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Approved delivery strategies for biopharmaceuticals

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ABSTRACT

In recent years, biopharmaceuticals have witnessed remarkable advancements, transforming the landscape of therapeutic interventions. Biopharmaceuticals encompassing therapeutics generated through cutting-edge biotechnological methods have shown promising therapeutic outcomes. However, their clinical success hinges significantly on overcoming drug delivery challenges related to stability, intracellular delivery, immunogenicity, and pharmacokinetic properties. Herein, we provide an overview of various marketed macromolecules, including nucleic acids, and immunotherapeutic agents such as cytokines and monoclonal antibodies, as well as other therapeutic peptides/proteins like enzymes, hormones, and coagulation factors. Our primary focus is on elucidating the delivery challenges associated with these macromolecules and highlighting the pivotal role played by drug delivery platforms in the development of currently marketed products, offering valuable insights for both scientific research and the pharmaceutical industry.

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1. Introduction

Biopharmaceuticals refer to therapeutics produced through advanced biotechnological methods based on recombinant DNA technology, including recombinant proteins and nucleic acids [1]. These therapeutics, often manufactured using living systems, offer superior specificity and homogeneity while posing lower risks of immunogenicity and contamination, in stark contrast to products sourced from human and animal blood or tissues [1,2]. Besides traditional small drugs, which still dominate the pharmaceutical market, the field of biopharmaceuticals has experienced rapid development since the first biopharmaceutical approval in 1982, revolutionizing the treatment of various diseases, including cancers, metabolic disorders, and autoimmune conditions [2,3]. The growth of this sector has been significantly hastened by the maturity of scientific technologies used in the manufacturing processes. In 2022, more than 40% of the newly approved drugs belonged to the realm of biopharmaceuticals. Notably, nine monoclonal antibodies, one antibody-drug conjugate (ADC), one oligonucleotide, four peptides, and five other therapeutic proteins have been authorized [4].

Unlike conventional small drugs (<1 kDa), biopharmaceuticals are typically larger and more complex in structure. They offer advantages such as increased specificity, enhanced effectiveness, and reduced side effects. However, biopharmaceuticals can potentially trigger immune responses and exhibit batch-to-batch variability due to biological differences in expression systems and manufacturing conditions [1,2]. Additionally, their susceptibility to enzymatic degradation and thermal sensitivity presents additional challenges, including formulation optimization, the use of stabilizing excipients and specialized drying techniques, and the maintenance of a continuous cold chain throughout their production and transportation [2]. While the delivery of small drugs primarily focused on enhancing solubility and monitoring release to enhance bioavailability and pharmacokinetic behavior, the advent of biological macromolecules introduced new delivery challenges, specifically related to stability, intracellular delivery, and safety. Consequently, drug delivery strategies had to evolve to overcome these issues [5,6].

Herein, we provide an overview of diverse categories of authorized macromolecules, encompassing nucleic acids, immunotherapeutic agents (cytokines and monoclonal antibodies), and the other therapeutic peptides and proteins (enzymes, hormones, and coagulation factors), with a specific focus on their delivery challenges. Our overarching objective throughout this manuscript is to provide insights into how drug delivery platforms led to the

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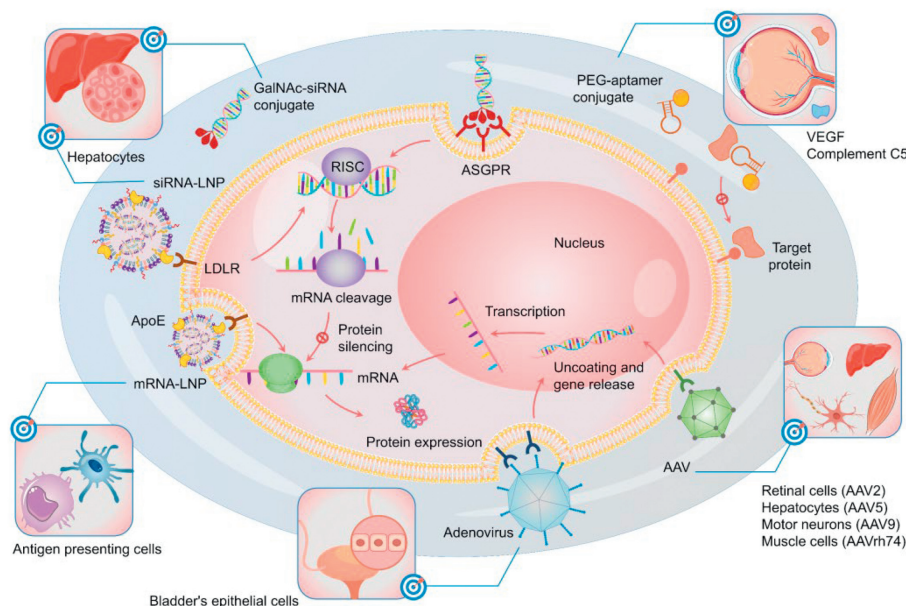


Fig. 1. Schematic illustration depicting the delivery mechanisms of nucleic acids using various delivery systems. Each system employs a distinct targeting strategy designed to interact with specific cells or tissues for targeted therapeutic purposes. Viral vectors are engineered to selectively deliver genes to specific cell types. LNPs and GalNAc-based drug delivery systems specifically bind to receptors expressed on the target cells to deliver siRNA/mRNA to the cytosol.

development of the currently marketed biopharmaceuticals. Additionally, we delve into how these platforms have significantly contributed to enhancing the effectiveness and safety profiles of these products.

2. Definition and classification of biopharmaceuticals

Biopharmaceuticals are a part of the broad category of biologics manufactured through biotechnological processes involving live organisms and/or bioprocessing [2]. These therapeutics are distinct from small drugs in various aspects, including their sources, manufacturing processes, structures, formulations, storage, transportation, regulations, and marketing [7]. Currently, biopharmaceuticals are primarily manufactured using prokaryotic systems such as *Escherichia coli* or eukaryotic systems like fungi, mammalian cells (e.g., Chinese hamster ovary cells), and insect cell lines. Plant and cell-free expression systems have also been explored [2]. Herein, biopharmaceuticals include nucleic acid-based treatments and amino-acid-based therapeutics, which can be peptides or recombinant proteins (Table S1 in Supporting information) [8,9]. Nucleic acid therapies can be categorized into DNA gene therapies and RNA-based therapies such as small interfering RNA (siRNA), microRNA (miRNA), and messenger RNA (mRNA) [10,11]. This category also includes anti-sense oligonucleotides and aptamers which can be either single-stranded synthetic DNA or RNA molecules. Recombinant proteins consist of monoclonal antibodies, cytokines (interferons (IFNs), interleukins (ILs), and growth factors), enzymes, hormones, and blood factors. It is noteworthy that some drugs such as oligonucleotides and peptide therapeutics, commonly referred to as TIDES (peptides and oligonucleotides), may reside in a gray area, being considered biopharmaceuticals due to their similarity to biological molecules, conventional drugs due to their synthetic origin, or both [7]. In this review, TIDES were included due to facing delivery challenges similar to large recombinant nucleic acids and/or proteins. Oligonucleotides are relatively large molecules (4–14 kDa) that do not easily traverse the cell membrane. As nucleic acid drugs, they must resist nuclease degradation, escape the endo-lysosomal system, and evade renal clearance to exert their therapeutic effects effectively [12]. Also, peptides of-

ten encounter challenges similar to those of proteins, such as instability and susceptibility to proteolytic enzymes.

3. Drug delivery of approved nucleic acid-based therapies

Combining gene therapies with drug delivery systems has expanded their biomedical applications to revolutionize the treatment of many diseases [13,14]. So far, several strategies have been approved by the relevant regulatory bodies for the delivery of nucleic acid-based therapeutics including DNA, siRNA, mRNA, and aptamers (Fig. S1 in Supporting information) [15–20]. The optimal nucleic acid carrier should shield the genetic material from nucleases, evade renal elimination, minimize immunotoxicity, reach the target cells, prevent off-target effects, facilitate the endosomal escape, liberate the payload in the cytosol, and if necessary, transport it into the nucleus [21]. Generally, each system possesses its unique targeting strategy tailored to effectively interact with particular cells or tissues for specific therapeutic purposes. This can be achieved by using viral vectors engineered to selectively transfect specific cell types, encapsulating and protecting the therapeutic agents, and functionalization with polymers or ligands to specifically bind to receptors expressed on the surface of target cells (Fig. 1) [22].

3.1. Drug delivery of DNA therapeutics

Viruses serve as naturally occurring gene delivery vectors that can be engineered to efficiently deliver genes of interest into specific cells. However, they have been criticized for their potential to cause mutations and immune reactions, as well as their high production costs, limited payload capacity, and difficulties in large-scale production [23]. Despite these challenges, many viral platforms, mainly constituted of recombinant adenovirus or adeno-associated virus (AAV), gained regulatory approval for the delivery of DNA-based therapeutics (Table S2 in Supporting information) [15–20]. The specific tropism, transduction efficiency, and safety profile of AAV are determined by its serotype [24]. The first registered AAV-based gene therapy was alipogene tiparvovec (Glybera), approved by the European Medicines Agency (EMA) in 2012

for the treatment of lipoprotein lipase deficiency [24]. However, it was withdrawn from the market in October 2017 due to commercial failure [25]. In December 2017, Food and Drug Administration (FDA) approved voretigene neparvec-rzyl (Luxturna), utilizing an AAV serotype 2 (AAV2) vector, to treat retinal dystrophy associated with biallelic retinal pigment epithelium-specific 65 kDa (RPE65) mutation [15]. The AAV2-based system could deliver the gene RPE65 encoding the functional human retinal protein to retinal cells after subretinal injection as a one-time therapy [26]. In May 2019, the FDA approved the use of AAV9-based DNA therapy, onasemnogene abeparvec-xioi (Zolgensma), to treat spinal muscular atrophy as a one-time intravenous treatment [16]. The AAV9 endowed with an inherent ability to cross the blood-brain barrier was leveraged for the neural delivery of the gene copy of SMN1 (survival motor neuron 1), essential for motor neuron function [27]. In August 2022, valoctocogene roxaparvec (Roctavian) received EMA approval as a one-time therapy for hemophilia A. Roctavian uses an AAV5 vector engineered to deliver the deficient factor VIII gene to the liver of hemophilia A patients, enabling continuous endogenous production of the coagulation factor by the hepatocytes [17,28]. In November 2022, another AAV5-based system carrying the gene of deficient factor IX, etranacogene dezaparvec-drlb (Hemgenix), was approved by the FDA for the treatment of hemophilia B [17].

Additionally, nadofarogene firadenovec-vncg (Adstiladrin), an adenovirus-based DNA therapy engineered to carry the human IFN- α 2b gene, was approved by the FDA in December 2022 for treating Bacillus Calmette-Guerin (BCG)-unresponsive non-muscle invasive bladder cancer [18]. Upon intravesical instillation, the gene-loaded vehicle transfects the bladder's epithelial cells leading to the incorporation of the functional gene into the cellular DNA [29]. In June 2023, the FDA approved another viral-based gene therapy, namely delandistrogene moxeparvec-rokl (Elevidys) [20]. It is an AAV rhesus isolate serotype 72 (AAVrh74)-based system developed to deliver a gene encoding micro-dystrophin protein, an engineered version of wild-type dystrophin that is normally expressed in muscle cells and whose mutation causes Duchenne muscular dystrophy [20]. Besides its tropism for muscle, the AAVrh74 vector, originally isolated from rhesus monkeys, exhibits lower immunogenicity than other serotypes isolated from humans (e.g., AAV2, AAV5, and AAV9) [30,31].

3.2. Drug delivery of siRNA

RNA interference (RNAi) therapeutics, particularly siRNA, have garnered significant interest owing to their potential in treating undruggable disorders and for their promising prospects for precision medicine [32]. These therapeutics leverage the innate cellular process of RNAi to regulate gene expression by interfering with mRNA through post-transcriptional repression [33]. siRNAs are 21–23 nucleotides-double-stranded RNA molecules used to silence specific genes encoding for the disease-causing protein by binding to the target mRNA leading to its cleavage by the RNA-induced silencing complex (RISC) [34].

Free siRNA has been shown unstable in the bloodstream and vulnerable to breakdown by nucleases with a half-life of a few minutes after systemic administration [35]. Also, it is rapidly cleared by the kidneys due to its small molecular weight that is readily excreted through the glomeruli (<50 kDa) [32]. At the cellular level, siRNA is hydrophobic, anionic, and large-sized (~13 kDa), which limits its diffusion through the phospholipid bilayer of cytomembranes to only 0.7% of the injected dose. Poor endosomal escape, immunogenicity, and off-target effects are other concerns that need to be considered while developing RNA therapies [32,36]. For safe and efficient delivery, lipid nanoparticles (LNPs) and *N*-acetylgalactosamine (GalNAc)-based drug delivery

systems have been primarily implemented to deliver siRNA (Table S3 in Supporting information) [37–46].

To date, six siRNA-based therapeutics have received approval for human use. In August 2018, the FDA approved patisiran for treating hereditary transthyretin amyloidosis. Patisiran, administered intravenously using LNP, marked a significant milestone [37]. Later, five siRNA-GalNAc conjugates, givosiran for acute hepatic porphyria, lumasiran for primary hyperoxaluria type 1, inclisiran for primary hypercholesterolaemia or mixed dyslipidaemia, vutrisiran for amyloid transthyretin-mediated amyloidosis, and nedosiran for primary hyperoxaluria, gained approval in November 2019, November 2020, December 2020, June 2022, and September 2023, respectively [40–44].

LNP consists of a spherical lipid bilayer containing a mixture of ionizable lipids, helper lipids such as distearyl phosphatidylcholine (DSPC), cholesterol, and polyethylene glycol (PEG)-lipids to reduce opsonization and prolong circulation time [47]. LNPs are used to encapsulate oligonucleotides in their core to protect them from nuclease degradation and renal clearance. Ionizable lipids are neutral at physiological pH to reduce the toxicity of LNPs but cationic at low pH to enable encapsulation of RNA molecules and facilitate the endosomal escape upon endosomal acidification [48]. LNPs adsorb apolipoprotein E (ApoE) in the circulation which mediates their attachment to low-density lipoprotein receptors (LDLR) expressed on hepatocytes, leading to their internalization via clathrin-mediated endocytosis [49]. After systemic administration, the preferential accumulation in the liver makes LNP a suitable vector for patisiran. However, it limits siRNA delivery to extra-hepatic tissues, thereby their application in cancer and neurodegenerative diseases [48].

Besides LNP technology, GalNAc-siRNA conjugates represent an efficient and safe delivery strategy for hepatic diseases. The GalNAc has a high affinity for the asialoglycoprotein receptor (ASGPR) enhancing the cellular uptake by hepatocytes expressing high levels of ASGPR, a lectin that specifically recognizes GalNAc (Fig. S2 in Supporting information) [41,50]. GalNAc-siRNA conjugates attach to ASGPR and are rapidly internalized through the clathrin-mediated pathway. At acidic endosomal pH, ASGPR is released from GalNAc-siRNA to be recycled and GalNAc molecules are then cleaved from siRNA by endosomal glycosidases. However, less than 1% of free siRNAs escape to the cytosol, making endosomal escape a rate-limiting step for efficient delivery of GalNAc-siRNA conjugates [51]. Comparatively, GalNAc-based siRNA conjugates are easier to produce, require less frequent dosing, can be administered subcutaneously, and do not require premedication, making them a promising alternative for targeted therapy in hepatic disorders [35,52].

3.3. Drug delivery of mRNA

mRNA is an emerging gene therapy that enabled the *in vivo* production of any peptide or protein of interest by introducing mRNA as a vaccine or therapeutic agent in infectious diseases, oncology, protein replacement, and regenerative medicine [53]. Unlike recombinant proteins, mRNA molecules can be easily produced by *in vitro* transcription of DNA templates, making manufacturing faster and more adaptable [54]. By mimicking the endogenous process, *in vitro* transcribed mRNA can be translated and processed in the target cell's cytoplasm, leading to efficient and long-lasting expression of the desired protein rather than transient replacement with conventional protein or peptide drugs [53]. However, mRNA macromolecules, which typically range from 300 kDa to 1500 kDa, are larger than siRNA and face similar drawbacks such as susceptibility to enzyme degradation, limited cellular uptake, and potential immunotoxicity. Hence, an appropriate delivery vehicle is necessary to transport mRNA to the target cell and facilitate the escape into the cytosol where it is translated into proteins [55,56].

In 2020, LNPs facilitated the approval of two mRNA-based drugs as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines, tozinameran (Comirnaty or Pfizer/BioNTech vaccine) and elasmomeran (Spikevax or Moderna vaccine) [38,39]. The LNPs were used to deliver mRNA encoding SARS-CoV-2 spike protein to antigen-presenting cells (APCs), triggering the generation of neutralizing antibodies. However, the mechanism of LNP uptake into APCs has not yet been fully understood. Although having a similar formulation to patisiran, the approved mRNA-LNP products need to be stored at lower temperatures of $-20\text{ }^{\circ}\text{C}$ or less to decelerate mRNA hydrolysis, in contrast to $4\text{ }^{\circ}\text{C}$ for siRNA-LNPs [49]. Hitherto, many clinical trials involving mRNA for different conditions are underway, which may lead to more approvals in the coming years.

3.4. Drug delivery of aptamers

Aptamers are single-stranded, non-coding DNA or RNA molecules, typically consisting of 25–80 bases, that fold into a unique 3D structure with antibody-like abilities to bind to specific targets. Unlike traditional biological drugs, aptamers can be fully engineered using systematic evolution of ligands by exponential enrichment (SELEX) [57]. These nucleic acid antibodies have been used for versatile applications (e.g., diagnostic tools, therapeutic agents, or drug delivery systems) due to their merits, including long-term storage stability, simplicity of synthesis with low batch-to-batch variability, enhanced tissue penetration, low immunogenicity, and high selectivity [45,58]. Still, aptamers suffer from nuclease-mediated hydrolysis and fast glomerular filtration because of their relatively small molecular weight (6–30 kDa) (Fig. S3 in Supporting information) [57]. Therefore, modifications are required to extend their half-lives, such as conjugation to polyethylene glycol (PEG), proteins, fatty acids, and organic or inorganic nanoparticles. So far, PEG is the only moiety used to increase the size of approved aptamers [57].

Pegaptanib (Macugen) is a 5'-end PEGylated RNA aptamer approved by the FDA in 2004 to treat neovascular age-related macular degeneration (AMD). It specifically inhibits vascular endothelial growth factor 165 (VEGF 165)-mediated pathological effects on vascular endothelial cells. The PEGylation of the aptamer with 40 kDa monomethoxyPEG delays its degradation and renal filtration, resulting in a longer half-life and improved efficacy [45]. In August 2023, another 5'-end PEGylated RNA aptamer, avacincaptad pegol (Izervay, formerly Zimura), received FDA authorization. It is a 39-nucleotide targeting the complement component 5 for treating geographic atrophy secondary to AMD [46]. Although PEGylated drugs have been widely incriminated by the accelerated blood clearance (ABC) phenomenon resulting from the production of anti-PEG antibodies, they were not associated with any specific pathology [59,60]. About 20 PEGylated biotherapeutics have gained approvals since 1990 and dozens of others are in preclinical or clinical stages [60]. Still, alternative coupling agents besides bulky PEG moieties are needed to develop long-acting aptamers without immune concerns.

4. Drug delivery of approved immunotherapeutic agents

The number of immunotherapeutics approvals has been increasing in recent years, with many treatments, mainly targeting cancer, in clinical and preclinical stages. Immunotherapeutics can be classified into cytokine immunotherapy, therapeutic monoclonal antibodies, adoptive cell therapy, tumor vaccines, and oncolytic virotherapy [61]. Despite the approval of numerous immunotherapeutics, their efficacy is hindered by various factors, including their large molecular size, instability, and limited tissue penetration. Additionally, frequent administration at high doses raises concerns

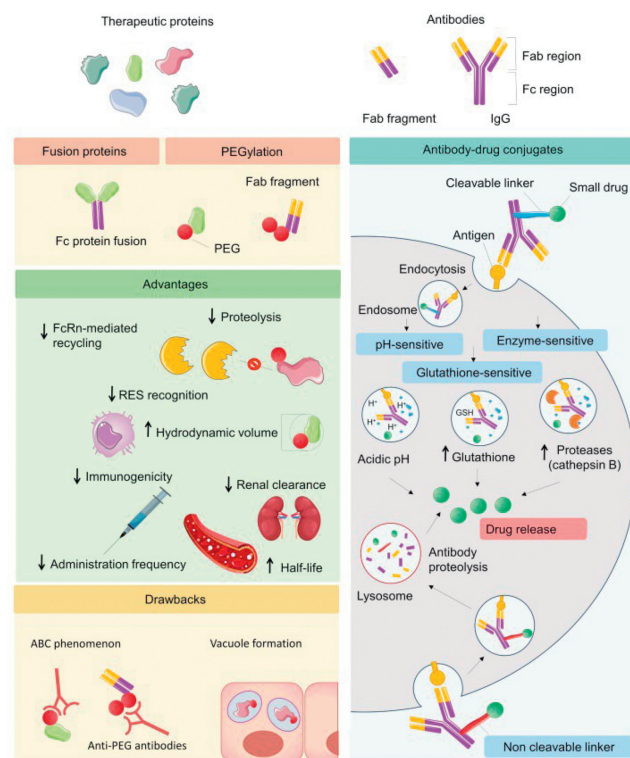


Fig. 2. Illustration of the delivery principles of different immunotherapeutic agents. Multiple delivery strategies have been used including PEG conjugates, fusion proteins, and ADCs.

about autoimmune-mediated side effects [62]. Moreover, while many drugs have demonstrated effectiveness in treating hematological tumors through intravenous administration, their efficacy remains limited against solid tumors due to the inaccessibility of tumor sites [61]. Recent advancements in the field of drug delivery afford new strategies that can improve both the safety and efficiency of immunotherapeutics [63]. Multiple drug carriers and delivery strategies have been proposed, including liposomes, polymeric nanoparticles, exosomes, hydrogels, living cells, and inorganic materials. Still, only polymer conjugates, fusion proteins, and ADCs have successfully transitioned to the market so far (Fig. 2) [62].

4.1. Cytokines

Cytokines, including IFNs, ILs, and growth factors, are polypeptides/glycoproteins that act as potent immunomodulators [64]. Cytokines were the first marketed class of immunotherapy by the FDA approval of recombinant IFN- α in 1986 for hairy cell leukemia [65]. In 1992, recombinant human IL-2 was approved for treating kidney cancer [61]. After these early approvals, cytokines clinical translation has been limited due to their pleiotropic action, suboptimal pharmacokinetics, and dose-dependent toxicity [66]. Due to cytokines' short half-life, the need for high doses and frequent administration often leads to adverse effects such as capillary leakage syndrome and cytokine storm [62]. Recent research efforts have revitalized interest in cytokine therapy, especially in oncology, where cytokines are explored as combinatory agents with checkpoint inhibitors or chimeric antigen receptor T cells [64]. Parallely, more explorations in cytokines delivery are needed to enhance the therapeutic efficacy and mitigate safety concerns. Various delivery systems, such as polymeric NPs, liposomes, and inorganic NPs, were suggested to protect protein drugs from enzyme degradation and promote their action [67]. Notably, PEG-protein conjugates are the

predominant approved approach in this domain thus far (Table S4 in Supporting information) [68–81].

The PEGylation of cytokines minimizes proteolytic digestion and shields them from the reticuloendothelial system, thereby extending their half-life. Additionally, the hydrodynamic volume of the biomolecule increases in the presence of the polymer which lowers renal clearance [60]. However, the covalent attachment of PEG molecules might impact the cytokines' ability to bind to their intended targets [59]. Apart from concerns related to the ABC phenomenon, another challenge associated with PEGylated proteins is the formation of vacuoles within cells. Nevertheless, carefully monitoring the polymer's molecular weight can mitigate this issue [82].

PEG conjugates have been leveraged to deliver IFNs, which are normally produced by immune cells in response to microbial pathogens, inducing the maturation of monocytes, natural killer cells, dendritic cells, and lymphocytes [61]. To date, four PEGylated IFNs, including peginterferon alfa-2b (PegIntron), peginterferon alfa-2a (Pegasys), peginterferon beta-1a (Plegridy), and ropeginterferon alfa-2b (Besremi), gained FDA/EMA first authorizations in 2001, 2002, 2014, and 2019, respectively [82]. Compared to conventional IFN molecules, PEG conjugates offer prolonged blood circulation, long-acting effects, and slower elimination, reducing administration frequency and consequently fewer side effects. PegIntron consists of an alpha IFN molecule conjugated to a mono-methoxy PEG chain (12 kDa), approved for monotherapy or combined with ribavirin to treat chronic hepatitis C [68]. Pegasys, a recombinant human alfa-2a IFN, is attached to a branched PEG (40 kDa) for the treatment of hepatitis C and hepatitis B. While free IFNs necessitate thrice-weekly administration, their PEG conjugates require subcutaneous injection once weekly [69]. Plegridy, a glycosylated recombinant human IFN beta-1a modified with methoxy-PEG-O-2-methylpropionaldehyde molecule (20 kDa), is indicated for multiple sclerosis once every 2 weeks [70,82]. Besremi is a long-acting proline-IFN alfa-2b conjugated with a branched PEG (40 kDa) at its N-terminal proline [83]. It has high tolerability and an improved pharmacokinetic profile, allowing a single injection every two weeks for the treatment of polycythemia vera [71].

Besides IFNs, pegfilgrastim (Neulasta), a PEGylated form of the recombinant methionyl human granulocyte-colony stimulating factor (G-CSF) called filgrastim, was approved by the FDA in 2002 for the treatment of neutropenia, a common post-chemotherapy adverse effect [84]. The covalent conjugation of the monomethoxy-PEG aldehyde chain (20 kDa) at the N-terminus of filgrastim increased the solubility and the circulation time by reducing enzymatic degradation and renal clearance, making a single subcutaneous injection of pegfilgrastim per chemotherapy cycle equivalent to daily administration of the parent molecule (filgrastim) [72]. Another long-acting form of filgrastim, lipegfilgrastim (Lonquex), was obtained through site-specific glycopegylation with a 20 kDa PEG at the O-glycosylation site of G-CSF, aiming to optimize the pharmacokinetic and pharmacodynamic profiles [74]. Several biosimilars of Neulasta gained approval demonstrating that PEGylated conjugates entered a new era by improving the conjugation chemistry and bioengineering processes [82]. Most recently, a more tolerable and convenient filgrastim, eflapegrastim (Rovedon), gained approval as a long-acting G-CSF. A bifunctional PEG (~3.4 kDa) was used as a crosslinker between filgrastim and the fragment crystallizable (Fc) of human IgG4 to increase its circulation time and penetration to the target site [73].

Epoetin β (EPO) is the recombinant form of erythropoietin, an essential erythropoiesis-stimulating factor. EPO has been conjugated to methoxy-PEG (30 kDa) via amide bonds, resulting in the creation of Mircera, which received approval in 2007 for treating anemia associated with chronic renal failure [84,85]. The methoxy PEG glycol-EPO prolonged the half-life to ~130 h compared to ~7 h of the native form. Consequently, the frequency of admin-

istration was reduced to one intravenous/subcutaneous injection per 2 weeks instead of 3 times a week for the free protein [75]. While other cytokines such as IL-2 and IL-10 have also undergone PEGylation, their clinical translation has been impeded due to low tolerability and the absence of improved efficacy compared to free cytokines or standard treatments [66]. Promisingly, antibody-cytokine-conjugates or cytokine-encoded plasmids represent alternative strategies for effectively delivering recombinant cytokines [86].

4.2. Therapeutic monoclonal antibodies

Monoclonal antibodies (mAbs) have revolutionized the field of drug therapy, offering targeted treatments for a wide array of diseases, including cancer, autoimmune disorders, and infectious diseases [87]. mAb-based therapeutics hold significant importance in biopharmaceutics due to their high specificity, extended duration in the bloodstream, immune-modulating capabilities, adaptability, and deference of their framework to protein engineering [88]. Lately, extensive research endeavors have been focused on optimizing the therapeutic outcomes and delivery of mAbs. Antibodies or their fragments can be conjugated to polymers, small drugs to create ADCs, or fused to other proteins to tailor their therapeutic applications for specific purposes [88].

4.2.1. Antibody fragment-polymer conjugates

The antibody fragment Fab (antigen-binding fragment) maintains the binding ability and targeting specificity of the parent antibody. It offers extra advantages, such as faster penetration rate, more cost-effective production, and simpler engineering owing to its smaller size (50 kDa) compared to the full-length IgG (150 kDa) [89]. However, antibody fragments are more susceptible to renal clearance. Additionally, the absence of Fc fragment and its mediated endosomal recycling accelerates the endosomal degradation of Fabs [88]. Therefore, appropriate modifications such as conjugation to polymers are required to avoid rapid clearance, especially when targeting chronic diseases.

The Fab fragment of the anti-tumor necrosis factor (TNF)- α mAb, certolizumab, has been PEGylated to produce certolizumab pegol, marketed as Cimzia for the treatment of inflammatory disorders such as Crohn's disease since 2008. The site-specific attachment of PEG (40 kDa) in Cimzia was facilitated by its engineered thiol after the insertion of a cysteine residue [90]. Resultantly, the half-life increased to 2 weeks, similar to that of the full-length anti-TNF- α mAbs [89]. Fortunately, the Fc-mediated cell-killing effects via complement activation or recruitment of effector cells are unnecessary for certolizumab pegol to exert its neutralizing therapeutic effect.

4.2.2. ADCs

ADC is typically composed of a monoclonal antibody covalently attached to a small drug via a synthetic linker to combine the two entities' targeting ability and therapeutic potency [87]. A pivotal aspect of ADC design lies in the choice of the linker, as it significantly impacts crucial attributes such as stability, pharmacokinetics, specificity, toxicity, and potency [91]. An optimal linker should possess high solubility to allow bioconjugation of hydrophobic drugs and prevent ADC aggregation, provide high serum stability to prevent premature drug release, not impede the mAb/drug binding ability and efficiency, reduce off-target toxicity, and selectively release the drugs at the target site [92]. The conjugation process used to attach the linker to the mAb is another critical approach. Conventional coupling methods involving lysine and cysteine residues often result in heterogeneous ADCs, posing challenges for quality control and potentially causing stability issues

leading to premature payload release. Novel site-specific conjugation strategies might be advantageous, such as incorporating engineered reactive cysteine residues or unnatural amino acids [93,94].

According to the type of linker used, ADCs can be divided into cleavable and non-cleavable categories. Cleavable ADCs remain intact while circulating in the bloodstream as the linkers are selectively cleaved in the diseased area/target cells releasing the payload. Various cleavable linkers have been exploited to selectively deliver anticancer agents. pH-sensitive linkers, commonly hydrazones, are stable under blood physiological pH but undergo hydrolysis in the acidic microenvironment and endo-lysosomal compartments [91]. However, these linkers may lead to premature release of drugs in the circulation resulting in non-specific delivery and systemic toxicity. Mylotarg, a hydrazone-based ADC approved in 2000, was withdrawn from the market in 2010 due to toxicity issues partially attributed to the non-selective drug release; although it was reapproved in 2017 [92]. CL2A is another hydrolyzable linker *via* pH sensitivity used in approved ADCs [95]. Similarly, glutathione-sensitive ADCs containing disulfide linkers leverage the high intracellular level of glutathione in target cells that reduce the linkers to enable drug release [93]. Succinimidyl 4-(pyridin-2-yl)disulfanyl (SPDB) and its more hydrophilic derivative sulfo-SPDB are glutathione-sensitive linkers that can be used for site-specific release of drugs in cancer cells [96]. Besides chemical cleavable linkers, dipeptide/tetrapeptide linkers have been harnessed to create enzyme-sensitive ADCs that remain stable in circulation but undergo cleavage by lysosomal proteases overexpressed in tumor cells, such as cathepsin B. This includes valine-citrulline, valine-alanine, and glycine-glycine-phenylalanine-glycine linkers. Chemically modified dipeptide linkers, like maleimidocaproyl (MC)-valine-citrulline-*p*-aminobenzylcarbamate (PABC), are frequently employed to facilitate protease access to the cleavage site [91]. Comparatively, enzyme-sensitive ADCs exhibit higher serum stability than their chemical-sensitive counterparts due to protease inhibitors in the bloodstream [96]. On the other hand, non-cleavable linkers, such as MC and the thioether linker succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), remain intact in circulation until complete lysosomal proteolysis of the mAb, enhancing ADCs plasma stability and reducing off-target effects [93,97].

Theoretically, most ADCs actively target and bind to specific surface antigens recognized by mAb before being internalized. Subsequently, the drug payload is released into the cytoplasm, either through chemical or enzymatic cleavage of the linker in the case of cleavable linkers, or *via* proteolysis of the mAb for those with non-cleavable linkers [91]. Apart from their specific targeting capabilities, certain mAbs used in oncology contribute to the anti-tumor action upon interactions with their target antigens. They can modulate survival-related pathways and/or activate immune responses through antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis [97]. ADCs that are not internalized can liberate their payload after enzymatic proteolysis or hydrolysis in the tumor microenvironment [96]. As of October 2023, 13 ADCs are available in the market after the recent withdrawal of moxetumomab pasudotox (Lumoxiti, an ADC approved by the FDA in 2018 for hairy cell leukemia). Additionally, hundreds of ADCs are under clinical investigations [97,98]. Approximately 85% of the currently approved ADCs are based on cleavable linkers, and 7 out of 13 ADCs utilize enzyme-sensitive linkers (Table S5 in Supporting information) [98,99].

4.3. Other immunomodulators

Fragment crystallizable (Fc) fusion proteins are a unique class of therapeutics that combine an active protein with an IgG-Fc. This

strategy is commonly used to extend the half-life of biomolecules due to the increased stability to proteolysis and neonatal Fc receptor-mediated recycling. Most of these Fc fusion proteins are generated through genetic fusion, connecting a biological moiety's C-terminus to the IgG-Fc domain's N-terminus. The Fc portion utilized undergoes mutation to eliminate its effector functions [100]. Notably, the inaugural Fc fusion protein to receive FDA approval was etanercept (Enbrel) in 1998, used for treating rheumatoid arthritis. Etanercept is a prominent TNF inhibitor, comprising two human TNF receptors linked to the IgG-1 Fc domain [76]. Subsequently, a series of Fc fusion proteins with immunomodulatory properties have gained approval, including abatacept (Orencia) and belatacept (Nulojix). Abatacept and belatacept both feature the IgG1-Fc domain linked to the extracellular domain of human cytotoxic T-lymphocyte antigen 4 (CTLA-4), a cell membrane molecule crucial for regulating T-cell activation. Initially approved for rheumatoid arthritis in 2005, abatacept serves as the foundational compound for belatacept. The latter, achieved through two amino-acid substitutions in the CTLA-4 region to enhance binding affinity, earned approval for preventing graft rejection after kidney transplantation in 2011 [77,78].

Cytokine traps, composed of more than one extracellular cytokine receptor domain, are more potent than monomeric neutralizers like etanercept. Rilonacept (Arcalyst) is a cytokine trap composed of the extracellular domains of the IL-1 receptor and IL-1 receptor accessory protein linked to IgG1-Fc. It was approved in 2008 to treat rare genetic conditions (cryopyrin-associated periodic syndromes) [79]. A similar approach has been employed in developing VEGF decoys, such as aflibercept (Eylea) and conbercept (Lumitin) to prevent the binding of the angiogenic cytokine VEGF and placental growth factor to their endogenous receptors. Aflibercept, FDA-approved in 2011 for treating wet age-related macular degeneration, results from the fusion of the 2nd domain of vascular endothelial growth factor receptor (VEGFR)-1 and the 3rd domain of VEGFR-2 to the Fc portion of human IgG1. Later, conbercept, which contains an additional extracellular domain 4 of VEGFR-2, was approved by the China Food and Drug Administration in 2013 [80,81].

5. Drug delivery of other approved therapeutic proteins

Endogenous proteins are multifunctional biomolecules involved in diverse biochemical reactions, including inflammatory and immune responses, cellular proliferation and differentiation, as well as metabolic and signaling pathways [101]. Since the groundbreaking success of recombinant insulin in 1982, dozens of proteins have gained clinical approval, highlighting the potential of protein-based therapies in addressing a wide spectrum of diseases [90]. Among proteins currently used as therapeutic agents in addition to immunotherapies are enzymes, hormones, and coagulation factors [101].

The complexity and specific conformation of proteins contribute to their high binding specificity. However, their large molecular weight, physicochemical instability, susceptibility to proteolysis, endosomal entrapment, and eventual exocytosis significantly diminish their bioavailability [102,103]. These factors restrict their administration to parenteral routes, shorten their half-lives, and can cause structural changes that impair their bioactivity. The challenge of oral delivery due to proteolytic degradation in the gastrointestinal tract and the required high and frequent doses compromise patient compliance and frequently lead to serious side effects [101]. To overcome these challenges, multiple approaches have been explored, including PEG, lipids or albumin conjugates, and Fc fusion proteins, as illustrated in Table S6 (Supporting information) [104–122].

5.1. Enzymes

Enzymes represent a profitable market among therapeutic proteins since they are the only treatment for certain illnesses [123]. A series of therapeutic enzymes have been marketed so far. Frequently, PEG was conjugated to the enzyme to increase stability, reduce immunogenicity, and prolong blood circulation [124]. Pegademase bovine (Adagen) was the first PEGylated therapeutic protein approved by the FDA in 1990 for adenosine deaminase (ADA) deficiency-severe combined immunodeficiency. The protein was extracted from calf intestines and thereby holds the risk of infection with bovine spongiform encephalopathy [104]. Later, a new version of the enzyme was produced by recombinant technology in *E. coli* and conjugated to 13×5 kDa PEG to form elapegamase (Revcovi), approved by the FDA in 2018 [104]. Besides the increased production yield and reduced risks of infection and immunogenicity, Revcovi could last longer in the bloodstream with more potency in elevating ADA levels than Adagen. These improvements could be attributed to PEGylation with a succinimidyl carbonate linker instead of succinimidyl succinate and the replacement of cysteine by serine [105]. Likewise, two PEGylated forms of *E. coli*-derived L-asparaginase, namely pegaspargase (Oncaspar) and calaspargase pegol (Asparlas), received approvals in 1994 and 2018, respectively, for the treatment of acute lymphoblastic leukemia [106,107]. Despite employing similar PEG chains (5 kDa), the half-life of Asparlas is three times longer than Oncaspar, which is ascribed to the utilization of succinimidyl carbamate linker instead of succinimidyl succinate [125].

Pegloticase (Krystexxa) is another PEGylated enzyme used clinically to treat gout since 2010. Like the non-PEGylated uricase (rasburicase), pegloticase reduces hyperuricemia by converting uric acid to allantoin, which is easily excreted in the urine. The presence of approximately nine 10 kDa monomethoxy-PEG aims to prevent the immunogenicity of the porcine-like uricase [108,126]. Also, the half-life increased to 2 weeks compared to 18 h for rasburicase, allowing for less frequent administrations [123]. Recombinant phenylalanine ammonia lyase derived from *Anabaena variabilis* was conjugated to nine 20 kDa PEG chains to lower its high immunotoxicity, generating pegvaliase (Palynziq), which was approved in 2018 for phenylketonuria as a once-daily subcutaneous injection for maintenance therapy [109]. Most recently, pegunigalsidase alfa (Elfabrio) has been authorized for Fabry disease, a deficiency of the enzyme α -galactosidase A (AGAL) [110]. Elfabrio is a recombinant protein composed of the two subunits of AGAL covalently bound by a PEG chain. Cross-linking the plant-derived macromolecule with bifunctional ~ 2 kDa PEG results in a more stable and long-circulating enzyme that can be infused once monthly [127].

Fc fusion proteins have also been leveraged to deliver recombinant enzyme replacement therapies, such as asfotase alfa (Strensiq), a recombinant human tissue-nonspecific alkaline phosphatase approved for the treatment of hypophosphatasia in 2015. The enzyme consists of a glycoprotein containing two identical polypeptide chains of 726 amino acids covalently linked by two disulfide bonds, an IgG1-Fc domain to facilitate the purification of the molecule and enhance the circulation half-life, and a deca-aspartate peptide used as a bone targeting moiety [111,128].

5.2. Hormones

The hypersecretion of growth hormone (GH) leads to gigantism in childhood and acromegaly in adults, conditions that can be treated with GH receptor antagonists such as B2036. Pegvisomant (Somavert) is a PEGylated form of B2036 approved by the FDA in 2003 for the treatment of acromegaly. The attachment of 5 kDa PEG molecules to B2036 significantly increases its half-life from

20 min to 72 h and mitigates its immunogenicity [112]. Conversely, GH deficiency has been treated by recombinant human growth hormone (rhGH) available since 1985. However, the need for daily injections impedes patient adherence, necessitating the development of long-acting forms of GH [129]. Interestingly, the reversible attachment of methoxyPEG to rhGH (somatropin) via TransCon linker generates the prodrug lonapegsomatropin (Skytrofa), which has been authorized for clinical use as a once-weekly treatment since 2021 [113]. The PEG temporarily shields the hormone and limits its renal elimination before the cleavage of the linker under physiological conditions in a controlled manner, liberating the active drug with a half-life of approximately 25 h instead of 3 h for somatropin [113,129]. Somapacitan-beco (Sogroya) is another approved long-acting rhGH developed by Novo Nordisk as a once-weekly subcutaneous injection for both children and adults. Compared to lonapegsomatropin, somapacitan incorporates a fatty acid chain attached to the hormone through a hydrophilic spacer, enabling reversible binding to endogenous serum albumin, thereby reducing the elimination rate and extending bloodstream circulation [114].

5.3. Coagulation factors

Coagulation factors play a crucial role in maintaining hemostasis. Replacement therapy with recombinant human coagulation factors including FVIII, FIX, and FVIIa has been the standard of care for patients with bleeding disorders such as hemophilia A and hemophilia B. Due to the short half-lives of these factors, PEGylation technology has been widely applied for their delivery [117]. Currently, there are three PEGylated recombinant FVIII (rFVIII) drugs available in the market for treating hemophilia A, along with one PEGylated recombinant FIX for hemophilia B. Ru-roctocog alfa pegol (Adynovate) was the first PEG-rFVIII approved by the FDA in 2015. The covalent attachment of a 20 kDa branched PEG in Adynovate extended its half-life to 14–19 h, compared to the 12 h half-life of its non-modified counterpart, octocog alfa (Advate) [115,130]. The second PEGylated rFVIII, damoctocog alfa pegol (Jivi), is site-specifically coupled to a single chain of 60 kDa branched PEG at cysteine residue inserted by mutation [116]. Recently, a new long-acting rFVIII, turoctocog alfa pegol (Esperoct), conjugated with a single 40 kDa branched PEG using glycoPEGylation technology, was approved [117]. These three extended half-lives PEG-rFVIII products display comparable pharmacokinetic and pharmacological properties but differ in safety profiles due to the proportional relationship between anti-PEG antibodies production and PEG size [60]. The extended half-life of PEGylated rFVIII is not attributed to the reduced renal elimination since FVIII (~ 280 kDa) is large enough to avoid glomerular filtration. Still, PEGylation decreases proteolysis and interferes with low-density lipoprotein receptor-mediated clearance [131]. Nonacog beta pegol (Rebinyn/Refixia) is a 40 kDa PEG recombinant FIX authorized for the treatment of hemophilia B since 2017. It is produced using an acceptor site-directed glycoPEGylation of FIX to overcome any loss of bioactivity. The glycoPEGylated version of FIX has 5 times longer half-life than the non-modified ones [118].

Along with PEGylation, other extended half-life recombinant coagulation factors developed using Fc fusion protein technology are in the market including rFVIII, such as efmoctocog alfa (Eloctate) and efanesoctocog alfa (Altuviio), as well as rFIX analogs such as eftrenonacog alfa (Alprolix) [119–121]. Fusion of the Fc domain to the coagulation factor prolongs its circulation time through neonatal Fc receptor-mediated recycling [132]. Interestingly, efanesoctocog alfa has a more complex structure leveraging both Fc fusion protein and XTEN technology. It contains a B-domain deleted FVIII-XTEN-Fc chain with an XTEN polypeptide inserted at the B-domain region, and a von Willebrand factor (VWF) D'D3-XTEN-Fc

chain with a second XTEN polypeptide inserted between D'D3 domain and Fc. In addition to the Fc domain facilitating recycling, the presence of two chains of XTEN polypeptides reduces protein degradation and clearance. The dependency of FVIII action on endogenous VWF, which has a half-life of about 15 h, has limited the half-life of long-acting rFVIII replacement therapies to 15–19 h. Unlike traditional FVIII therapies, efanesoctocog alfa is not dependent on VWF for its activity due to the presence of a VWF D'D3 FVIII binding domain, offering a potential advantage in terms of circulation time and treatment predictability. The combination of these strategies resulted in a half-life of 43 h after intravenous administration [133]. Fusion with human albumin is another approach used to develop long-acting coagulation factors. Albutrepenonacog alfa (Idelvion) consists of rFIX genetically fused with recombinant human albumin *via* a cleavable linker peptide. During coagulation, the linker is cleaved by the same proteases that activate FIX to detach the albumin moiety. The presence of albumin prolongs the half-life, allowing once or twice weekly administrations for routine prophylaxis [122].

6. Drug delivery of approved therapeutic peptides

Peptides are a unique class of pharmaceutical agents with a molecular weight of 0.5–5 kDa, acting as neurotransmitters, hormones, growth factors, antimicrobials, and immunomodulators [134]. Compared to conventional small drugs, they have high affinity and specificity, with a similar mode of action to therapeutic proteins and antibodies. However, compared with biologics, therapeutic peptides show less immunogenicity and production costs [134]. The progress in protein purification and synthesis technologies accelerated the development of peptide therapeutics, leading to the approval of dozens of synthetic peptides such as oxytocin, vasopressin, and recombinant peptides such as insulin and analogs. However, peptide drugs had limitations such as proteolysis, membrane impermeability, instability, poor bioavailability, and short half-life [135]. Various strategies such as conjugation to PEG, lipids, or larger proteins have been employed to decrease renal clearance, lower proteolysis, and increase residence time (Table S7 in Supporting information) [136–145].

6.1. PEG-peptide conjugates

Similar to proteins, PEGylation is a well-established strategy applied to extend the half-life of various approved therapeutic peptides [146]. The erythropoiesis stimulator peginesatide (Omontys) was the first approved PEGylated peptide in 2012; however, it was withdrawn from the market in 2013 due to concerns over severe acute hypersensitivity reactions [115]. Despite this setback, there has been a resurgence of interest in PEG peptides, exemplified by the recent approval of pegcetacoplan (Empaveli and Syfovre). Pegcetacoplan, a complement C3 inhibitor, contains two copies of a disulfide cyclic peptide and a 40 kDa PEG molecule. It was approved in 2021 for the treatment of paroxysmal nocturnal haemoglobinuria and geographic atrophy [136,147]. Glucagon-like peptide-1 (GLP-1) analogs are used for the treatment of type 2 diabetes mellitus (T2DM). The unmodified GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) and cleared within a few minutes [148]. The PEGylated GLP-1 analog, PEG-loxanatide, developed by Jiangsu Hansoh Pharmaceuticals in 2019 improved the pharmacokinetic profile of the peptide to make a once-weekly injection possible instead of a twice-daily injection of the non-modified GLP-1 analog (exenatide) [137].

6.2. Lipid-peptide conjugates

Peptide lipidation is a process that involves conjugating a peptide molecule with a fatty acid, such as caprylic acid (C_8), capric

acid (C_{10}), lauric acid (C_{12}), myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), and eicosanedioic acid (C_{20}), either directly to the peptide backbone or *via* a linker/spacer. The incorporation of fatty acids can improve stability and increase the drug's liposolubility, thereby enhancing membrane permeability. Moreover, it allows a depot formation at the injection site after subcutaneous administration, offering delayed drug absorption. The reversible binding of fatty acids to human serum albumin after *in vivo* administration acts as a protraction mechanism by limiting the access of proteases to the peptide drugs and lowering their renal clearance [138,149]. This strategy has been applied to formulate long-lasting peptide therapeutics. For instance, the insulin analog, detemir (Levemir), was developed by the removal of the threonine at residue 30 and attaching myristic acid to lysine 29 of the B chain of insulin, resulting in a slower release and long-acting hypoglycemic effect [139]. Degludec (Tresiba) is another long-acting insulin analog obtained by removing threonine at residue 30 and incorporating a C_{16} fatty acid at residue 29 of the B chain through a glutamic acid spacer [150]. Detemir and degludec form stable di/multi-hexamers in the subcutaneous tissue, thereby slowing the absorption [139].

The conjugation of palmitic acid to the peptide at lysine 26 through γ -glutamic acid spacer, resulted in the creation of a long-acting GLP-1 analog, liraglutide (Victoza/Saxenda), with a half-life of ~ 12 h after subcutaneous injection, allowing once-daily administration. Liraglutide received its initial approval in 2009 for the treatment of T2DM and in 2014 for obesity [140]. The linkage of a longer fatty acid (C_{18}) to lysine 26 of GLP-1 peptide in semaglutide structure contributed to the extension of its half-life to almost one week, allowing a once-weekly subcutaneous injection or once-daily oral administration [141]. It should be noted that the oral delivery of semaglutide was facilitated by its co-formulation with sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) to form a peptide/SNAC complex with enhanced lipophilicity, enabling it to cross the gastrointestinal epithelium [148]. Tirzepatide (Mounjaro) is another marketed peptide drug incorporating a long C_{20} fatty acid in its structure to increase its *in vivo* stability and circulation time. Tirzepatide is a bispecific agonist of gastric inhibitory polypeptide and GLP-1 receptors that has been authorized in 2022 for the treatment of T2DM as a once-weekly regimen [142]. Most recently, zilucoplan (Zilbrysq), an inhibitor of complement component 5, has been approved for the treatment of myasthenia gravis. It is a macrocyclic peptide with a main backbone of 15 amino acids and a side chain containing palmitic acid, which led to pharmacokinetic improvement and allowed a once-daily subcutaneous administration [143].

6.3. Protein-peptide conjugates

Selective conjugation of small peptides to larger and longer half-life carrier proteins such as serum albumin or Fc fragments of immunoglobulins endow peptide drugs with higher *in vivo* stability and longer circulation times [100]. This approach has been applied to extend the half-life of dulaglutide and albiglutide, enabling their once-weekly injections for the treatment of T2DM. Albiglutide (Tanzeum), approved by the FDA in 2014, is composed of two copies of GLP-1 analogs genetically fused to recombinant human albumin. This modification improved the resistance to DPP-4 degradation and prolonged its half-life to one week [151]. However, albiglutide was globally withdrawn from the market in 2018 for commercial reasons [152]. Dulaglutide (Trulicity) consists of two copies of GLP-1 analogs covalently linked to a modified human IgG4 Fc fragment by a small peptide linker. This modification reduced the immunogenicity of the peptide and made once-weekly subcutaneous injection possible [144]. A similar strategy was previously employed for the delivery of romiplostim (Nplate), which received approval in 2008 to treat chronic immune thrombocy-

topenia. Romiplostim comprises a recombinant IgG1 Fc fragment linked with two tandem copies of thrombopoietin peptidomimetics via glycine linker [145]. However, peptide-Fc products may hold potential safety concerns arising from the ability of the Fc fragment to trigger antibody-dependent cell-mediated cytotoxicity and complement-mediated cell lysis [148].

7. Conclusions and prospect view

Biopharmaceuticals represent a transformative paradigm in therapeutics, as evidenced by the remarkable surge in their market in recent years. Despite the high production costs, analysts anticipate that this market will continue to grow, regarding the ongoing advancements in molecular biology, enhanced understanding of expression systems, and improved comprehension of the technological factors influencing the scaling up of biologics. In response to the evolving therapeutic landscape, delivery technologies have rapidly adapted to fulfill drug delivery needs, helping to convert biotechnology-derived products into successful therapies. An insightful analysis of approved biotherapeutics over the past decades unveils numerous pioneering paradigms that have been employed to overcome drug delivery challenges.

In the field of gene therapy, viral vectors, mainly recombinant adeno-associated viruses, are still the gold standard delivery vectors for DNA-based therapeutics, due to their high transfection ability and efficiency in delivering genes into specific cells by harnessing the specific tropism of different serotypes. Besides, non-viral carriers like LNPs are drawing more attention for the delivery of oligonucleotides especially after the clinical success of the siRNA-based drug, Onpattro, and the recent approval of two mRNA-based COVID-19 vaccines. GalNAc conjugates represent another breakthrough in siRNA therapy where the latest five approved siRNAs were GalNAc conjugates. However, all the approved siRNA therapeutics target the hepatocytes. The effective targeting of siRNA therapies might be partially attributed to the liver's intrinsic properties, such as its high perfusion and the presence of a discontinuous sinusoidal endothelium, as well as the abundance of receptors facilitating the uptake of these agents. Therefore, the development of advanced strategies for effectively delivering siRNA therapies to extrahepatic tissues remains a significant challenge.

While significant progress has been made in the field of targeted drug delivery, some biotherapeutics still rely on traditional methods such as PEGylation, despite growing concerns raised by critical studies pointing to the ABC phenomenon and vacuole formation. This divergence in development highlights the complex landscape of biotherapeutics, where both traditional and advanced approaches coexist, and underscores the need for continuous innovation to optimize the delivery and efficacy of these therapies. This is notably the case for certain recombinant proteins, where PEGylation has been commonly employed to extend their circulation time, limit renal excretion, enhance stability, and reduce immunogenicity. Additionally, it remains a popular choice due to its cost-effectiveness when compared to alternative polymer technologies. Promisingly, recent advancements, such as the development of heterobifunctional PEG derivatives like the recently approved Elfabrio, innovative strategies like antibody-protein-conjugates, and ongoing research in conjugation methods such as site-specific PEGylation, promise the emergence of more PEGylated drugs. These developments are set to expand the scope of protein drug candidates, enabling the production of proteins with superior properties compared to their native counterparts. Furthermore, PEGylation might have a significant impact on the development of innovative combination therapies by crosslinking various therapeutic agents through a single PEG molecule. Conjugation with fatty acids, which inherently bind to albumin *in vivo*, or fusion to larger proteins such as serum albumin and Fc fragments of immunoglob-

ulins, has also been used for the delivery of certain biotherapeutics to endow them with higher *in vivo* stability and longer circulation times.

Conversely, other therapeutics are witnessing a transformative evolution where the delivery strategy becomes an integral, inseparable part of the therapeutic agent, emphasizing the symbiotic relationship between drug and carrier. This paradigmatic shift not only enhances the potency of drug delivery but also underscores the pivotal role of integrated delivery moieties in shaping the future landscape of pharmaceutical therapies, as exemplified by ADCs. These intelligent carriers can respond to specific physiological cues and release therapeutic agents in a controlled and selective manner. The remarkable surge in this modality is evidenced by the approval of 13 ADCs in recent years, with hundreds more undergoing clinical investigations. The majority of the approved ADCs are based on enzyme-sensitive linkers. Advances in linker stability and specificity should be achieved for improved efficacy and lower toxicity. Furthermore, the prevalent use of random chemical conjugation, primarily involving lysine and cysteine residues, poses challenges in terms of quality control and stability. Exploring novel site-specific conjugation strategies, such as incorporating engineered reactive cysteine residues or unnatural amino acids, holds considerable promise. The future of ADCs in cancer therapy, as well as other fields, holds immense promise, driven by continuous research in targeted drug delivery, innovative linker technologies, combination therapies, and personalized medicine.

Biopharmaceuticals are delivered through various administration routes to optimize delivery, efficacy, and patient convenience. Gene therapies are typically administered through the parenteral route. However, topical administration is preferred for gene therapeutics targeting localized diseases, like Luxturna, to reduce systemic exposure and maximize the local therapeutic effect, albeit requiring specialized equipment and expertise. While DNA gene therapies are administered intravenously as a one-time treatment, subcutaneous injection is more convenient for RNA therapeutics such as GalNAc-based siRNA conjugates, improving patient adherence, particularly in chronic therapies. The majority of approved proteins and peptides are administered subcutaneously to be absorbed slowly and steadily into the bloodstream from the subcutaneous tissue, allowing for extended release. However, certain peptides, including GLP-1 receptor agonists, are currently available in oral formulations. This is achieved through the incorporation of permeation enhancers that increase their lipophilicity, enabling them to cross the gastrointestinal epithelium. In parallel, the market for packaging innovations is expanding rapidly to meet the rising demand for approved biopharmaceuticals. Parenteral packaging devices, such as pen injectors, auto-injectors, and prefilled syringes designed for self-administration and subcutaneous delivery of biotherapeutics, have made it easier and more convenient for patients to receive accurate doses. This is critical for maintaining therapeutic efficacy and safety.

For successful clinical translation of biopharmaceuticals, maintaining a balance between innovation, efficiency, and safety is of utmost importance. The International Council for Harmonization (ICH) offers essential guidelines for the safety evaluation of biotechnology-derived drugs. Still, specific instructions on assessing certain biologics toxicities are lacking. Besides the previously mentioned issues, modified versions of biotherapeutics, including the PEGylated ones, are generally considered new biologics requiring clinical evaluations and a rigorous and costly regulatory approval process, which may represent a substantial barrier to the clinical advancement of biopharmaceuticals drug delivery. Furthermore, integrating drug delivery approaches with biopharmaceuticals may increase their manufacturing complexity. Issues such as loss of activity due to the incorporation of polymers, increased aggregation, heterogeneity, and low encapsulation efficacy, necessi-

tate more control studies to ensure stability, quality, and safety. Consequently, these factors contribute to elevated production costs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

Makhloufi Zoulikha: Writing – original draft. **Zhongjian Chen:** Writing – review & editing. **Jun Wu:** Writing – review & editing. **Wei He:** Writing – review & editing, Supervision, Conceptualization.

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Supplementary materials

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