of full-thickness, excisional wounds in the (db/db) diabetic mouse. *J. Control. Release* **152**, 411–417 (2011).
82. Vanparijs, N. et al. Transiently responsive protein-polymer conjugates via a 'grafting-from' RAFT approach for intracellular co-delivery of proteins and immune-modulators. *Chem. Commun.* **51**, 13972–13975 (2015).

83. Le Droumaguet, B. & Velonia, K. In situ ATRP-mediated hierarchical formation of giant amphiphile bionanoreactors. *Angew. Chem. Int. Ed.* **47**, 6263–6266 (2008).
84. Jatzkewitz, H. Peptamin (glycyl-l-leucyl-mescaline) bound to blood plasma expander (polyvinylpyrrolidone) as a new depot form of a biologically active primary amine (mescaline). *Z. Naturforsch.* **10B**, 27–31 (1955).
85. Matsumura, Y. & Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Res.* **46**, 6387–6392 (1986).
86. Stirland, D. L., Nichols, J. W., Miura, S. & Bae, Y. H. Mind the gap: a survey of how cancer drug carriers are susceptible to the gap between research and practice. *J. Control. Release* **172**, 1045–1064 (2013).
87. Vasey, P. A. et al. Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates. Cancer Research Campaign Phase I/II Committee. *Clin. Cancer Res.* **5**, 83–94 (1999).
88. Seymour, L. W. et al. Phase II studies of polymer-doxorubicin (PK1, FCE28068) in the treatment of breast, lung and colorectal cancer. *Int. J. Oncol.* **34**, 1629–1636 (2009).
89. Duncan, R. & Vicent, M. J. Do HPMA copolymer conjugates have a future as clinically useful nanomedicines? A critical overview of current status and future opportunities. *Adv. Drug Deliv. Rev.* **62**, 272–282 (2010).
90. Hoch, U., Staschen, C. M., Johnson, R. K. & Eldon, M. A. Nonclinical pharmacokinetics and activity of etirinotecan pegol (NKTR-102), a long-acting topoisomerase 1 inhibitor, in multiple cancer models. *Cancer Chemother. Pharmacol.* **74**, 1125–1137 (2014).
91. Jameson, G. S. et al. A multicenter, phase I, dose-escalation study to assess the safety, tolerability, and pharmacokinetics of etirinotecan pegol in patients with refractory solid tumors. *Clin. Cancer Res.* **19**, 268–278 (2013).
92. Perez, E. A. et al. Etirinotecan pegol (NKTR-102) versus treatment of physician's choice in women with advanced breast cancer previously treated with an anthracycline, a taxane, and capecitabine (beacon): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol.* **16**, 1556–1568 (2015).
93. Cortes, J. et al. Prolonged survival in patients with breast cancer and a history of brain metastases: results of a preplanned subgroup analysis from the randomized phase III beacon trial. *Breast Cancer Res. Treat.* **165**, 329–341 (2017).
94. Garnock-Jones, K. P. Naloxegol: a review of its use in patients with opioid-induced constipation. *Drugs* **75**, 419–425 (2015).
95. Miyazaki, T. et al. NKTR-181: a novel mu-opioid analgesic with inherently low abuse potential. *J. Pharmacol. Exp. Ther.* **363**, 104–113 (2017).
96. Webster, L. et al. Human abuse potential of the new opioid analgesic molecule NKTR-181 compared with oxycodone. *Pain Med.* **19**, 307–318 (2018).
97. El Mehdil, D. et al. APL-2, a complement C3 inhibitor, may potentially reduce both intravascular and extravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria. *Mol. Immunol.* **89**, 115 (2017).
98. Roblin, D. et al. Topical TrkA kinase inhibitor CT327 is an effective, novel therapy for the treatment of pruritus due to psoriasis: results from experimental studies, and efficacy and safety of CT327 in a phase 2b clinical trial in patients with psoriasis. *Acta Derm. Venereol.* **95**, 542–548 (2015).
99. Duro-Castano, A., Conejos-Sanchez, I. & Vicent, M. J. Peptide-based polymer therapeutics. *Polymers* **6**, 515–551 (2014).
100. Yang, J. et al. Backbone degradable N-(2-hydroxypropyl)methacrylamide copolymer conjugates with gemcitabine and paclitaxel: impact of molecular weight on activity toward human ovarian carcinoma xenografts. *Mol. Pharm.* **14**, 1384–1394 (2017).
101. Pan, H. et al. Efficiency of high molecular weight backbone degradable HPMA copolymer-prostaglandin E1 conjugate in promotion of bone formation in ovariectomized rats. *Biomaterials* **34**, 6528–6538 (2013).
102. Zhang, R., Yang, J., Sima, M., Zhou, Y. & Kopecek, J. Sequential combination therapy of ovarian cancer with degradable N-(2-hydroxypropyl)methacrylamide copolymer paclitaxel and gemcitabine conjugates. *Proc. Natl Acad. Sci. USA* **111**, 12181–12186 (2014).
103. Tsai, F. T., Wang, Y. & Darensbourg, D. J. Environmentally benign CO_2-based copolymers: degradable polycarbonates derived from dihydroxybutyric acid and their platinum-polymer conjugates. *J. Am. Chem. Soc.* **138**, 4626–4633 (2016).
This report describes the synthesis of a novel, water-soluble and biodegradable polymer drug carrier.
104. Cho, S. et al. Functionalizable hydrophilic polycarbonate, poly(5-methyl-5-(2-hydroxypropyl) aminocarbonyl-1,3-dioxan-2-one), designed as a degradable alternative for PHPMA and PEG. *Macromolecules* **48**, 8797–8805 (2015).
105. Dubikovskaya, E. A., Thorne, S. H., Pillow, T. H., Contag, C. H. & Wender, P. A. Overcoming multidrug resistance of small-molecule therapeutics through conjugation with releasable octaarginine transporters. *Proc. Natl Acad. Sci. USA* **105**, 12128–12133 (2008).
106. Wang, Y. et al. Peptide-drug conjugates as effective prodrug strategies for targeted delivery. *Adv. Drug Deliv. Rev.* **110–111**, 112–126 (2017).
107. Zhang, P., Cheetham, A. G., Lock, L. L. & Cui, H. Cellular uptake and cytotoxicity of drug-peptide conjugates regulated by conjugation site. *Bioconjug. Chem.* **24**, 604–613 (2013).
108. Birke, A., Ling, J. & Barz, M. Polysarcosine-containing copolymers: synthesis, characterization, self-assembly, and applications. *Prog. Polym. Sci.* **81**, 163–208 (2018).
109. Nasongkla, N. et al. Dependence of pharmacokinetics and biodistribution on polymer architecture: effect of cyclic versus linear polymers. *J. Am. Chem. Soc.* **131**, 3842–3843 (2009).
110. Etrych, T. et al. HPMA copolymer-doxorubicin conjugates: the effects of molecular weight and architecture on biodistribution and in vivo activity. *J. Control. Release* **164**, 346–354 (2012).
In this study, the polymer–drug conjugate in vivo residence time is extended through polymer branching or increased molecular mass.
111. Quan, L. et al. Nanomedicines for inflammatory arthritis: head-to-head comparison of glucocorticoid-containing polymers, micelles, and liposomes. *ACS Nano* **8**, 458–466 (2014).
112. Zhou, Y., Yang, J. & Kopecek, J. Selective inhibitory effect of HPMA copolymer-cyclopamine conjugate on prostate cancer stem cells. *Biomaterials* **33**, 1863–1872 (2012).
113. England, R. M., Masia, E., Gimenez, V., Lucas, R. & Vicent, M. J. Polyacetal-stilbene conjugates — the first examples of polymer therapeutics for the inhibition of HIF-1 in the treatment of solid tumours. *J. Control. Release* **164**, 314–322 (2012).
114. Chu, T. W., Yang, J. & Kopecek, J. Anti-CD20 multivalent HPMA copolymer-Fab' conjugates for the direct induction of apoptosis. *Biomaterials* **33**, 7174–7181 (2012).
A drug-free macromolecular therapeutic comprising anti-CD20 Fab' conjugated to a polymer carrier exhibits multivalent binding and enhanced apoptosis induction in malignant B cells.
115. Chu, T. W., Yang, J., Zhang, R., Sima, M. & Kopecek, J. Cell surface self-assembly of hybrid nanoconjugates via oligonucleotide hybridization induces apoptosis. *ACS Nano* **8**, 719–730 (2014).
116. Wu, K., Liu, J., Johnson, R. N., Yang, J. & Kopecek, J. Drug-free macromolecular therapeutics: induction of apoptosis by coiled-coil-mediated cross-linking of antigens on the cell surface. *Angew. Chem. Int. Ed.* **49**, 1451–1455 (2010).
117. Tomalia, D. A. et al. A new class of polymers: starburst-dendritic macromolecules. *Polym. J.* **17**, 117–132 (1985).
118. Newkome, G. R., Yao, Z., Baker, G. R. & Gupta, V. K. Micelles. Part 1. Cascade molecules: a new approach to micelles. A [27]-arborol. *J. Org. Chem.* **50**, 2003–2004 (1985).
119. Sadekar, S. et al. Comparative pharmacokinetics of PAMAM-OH dendrimers and HPMA copolymers in ovarian tumor-bearing mice. *Drug Deliv. Transl Res.* **3**, 260–271 (2013).
120. Nanaware-Kharade, N. et al. Therapeutic anti-methamphetamine antibody fragment-nanoparticle conjugates: synthesis and in vitro characterization. *Bioconjug. Chem.* **23**, 1864–1872 (2012).
121. Wang, X., Inapagolla, R., Kannan, S., Lieh-Lai, M. & Kannan, R. M. Synthesis, characterization, and in vitro activity of dendrimer-streptokinase conjugates. *Bioconjug. Chem.* **18**, 791–799 (2007).
122. McCarthy, T. D. et al. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol. Pharm.* **2**, 312–318 (2005).
123. Starpharma Holdings Limited. DEP® docetaxel positive phase 1 results; phase 2 commences. *Starpharma* https://www.starpharma.com/news/339 (2017).
124. McNerny, D. Q., Leroueil, P. R. & Baker, J. R. Understanding specific and nonspecific toxicities: a requirement for the development of dendrimer-based pharmaceuticals. *WIREs Nanomed. Nanobiotechnol.* **2**, 249–259 (2010).
125. Mishra, M. K. et al. Dendrimer brain uptake and targeted therapy for brain injury in a large animal model of hypothermic circulatory arrest. *ACS Nano* **8**, 2134–2147 (2014).
Systemically administered dendrimer–drug conjugates traverse the impaired blood–brain barrier and localize in activated microglia and injured neurons, affording improved therapeutic efficacy and enhanced safety in a large animal model of brain injury.
126. Etrych, T. et al. Biodegradable star HPMA polymer-drug conjugates: biodegradability, distribution and anti-tumor efficacy. *J. Control. Release* **154**, 241–248 (2011).
127. Restani, R. B. et al. Biocompatible polyurea dendrimers with pH-dependent fluorescence. *Angew. Chem. Int. Ed.* **51**, 5162–5165 (2012).
128. Kaminskas, L. M. et al. Pulmonary administration of a doxorubicin-conjugated dendrimer enhances drug exposure to lung metastases and improves cancer therapy. *J. Control. Release* **183**, 18–26 (2014).
129. Pu, Y. et al. The anti-tumor efficiency of poly(l-glutamic acid) dendrimers with polyhedral oligomeric silsesquioxane cores. *Biomaterials* **34**, 3658–3666 (2013).
130. Carnahan, M. A. & Grinstaff, M. W. Synthesis and characterization of polyether-ester dendrimers from glycerol and lactic acid. *J. Am. Chem. Soc.* **123**, 2905–2906 (2001).
131. Carnahan, M. A. & Grinstaff, M. W. Synthesis and characterization of poly(glycerol–succinic acid) dendrimers. *Macromolecules* **34**, 7648–7655 (2001).
132. Mintzer, M. A. & Grinstaff, M. W. Biomedical applications of dendrimers: a tutorial. *Chem. Soc. Rev.* **40**, 173–190 (2011).
133. Morgan, M. T. et al. Dendritic molecular capsules for hydrophobic compounds. *J. Am. Chem. Soc.* **125**, 15485–15489 (2003).
134. Iezzi, R. et al. Dendrimer-based targeted intravitreal therapy for sustained attenuation of neuroinflammation in retinal degeneration. *Biomaterials* **33**, 979–988 (2012).
135. Zhou, Z. et al. Molecularly precise dendrimer-drug conjugates with tunable drug release for cancer therapy. *Angew. Chem. Int. Ed.* **53**, 10949–10955 (2014).
The release kinetics of a therapeutic buried within a dendrimer carrier are tuned by modulating the number of generations or altering the surface chemistry.
136. Kim, S. H. et al. Ligand accessibility and bioactivity of a hormone-dendrimer conjugate depend on pH and pH History. *J. Am. Chem. Soc.* **137**, 10326–10335 (2015).
137. Pearson, R. M. et al. Tuning the selectivity of dendron micelles through variations of the poly(ethylene glycol) corona. *ACS Nano* **10**, 6905–6914 (2016).
The cellular interaction of targeted dendron micelles is tuned via modulation of PEG corona length and targeting ligand content.
138. Choi, S. K. et al. Dendrimer-based multivalent vancomycin nanoplatform for targeting the drug-resistant bacterial surface. *ACS Nano* **7**, 214–228 (2013).
A multivalent dendrimer–vancomycin conjugate affords enhanced avidity, restoring the ability of vancomycin to bind vancomycin-resistant bacterial cell walls.
139. Myung, J. H., Gajjar, K. A., Saric, J., Eddington, D. T. & Hong, S. Dendrimer-mediated multivalent binding for the enhanced capture of tumor cells. *Angew. Chem. Int. Ed.* **50**, 11769–11772 (2011).
140. Skwarczynski, M. et al. Polyacrylate dendrimer nanoparticles: a self-adjuvanting vaccine delivery system. *Angew. Chem. Int. Ed.* **49**, 5742–5745 (2010).

141. Sweet, D. M., Kolhatkar, R. B., Ray, A., Swaan, P. & Ghandehari, H. Transepithelial transport of PEGylated anionic poly(amidoamine) dendrimers: implications for oral drug delivery. *J. Control. Release* **138**, 78–85 (2009).
142. Kannan, S. et al. Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. *Sci. Transl Med.* **4**, 130ra146 (2012).
143. Goldberg, D. S., Vijayalakshmi, N., Swaan, P. W. & Ghandehari, H. G3.5 PAMAM dendrimers enhance transepithelial transport of SN38 while minimizing gastrointestinal toxicity. *J. Control. Release* **150**, 318–325 (2011).
144. Wu, W., Driessen, W. & Jiang, X. Oligo(ethylene glycol)-based thermosensitive dendrimers and their tumor accumulation and penetration. *J. Am. Chem. Soc.* **136**, 3145–3155 (2014).
145. Li, H. J. et al. Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. *Proc. Natl Acad. Sci. USA* **113**, 4164–4169 (2016).
146. Zhang, S. et al. Mimicking biological membranes with programmable glycan ligands self-assembled from amphiphilic janus glycodendrimers. *Angew. Chem. Int. Ed.* **53**, 10899–10903 (2014).
147. Kopf, H., Joshi, R. K., Soliva, M. & Speiser, P. Study on micelle polymerization in the presence of lowmolecular-weight drugs. 1. Production and isolation of nanoparticles, residual monomer determination, physical–chemical data. *Pharm. Ind.* **38**, 281–284 (1976).
148. Petros, R. A. & DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug Discov.* **9**, 615–627 (2010).
149. Young, C., Schluep, T., Hwang, J. & Eliasof, S. CRLX101 (formerly IT-101) — a novel nanopharmaceutical of camptothecin in clinical development. *Curr. Bioact. Compd.* **7**, 8–14 (2011).
150. Markman, B. et al. A phase 1 study of CRLX301, a novel nanoparticle-drug conjugate (NDC) containing docetaxel (DOC), in patients with refractory solid tumors. *J. Clin. Oncol.* **34**, 2 (2016).
151. Griset, A. P. et al. Expansile nanoparticles: synthesis, characterization, and in vivo efficacy of an acid-responsive polymeric drug delivery system. *J. Am. Chem. Soc.* **131**, 2469–2471 (2009).
152. Musumeci, T. et al. PLA/PLGA nanoparticles for sustained release of docetaxel. *Int. J. Pharm.* **325**, 172–179 (2006).
153. Mu, L. & Feng, S. S. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. *J. Control. Release* **86**, 33–48 (2003).
154. Cabral, H. & Kataoka, K. Progress of drug-loaded polymeric micelles into clinical studies. *J. Control. Release* **190**, 465–476 (2014).
155. Matsumura, Y. et al. Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *Br. J. Cancer* **91**, 1775–1781 (2004).
156. Svenson, S. What nanomedicine in the clinic right now really forms nanoparticles? *WIREs Nanomed. Nanobiotechnol.* **6**, 125–135 (2014).
157. Cristal Therapeutics. CriPec platform. *Cristal Therapeutics* https://cristaltherapeutics.com/technology/cripec-platform (2015).
158. Hu, Q. et al. Complete regression of breast tumour with a single dose of docetaxel-entrapped core-cross-linked polymeric micelles. *Biomaterials* **53**, 370–378 (2015).
159. Harrisson, S. et al. Nanoparticles with in vivo anticancer activity from polymer prodrug amphiphiles prepared by living radical polymerization. *Angew. Chem. Int. Ed.* **52**, 1678–1682 (2013).
160. Liu, J. et al. Ring-opening polymerization of prodrugs: a versatile approach to prepare well-defined drug-loaded nanoparticles. *Angew. Chem. Int. Ed.* **54**, 1002–1006 (2015).
161. Louage, B. et al. Well-defined polymer-paclitaxel prodrugs by a grafting-from-drug approach. *Angew. Chem. Int. Ed.* **55**, 11791–11796 (2016).
Drug-initiated in situ polymerization affords synthetically precise amphiphilic polymer–drug conjugates.
162. Zhang, H. & Grinstaff, M. W. Synthesis of atactic and isotactic poly(1,2-glycerol carbonate)s: degradable polymers for biomedical and pharmaceutical applications. *J. Am. Chem. Soc.* **135**, 6806–6809 (2013).
163. Ekladious, I. et al. Synthesis of poly(1,2-glycerol carbonate)-paclitaxel conjugates and their utility as a single high-dose replacement for multi-dose treatment regimens in peritoneal cancer. *Chem. Sci* **8**, 8443–8450 (2017).
A high-drug-density, biodegradable polymer–drug conjugate nanocarrier exhibits sustained drug release, eliminating the need for frequent, repeated administrations of the small-molecule chemotherapeutic.
164. Geschwind, J. & Frey, H. Poly(1,2-glycerol carbonate): a fundamental polymer structure synthesized from CO_2 and glycidyl ethers. *Macromolecules* **46**, 3280–3287 (2013).
165. MacKay, J. A. et al. Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection. *Nat. Mater.* **8**, 993–999 (2009).
166. Kim, W. et al. Targeted antithrombotic protein micelles. *Angew. Chem. Int. Ed.* **54**, 1461–1465 (2015).
167. Bhattacharyya, J. et al. A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms abraxane in multiple murine cancer models. *Nat. Commun.* **6**, 7939 (2015).
168. Zhang, S. et al. Poly(ethylene oxide)-block-polyphosphester-based paclitaxel conjugates as a platform for ultra-high paclitaxel-loaded multifunctional nanoparticles. *Chem. Sci.* **4**, 2122–2126 (2013).
169. Ekladious, I. et al. Reinforcement of polymeric nanoassemblies for ultra-high drug loadings, modulation of stiffness and release kinetics, and sustained therapeutic efficacy. *Nanoscale* **10**, 8360–8366 (2018).
170. Wilson, J. T. et al. pH-responsive nanoparticle vaccines for dual-delivery of antigens and immunostimulatory oligonucleotides. *ACS Nano* **7**, 3912–3925 (2013).
171. McDaniel, J. R. et al. Rational design of "heat seeking" drug loaded polypeptide nanoparticles that thermally target solid tumors. *Nano Lett.* **14**, 2890–2895 (2014).
172. Zhou, K. et al. Tunable, ultrasensitive pH-responsive nanoparticles targeting specific endocytic organelles in living cells. *Angew. Chem. Int. Ed.* **50**, 6109–6114 (2011).
173. Zhang, Y. et al. Chain-shattering polymeric therapeutics with on-demand drug-release capability. *Angew. Chem. Int. Ed.* **52**, 6435–6439 (2013).
174. Duan, X. et al. Smart pH-sensitive and temporal-controlled polymeric micelles for effective combination therapy of doxorubicin and disulfiram. *ACS Nano* **7**, 5858–5869 (2013).
175. Tong, R. & Cheng, J. Paclitaxel-initiated, controlled polymerization of lactide for the formulation of polymeric nanoparticulate delivery vehicles. *Angew. Chem. Int. Ed.* **47**, 4830–4834 (2008).
176. Callari, M., De Souza, P. L., Rawal, A. & Stenzel, M. H. The effect of drug loading on micelle properties: solid-state NMR as a tool to gain structural insight. *Angew. Chem. Int. Ed.* **56**, 8441–8445 (2017).
177. Namgung, R. et al. Poly-cyclodextrin and poly-paclitaxel nano-assembly for anticancer therapy. *Nat. Commun.* **5**, 3702 (2014).
178. Mochida, Y. et al. Bundled assembly of helical nanostructures in polymeric micelles loaded with platinum drugs enhancing therapeutic efficiency against pancreatic tumor. *ACS Nano* **8**, 6724–6738 (2014).
179. Benny, O. et al. An orally delivered small-molecule formulation with antiangiogenic and anticancer activity. *Nat. Biotechnol.* **26**, 799–807 (2008).
A polymer–drug conjugate nanoparticle affords oral bioavailability of the conjugated therapeutic, mitigating the neurotoxicity associated with systemic administration of the active agent.
180. Lee, U. Y. et al. Facile synthesis of multimeric micelles. *Angew. Chem. Int. Ed.* **51**, 7287–7291 (2012).
181. Shen, Y. et al. Prodrugs forming high drug loading multifunctional nanocapsules for intracellular cancer drug delivery. *J. Am. Chem. Soc.* **132**, 4259–4265 (2010).
The self-assembly of polymer–drug conjugates into liposome-like nanocapsules enables the simultaneous encapsulation of a hydrophilic agent in the aqueous core.
182. Boott, C. E., Gwyther, J., Harniman, R. L., Hayward, D. W. & Manners, I. Scalable and uniform 1D nanoparticles by synchronous polymerization, crystallization and self-assembly. *Nat. Chem.* **9**, 785–792 (2017).
183. Yang, M. et al. Biodegradable nanoparticles composed entirely of safe materials that rapidly penetrate human mucus. *Angew. Chem. Int. Ed.* **50**, 2597–2600 (2011).
184. Ahn, S. et al. Oral delivery of an anti-diabetic peptide drug via conjugation and complexation with low molecular weight chitosan. *J. Control. Release* **170**, 226–232 (2013).
185. Colson, Y. L. & Grinstaff, M. W. Biologically responsive polymeric nanoparticles for drug delivery. *Adv. Mater.* **24**, 3878–3886 (2012).
186. Sprogoe, K., Mortensen, E., Karpf, D. B. & Leff, J. A. The rationale and design of transcon growth hormone for the treatment of growth hormone deficiency. *Endocr. Connect.* **6**, R171–R181 (2017).
187. Gilfoyle, D., Mortensen, E., Christoffersen, E. D., Leff, J. A. & Beckert, M. A first-in-man phase 1 trial for long-acting TransCon Growth Hormone. *Growth Horm. IGF Res.* **39**, 34–39 (2018).
188. Chan, J. M. et al. Spatiotemporal controlled delivery of nanoparticles to injured vasculature. *Proc. Natl Acad. Sci. USA* **107**, 2213–2218 (2010).
189. Crielaard, B. J. et al. Glucocorticoid-loaded core-cross-linked polymeric micelles with tailorable release kinetics for targeted therapy of rheumatoid arthritis. *Angew. Chem. Int. Ed.* **51**, 7254–7258 (2012).
190. Peng, Z. H. & Kopecek, J. Enhancing accumulation and penetration of HPMA copolymer-doxorubicin conjugates in 2D and 3D prostate cancer cells via iRGD conjugation with an MMP-2 cleavable spacer. *J. Am. Chem. Soc.* **137**, 6726–6729 (2015).
191. Miller, K., Erez, R., Segal, E., Shabat, D. & Satchi-Fainaro, R. Targeting bone metastases with a bispecific anticancer and antiangiogenic polymer-alendronate-taxane conjugate. *Angew. Chem. Int. Ed.* **48**, 2949–2954 (2009).
192. Wang, T. et al. Intracellularly acid-switchable multifunctional micelles for combinational photo/chemotherapy of the drug-resistant tumor. *ACS Nano* **10**, 3496–3508 (2016).
193. Zhu, L., Wang, T., Perche, F., Taigind, A. & Torchilin, V. P. Enhanced anticancer activity of nanopreparation containing an MMP2-sensitive PEG-drug conjugate and cell-penetrating moiety. *Proc. Natl Acad. Sci. USA* **110**, 17047–17052 (2013).
194. Pang, X. et al. pH-responsive polymer-drug conjugates: design and progress. *J. Control. Release* **222**, 116–129 (2016).
195. Quan, L. D. et al. Development of a macromolecular prodrug for the treatment of inflammatory arthritis: mechanisms involved in arthrotropism and sustained therapeutic efficacy. *Arthritis Res. Ther.* **12**, R170 (2010).
196. Zhu, S. et al. Partly PEGylated polyamidoamine dendrimer for tumor-selective targeting of doxorubicin: the effects of PEGylation degree and drug conjugation style. *Biomaterials* **31**, 1360–1371 (2010).
197. Du, J. Z., Du, X. J., Mao, C. Q. & Wang, J. Tailor-made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery. *J. Am. Chem. Soc.* **133**, 17560–17563 (2011).
The triggered switch of nanocarrier surface charge from positive to negative promotes cancer cell internalization.
198. Takahashi, A. et al. NC-6300, an epirubicin-incorporating micelle, extends the antitumor effect and reduces the cardiotoxicity of epirubicin. *Cancer Sci.* **104**, 920–925 (2013).
199. Mukai, H. et al. A first-in-human phase 1 study of epirubicin-conjugated polymer micelles (K-912/NC-6300) in patients with advanced or recurrent solid tumors. *Invest. New Drugs* **35**, 307–314 (2017).
200. Hu, X., Liu, G., Li, Y., Wang, X. & Liu, S. Cell-penetrating hyperbranched polyprodrug amphiphiles for synergistic reductive milieu-triggered drug release and enhanced magnetic resonance signals. *J. Am. Chem. Soc.* **137**, 362–368 (2015).
Upon intracellular release of disulfide-linked camptothecin, a hydrophobic-to-hydrophilic transition of the nanocarrier polymer matrix enables the exchange of water for magnetic resonance imaging.
201. Luo, C. et al. Self-assembled redox dual-responsive prodrug-nanosystem formed by single thioether-bridged paclitaxel-fatty acid conjugate for cancer chemotherapy. *Nano Lett.* **16**, 5401–5408 (2016).
202. Davis, M. E., Chen, Z. G. & Shin, D. M. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov.* **7**, 771–782 (2008).
203. Arroyo-Crespo, J. J. et al. Anticancer activity driven by drug linker modification in a polyglutamic acid-based combination-drug conjugate. *Adv. Funct. Mater.* **28**, 13 (2018).
204. Tong, R., Hemmati, H. D., Langer, R. & Kohane, D. S. Photoswitchable nanoparticles for triggered tissue

penetration and drug delivery. *J. Am. Chem. Soc.* **134**, 8848–8855 (2012).
205. Li, H. J. et al. Smart superstructures with ultrahigh pH-sensitivity for targeting acidic tumor microenvironment: instantaneous size switching and improved tumor penetration. *ACS Nano* **10**, 6753–6761 (2016).
Upon exposure to the mildly acidic tumour microenvironment, trigger-responsive nanoassemblies dissociate into smaller dendrimer components, enabling the optimization of both plasma half-life and tumour penetration.
206. Beck, A., Goetsch, L., Dumontet, C. & Corvaia, N. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat. Rev. Drug Discov.* **16**, 315–337 (2017).
207. Yang, J. & Kopecek, J. The light at the end of the tunnel-second generation HPMA conjugates for cancer treatment. *Curr. Opin. Colloid Interface Sci* **31**, 30–42 (2017).
208. Seymour, L. W. et al. Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. *J. Clin. Oncol.* **20**, 1668–1676 (2002).
209. Duncan, R. Development of HPMA copolymer-anticancer conjugates: clinical experience and lessons learnt. *Adv. Drug Deliv. Rev.* **61**, 1131–1148 (2009).
210. Williams, R. Discontinued drugs in 2008: oncology drugs. *Expert Opin. Investig. Drugs* **18**, 1581–1594 (2009).
211. Xu, H. et al. Targeted polymer-drug conjugates: current progress and future perspective. *Colloids Surf. B Biointerfaces* **136**, 729–734 (2015).
212. Yao, V. J. et al. Ligand-targeted theranostic nanomedicines against cancer. *J. Control. Release* **240**, 267–286 (2016).
213. Srinivasarao, M. & Low, P. S. Ligand-targeted drug delivery. *Chem. Rev.* **117**, 12133–12164 (2017).
214. Miura, Y. et al. Cyclic RGD-linked polymeric micelles for targeted delivery of platinum anticancer drugs to glioblastoma through the blood-brain tumor barrier. *ACS Nano* **7**, 8583–8592 (2013).
215. Oh, S. S. et al. Synthetic aptamer-polymer hybrid constructs for programmed drug delivery into specific target cells. *J. Am. Chem. Soc.* **136**, 15010–15015 (2014).
216. Park, J. et al. Polydopamine-based simple and versatile surface modification of polymeric nano drug carriers. *ACS Nano* **8**, 3347–3356 (2014).
217. Lee, H., Dellatore, S. M., Miller, W. M. & Messersmith, P. B. Mussel-inspired surface chemistry for multifunctional coatings. *Science* **318**, 426–430 (2007).
218. Wei, X. et al. A D-peptide ligand of nicotine acetylcholine receptors for brain-targeted drug delivery. *Angew. Chem. Int. Ed.* **54**, 3023–3027 (2015).
219. Zhang, C. et al. Dual-functional nanoparticles targeting amyloid plaques in the brains of Alzheimer's disease mice. *Biomaterials* **35**, 456–465 (2014).
220. Morris, C. J., Smith, M. W., Griffiths, P. C., McKeown, N. B. & Gumbleton, M. Enhanced pulmonary absorption of a macromolecule through coupling to a sequence-specific phage display-derived peptide. *J. Control. Release* **151**, 83–94 (2011).
221. Ma, K. et al. Pamam-triamcinolone acetonide conjugate as a nucleus-targeting gene carrier for enhanced transfer activity. *Biomaterials* **30**, 6109–6118 (2009).
222. Cuchelkar, V., Kopeckova, P. & Kopecek, J. Novel HPMA copolymer-bound constructs for combined tumor and mitochondrial targeting. *Mol. Pharm.* **5**, 776–786 (2008).
223. Greco, F. & Vicent, M. J. Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. *Adv. Drug Deliv. Rev.* **61**, 1203–1213 (2009).
224. Conway, D. & Cohen, J. A. Combination therapy in multiple sclerosis. *Lancet Neurol.* **9**, 299–308 (2010).
225. Cahn, A. & Cefalu, W. T. Clinical considerations for use of initial combination therapy in type 2 diabetes. *Diabetes Care* **39**, (Suppl. 2), S137–S145 (2016).
226. Tallarida, R. J. Drug synergism: its detection and applications. *J. Pharmacol. Exp. Ther.* **298**, 865–872 (2001).
227. Xiao, H. et al. Co-delivery of daunomycin and oxaliplatin by biodegradable polymers for safer and more efficacious combination therapy. *J. Control. Release* **163**, 304–314 (2012).
228. Aryal, S., Hu, C. M. & Zhang, L. Polymeric nanoparticles with precise ratiometric control over drug loading for combination therapy. *Mol. Pharm.* **8**, 1401–1407 (2011).
229. Pathak, R. K. & Dhar, S. A nanoparticle cocktail: temporal release of predefined drug combinations. *J. Am. Chem. Soc.* **137**, 8324–8327 (2015).
A polymer–drug conjugate nanocarrier is developed for the concurrent delivery of a cocktail of therapeutic agents at predefined ratios and with differential release kinetics.
230. Luo, S. et al. Precise ratiometric control of dual drugs through a single macromolecule for combination therapy. *Mol. Pharm.* **12**, 2318–2327 (2015).
231. Yuan, Y., Liu, J. & Liu, B. Conjugated-polyelectrolyte-based polyprodrug: targeted and image-guided photodynamic and chemotherapy with on-demand drug release upon irradiation with a single light source. *Angew. Chem. Int. Ed.* **53**, 7163–7168 (2014).
This report describes the rational design of a sophisticated multifunctional polymer therapeutic that enables targeted and image-guided chemotherapy, as well as photodynamic therapy.
232. Li, Y. et al. A smart and versatile theranostic nanomedicine platform based on nanoporphyrin. *Nat. Commun.* **5**, 4712 (2014).
233. Janib, S. M., Moses, A. S. & MacKay, J. A. Imaging and drug delivery using theranostic nanoparticles. *Adv. Drug Deliv. Rev.* **62**, 1052–1063 (2010).
234. Huang, C. H., Nwe, K., Al Zaki, A., Brechbiel, M. W. & Tsourkas, A. Biodegradable polydisulfide dendrimer nanoclusters as mri contrast agents. *ACS Nano* **6**, 9416–9424 (2012).
235. Wang, Y. et al. A nanoparticle-based strategy for the imaging of a broad range of tumours by nonlinear amplification of microenvironment signals. *Nat. Mater.* **13**, 204–212 (2014).
236. Cheng, Z., Thorek, D. L. & Tsourkas, A. Gadolinium-conjugated dendrimer nanoclusters as a tumor-targeted T1 magnetic resonance imaging contrast agent. *Angew. Chem. Int. Ed.* **49**, 346–350 (2010).
237. Wu, C. et al. Design of highly emissive polymer dot bioconjugates for in vivo tumor targeting. *Angew. Chem. Int. Ed.* **50**, 3430–3434 (2011).
238. Rong, Y. et al. Multicolor fluorescent semiconducting polymer dots with narrow emissions and high brightness. *ACS Nano* **7**, 376–384 (2013).
239. Li, X., Takashima, M., Yuba, E., Harada, A. & Kono, K. PEGylated pamam dendrimer-doxorubicin conjugate-hybridized gold nanorod for combined photothermal-chemotherapy. *Biomaterials* **35**, 6576–6584 (2014).
240. Swierczewska, M., Lee, K. C. & Lee, S. What is the future of PEGylated therapies? *Expert Opin. Emerg. Drugs* **20**, 531–536 (2015).
241. Zhang, P., Sun, F., Liu, S. & Jiang, S. Anti-PEG antibodies in the clinic: current issues and beyond PEGylation. *J. Control. Release* **244**, 184–193 (2016).
242. Baumann, A., Tuerck, D., Prabhu, S., Dickmann, L. & Sims, J. Pharmacokinetics, metabolism and distribution of PEGs and PEGylated proteins: quo vadis? *Drug Discov. Today* **19**, 1623–1631 (2014).
243. Shi, J., Kantoff, P. W., Wooster, R. & Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **17**, 20–37 (2017).
244. Prabhakar, U. et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res.* **73**, 2412–2417 (2013).
245. Danhier, F. To exploit the tumor microenvironment: since the EPR effect fails in the clinic, what is the future of nanomedicine? *J. Control. Release* **244**, 108–121 (2016).
246. Hansen, A. E. et al. Positron emission tomography based elucidation of the enhanced permeability and retention effect in dogs with cancer using copper-64 liposomes. *ACS Nano* **9**, 6985–6995 (2015).
247. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* **1**, 16014 (2016).
248. Miller, M. A. et al. Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle. *Sci. Transl Med.* **7**, 314ra183 (2015).
249. Fang, J., Nakamura, H. & Maeda, H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Adv. Drug Deliv. Rev.* **63**, 136–151 (2011).
250. Arrieta, O. et al. High liposomal doxorubicin tumour tissue distribution, as determined by radiopharmaceutical labelling with (99m)Tc-LD, is associated with the response and survival of patients with unresectable pleural mesothelioma treated with a combination of liposomal doxorubicin and cisplatin. *Cancer Chemother. Pharmacol.* **74**, 211–215 (2014).
251. Ramanathan, R. K. et al. Correlation between ferumoxytol uptake in tumor lesions by MRI and response to nanoliposomal irinotecan in patients with advanced solid tumors: a pilot study. *Clin. Cancer Res.* **23**, 3638–3648 (2017).
252. Lee, H. et al. ^{64}Cu-MM-302 positron emission tomography quantifies variability of enhanced permeability and retention of nanoparticles in relation to treatment response in patients with metastatic breast cancer. *Clin. Cancer Res.* **23**, 4190–4202 (2017).
253. Lee, H. et al. Companion diagnostic ^{64}Cu-liposome positron emission tomography enables characterization of drug delivery to tumors and predicts response to cancer nanomedicines. *Theranostics* **8**, 2300–2312 (2018).
254. Gabizon, A. et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.* **54**, 987–992 (1994).
255. Symon, Z. et al. Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. *Cancer* **86**, 72–78 (1999).
256. Lammers, T. et al. Cancer nanomedicine: is targeting our target? *Nat. Rev. Mater.* **1**, 16069 (2016).
257. Blanco, E., Shen, H. & Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **33**, 941–951 (2015).
258. MacEwan, S. R. & Chilkoti, A. From composition to cure: a systems engineering approach to anticancer drug carriers. *Angew. Chem. Int. Ed.* **56**, 6712–6733 (2017).
259. Duncan, R. Polymer therapeutics at a crossroads? Finding the path for improved translation in the twenty-first century. *J. Drug Target.* **25**, 759–780 (2017).
260. Tsuchiya, K. et al. Tumor-targeted chemotherapy with SMANCS in lipiodol for renal cell carcinoma: longer survival with larger size tumors. *Urology* **55**, 495–500 (2000).
261. Dijkgraaf, E. M. et al. A phase 1/2 study combining gemcitabine, PEGintron and p53 SLP vaccine in patients with platinum-resistant ovarian cancer. *Oncotarget* **6**, 32228–32243 (2015).
262. Chen, X. et al. Extended PEGinterferon alfa-2a (pegasys) therapy in Chinese patients with HBeAg-negative chronic hepatitis B. *J. Med. Virol.* **86**, 1705–1713 (2014).
263. Kosaka, Y. et al. Phase III placebo-controlled, double-blind, randomized trial of PEGfilgrastim to reduce the risk of febrile neutropenia in breast cancer patients receiving docetaxel/cyclophosphamide chemotherapy. *Support. Care Cancer* **23**, 1137–1143 (2015).
264. Freda, P. U. et al. Long-term treatment with pegvisomant as monotherapy in patients with acromegaly: experience from ACROSTUDY. *Endocr. Pract.* **21**, 264–274 (2015).
265. Autrata, R. et al. Intravitreal pegaptanib combined with diode laser therapy for stage 3+retinopathy of prematurity in zone I and posterior zone II. *Eur. J. Ophthalmol.* **22**, 687–694 (2012).
266. Mease, P. J. et al. Effect of certolizumab pegol on signs and symptoms in patients with psoriatic arthritis: 24-week results of a phase 3 double-blind randomised placebo-controlled study (RAPID-PsA). *Ann. Rheum. Dis.* **73**, 48–55 (2014).
267. Baraf, H. S. et al. Tophus burden reduction with pegloticase: results from phase 3 randomized trials and open-label extension in patients with chronic gout refractory to conventional therapy. *Arthritis Res. Ther.* **15**, R137 (2013).
268. Kieseier, B. C. et al. PEGinterferon beta-1a in multiple sclerosis: 2-year results from ADVANCE. *Mult. Scler.* **21**, 1025–1035 (2015).
269. Konkle, B. A. et al. PEGylated, full-length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A. *Blood* **126**, 1078–1085 (2015).
270. Thomas, J. et al. Pegvaliase for the treatment of phenylketonuria: results of a long-term phase 3 clinical trial program (PRISM). *Mol. Genet. Metab.* **124**, 27–38 (2018).
271. Coyle, T. E. et al. Phase I study of BAY 94-9027, a PEGylated B-domain-deleted recombinant factor VIII with an extended half-life, in subjects with hemophilia A. *J. Thromb. Haemost.* **12**, 488–496 (2014).
272. Giangrande, P. et al. Clinical evaluation of glycoPEGylated recombinant FVIII: efficacy and safety in severe haemophilia A. *Thromb. Haemost.* **117**, 252–261 (2017).
273. Angiolillo, A. L. et al. Pharmacokinetic and pharmacodynamic properties of calaspargase pegol Escherichia coli L-asparaginase in the treatment of patients with acute lymphoblastic leukemia: results from Children's Oncology Group Study AALL07P4. *J. Clin. Oncol.* **32**, 3874–3882 (2014).

274. Bowser, J. L., Lee, J. W., Yuan, X. & Eltzschig, H. K. The hypoxia-adenosine link during inflammation. *J. Appl. Physiol.* **123**, 1303–1320 (2017).
275. Hingorani, S. R. et al. HALO 202: randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine versus nab-paclitaxel/gemcitabine in patients with untreated, metastatic pancreatic ductal adenocarcinoma. *J. Clin. Oncol.* **36**, 359–366 (2018).
276. Szlosarek, P. W. et al. Arginine deprivation with PEGylated arginine deiminase in patients with argininosuccinate synthetase 1-deficient malignant pleural mesothelioma: a randomized clinical trial. *JAMA Oncol.* **3**, 58–66 (2017).
277. Mussai, F. et al. Arginine dependence of acute myeloid leukemia blast proliferation: a novel therapeutic target. *Blood* **125**, 2386–2396 (2015).
278. Sands, E., Kivitz, A. J., DeHaan, W., Johnston, L. & Kishimoto, T. K. Initial phase 2 clinical data of SEL-212 in symptomatic gout patients: monthly dosing of a PEGylated uricase (PEGsiticase) with SVP-rapamycin enables sustained reduction of serum uric acid levels by mitigating formation of anti-drug antibodies. *Arthritis Rheumatol.* **69**, 2 (2017).
279. Misra, H., Lickliter, J., Kazo, F. & Abuchowski, A. PEGylated carboxyhemoglobin bovine (SANGUINATE): results of a phase I clinical trial. *Artif. Organs* **38**, 702–707 (2014).
280. Burrage, L. C. et al. Human recombinant arginase enzyme reduces plasma arginine in mouse models of arginase deficiency. *Hum. Mol. Genet.* **24**, 6417–6427 (2015).
281. Chamberlain, C. et al. Repeated administration of dapirolizumab pegol in a randomised phase I study is well tolerated and accompanied by improvements in several composite measures of systemic lupus erythematosus disease activity and changes in whole blood transcriptomic profiles. *Ann. Rheum. Dis.* **76**, 1837–1844 (2017).
282. Drolet, D. W., Green, L. S., Gold, L. & Janjic, N. Fit for the eye: aptamers in ocular disorders. *Nucleic Acid. Ther.* **26**, 127–146 (2016).
283. Chhabra, E. S. et al. Evaluation of enhanced in vitro plasma stability of a novel long acting recombinant FVIIIFc-VWF-XTEN fusion protein. *Blood* **126**, 3 (2015).
284. Ludwig, H. et al. Olaptesed pegol, an anti-CXCL12/SDF-1 Spiegelmer, alone and with bortezomib-dexamethasone in relapsed/refractory multiple myeloma: a phase IIa study. *Leukemia* **31**, 997–1000 (2017).
285. Jaffe, G. J. et al. A phase 1 study of intravitreous E10030 in combination with ranibizumab in neovascular age-related macular degeneration. *Ophthalmology* **123**, 78–85 (2016).
286. Chen, X. et al. Polyethylene glycol loxenatide injections added to metformin effectively improve glycemic control and exhibit favorable safety in type 2 diabetic patients. *J. Diabetes* **9**, 158–167 (2017).
287. Plummer, R. et al. A phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours. *Br. J. Cancer* **104**, 593–598 (2011).
288. Paz-Ares, L. et al. Phase III trial comparing paclitaxel poliglumex versus docetaxel in the second-line treatment of non-small-cell lung cancer. *Br. J. Cancer* **98**, 1608–1613 (2008).
289. Pham, E. et al. Translational impact of nanoparticle-drug conjugate CRLX101 with or without bevacizumab in advanced ovarian cancer. *Clin. Cancer Res.* **21**, 808–818 (2015).
290. Hamaguchi, T. et al. Phase I study of NK012, a novel SN-38-incorporating micellar nanoparticle, in adult patients with solid tumors. *Clin. Cancer Res.* **16**, 5058–5066 (2010).
291. Thellenberg-Karlsson, C. et al. Bone-targeted novel cytotoxic polybisphosphonate conjugate in castration-resistant prostate cancer: a multicenter phase 1 study. *Anticancer Res.* **36**, 6499–6504 (2016).
292. Liu, Z., Marquez, M., Nilsson, S. & Holmberg, A. R. Incubation with somatostatin, 5-aza decitabine and trichostatin up-regulates somatostatin receptor expression in prostate cancer cells. *Oncol. Rep.* **20**, 151–154 (2008).
293. Fares, F., Azzam, N., Fares, B., Larsen, S. & Lindkaer-Jensen, S. Benzene-poly-carboxylic acid complex, a novel anti-cancer agent induces apoptosis in human breast cancer cells. *PLOS ONE* **9**, e85156 (2014).
294. Starpharma Holdings Limited. Starpharma to commence DEP® cabazitaxel phase 1/2 trial. *Starpharma* http://www.starpharma.com/news/356 (2018).
295. Bhasi, K. et al. Modeling suggests synergistic treatment effect following combination therapy of NKTR-214 and NKTR-262 in tumor bearing mice. *J. Pharmacokinet. Pharmacodyn.* **44**, S89 (2017).
296. Ueno, T. et al. Assessment of antitumor activity and acute peripheral neuropathy of 1,2-diaminocyclohexane platinum (II)-incorporating micelles (NC-4016). *Int. J. Nanomed.* **9**, 3005–3012 (2014).
297. Anselmo, A. C. & Mitragotri, S. Nanoparticles in the clinic. *Bioeng. Transl Med.* **1**, 10–29 (2016).
298. Eskow Jaunarajs, K. L. et al. Rotigotine polyoxazoline conjugate SER-214 provides robust and sustained antiparkinsonian benefit. *Mov. Disord.* **28**, 1675–1682 (2013).
299. Santi, D. V., Schneider, E. L. & Ashley, G. W. Macromolecular prodrug that provides the irinotecan (CPT-11) active-metabolite SN-38 with ultralong half-life, low C(max), and low glucuronide formation. *J. Med. Chem.* **57**, 2303–2314 (2014).

Acknowledgements
This work was supported in part by funding from the National Institutes of Health (R01EB017722, R01CA227433), the National Science Foundation (DGE-1247312 (I.E.), DMR-1507081), the Michael A. Bell Family Distinguished Chair in Healthcare Innovation at Brigham and Women's Hospital (Y.L.C.) and the Distinguished Professor of Translational Research Chair at Boston University (M.W.G.).

Competing interests
M.W.G. and I.E. are co-inventors on a patent application describing a new polymer for drug conjugation (US20170369643A1) owned by Boston University and available for license.

Publisher's note