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Glycosylation of Therapeutic Proteins: An Effective Strategy to Optimize Efficacy

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Abstract

During their development and administration, protein-based drugs routinely display suboptimum therapeutic efficacies due to their poor physicochemical and pharmacological properties. These innate liabilities have driven the development of molecular level strategies to improve the therapeutic behavior of protein drugs. Among, the currently developed approaches, glycoengineering is one of the most promising due fact that it has been shown to simultaneously afford improvements over most of the parameters necessary for optimization of protein drug *in vivo* efficacy (e.g., *in vitro* and *in vivo* molecular stability, pharmacodynamic responses, and pharmacokinetic profiles) while allowing for targeting to the desired site of action. The intent of this article is to provide an account of the effects that glycosylation has on the therapeutic efficacy of protein drugs and to describe the current understanding of the mechanisms by which glycosylation leads to such effects.

In recent decades there has been an accelerated drive towards the increased development of protein based drugs due to their great economic and clinical importance. Although proteins display multiple therapeutically favorable properties (e.g., higher target specificities, pharmacological potencies, and frequently lower side effects) their development and employment is often hindered as these routinely display suboptimum therapeutic efficacies due to intrinsic limitations in their physicochemical and pharmacological properties. (1–17) As a result, there is great interest in the development and employment of molecular level approaches to improve the therapeutic efficacy of protein drugs by engineering their physicochemical and pharmacological properties.(17–23) A promising approach being currently employed involves the strategic manipulation of the protein's surface glycosylation patterns through glycoengineering.(13,24–29) Even though a vast amount of studies have demonstrated that glycosylation can lead to enhanced therapeutic efficacies for protein drugs, many aspects regarding the mechanisms by which glycosylation induces such effects remain unclear. The intent of this article is therefore to provide an account of the current understanding of the mechanisms by which glycosylation improves the therapeutic efficacy of protein drugs. This is achieved by presenting a survey of the principal physicochemical and pharmacological aspects limiting the therapeutic effectiveness of protein drugs, by addressing which of these can be improved by glycosylation, and by discussing the currently proposed mechanisms for such effects.

1. Intrinsic Limitations to Protein Therapeutic Efficacy

Protein drugs routinely display suboptimum therapeutic efficacies due to their inherently poor physicochemical and pharmacological properties. While poor physicochemical properties for

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protein drugs mainly arise from low *in vitro* and *in vivo* molecular stabilities their poor pharmacological properties are due to adverse pharmacodynamic (PD) responses and limited pharmacokinetic (PK) profiles.(9,14–16) All of these liabilities can diminish the clinical effectiveness of protein drugs by affecting their systemic bioavailability.

1.1 Molecular Instability

Proteins drugs generally display low *in vitro* molecular stabilities during their pharmaceutical development lifecycle due to their inherently liable structural elements coupled with several innate physical and chemical instabilities.(13) This problem is further compounded in a pharmaceutical setting as protein drugs are routinely exposed to several destabilizing environments during their production, purification, storage, and delivery. (4,11,30,31) For example, the backbone and amino acid side chains of protein drugs can be subject to several chemical instability issues (e.g., chemical hydrolysis, fragmentation, crosslinking, oxidation, deamidation, β-elimination, and racemization) due to their potential to undergo acid-base and redox chemistries. (9,32–34) Additionally, the secondary and tertiary structural elements of proteins which are requisite for function can also be affected by physical instability issues; such as, irreversible conformational changes, local and global unfolding, due to their non-covalent nature.(4,6,30,35) Protein drugs are also prone to pH, temperature, and concentration dependant precipitation, surface adsorption, and non-native supramolecular aggregation as a result of their colloidal properties.(3,11,36–40) Furthermore, once administered their *in vivo* molecular stability becomes a limiting issue as their structure is susceptible to extra- and intra-cellular enzymatic degradation.(16) If left unaddressed these *in vitro* and *in vivo* molecular instability issues can adversely impact the therapeutic efficacy of protein drugs due to the direct dependency of pharmacological properties (PK/PD) on the amount of functionally active protein that is administered and persistent in circulation.(4,13,16)

1.2 Adverse Pharmacodynamic and Pharmacokinetic Profiles

Achieving optimum therapeutic efficacy is dependant on maintaining a proper balance between drug exposure and effect. Therefore, the PK and PD parameters for therapeutics are often tuned through the drug design lifecycle in a manner assuring that desired *in vivo* responses are achieved. PK refers to the time dependency of drug action (dose/metabolic profiles) and is influenced by drug absorption, distribution, and excretion as well as initial response times and duration of effects. PK parameters usually determined for protein drugs include circulatory half-lifes, volumes of distribution, clearance rates, and total bioavailability. PD examines the potency of drugs (dose/response profiles) through the study of *in vitro* activities. For protein based drugs PD parameters usually determined are enzymatic rates and receptor binding affinities. Protein based drugs usually display limited PK profiles and sharp PD responses as a result of their poor physicochemical and pharmacological properties.(15) Limited PK profiles are mainly evidenced by adverse local adsorption and systemic distribution patterns for subcutaneously (SC) administered protein drugs as result of variable protein hydropathy (hydrophilic/hydrophobic surface balance) and by fast excretion rates (e.g., short circulation half-lifes) for intravenously (IV) administered ones due to rapid elimination from the body through proteolytic, renal, hepatic, and receptor mediated clearance mechanisms.(15,16)

All protein drugs are susceptible to some level of clearance through non-specific proteolytic degradation due to the ubiquitous nature and systemic distribution of proteases. Therefore, protein catabolism is not limited to the gastrointestinal, renal, and hepatic tissues but can also occur in the blood and at other tissues. (16) Such non-specific cleavage events negatively affect efficacy through inactivation of the protein drug.(13) For smaller sized protein drugs clearance occurs mainly at the kidneys with renal clearance occurring through three main routes.(16, 41) The first route, glomerular filtration, is controlled primarily by the kidney's size permeaselectivity with the urine/blood filterability being greatly reduced for proteins with

molecular weights and hydrodynamic radius greater that 50 kDa and 60 A.(42–44) Proteins exceeding this molecular weight limit are eliminated by other pathways; mainly proteolytic degradation, hepatic uptake, and immune clearance. The second factor affecting kidney permeaselectivity is the protein surface charge. Filtration of highly charged proteins (both anionic and cationic) is retarded by the presence of negatively and positively charged proximal and distal elements within the renal glomerular basement membrane and epithelium.(16,43) The second route of renal elimination; which applies mainly to small linear peptides, occurs through hydrolysis by brush border enzymes located on the luminal membrane followed by reabsorption. A third and less frequent route involves peritubular extraction from postglomerular capillaries followed by intracellular degradation. For larger sized proteins clearance occurs mainly at the liver through both specific and non-specific hepatic uptake mechanisms.(16) Specific clearance occurs through receptor-mediated (e.g. asialoglycoprotein and low density lipoprotein receptors) endocytosis at the hepatocytes. This process is interestingly regulated by the glycosylation state of proteins (discussed further on). Alternatively, non-specific hepatic clearance of proteins can also occur through phagocytosis in the reticuloendothelial system. Additional non-hepatic receptor mediated specific elimination mechanisms can occur whereas the protein drug is removed by endocytosis after binding to its therapeutic target receptor. This process is influenced by the protein drug potency with stronger receptor binders being removed from the systemic circulation faster.

As a result of the innate susceptibility of proteins to all of these clearance mechanisms, protein drugs generally display limited plasma persistence lifetimes.(15) Higher protein concentrations and increased dosing frequencies are therefore often employed to achieve favorable therapeutic responses. Ironically, such frequent treatment regimes coupled with the high target specificities and potencies of protein drugs can lead to inappropriately sharp dose/response profiles. This can lead to overstimulation of the targeted pathway and in many instances trigger autoregulatory feedback inhibition mechanisms that can be therapeutically counterproductive in the long run by leading to loss of *in vivo* efficacy.(45,46) To counteract these innate limitations it has become routine practice to integrate molecular level technologies to engineer the physicochemical and pharmacological properties of protein drugs (second-generation biopharmaceuticals) in the early stages of their development lifecycle.(47) Established technologies that have been shown to significantly improve the efficacy of protein drugs by increasing their molecular stabilities and plasma persistence times and by decreasing their PD responses through various mechanisms include: targeted mutations, generation of fusion proteins and conjugates, glycosylation engineering, and pegylation.(17–19,21–24,48) Of these, engineered glycosylation is one of the most promising due fact that it has been shown to simultaneously afford improvements over most of the molecular parameters necessary for optimization of therapeutic efficacy while allowing for targeting to the desired site of action.(13,24–29)

2. Protein Glycosylation

A substantial fraction of the currently approved protein pharmaceuticals need to be properly glycosylated to exhibit optimum therapeutic efficacy (Table I). This is due to the fact that glycosylation can influence a variety of physiological processes at both the cellular (e.g. intracellular targeting) and protein levels (e.g. protein-protein binding, protein molecular stability).(24,49,50) Glycosylation refers to the covalent attachment of carbohydrate based molecules (glycans) to the protein surface. Glycosylation is the most prevalent and structurally complex of the chemical modifications that occur naturally in proteins.(51–56) In this context glycosylation can display structural heterogeneity with respect to both the site of glycan attachment (macro-heterogeneity) and with respect to the glycan's structure (micro-heterogeneity). Additionally, since all of the potential glycosylation sites are not occupied

simultaneously this can lead to the formation of glycoforms with differences in the number of attached glycans.

In humans the most prevalent glycosylation sites occur at asparagine residues (N-linked glycosylation through the Asn-X-Thr/Ser consensus sequence) and at serine or threonine residues (O-linked glycosylation).(57,58) Further structural complexity occurs due to variability in the glycan's monosaccharide sequence order, branching pattern, and length. Nonetheless, certain glycan core structures have been identified with these being formed by the enzymatic bridging and remodeling of the following monosaccharides: fucose (Fuc), galactose (Gal), mannose (Man), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and N-acetylneuraminic acid (sialic acid).(55,59) In humans three principal N-linked core glycan structures are formed with these being classified according to their monosaccharide content and structure: high mannose type ($Man_{2\text{-}6}Man_3GlcNAc_2$), mixed type ($GlcNAc_2Man_3GlcNAc_2$), and hybrid type ($Man_3GlcNAcMan_3GlcNAc_2$).(59) For O-linked glycans four principal core structures have been identified: core 1 (GalGalNAc), core 2 (GalGlcNAcGalNAc), core 3 (GlcNAcGalNAc), and core 4 ($GlcNAc_2GalNAc$). The terminal ends of these glycan core structures are often further functionalized (e.g., phosphates, sulfates, carboxylic acids) with chemically charged glycans (e.g., sialic acid) in human glycoproteins, leading to even greater structural diversity. These functionalized terminal glycans can alter the protein's surface charge and isoelectric point (pI) which have been related to the increased circulatory lifetimes for glycoproteins.(23) Such structural diversity poses certain problems for glycoprotein based drugs as it has been found that variations in the expression system (Table I) or changes to the manufacturing process can lead to changes in glycosylation patterns.(60) Accordingly, alternative methods of glycoprotein production are being explored to design protein drugs with homogeneous structurally defined glycosylation patterns through genetic, enzymatic, and chemo enzymatic methods.(48,61–75) The reader is referred to the following recent reviews for extensive discussions on the details of different glycoprotein production systems.(62,76–88)

3. Optimization of Protein Therapeutic Efficacy by Glycosylation

3.1 Effects of Glycosylation on Protein Molecular Instability

A vast amount of studies have demonstrated that natural glycosylation increases the molecular stability of proteins (for a detailed mechanistic account refer to the recent review on glycoprotein biophysics by Solá *et al.*).(49) Furthermore, engineered glycosylation has been shown to stabilize a variety of protein drugs against almost all of the major physicochemical instabilities encountered during their pharmaceutical employment thus leading to enhanced *in vitro* molecular stabilities.(13) Pharmaceutically relevant protein instabilities which are improved by glycosylation include: oxidation; cross-linking; pH, chemical, heating, and freezing induced unfolding/denaturation; precipitation; kinetic inactivation; and aggregation. (13) Furthermore, these stabilization effects appear to be of a generalized nature since they have been shown to occur in a variety of structurally unrelated proteins.(13) Protein drugs whose stability has been reported to be increased by glycosylation include agalsidase alfa (REPLAGAL®, Shire) (aggregation, precipitation),(89) alglucosidase (MYOZYME®; Shire) (thermal denaturation),(90) alpha 1-antitrypsin (PROLASTIN®; Talecris Biotherapeutics) (chemical/thermal denaturation),(91) chymotrypsin (WOBE MUGOS®; Marlyn Nutraceuticals) (chemical/thermal/kinetic denaturation, aggregation),(92,93) choriogonadotropin alfa (OVIDREL®; EMD Serono) (thermal denaturation),(94) epoetin alfa (EPOGEN®/PROCRIT®; Amgen/Ortho Biotech) (thermal/pH/chemical/kinetic denaturation, oxidation, aggregation),(95–97) interferon beta (AVONEX®/REBIF®; Biogen/Pfizer EMD Serono) (disulfide crosslinking, precipitation, thermal denaturation, aggregation),(98–100) ranpirnase (ONCONASE®; Alfacell) (thermal denaturation),(101,102) lenograstim (GRANOCYTE®/NEUTROGIN®; Sanofi Aventis/Chugai Pharma) (thermal/pH/kinetic

denaturation, disulfide crosslinking),(103–105) thyrotropin alfa (THYROGEN®; Genzyme) (aggregation),(106) urokinase alfa (ABBOKINASE®; ImaRx Therapeutics) (thermal denaturation),(107) insulin (nondisulfide crosslinking, aggregation),(108) and various IgG-like antibodies (thermal denaturation).(109,110) These *in vitro* stabilization effects as a result of protein glycosylation have been directly related to the amount of glycans present in the protein drug (for a detailed account of each of the different stabilization mechanisms see the recent review by Solá and Griebenow).(13) Many of these *in vitro* stabilization effects have been proposed to lead to increased *in vivo* efficacies by possibly allowing for a greater amount of functional protein to be administered to the patient and by minimizing the formation of neutralizing antibodies due to the lack of conformationally altered and aggregated species in the end formulation.(13)

In addition to these *in vitro* stabilizing effects, glycosylation can also result in increased *in vivo* molecular stability for protein drugs once administered by leading to increased lifetimes for the functional forms of these proteins due to its prevention of proteolytic degradation.(13, 111–114) Some examples of therapeutically relevant proteins whose proteolytic susceptibility has been reported to be decreased by glycosylation include glucagons-like peptide 1,(115) lenograstim,(103,116) bucelipase alfa,(117) drotrecogin alfa (XIGRIS®; Eli Lilly),(118) ranpirnase,(101,102) thyrotropin alfa,(106) urokinase alfa,(119) interferon-γ (ACTIMMUNE®; Intermune),(120) and various IgG-like antibodies.(121) Proteolytic stabilization of proteins has also been related to the number of glycans bound to the protein surface; their length and branching; and the charges of their terminal end glycans therefore this effect can be influenced by both from steric and electrostatic repulsions induced by the surface bound glycans.(13,122) In this context, it was recently shown by Raju *et al.* that negatively charged glycans (e.g., those ending with sialic acids) are more efficient in preventing antibody proteolysis.(121)

3.2 Effects of Glycosylation on Protein Pharmacodynamics and Pharmacokinetics

The initial understanding of the role of glycans on protein *in vivo* circulatory behavior can be attributed in large part to the discovery of the hepatic asialoglycoprotein receptor by Aswell and Morell in the 1960's.(123–126) While studying the mechanisms controlling the circulatory turnover of ceruloplasmin, a protein involved in hepatolenticular degeneration (Wilson disease), Aswell *et al.* noticed dramatic differences in the circulatory lifetimes between the natively glycosylated protein (sialic acid terminated glycans) ($t_{1/2}$: ~ 56 hrs) and a partially deglycosylated variant of the protein (galactose terminated glycans) ($t_{1/2}$: < 30 min).(127) Subsequent studies by them and others extended these findings to other proteins (α_1-acid glycoprotein, α_2-macroglobulin, thyroglobulin, haptoglobin, fetuin, orosomucoid, ribonuclease) validating the generality of this specific hepatic clearance mechanism.(128–130) It was found that exposure of galactose terminating glycans through desialylation led to fast removal of the partially deglycosylated proteins from the circulation due to specific endocytosis mediated by asialoglycoprotein receptors expressed in the hepatocytes.(123,124, 129,131) Subsequently it was found that glycoproteins exposing glycans terminating in mannose, N-acetyl-glucosamine, or fucose could be also removed from the circulation due to specific interactions with other mammalian lectin-like receptors expressed at different cell types.(132–136) These pioneering studies highlighted several important facts about the effects of surface glycans on the circulatory behavior of glycoproteins: (i) improperly glycosylated proteins are rapidly removed from the circulation by specific receptor-based mechanisms, (ii) natively glycosylated proteins (sialic acid terminated) have longer circulating lifetimes than non-glycosylated proteins and partially glycosylated proteins, (iii) increased sialic acid and glycan content correlates with increased circulating lifetimes, (iv) depending on their glycosylation patterns proteins can be targeted to certain tissue types and organs. Accordingly most studies on the effects of glycosylation on the *in vivo* efficacy of proteins have emphasized

on the role of increased glycan content and glycan structure.(23) It is important to note that these lectin-like receptor based clearance mechanisms do not apply to all glycosylated biopharmaceuticals. An exception being IgG-like antibodies whose clearance is mediated via the neonatal Fc receptor (FcRn) and is not influenced by antibody glycosylation or glycoforms.(137) Glycosylation of protein drugs has been found to lead to improved therapeutic efficacy by increasing *in vivo* bioavailability, ambient circulating levels, and duration of effects through the modulation of their PK/PD properties. Changes to protein PK parameters induced by glycosylation include: improved absorption for small peptides,(138–142) modulated absorption for larger proteins,(143) improved distribution,(26,144) longer circulation lifetimes,(26,27,130,145–153) and decreased clearance rates.(24–27,29,42,[82],130,140,145–171) Alternatively glycosylation modulates protein PK parameters by leading to altered potencies as a result of diminished *in vitro* enzymatic activities and altered receptor binding affinities.(49,137,140,172,173) Therefore similarly as to what has been described to occur for other protein engineering methodologies (e.g. pegylation), glycosylation appears to modulate the *in vivo* efficacy of protein drugs by altering the balance between their potencies (PD) and exposure times (PK).(174) In the specific case of IgG-like antibody based therapeutics, glycosylation has been shown to improve their *in-vivo* therapeutic efficacy by altering their effector functions through modulated binding affinities for the FcγR receptor (for a mechanistic discussion see the following reviews).(21,175,176)

Examples of therapeutically relevant proteins whose *in vivo* efficacies have been reported to be increased by their natural glycosylation include: agalsidase alfa,(177–179) agalsidase beta (FABRAZYME®; Genzyme),(177,179–181) epoetin alfa and epoetin beta,(143,168,172,182,183) follitropin alfa (GONAL-F®; Merck/Serono) and follitropin beta (FOLLISTIM®; Schering-Plough),(159,160,169,184,185) insulin growth factor binding protein 6 (IGFBP-6),(163) lutropin alfa (LUVERIS®; Merck/Serono),(186–191) transforming growth factor β1,(192) antithrombin (ATryn®/TROMBATE-III®; Genzyme/Talecris Biotherapeutics),(162) thyrotropin alfa (THYROGEN®; Genzyme),(166) lenograstim,(103,193) sargramostim (LEUKINE®; Genzyme),(154,194,195) interleukin-3,(196) prourokinase,(151) lymphotoxin,(152,197) C1-esterase inhibitor (Berinert®; CSL),(198–200) IgG-like antibodies,(72,121,201) interferon beta,(98,202,203) coagulation factor VIIa (NOVOSEVEN®; Novo Nordisk),(204) coagulation factor VIII (moroctocog alfa),(155,156,205) coagulation factor IX (nonacog alfa) (BENEFIX®; Wyeth), and the p55 tumor necrosis receptor fusion protein.(158) In most of these studies increased circulatory lifetimes and improved *in vivo* activities have been attributed to reduced hepatic and renal clearance as well as diminished proteolytic degradation as a result of the presence of the charged glycans (terminal sialic acid). Expanding on this concept several studies have shown that increasing the number of sialic acid containing glycans beyond those of the native protein through engineered hyperglycosylation can effectively be employed as a technology to further optimize the circulatory half-life and *in vivo* activity of proteins.(24,27,206–208) Examples of pharmaceutically relevant proteins whose circulatory half-lifes were shown to be increased by hyperglycosylation include: interferon alfa and gamma,(26,120) luteinizing hormone,(149) Fv antibody fragments,(209) asparaginase,(210,211) cholinesterase,(164,165) darbepoetin alfa (AraNESP®; Amgen),(25,27,161,212–214) trombopoietin,(25,27,215) leptin,(25,27) FSH,(159,184,216,217) IFN-α2,(26) serum albumin,(145) and corifollitropin alfa.(218–222) Engineered glycosylation has been also employed to further optimize the *in vivo* pharmacological behavior of protein drugs by allowing for targeted delivery to disease-affected tissues.(48) This methodology has been mainly applied to treat the lysosomal storage diseases (e.g. Gaucher, Pompe, and Fabry disease; Hurler and Maroteaux-Lamy syndrome).(223) The employed strategy involves enzyme transport to the lysosomes by receptor-mediated endocytosis after targeting the mannose and IGF-II/cation-independent mannose 6-phosphate receptors.(224–228) This is achieved through glycoengineering of the protein glycans to expose at their terminal either mannose or mannose 6-phosphate. This strategy has been employed successfully with the following enzymes: β-glucocerebrosidase

(CEREZYME®; Genzyme),(229–231) α-glucosidase (MYOZYME®; Genzyme),(232) α-galactosidase (FABRAZYME®/REPLAGAL® Genzyme/Shire),(177,178) galsulfase (NAGLAZYME®; Biomarin Pharmaceuticals),(233) and α-L-iduronidase (ALDURAZYME®; Genzyme/Biomarin Pharmaceuticals).(234) For all of these enzymes increase therapeutic efficacy has been achieved by targeting the protein drug to the desired site of action.

4. Conclusions and Future Prospects

Design of protein therapeutics with optimized *in vivo* efficacy can be achieved through the simultaneous optimization of drug molecular stability, pharmacokinetics, pharmacodynamics, and targeting by engineered glycosylation. This technology can be employed to ameliorate a multitude of pharmaceutically relevant physicochemical and pharmacological problems. Mechanistically, it appears that the different glycosylation parameters (e.g., number of glycans attached, glycan's molecular size, sequence, and charge) can modulate the pharmacological properties of protein drugs to different extents. Engineered glycosylation could therefore provide ample future opportunities towards the improvement of protein drugs since in principle all of these glycosylation parameters can be simultaneously optimized.

While the pharmaceutical application of glycosylation still suffers from some technical challenges due to the intrinsically complex nature of glycan structures and the difficulties related to glycoprotein production in host-expression systems (e.g., low glycoprotein expression yields, glycan structural macro- and micro-heterogeneity), further advancements in the understanding of chemical- and enzyme- based glycan remodeling strategies being currently pursued by glycoengineering companies, will allow for the rational design of targeted glycoprotein structures. The significant potential that glycosylation engineering holds towards improving the therapeutic efficacy of protein drugs should lead to further research towards the understanding of the fundamental effects that glycans have on protein physicochemical and pharmacological properties.

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Table I

Partial List of Approved Glycoprotein-based BioDrugs

INN	Brand Name (Company)	Indication	Number of Glycans	Production System
Agalsidase alfa (galactosidase)	Replagal® (Shire)	Treatment of Fabry disease	6 N-Linked	HF cells
Agalsidase beta (galactosidase)	Fabrazyme® (Genzyme)	Treatment of Fabry disease	6 N-Linked	CHO cells
Alglucosidase alfa (glucosidase)	Myozyme® (Shire)	Treatment of Pompe disease	7 N-Linked	CHO cells
Alpha 1-antitrypsin (α1AT)	Prolastin® (Talecris Biotherapeutics)	Treatment of congenital α1AT deficiency with emphysema	3 N-Linked	Tissue fractionation (human placenta)
Antithrombin III	Atryn® (Ovation Pharmaceutics) Berinert® (CSL)	Prevention of peri-operative and peri-partum thromboelitic events	3–4 N-Linked	Milk fractionation (transgenic goats)
C1-esterase-inhibitor	Cinryze® (CSL)	Treatment of hereditary angioedema	6 N-Linked 7 O-Linked	Plasma fractionation (human)
Choriogonadotropin alfa	Ovidrel® (EMD Serono)	Treatment of female infertility	4 N-Linked 4 O-Linked	CHO cells
Darbopoetin alfa	ARANESP® (Amgen)	Treatment of anemia associated with chronic renal failure	5 N-Linked 1 O-Linked	CHO cells
Dornase alfa (Dnase)	Pulmozyme® (Genzyme)	Treatment of cystic fibrosis	2 N-Linked	CHO cells
Drotrecogin alfa (CF-XIV, Protein C)	Xigris® (Eli Lilly)	Treatment of severe sepsis	4 N-Linked	HEK cells
Epoetin alfa, beta, delta, omega, zeta	*	Treatment of anemia associated with chronic renal failure (CRF)	3 N-Linked 1 O-Linked	CHO cells
Eptacog alfa (CF VIIa)	NovoSeven® (Novo Nordisk)	Treatment of spontaneous and surgical bleedings in haemophilia A and B	2 N-Linked 2 O-Linked	BHK cells
Fibrinogen	Haemocomplettan® (CSL)	Haemorrhagic diatheses in hypo-, dys-, or afibrinogenaemia	5 N-Linked	Plasma fractionation (human)
Follitropin alfa	Gonal-F® (EMD Serono)	Treatment of female infertility	4 N-Linked	CHO cells
Follitropin beta	Follistim AQ® (Schering Plough)	Treatment of female infertility	4 N-Linked	CHO cells
Galsulfase	Naglazyme® (Genzyme)	Treatment of Maroteaux-Lamy syndrome	6 N-Linked	CHO cells
Glucocerebrosidase	Cerezyme® (Genzyme)	Treatment of Type I Gaucher disease	4 N-Linked	CHO cells
Hyaluronidase	Hylenex® (Baxter/Halozyme Therapeutics)	Ophthalmic surgery	3 N-Linked	CHO cells
Iduronidase alfa (laronidase)	Aldurazyme® (Genzyme)	Treatment of Mucopolysaccharidosis I	6 N-Linked	CHO cells
Idursulfase	Elaprase® (Shire)	Treatment of Mucopolysaccharidosis I	8 N-Linked	Human cells
Imiglucerase	Ceredase® (Genzyme)	Treatment of Type I Gaucher disease	4 N-Linked	Tissue fractionation (human placenta)
Insulin	*	Treatment of diabetes	*	*
Interferon-alfa-n3	Alferon N® (Hemisphere Rx) Avonex® (Biogen)	Treatment of external condylomata acuminata	1 O-Linked	HL cells

INN	Brand Name (Company)	Indication	Number of Glycans	Production System
Interferon beta-1a	Rebif® (Pfizer/EMD Serono)	Treatment of multiple sclerosis	1 N-Linked	CHO cells
Lenograstim (G-CSF)	Granocyte® (Chugai Pharma)	Treatment of chemotherapy induced neutropenia	1 O-Linked	CHO cells
Lutropin alfa	Luveris® (EMD Serono)	Treatment of female infertility	3 N-Linked	CHO cells
Monoclonal antibodies	*	Multiple indications	*	*
Moroctocog alfa (CF VIII)	*	Prevention and control of hemorrhagic episodes in patients with haemophilia B	7 N-Linked	CHO cells BHK cells
Nonocog alfa (CF IX)	Benefix® (Wyeth)	Treatment of hemorrhagic episodes in patients with haemophilia B	2 N-Linked 4 O-Linked	CHO cells
Sargramostim (G-CSF)	Leukine® (Genzyme)	Treatment after induction chemotherapy with acute myelogenus leukemia	2 N-Linked 4 O-Linked	Yeast cells
Tenecteplase	TNKase® (Genentech)	Treatment of acute myocardial infarction	3 N-Linked 1 O-Linked	CHO cells
Thyrotropin alfa	Thyrogen® (Genzyme)	Detection of thyroid cancer and hypothyroidism	3 N-Linked	CHO cells
Tissue plasminogen activator	Alteplase® (Genentech)	Treatment of acute myocardial infarction	3 N-Linked	CHO cells
Urokinase alfa	Abbokinase® (ImaRx Therapeutics)	Treatment of acute massive pulmonary emboli	1 N-Linked 1 O-Linked	HK cells

Information was obtained from the Prescribing Information (PI) for each product. Further information available at www.fda.gov and www.emea.europa.eu.

*Multiple approved products. Further information available at www.biopharma.com.

INN = International nonproprietary name.

CHO = Chinese hamster ovary

BHK = Baby hamster kidney

HEK = Human embryonic kidney

HF = Human fibroblast

HL = Human leukocytes