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What is the future of PEGylated therapies?

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Abstract

The tremendous potential of biologic drugs is hampered by short half-lives in vivo, resulting in significantly lower potency than activity seen in vitro. These short-acting therapeutic agents require frequent dosing profiles that can reduce applicability to the clinic, particularly for chronic conditions. Therefore, half-life extension technologies are entering the clinic to enable improved or new biologic therapies. PEGylation is the first successful technology to improve pharmacokinetic (PK) profiles of therapeutic agents and has been applied in the clinic for over 25 years. Over 10 PEGylated therapeutics have entered the clinic since the early 1990s, and new PEGylated agents continue to expand clinical pipelines and drug patent life. PEGylation is the most established half-life extension technology in the clinic with proven safety in humans for over two decades. Still, it is one of the most evolving and emerging technologies that will be applied for the next two decades.

1. Background

PEGylation is a commonly utilized technique for bioactive molecules (proteins, peptides, enzymes, antibody fragments, oligonucleotides, small synthetic drugs, etc.) that are generally limited by poor physiochemical and PK properties in vivo. After covalent attachment of PEG, molecules can have prolonged blood circulation half-lives, improved drug solubility and stability, and reduced immunogenicity [1]. Each ethylene glycol subunit in PEG associates with two to three water molecules making PEGylated molecules about five to ten times larger than a soluble protein of a similar molecular mass [2]. Because the kidneys filter substances based on size, PEGylated molecules that have a higher molecular weight and larger hydrodynamic radius than the parent molecule are cleared from the body at a much slower rate. This decreased rate increases the half-life of the PEGylated molecule in vivo. In addition to having a fast clearance in vivo, many native type proteins and peptides are also rapidly degraded by circulating enzymes via proteolysis. The hydrated PEG chain

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protects the conjugated compound from access to proteases and peptidases by steric hindrance and therefore reduces the drug's nonspecific degradation. These positive advantages have made PEGylation a common technique in the pharmaceutical industry. On the other hand, these properties can greatly reduce the drug's potency.

Since its discovery, PEG chemistry experienced enormous enhancements. First-generation PEGylation involved random PEG conjugation along the biomolecule. Even with extensive purification, non-specific PEGylation resulted in multiple isoforms of products with varying physicochemical and pharmaceutical properties. Second-generation PEGylation experienced the benefits from an array of PEG derivatives – activated PEG molecules, higher molecular weight PEG polymers, branched PEG structures. Activated PEG molecules enabled site-specific PEGylation that reduced the amount of impurities and side products during synthesis of PEGylated therapeutics [3]. Branched PEGylation decreases immunogenicity and increases half-life better than linear PEG, but sacrifices activity of the biomolecules. Third generation technologies aim to preserve the drug's bioactivity.

2. Marketed PEGylated therapeutic agents

PEGylated drugs are estimated to be a multi-billion dollar market [4]. Over 10 PEGylated drugs have been approved by the FDA to date with over 20 drugs in clinical trials (**Table 1**). Adagen® and Oncaspar® make up the first generation of PEGylated products that were FDA approved in the 1990s. They are both randomly PEGylated enzymes at multiple sites (>60 sites) along the protein using small molecular weight PEGs (5 kDa). Enzon Pharmaceuticals, Inc., who marketed the drugs, successfully branded PEGylation as an effective half-life extension technology. At the turn of the millennium, an explosion of PEGylated products entered the market. Matched with the discoveries of 2nd generation PEG, the FDA-approved PEGylated drugs were diverse in type (proteins, aptamers antibodies) and PEG characteristic (size, shape). Branched PEG motivates a single site on the molecule for PEG binding and enables a more homogenous mixture. Examples of successful, site-specific PEGylation sites include the N-terminus via aldehyde-functionalized PEG molecules (e.g. Neulasta®), free cysteine that is either naturally present or genetically introduced via thiol-selective PEG molecules (e.g. Cimzia®), a native disulfide bond or histidine tag. Although some PEGylated pharmaceuticals are still made as isomeric mixtures (e.g. PegIntron®, Somavert®), site-specific PEGylation with 40 kDa is now considered the gold standard for PEGylation of therapeutic molecules.

3. PEGylated agents in clinical trials

There are over 20 PEGylated drugs undergoing clinical trials (**Table 2**). Most commonly these drugs are PEGylated with larger PEGs (40 kDa) than the first-generation therapeutics, but are similarly applied to chronic conditions where frequent injections limit patient compliance and diminish treatment results. Bayer, Baxter-spin off Baxalta, and Novo Nordisk have a total of four long-acting hemophilia drugs based on PEGylated recombinant coagulation factors in Phase III clinical trials. Hemophilia is a rare genetic disorder where the blood cannot clot effectively because it lacks certain blood clotting factors. As an on-demand or prophylaxis treatment, those blood clotting factors are substituted to the patient

by injections and infusions. Longer-acting solutions are needed to avoid frequent dosing. Bayer's BAY94-9027 has been investigated as a prophylactic treatment at one to two infusions per week, while the majority of replacement clotting factors require infusions up to three times a week. The long-acting strategy requires site-specific PEGylation via a maleimide linker using a 60 kDa branched PEG to a B-domain deleted recombinant factor VIII that offers a 19 h half-life in humans. Just like BA94-9027, Baxalta's Bax855 and Novo Nordisk's N8-GP and N9-GP are undergoing a Phase III clinical for on-demand or prophylaxis treatment to investigate complete pharmacokinetic properties. The first FDA-approved long-acting Hemophilia A and B drugs utilized Fc fusion technology with the coagulation factor. Half-life extension technologies other than PEGylation will be discussed in **Section 4**. In addition to long-lasting antihemophilic agents, a number of PEGylated aptamers are in clinical trials, after the first, Pfizer's Macugen®, was FDA-approved in 2004. Noxxon Pharma developed synthetic oligonucleotides using non-natural L-nucleotides and PEGylated the aptamers with 40 kDa branched PEG. The PEGylated agents are in Phase II clinical trials for cancer, anemia and damage to kidneys due to diabetes. Ophthotech is also investigating PEGylated aptamers using 40 kDa branched PEG for wet and drug macular degeneration. If marketed, these drugs may prevent frequent injections into the eye and avoid numerous outpatient visits. Nektar's Movantik -181 and -171 use small molecular weight PEGs (<10 kDa) to prevent blood-brain penetration while maintaining the drug's oral bioavailability. By restricting central nervous system uptake of the active pharmaceutical ingredients (APIs) (i.e. opioid analgesic and sodium channel blocker), Nektar's PEGylation approach reduces abuse liability and side effects like dizziness and drowsiness. PEGylated agents under clinical investigation are more diverse in PEG and drug type over the first FDA-approved PEGylated drugs because of the increased control and confidence of PEGylation. Although our focus here is half-life extension, PEGylation is applied to therapeutics for ionic repulsion in proteins or surface charge shielding.

4. Long-acting agents in clinical pipelines using other half-life extension technologies

Based on the successful pharmaceutical application of PEGylation and the growing interest in complex therapeutic biologics, new half-life extension strategies have been developed as an alternative to PEGylation. After PEGylation, albumin and Fc fusion technologies are common techniques to increase biological drug's persistence in vivo. They take advantage of the intrinsic properties of albumin and antibodies that have extraordinary long half-lives in vivo because they bind to neonatal Fc receptors (FcRn) to prevent endosomal degradation and have large hydrodynamic diameters to prevent clearance. In Fc fusion technology, the Fc region of an antibody is genetically linked to a protein of interest to increase the molecules' circulation half-life, stability and solubility. Many questions remain for the long-term use of Fc fusion proteins because of the immunological consequences. Nonetheless, 11 Fc-fusion proteins have been FDA approved since 1998, including blockbusters like Regeneron's Eylea and Amgen/Pfizer's Enbrel. Wild-type or engineered albumin can be fused (e.g. Novozyme's Albufuse®) or conjugated to the therapeutic protein. Additionally, albumin-binding sites, such as peptides, antibodies or fatty acid chains, can be introduced to the pharmaceutical agent to promote albumin attachment in vivo. More recently, half-life extension technologies

in clinical trials depend upon unique polypeptides. Versartis' XTEN technology requires the fusion or chemical conjugation of a large polypeptide consisting of over 800 natural, hydrophilic amino acids that are designed to be non-immunogenic and biodegradable [5]. PhaseBio's technology relies on elastin-like polypeptides (ELPs) that utilize the transition temperature of elastin to deliver drugs using a depot-release strategy. These protein and polypeptide-based alternatives cannot offer the same increase in apparent molecular weight as PEG (PEG is 5-10x larger than proteins of similar molecular mass). Therefore, such technologies may require molecules at a higher size, e.g. over 80 kDa, to increase half-life, which can greatly reduce potency for proteins or peptides less than 80 kDa in size. As a result, large doses of peptide and protein drugs may be administered with alternative technologies.

A loss of potency to the API is shared among most half-life extension technologies. The conjugation or fusion of a large molecular weight molecule to a drug, especially when the drug is comparatively equal or smaller in size like a peptide, can impede on the biomolecule's active site and reduce binding events to its intended target. Therefore, a large dose, which may carry increased risks of adverse effects, is generally required to achieve the intended pharmaceutical effect. Site-specific PEGylation or carefully designed genetic sequences are developed to reduce this effect.

5. Expert Opinion – Future of PEGylation

The first PEGylated drug was FDA-approved 25 years ago. Since that time, PEGylation technology progressed from random to site-specific PEGylation, from the use of multiple linear PEGs to singular and branched PEGs, from small 5 kDa PEGs to 40 kDa PEGs. In addition, PEGylation is now being applied to a more diverse set of therapeutic agents, like aptamers, peptides and even small molecules (Table 1 and 2). As a result, PEGylated therapeutic agents are expanding clinical pipelines for many biotech companies.

Because PEGylated drugs have a long track-record in the clinic, PEGylation will drive the market demand for low dose, longer-acting therapies. The demand is particularly necessary for chronic conditions where frequent dosing impedes patient compliance to treatment. With partnerships between half-life extension platform companies and biotech companies, long-acting therapies in a number of indications may be discovered to meet these demands. Also, applying these technologies to FDA-approved drugs can prolong the company's competitive advantage (i.e. patent life) of blockbuster drugs.

Third-generation PEGylation looks to minimize the current trade-off between potency and circulation half-life for longer-lived drugs. One technique is to utilize releasable PEG conjugates as a prodrug approach, pioneered by Enzon Pharmaceuticals. Customized linkers between the PEG conjugate and the API can reversibly bind the PEG conjugate with the therapeutic molecules and transform the drug to its active form after delivery in vivo (e.g. NKTR-102). Another approach is to create customizable PEGylation sites on proteins, like Ambrx's ReCODE and EuCODE technologies, to reduce steric hindrance of the API. Both companies are partnering with large pharma to enable drug candidates that suffer from poor PK profiles. Next-generation techniques, like ones we have developed, introduce unique

PEG shapes that have high hydrodynamic radii but reduce steric hindrance of the API's active site. PEG size and shape, like choosing the site of PEGylation, can greatly affect therapeutic potency of the parent molecule and require careful bioactivity screening during drug development. With an influx of new techniques, assays to track and characterize the released PEG molecules are increasingly requested by regulatory bodies [6].

Therapies utilizing new half-life extension strategies will undoubtedly follow PEGylated products onto the market. However, immunogenicity must be de-risked for longer-acting fusion proteins, as well as PEGylated drug. Numerous repeated doses over the lifetime of a patient can greatly increase the risk of an immune response to the “new” fused protein, even if it consists of proteins that may pose no risk separately. Just as fusion proteins may have unique effects in the body, so do PEGylated drugs. Biodistribution and accumulation of PEG is directed by the biomolecule it binds. Longer-acting therapies may impart additional effects in vivo, besides just improved pharmacokinetic parameters that require long-term investigation. The development of antibodies against PEG has been induced in humans after certain PEG-drug treatment (89% of gout patients treated with Krystexxa) [7] or pre-existing (healthy donor prevalence up to 25% [8]). The presence of PEG antibodies may induce rapid clearance of certain drugs, like Oncaspar [9]; while in other cases, it can show no impairment to the therapy. It seems that the development of PEG antibodies greatly depends on the protein itself, rather than PEG alone [7]. Therefore immunogenicity of approved PEGylated compounds (Table 1) should be carefully monitored on a drug-by-drug basis over the long-term, and patients may need to be pre-screened before treatment with new drugs (Table 2). Although some clinical concerns may have been raised for high molecular weight PEGylated drugs, available safety data in animal models and humans do not indicate concerns [10]. Vacuolation in macrophages has been observed with the use of Cimzia and other PEGylated proteins at high doses over a long time. However, macrophage vacuolation has caused no functional effects in animal models and there have been no PEG-related adverse effect in clinical studies of Cimzia. Generally, clinical doses of PEGylated drugs are drastically lower than required for PEG toxicity [11]. Compared to emerging non-PEGylation half-life extension technologies, PEGylation holds a clear advantage for clinical development – its 20-year utility in humans with proven safety. No other half-life extension technology has been applied in the clinic for such a long time. The benefits of pharmaceutical agents come with certain caveats and cannot be absolutely safe; but, the extended application of PEGylated agents has demonstrated therapy with little tradeoff.

The growing investment of PEGylated products by many pharma companies exemplifies the potential PEGylation holds for future drug development. As PEGylation techniques continue to evolve, new PEGylated agents will address the growing demand for stable, longer-lasting drugs. PEGylation is the most established half-life extension platform in the clinic, yet, it also remains one of the most evolving and emerging. PEGylation will continue to expand not only the biologics market but also drug development as a whole for the next two decades.

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Table 1

PEGylated Drugs Approved by FDA

Brand Name	Company	Year Approved	PEGylated Molecule	Indication(s)	PEG#
Adagen	Enzon	1990	enzyme	Severe combined immunodeficiency disease	multiple × 5 kDa
Oncaspar	Enzon	1994	enzyme	Acute lymphoblastic leukemia	69-82 × 5 kDa
Doxil	Ortho/Schering-Plough	1995	liposome	Cancer	
PEGasys	Roche	2001	protein	Hepatitis B&C	40 kDa, branched
PegIntron	Schering-Plough	2001	protein	Hepatitis C	12 kDa
Neulasta	Amgen	2002	protein	Chemotherapy induced neutropenia	20 kDa
Somavert	Pfizer	2003	protein	Acromegaly	4-6 × 5 kDa
Macugen	Pfizer	2004	aptamer	Neovascular age-related macular degeneration	2 × 20 kDa
Mircera	Roche	2007	protein	Anemia associated with chronic kidney disease	30 kDa
Cimzia	UCB	2008	FAB' fragment	Crohn's disease, Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	40 kDa, branched
Krystexxa	Savient	2010	enzyme	Gout	9-11 × 10 kDa
Sylatron	Merck	2011	protein	Melanoma [*]	1 × 12 kDa
Omontys	Affymax/Takeda ^{**}	2012	peptide	Anemia associated with chronic kidney disease	1 × 40 kDa, branched
Movantik	AstraZeneca/Nektar	2014	small molecule, oral	Non-cancer opioid-induced constipation	<1 kDa

[#] PEG is linear unless otherwise noted

^{*} with nodal involvement after surgical resection

^{**} recalled for hypersensitivity

Table 2

PEGylated Drugs in Active Clinical Trials

Name	Company	Phase	PEGylated Molecule	Indication(s)	PEG #
BAY94-9027	Bayer	3	protein	Hemophilia A	60 kDa, branched
Bax855	Baxalta	3 ^{***} 3	protein	Hemophilia A	2-20 kDa
Peglispro	Eli Lilly	3	protein	Type 1 and 2 Diabetes	20 kDa
N8-GP	Novo Nordisk	3	protein	Hemophilia A	40 kDa, branched
N80-GP	Novo Nordisk	3	protein	Hemophilia B	40 kDa, branched
ADI PEG-20	Polaris Group	3	enzyme	Cancer	20 kDa
Revolixys kit (pegnivacogin)	Regado biosciences	3 [◆]	aptamer	Percutaneous coronary intervention	40 kDa, branched
NKTR-181	Nektar	3	small molecule, oral	Chronic pain	<1 kDa
Fovista	Ophthotech/Nektar	2	aptamer	Wet age-related macular degeneration	40 kDa, branched
NOX-A12	NOXXON Pharma	2	aptamer	Multiple myeloma and chronic lymphocytic leukemia	40 kDa, branched
NOX-E36	NOXXON Pharma	2	aptamer	Diabetic nephropathy	40 kDa, branched
NOX-H94	NOXXON Pharma	2	aptamer	Erythropoiesis-stimulating agent-hyporesponsive anemia	40 kDa, branched
Zimura, ARC1905	Ophthotech	2	aptamer	Age-related macular degeneration	40 kDa
PEGPH20	Halozyme	2	enzyme	Pancreatic cancer, orphan drug	20 kDa
pegvaliase	BioMarin	2	enzyme	Phenylketonuria	20 kDa
BMS-986036	Bristol-Myers Squibb/Ambix	2	proteins	Type 2 diabetes, NASH	30 kDa
Peg adreomedullin	Bayer	1	peptide	Lung Disease	40 kDa
NKTR-102	Nektar	1-3	small molecule	Many cancer indications	20 kDa, 4-arm
NKTR-171	Nektar	1	small molecule	Neuropathic pain	<1 kDa
PEG-SN-38	Prolynx	1	small molecule	Solid tumors	40 kDa, 4-arm

PEG is linear unless otherwise noted

*** Biologics License Agreement filed in US

◆ Clinical Hold