REVIEW

Recent advances in (therapeutic protein) drug development
[version 1; peer review: 2 approved]

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Abstract
Therapeutic protein drugs are an important class of medicines serving patients most in need of novel therapies. Recently approved recombinant protein therapeutics have been developed to treat a wide variety of clinical indications, including cancers, autoimmunity/inflammation, exposure to infectious agents, and genetic disorders. The latest advances in protein-engineering technologies have allowed drug developers and manufacturers to fine-tune and exploit desirable functional characteristics of proteins of interest while maintaining (and in some cases enhancing) product safety or efficacy or both. In this review, we highlight the emerging trends and approaches in protein drug development by using examples of therapeutic proteins approved by the U.S. Food and Drug Administration over the previous five years (2011–2016, namely January 1, 2011, through August 31, 2016).

Keywords
therapeutic protein drugs, protein therapeutics, cancer therapeutics, biosimilar, recombinant DNA-derived therapeutic proteins

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2 Yusuf Tutar, Cumhuriyet University, Sivas, Turkey

Any comments on the article can be found at the end of the article.
Protein engineering

The manufacturing and production of therapeutic proteins are highly complex processes. For example, a typical protein drug may include in excess of 5,000 critical process steps, many times greater than the number required for manufacturing a small-molecule drug (Figure 1a).

Similarly, protein therapeutics, which include monoclonal antibodies as well as large or fusion proteins, can be orders-of-magnitude larger in size than small-molecule drugs, having molecular weights exceeding 100 kDa (Figure 1b). In addition, protein therapeutics exhibit complex secondary and tertiary structures that must be maintained. Protein therapeutics cannot be completely synthesized by chemical processes and have to be manufactured in living cells or organisms; consequently, the choices of the cell line, species origin, and culture conditions all affect the final product characteristics. Moreover, most biologically active proteins require post-translational modifications that can be compromised when heterologous expression systems are used. Additionally, as the products are synthesized by cells or organisms, complex purification processes are involved. Furthermore, viral clearance processes such as removal of virus particles by using filters or resins, as well as inactivation steps by using low pH or detergents, are implemented to prevent the serious safety issue of viral contamination of protein drug substances. Given the complexity of therapeutic proteins with respect to their large molecular size, post-translational modifications, and the variety of biological materials involved in their manufacturing process, the ability to enhance particular functional attributes while maintaining product safety and efficacy achieved through protein-engineering strategies is highly desirable.

While the integration of novel strategies and approaches to modify protein drug products is not a trivial matter, the potential therapeutic advantages have driven the increased use of such strategies during drug development. A number of protein-engineering platform technologies are currently in use to increase the circulating half-life, targeting, and functionality of novel therapeutic protein drugs as well as to increase production yield and product purity (Table 1). For example, protein conjugation and derivatization approaches, including Fc-fusion, albumin-fusion, and PEGylation, are currently being used to extend a drug’s circulating half-life. Longer in vivo half-lives are of particular importance to patients undergoing factor/enzyme/hormone replacement therapy, in which frequent dosing regimens can result in substantial negative impacts on patient well-being in terms of ease of administration and compliance, especially in young children. Protein-engineering approaches have also been employed to target drugs through the addition of signaling peptides or the generation of antibody-drug conjugates, thereby limiting toxicity and increasing drug efficacy. Additionally, exploiting particular functional characteristics of a protein drug can be accomplished through protein engineering. For example, influencing a protein’s glycosylation pattern through engineering strategies can impact the protein’s receptor-binding properties and overall effector function. In Table 1, we have highlighted a few examples of the many technological innovations and protein-engineering platform technologies incorporated by recently approved therapeutic proteins.

Overview of recently approved protein therapeutics (2011–2016*)

Since 2011, the U.S. Food and Drug Administration Center for Drug Evaluation and Review (CDER) and the Center for Biologics Evaluation and Review (CBER) combined have approved 62 recombinant therapeutic proteins (*January 1, 2011, through August 31, 2016; “Purple Book” list of licensed biological products, including biosimilar and interchangeable biological products) (Figure 2a). Of these 62 therapeutic proteins, almost...
### Table 1. Protein-engineering platform technologies.

<table>
<thead>
<tr>
<th>Platform technology</th>
<th>Example of U.S. Food and Drug Administration-approved therapeutic protein</th>
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<tbody>
<tr>
<td><strong>Protein production technologies</strong></td>
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<tr>
<td>Production of proteins in transgenic animals</td>
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<td>Production of proteins in transgenic plants</td>
<td>Human glucocerebrosidase (Elysolso) produced in carrot root cells</td>
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<td><strong>Rational protein structure/function technologies</strong></td>
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<td>Glyco-engineering</td>
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<td>Fc fusion</td>
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<td>PEGylation</td>
<td>PEGylated IFNβ-1a (Plegridy)</td>
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<td>Antibody-drug conjugates</td>
<td>Humanized anti-HER2/neu conjugated to emtansine (Kadcyla)</td>
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<tr>
<td>mAb humanization/chimerism</td>
<td>Mouse/human chimeric anti-CD30 (Adcetris)</td>
</tr>
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</table>

**Listing of commonly used protein-engineering platform technologies and examples of U.S. Food and Drug Administration-approved therapeutic proteins (2011–2016, namely January 1, 2011, through August 31, 2016) that employ each strategy.**

- CD, cluster of differentiation
- CTLA-4, cytotoxic T-lymphocyte-associated protein 4
- Fc, fragment crystallizable
- GD2, disialoganglioside
- GLP-1, glucagon-like peptide-1
- HER2, human epidermal growth factor receptor 2
- IFNβ, interferon beta
- IL, interleukin
- LPAM-1, lymphocyte Peyer’s Patch adhesion molecule
- mAb, monoclonal antibody
- PD-1, programmed death receptor-1
- PEG, polyethylene glycol
- SLAMF7, SLAM family member 7
- TNFα, tumor necrosis factor alpha
- VEGFR, vascular endothelial growth factor receptor.
Figure 2. U.S. Food and Drug Administration (FDA)-approved therapeutic proteins (2011–2016*). (a) Bar graph showing the number of therapeutic protein FDA approvals by year (2011–2016*). (b) Pie chart showing the distribution of FDA-approved therapeutic proteins (2011–2016*) by drug class. (c) (Left) Pie chart showing the distribution of FDA-approved therapeutic proteins (2011–2016*) by therapeutic area. (Right) Pie chart showing the distribution of secondary therapeutic area for oncology drugs. *January 1, 2011, through August 31, 2016.
half (48%) were monoclonal antibodies (for this analysis, we included antibody-drug conjugates and antibody fragment antigen binding in this group). Coagulation factors were the next largest class (19%) of approved protein drugs over this time period. Replacement enzymes comprised 11% of all approvals. Remaining approvals (22%) were divided among fusion proteins, hormones, growth factors, and plasma proteins (Figure 2b). These U.S. Food and Drug Administration (FDA)-approved therapeutic proteins are indicated for a wide variety of therapeutic areas. Over half of the approved therapeutic proteins were indicated for oncology (26%) and hematology (29%), whereas the remaining 45% had primary indications in cardiology/vascular disease (5%), dermatology (3%), endocrinology (6%), gastroenterology (2%), genetic disease (2%), immunology (6%), infectious diseases (3%), musculoskeletal (8%), nephrology (2%), ophthalmology (3%), pulmonary/respiratory disease (3%), and rheumatology (2%) (Figure 2c, left). Of the 16 oncology drugs, six were approved to treat hematologic malignancies, whereas the remaining therapeutics were indicated for dermatology (3), gastroenterology (2), obstetrics/gynecology (2), pediatrics (1), pulmonary/respiratory disease (1), and urology (1) (Figure 2c, right). For a complete listing of the approved products, see Table 2. It is evident that recently approved therapeutic proteins serve a wide spectrum of patient populations and are of great benefit to public health.

Pathways for the development of novel therapeutics

The rapid advances in biomedical science and technology to address unmet medical needs also require that regulatory agencies ensure that such products are safe and effective. Several new pathways have emerged or have been finalized since 2011 and are summarized below (Table 3).

Breakthrough therapy designation

The Food and Drug Administration Safety and Innovation Act (FDASIA) was signed on July 9, 2012. FDASIA Section 902 provided the FDA with the ability to establish breakthrough therapy designation (BTD) as a new program within the Expedited Programs for Serious Conditions (19). BTD was designed to be available for drugs intended to treat a serious condition and that have been shown to exhibit initial clinical evidence of considerable improvement over pre-existing therapies. The BTD program joined other expedited development and review programs, including fast track designation (1997), accelerated approval (1992), and priority review designation (1992), which have promoted innovation by facilitating the expedited development and review of novel medicines. Since FDASIA was signed in July 2012, CDER has approved 30 original BTDs, one third (10) of which were protein drugs. Over this same period, 26% of CDER-approved biologics (10 out of 39) have been granted BTD designation (Table 4). Since July 2012, CBER has also approved two drugs under the BTD designation, but neither of these was a recombinant protein.

Orphan designation

Rare diseases substantially impact public health, as an estimated 7,000 different disorders collectively affect approximately 10% of the U.S. population, young children particularly, and many lack effective treatments (19). To promote the development of medicines that specifically address unmet medical needs, an orphan designation is given for drugs indicated for the treatment of fewer than 200,000 patients in the U.S. On June 12, 2013, the final regulations amending the 1992 Orphan Drug Regulations were issued (20). These amendments clarified and instituted minor changes to regulatory language, such as defining the term “orphan subsets”, the eligibility of designation for previously approved drugs, and the scope of orphan exclusive approval (https://www.gpo.gov/fdsys/pkg/FR-2013-06-12/pdf/2013-13930.pdf). Under the Orphan Drug Act (ODA) Final Rule, orphan designation grants incentives such as orphan exclusivity for a specific indication (7-year protection from competition), thereby promoting innovation in drug development to help treat patients in greatest need of novel medicines. This program appears to have fulfilled a real need, as 50% of the recently approved therapeutic proteins (31 out of 62) were licensed under the orphan designation (Table 5).

Biosimilars

Therapeutic protein drugs are now a critical component of the overall health-care industry and have revolutionized therapy options in many disease areas. These medications, however, are also some of the most expensive in the marketplace. It had become imperative to extend the generic concept for the licensure of therapeutic protein drugs because, while there existed only a few biopharmaceuticals when the Drug Price Competition and Patent Term Restoration Act of 1984 (Waxman-Hatch Act) was passed, these now account for almost a third of pharmaceutical sales, represent a core component of modern pharmacotherapy, and include some of the most expensive drugs on the market (21). As many biopharmaceuticals are poised to go off-patent, it has been recognized in both the U.S. and Europe that replicating the highly successful model of generic drugs to contain the costs of these therapeutics is a desirable goal, because biosimilar biological products (biosimilars) could potentially reduce the costs of therapeutic protein drugs.

In 2010, as part of the Patient Protection and Affordable Care Act (Affordable Care Act), biologics deemed to be “biosimilar” to an existing FDA-approved reference product were granted an abbreviated drug review and licensure pathway. Over the last few years, the FDA has issued several guidances to help sponsors navigate this novel regulatory pathway (22-24). As of August 31, 2016, three biosimilars have been approved by the FDA (Purple Book (25)). The first FDA-approved biosimilar, filgrastim-sndz (Zarzio; Sandoz), a biosimilar of filgrastim (Neupogen; Amgen), was approved on March 6, 2015, as a leukocyte growth factor for use in several neutropenia-related indications (26). This landmark approval was followed by the approval of infliximab-dyyb (Inflectra; Celltrion), a biosimilar of infliximab (Remicade; Janssen), on April 5, 2016 (27), and the approval of etanercept-szzs (Erelzi; Sandoz), a biosimilar of etanercept (Enbrel; Amgen), on August 30, 2016 (28); both are tumor necrosis factor (TNF) blockers indicated for use in inflammatory conditions. With the upcoming patent cliff approaching for several “blockbuster” therapeutic protein drugs, there is the expectation that the number of approved biosimilars will increase over the coming years. This trend has already been observed in Europe, where 21 biosimilar medicines have been authorized by the European Medicines Agency since 2006.

Although a drug product is off-patent, competitors do not get direct access to the originator company’s proprietary data or to resources such as the DNA sequence or cell lines used during the

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<table>
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<tr>
<th>#</th>
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<td>9</td>
<td>9/4/2015</td>
<td>antihemophilic factor (recombinant) [Niwiq; Octapharma USA]</td>
<td>coagulation factor [recombinant factor VIII]</td>
<td>hematology [hemophilia A]</td>
</tr>
<tr>
<td>13</td>
<td>3/16/2016</td>
<td>antihemophilic factor (recombinant) [Kovaltry; Bayer HealthCare]</td>
<td>coagulation factor [recombinant factor VIII full-length]</td>
<td>hematology [hemophilia A]</td>
</tr>
<tr>
<td>14</td>
<td>5/25/2016</td>
<td>antihemophilic factor (recombinant) [Alfalfa; CSL Behring]</td>
<td>coagulation factor [recombinant factor VIII]</td>
<td>hematology [hemophilia A]</td>
</tr>
</tbody>
</table>

Comprehensive listing of all FDA-approved therapeutic proteins granted orphan designation upon original submission from January 1, 2011, through August 31, 2016, listed in chronological order of FDA approval. In addition, the class of protein, a brief description, and orphan designation are included. CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; Fab, fragment antigen binding; Fc, fragment crystallizable; GD2, disialoganglioside; IL, interleukin; mAb, monoclonal antibody; PD-1, programmed death receptor-1; VEGFR, vascular endothelial growth factor receptor.
Table 3. Pathways for the development of novel therapeutics.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Description and relevant U.S. Food and Drug Administration (FDA) guidances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakthrough therapy</td>
<td>&quot;process designed to expedite the development and review of drugs that are intended to treat a serious condition and preliminary clinical evidence indicates that the drug may demonstrate substantial improvement over available therapy on a clinically significant endpoint(s)&quot;[^56][^57].</td>
</tr>
<tr>
<td>designation</td>
<td>Guidance for industry:</td>
</tr>
<tr>
<td></td>
<td>Expedited Programs for Serious Conditions – Drugs and Biologics[^56]</td>
</tr>
<tr>
<td>Orphan designation</td>
<td>Rare disease or condition that affects 200,000 people or fewer per year in the U.S.[^59]</td>
</tr>
<tr>
<td></td>
<td>Guidance for industry:</td>
</tr>
<tr>
<td></td>
<td>(Draft) Rare Diseases: Common Issues in Drug Development[^59]</td>
</tr>
<tr>
<td>Biosimilar</td>
<td>‘an abbreviated licensure pathway for biological products that are demonstrated to be “biosimilar” to or “interchangeable” with an FDA-licensed biological product… a biological product may be demonstrated to be “biosimilar” if data show that, among other things, the product is &quot;highly similar” to an already-approved biological product[^61].</td>
</tr>
<tr>
<td></td>
<td>Guidance for industry:</td>
</tr>
<tr>
<td></td>
<td>Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product[^69]</td>
</tr>
<tr>
<td></td>
<td>Scientific Considerations in Demonstrating Biosimilarity to a Reference Product[^67]</td>
</tr>
<tr>
<td></td>
<td>Formal Meetings Between the FDA and Biosimilar Biological Product Sponsors or Applicants[^69]</td>
</tr>
</tbody>
</table>

Summary of three pathways for the development of novel therapeutics that have emerged or have been finalized since 2011.

Table 4. Therapeutic proteins granted breakthrough therapy designation upon original submission.

<table>
<thead>
<tr>
<th>#</th>
<th>Approval date</th>
<th>Drug name (Market name)</th>
<th>Class</th>
<th>Description</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/1/2013</td>
<td>Obinutuzumab (Gazyva)</td>
<td>mAb</td>
<td>Humanized anti-CD20</td>
<td>Treatment of patients with previously untreated chronic lymphocytic leukemia in combination with chlorambucil</td>
</tr>
<tr>
<td>2</td>
<td>9/4/2014</td>
<td>Pembrolizumab (Keytruda)</td>
<td>mAb</td>
<td>Humanized anti-PD-1</td>
<td>Treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor</td>
</tr>
<tr>
<td>3</td>
<td>12/3/2014</td>
<td>Blinatumomab (Blincyto)</td>
<td>mAb</td>
<td>Mouse bispecific anti-CD19/anti-CD3</td>
<td>Treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL)</td>
</tr>
<tr>
<td>4</td>
<td>12/22/2014</td>
<td>Nivolumab (Opdivo)</td>
<td>mAb</td>
<td>Human anti-PD-1</td>
<td>Treatment of unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor</td>
</tr>
<tr>
<td>5</td>
<td>10/16/2015</td>
<td>Idarucizumab (Praxbind)</td>
<td>Fab</td>
<td>Humanized anti-dabigatran</td>
<td>Treatment of patients treated with Pradaxa when reversal of the anticoagulant effects of dabigatran is needed for emergency surgery/urgent procedures and in life-threatening or uncontrolled bleeding</td>
</tr>
<tr>
<td>6</td>
<td>10/23/2015</td>
<td>Asfotase-alfa (Strensiq)</td>
<td>Enzyme/fusion protein</td>
<td>Tissue non-specific alkaline phosphatase/Fc fusion/deca-aspartate (D10) peptide</td>
<td>Treatment of patients with perinatal/infantile- and juvenile-onset hypophosphatasia</td>
</tr>
<tr>
<td>#</td>
<td>Approval date</td>
<td>Drug name (Market name)</td>
<td>Class</td>
<td>Description</td>
<td>Use</td>
</tr>
<tr>
<td>----</td>
<td>----------------</td>
<td>--------------------------------------------</td>
<td>-----------</td>
<td>--------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7</td>
<td>11/16/2015</td>
<td>Daratumumab (Darzalex)</td>
<td>mAb</td>
<td>Human anti-CD38</td>
<td>Treatment of patients with multiple myeloma who have received at least three prior lines of therapy, including a proteasome inhibitor and an immunomodulatory agent, or are double-refractory to a proteasome inhibitor and an immunomodulatory agent.</td>
</tr>
<tr>
<td>8</td>
<td>11/30/2015</td>
<td>Elotuzumab (Empliciti)</td>
<td>mAb</td>
<td>Humanized anti-CD319(SLAMF7)</td>
<td>Treatment of patients with multiple myeloma who have received one to three prior therapies.</td>
</tr>
<tr>
<td>9</td>
<td>12/08/2015</td>
<td>Sebelipase alfa (Kanuma)</td>
<td>Enzyme</td>
<td>Lysosomal acid lipase</td>
<td>Treatment of patients with a diagnosis of lysosomal acid lipase deficiency.</td>
</tr>
<tr>
<td>10</td>
<td>5/18/2016</td>
<td>Atezolizumab (Tecentriq)</td>
<td>mAb</td>
<td>Humanized anti-PD-L1</td>
<td>Treatment of locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.</td>
</tr>
</tbody>
</table>

Comprehensive listing of all FDA-approved therapeutic proteins granted breakthrough therapy designation upon original submission from July 9, 2012, through August 31, 2016, listed in chronological order of FDA approval. In addition, the class of protein, a brief description, and use are included. BRAF, B-Raf proto-oncogene, serine/threonine kinase; CD, cluster of differentiation; Fab, fragment antigen binding; mAb, monoclonal antibody; PD-1, programmed death receptor-1; PD-L1, programmed death-ligand 1; SLAMF7, SLAM family member 7.

Table 5. Therapeutic proteins granted orphan designation upon original submission (2011–2016).

<table>
<thead>
<tr>
<th>#</th>
<th>Approval date</th>
<th>Drug name (Market name)</th>
<th>Class</th>
<th>Description</th>
<th>Orphan designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/25/2011</td>
<td>Ipilimumab (Yervoy)</td>
<td>mAb</td>
<td>Human anti-CTLA-4</td>
<td>Treatment of high-risk stage II, stage III, and stage IV melanoma.</td>
</tr>
<tr>
<td>2</td>
<td>6/15/2011</td>
<td>Belatacept (Nulojix)</td>
<td>Fusion (Fc)</td>
<td>CTLA-4 Fc-fusion</td>
<td>Prophylaxis of organ rejection in renal allograft recipients.</td>
</tr>
<tr>
<td>4</td>
<td>1/17/2012</td>
<td>Glucarpidase (Voraxaze)</td>
<td>Enzyme</td>
<td>Glucarpidase</td>
<td>Treatment of patients at risk of methotrexate toxicity.</td>
</tr>
<tr>
<td>5</td>
<td>5/1/2012</td>
<td>Taliglucerase alfa (Elelyso)</td>
<td>Enzyme</td>
<td>Taliglucerase</td>
<td>Treatment of Gaucher's disease.</td>
</tr>
<tr>
<td>6</td>
<td>12/14/2012</td>
<td>Raxibacumab (raxibacumab)</td>
<td>mAb</td>
<td>Human anti-anthrax protective antigen (PA)</td>
<td>Treatment of anthrax.</td>
</tr>
<tr>
<td>7</td>
<td>6/26/2013</td>
<td>Coagulation factor IX recombinant human (Rixubis)</td>
<td>Coagulation factor</td>
<td>Recombinant factor IX</td>
<td>Prophylactic use to prevent or reduce the frequency of bleeding episodes in patients with hemophilia B (routine prophylaxis in patients where there is no evidence or suspicion of bleeding).</td>
</tr>
<tr>
<td>8</td>
<td>11/1/2013</td>
<td>Obinutuzumab (Gazyva)</td>
<td>mAb</td>
<td>Humanized anti-CD20</td>
<td>Treatment of chronic lymphocytic leukemia.</td>
</tr>
<tr>
<td>10</td>
<td>2/14/2014</td>
<td>Elosulfase alfa (Vimizim)</td>
<td>Enzyme</td>
<td>Elosulfase alfa</td>
<td>Treatment of mucopolysaccharidosis type IV A (Morquio A syndrome).</td>
</tr>
<tr>
<td>11</td>
<td>2/24/2014</td>
<td>Metreleptin (Myalept)</td>
<td>Hormone</td>
<td>Metreleptin</td>
<td>Treatment of metabolic disorders secondary to lipodystrophy.</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>#</th>
<th>Approval date</th>
<th>Drug name (Market name)</th>
<th>Class</th>
<th>Description</th>
<th>Orphan designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>4/21/2014</td>
<td>Ramucirumab (Cyramza)</td>
<td>mAb</td>
<td>Human anti-VEGFR2 (KDR)</td>
<td>Treatment of gastric cancer</td>
</tr>
<tr>
<td>14</td>
<td>4/23/2014</td>
<td>Siltuximab (Sylvant)</td>
<td>mAb</td>
<td>Mouse/human chimeric anti-IL-6</td>
<td>Treatment of Castleman's disease</td>
</tr>
<tr>
<td>15</td>
<td>4/23/2014</td>
<td>Pembrolizumab (Keytruda)</td>
<td>mAb</td>
<td>Humanized anti-PD-1</td>
<td>Treatment of stage IIB through IV malignant melanoma</td>
</tr>
<tr>
<td>16</td>
<td>6/6/2014</td>
<td>Anthemophilic factor (recombinant), Fc fusion protein (Eloctate)</td>
<td>Coagulation factor</td>
<td>Recombinant factor VIII Fc-fusion</td>
<td>Treatment of hemophilia A</td>
</tr>
<tr>
<td>17</td>
<td>7/16/2014</td>
<td>C1 esterase inhibitor recombinant (Ruconest)</td>
<td>Plasma protein</td>
<td>Recombinant C1 esterase inhibitor</td>
<td>Treatment of (acute attacks of) angioedema caused by hereditary or acquired C1-esterase inhibitor deficiency</td>
</tr>
<tr>
<td>18</td>
<td>10/23/2014</td>
<td>Anthemophilic factor porcine, B-domain truncated recombinant (Obizur)</td>
<td>Coagulation factor</td>
<td>Recombinant factor VIII (porcine)</td>
<td>Treatment and prevention of episodic bleeding in patients with inhibitor antibodies to human coagulation factor VIII</td>
</tr>
<tr>
<td>19</td>
<td>12/3/2014</td>
<td>Blinatumomab (Blinlyco)</td>
<td>mAb</td>
<td>Mouse bispecific anti-CD19/anti-CD3</td>
<td>Treatment of acute lymphocytic leukemia</td>
</tr>
<tr>
<td>20</td>
<td>12/22/2014</td>
<td>Nivolumab (Opdivo)</td>
<td>mAb</td>
<td>Human anti-PD-1</td>
<td>Treatment of stage Iib to IV melanoma</td>
</tr>
<tr>
<td>21</td>
<td>1/23/2015</td>
<td>Parathyroid hormone (Natpara)</td>
<td>Hormone</td>
<td>Parathyroid hormone</td>
<td>Treatment of hypoparathyroidism</td>
</tr>
<tr>
<td>22</td>
<td>3/10/2015</td>
<td>Dinutuximab (Unituxin)</td>
<td>mAb</td>
<td>Mouse/human chimeric anti-GD2</td>
<td>Treatment of neuroblastoma</td>
</tr>
<tr>
<td>23</td>
<td>8/27/2015</td>
<td>Evolocumab (Repatha)</td>
<td>mAb</td>
<td>Human anti-proprotein convertase subtilisin/kexin type 9 (PCSK9)</td>
<td>Treatment of homozygous familial hypercholesterolemia</td>
</tr>
<tr>
<td>24</td>
<td>10/16/2015</td>
<td>Idarucizumab (Praxbind)</td>
<td>Fab</td>
<td>Humanized anti-dabigatran</td>
<td>To reverse the anticoagulant effect of dabigatran due to uncontrolled life-threatening bleeding requiring urgent intervention or a need to undergo an emergency surgery/urgent invasive procedure</td>
</tr>
<tr>
<td>25</td>
<td>10/23/2015</td>
<td>Asfotase-alfa (Strensiq)</td>
<td>Enzyme/fusion protein</td>
<td>Tissue non-specific alkaline phosphatase/Fc fusion/deca-aspartate (D10) peptide</td>
<td>Treatment of hypophosphatasia</td>
</tr>
<tr>
<td>26</td>
<td>11/16/2015</td>
<td>Daratumumab (Darzalex)</td>
<td>mAb</td>
<td>Human anti-CD38</td>
<td>Treatment of multiple myeloma</td>
</tr>
<tr>
<td>27</td>
<td>11/24/2015</td>
<td>Necitumumab (Portrazza)</td>
<td>mAb</td>
<td>Human anti-epidermal growth factor receptor</td>
<td>Treatment of squamous non-small cell lung cancer</td>
</tr>
<tr>
<td>28</td>
<td>12/8/2015</td>
<td>Sebelipase alfa (Kanuma)</td>
<td>Enzyme</td>
<td>Lysosomal acid lipase</td>
<td>Treatment of lysosomal acid lipase deficiency</td>
</tr>
<tr>
<td>30</td>
<td>3/4/2016</td>
<td>Coagulation factor IX recombinant human (Idelvion)</td>
<td>Coagulation factor</td>
<td>Recombinant factor IX albumin fusion</td>
<td>Treatment of patients with congenital factor IX deficiency (hemophilia B)</td>
</tr>
<tr>
<td>31</td>
<td>3/18/2016</td>
<td>Oblitoxaximab (Anthem)</td>
<td>mAb</td>
<td>Mouse/human chimeric anti-Bacillus anthracis spores</td>
<td>Treatment of exposure to B. anthracis spores</td>
</tr>
</tbody>
</table>

Comprehensive listing of all FDA-approved therapeutic proteins granted orphan designation upon original submission from January 1, 2011, through August 31, 2016, listed in chronological order of FDA approval. In addition, the class of protein, a brief description, and orphan designation are included. CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; Fab, fragment antigen binding; Fc, fragment crystallizable; GD2, disialoganglioside; IL, interleukin; mAb, monoclonal antibody; PD-1, programmed death receptor-1; VEGFR, vascular endothelial growth factor receptor.
manufacture. Typically, the developer of a biosimilar has to retrieve the reference protein as a finished drug product, purify the drug substance, and reverse-engineer the process. Consequently, the FDA biosimilar framework does not require an identical manufacturing process for the innovator product and the biosimilar. Therefore, it is not expected that, in the demonstration of biosimilarity, quality attributes such as protein structure and post-translational modifications measured in comparative physicochemical and functional studies will be identical between the biosimilar and reference product, but highly similar. It has, on the contrary, been argued that deviations from the technology of the innovator may actually be desirable. This is because since the introduction of the first recombinant DNA-derived therapeutic proteins, the technology to produce and purify these products has greatly improved. An increased focus upon innovation and streamlining of upstream and downstream processing has been reported by the widely respected “Biopharmaceutical benchmarks” series.

Thus, it is not necessary for manufacturers of biosimilars to be locked into the obsolete technologies of the manufacturers of the original products for whom changing methods have major financial and regulatory consequences. With this in mind, it seems much more desirable for biosimilars to be produced and analyzed by the best technology on offer.

**Emerging trends, challenges, and opportunities**

As protein-engineering technologies and regulatory frameworks evolve over time, so do protein therapeutics. Optimized versions of existing therapies can be achieved through better drug targeting as well as enhancing potency and functionality. By understanding the mechanism of action as well as the structure-function relationship of a protein, rational design and engineering strategies allow the modification of its activity or the introduction of new activities, leading to customization of existing proteins or the generation of novel therapeutics for specific clinical applications. Here, we will highlight just two examples of rational modifications to existing protein drugs achieved through protein engineering that have led to the approval of novel second-generation therapeutics (see Box 1 and Box 2).

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### Box 1. Engineering a second-generation cytotoxic T lymphocyte-associated protein 4 (CTLA4)-Fc fusion

Abatacept, a CTLA4-Fc fusion protein therapeutic, was developed by Bristol-Myers Squibb and approved by the U.S. Food and Drug Administration (FDA) in December 2005 as the first selective modulator of co-stimulation for the treatment of rheumatoid arthritis. CTLA4-Fc competitively inhibits CD28 on the surface of T cells for binding to the B7 family co-stimulatory receptors CD80 (B7-1) and CD86 (B7-2) expressed on the surface of antigen-presenting cells. Although this Fc-fusion has been shown to be efficacious in the treatment of rheumatoid arthritis, abatacept was not as effective when tested pre-clinically in non-human primate transplant models. Experimental data suggested that abatacept did not completely block B7 co-stimulatory receptor-mediated T-cell activation; therefore, a similar molecule with enhanced affinity for CD80/CD86 could be of therapeutic benefit as an immunosuppressant to prevent organ transplant rejection. Belatacept, formerly known as LEA29Y, was therefore developed by Bristol-Myers Squibb as a second-generation CTLA4-Fc. Two amino acid substitutions in the CTLA4-4 ligand-binding region (L104E and A29Y) resulted in enhanced in vitro binding to CD80 (about two fold more avidly) and CD86 (about four fold more avidly) in addition to greater immunosuppression of T-cell activation in vitro (about 10-fold) as compared with the parent molecule (abatacept). Belatacept’s enhanced activity was also observed in vivo with prolonged renal allograft survival in non-human primates (rhesus monkeys) compared with abatacept. In clinical studies of kidney allograft recipients, belatacept was shown to be associated with similar levels of patient and graft survival but superior renal function and reduced renal and non-renal toxicities compared with cyclosporine at 12 months after transplant. The rationally designed analog with enhanced CD80 and CD86 binding, belatacept, a second-generation selective co-stimulation blocker, was approved by the FDA on June 15, 2011, for the prophylaxis of kidney transplant rejection.

### Box 2. Engineering a second-generation anti-CD20 monoclonal antibody (mAb)

Rituximab, a chimeric mouse/human type I anti-CD20 mAb, was developed by Genentech and approved by the U.S. Food and Drug Administration (FDA) in November 1997 for the treatment of B-cell non-Hodgkin’s lymphoma. Rituximab binds to CD20 expressed on the surface of many B cells (but not plasma cells), resulting in B-cell depletion via antibody-dependent cellular cytotoxicity (ADCC), complement-mediated cytotoxicity (CMA), and the induction of direct cell death (apoptosis). Both ADCC and CMA are dependent upon the Fc region of the mAb interacting with Fc gamma receptor IIIa (FcγRIIIa) or complement component 1q (C1q), respectively. In the case of ADCC, antibody-bound CD20 B cells are targeted for cellular depletion by FcγRIIIa-expressing monocytes, macrophages, and natural killer cells. Given the importance of FcγRIIIa engagement and signaling in the mechanism of B-cell depletion, specifically engineering a next-generation mAb with enhanced functional activity would be of clinical benefit. Gazyva, formerly known as GA101, a type II anti-CD20 mAb, was engineered and developed by Genentech. This second-generation medicine contains a CD20-binding variable region introduced through protein engineering to take advantage of the potent induction of direct cell death and limited C1q binding typical of type II anti-CD20 antibodies. The Fc region of this mAb has also been glyco-engineered by producing the protein drug in an expression cell line that overexpresses the glycosylation enzymes β1,4-N-acetylgalactosaminyltransferase III (GnTIII) and Golgi α-mannosidase II (ManII), thereby enriching for afucosylated oligosaccharides. Changes to the Fc glycosylation at Asn297 can lead to changes in FcγR binding, phagocytosis, and cytotoxicity. In fact, afucosylated antibodies have higher-affinity FcγRIIIa binding and enhanced ADCC activity compared with parent fucosylated counterparts. The effects of these protein-engineering strategies can be observed in pre-clinical studies as Gazyva demonstrated higher affinity to FcγRIIα by SPR and induced more potent ADCC when compared with rituximab. These significant improvements were also observed in phase 3 clinical studies as patients with CD20+ chronic lymphocytic leukemia treated with Gazyva with chlorambucil had prolonged median progression-free survival time (26.7 months) when compared with patients treated with rituximab with chlorambucil (15.2 months). On November 1, 2013, Gazyva became the first glyco-engineered mAb drug to be approved by the FDA. This second-generation anti-CD20 mAb for treatment of chronic lymphocytic leukemia was also the first therapeutic protein to receive breakthrough therapy designation. In addition, Gazyva was granted orphan designation upon approval and contains pharmacogenetics information included on the drug label.
Over this examination of the recently approved therapeutic protein drug landscape, several emerging trends have become apparent. We anticipate that the inclusion of pharmacogenetics information in drug labeling and the importance of “companion diagnostics” will become the focus of increased attention. The FDA has been encouraging drug developers to collect and submit pharmacogenomics data through a guidance, “Pharmacogenic Data Submissions”, issued in March 2005. Pharmacogenetics can play an important role in identifying responders and non-responders to medications, avoiding adverse events, and optimizing drug dose. Drug labeling may contain information on genomic biomarkers and can describe drug exposure and clinical response variability, risk for adverse events, genotype-specific dosing, mechanisms of drug action, and polymorphic drug target and disposition genes. Therefore, pharmacogenetic profiling is of particular importance when potential drug candidates exhibit highly variable safety, efficacy, or pharmacokinetics profiles. In January 2013, the FDA issued a guidance, titled “Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling”, to assist drug developers with conducting exploratory pharmacogenomic investigations, enrichment strategies for clinical trials, adaptive trial designs, or companion diagnostics. Pharmacogenetic information and changes in drug labeling can lead to drugs targeted for different populations, personalized dosing regimens, and companion diagnostics. There have been 11 therapeutic proteins approved since 2011 that have included pharmacogenetic biomarkers in their drug labels (Table 6). For a complete listing of drugs with available pharmacogenetics information, see the FDA’s Table of Pharmacogenic Biomarkers in Drug Labeling. The FDA’s review process will continue to adapt as the incorporation of pharmacogenetic information becomes more commonplace. This will also require a coordinated cross-center review to incorporate the companion diagnostic/sequencing in the drug development/licensure process.

### Table 6. Therapeutic proteins with pharmacogenetic biomarkers in drug labeling.

<table>
<thead>
<tr>
<th>#</th>
<th>Approval date</th>
<th>Drug name (Market name)</th>
<th>Class</th>
<th>Description</th>
<th>Pharmacogenetic biomarker</th>
<th>Therapeutic area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/8/2012</td>
<td>Pertuzumab (Perjeta)</td>
<td>mAb</td>
<td>Humanized anti-human epidermal growth factor receptor 2 (HER2)</td>
<td>HER2 protein overexpression positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>2</td>
<td>2/22/2013</td>
<td>Ado-trastuzumab emtansine (Kadcyla)</td>
<td>Antibody-drug conjugate</td>
<td>Humanized anti-HER2/neu conjugated to emtansine</td>
<td>HER2 protein overexpression or gene amplification positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>3</td>
<td>11/1/2013</td>
<td>Obinutuzumab (Gazyva)</td>
<td>mAb</td>
<td>Humanized anti-CD20</td>
<td>CD20 antigen positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>4</td>
<td>2/14/2014</td>
<td>Elosulfase alfa (Vimizim)</td>
<td>Enzyme</td>
<td>Elosulfase alfa</td>
<td>N-acetylgalactosamine-6-sulfatase deficient</td>
<td>Inborn errors of metabolism</td>
</tr>
<tr>
<td>5</td>
<td>9/4/2014</td>
<td>Pembrolizumab (Keytruda)</td>
<td>mAb</td>
<td>Humanized anti-PD-1</td>
<td>(1) BRAF V600 mutation positive, (2) PD-L1 protein expression positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>6</td>
<td>12/3/2014</td>
<td>Blinatumomab (Blincoyo)</td>
<td>mAb</td>
<td>Mouse bispecific anti-CD19/anti-CD3</td>
<td>Philadelphia chromosome negative</td>
<td>Oncology</td>
</tr>
<tr>
<td>7</td>
<td>12/22/2014</td>
<td>Nivolumab (Opdivo)</td>
<td>mAb</td>
<td>Human anti-PD-1</td>
<td>(1) BRAF V600 mutation positive, (2) PD-L1 protein expression positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>8</td>
<td>1/23/2015</td>
<td>Parathyroid hormone (Natpara)</td>
<td>Hormone</td>
<td>Parathyroid hormone</td>
<td>Calcium sensing receptor mutation positive</td>
<td>Inborn errors of metabolism</td>
</tr>
<tr>
<td>9</td>
<td>3/10/2015</td>
<td>Dinutuximab (Unituxin)</td>
<td>mAb</td>
<td>Mouse/human chimeric anti-GD2</td>
<td>MYCN amplification positive</td>
<td>Oncology</td>
</tr>
<tr>
<td>10</td>
<td>7/24/2015</td>
<td>Alirocumab (Praluent)</td>
<td>mAb</td>
<td>Human anti-proprotein convertase subtilisin/kexin type 9 (PCSK9)</td>
<td>LDL receptor mutation heterozygotes</td>
<td>Endocrinology</td>
</tr>
<tr>
<td>11</td>
<td>8/27/2015</td>
<td>Evolocumab (Repatha)</td>
<td>mAb</td>
<td>Human anti-proprotein convertase subtilisin/kexin type 9 (PCSK9)</td>
<td>LDL receptor mutation heterozygotes and homozygotes</td>
<td>Endocrinology</td>
</tr>
</tbody>
</table>

Comprehensive listing of all FDA-approved therapeutic proteins with pharmacogenetics biomarkers in drug labeling from January 1, 2011, through August 31, 2016, listed in chronological order of FDA approval. In addition, the class of protein, a brief description, pharmacogenetics biomarker, and therapeutic area are included. BRAF, B-Raf proto-oncogene, serine/threonine kinase; CD, cluster of differentiation; GD2, disialoganglioside; LDL, low-density lipoprotein; mAb, monoclonal antibody; MYCN, v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; PD-1, programmed death receptor-1; PD-L1, programmed death-ligand 1.
It is reasonable to anticipate that proteins will be more extensively engineered in the future. This means that the new generation of therapeutic proteins will carry neo-sequences not found in nature. Thus, the potential risks of immunogenicity (undesirable immune responses to therapeutic proteins) will also increase and, in turn, demand new technologies for immunogenicity risk assessment and mitigation. Protein engineering is no longer restricted to altering the primary sequence of proteins. On the other hand, the rapidly growing trend of codon optimization involves the substitution of synonymous codons to improve protein synthesis and increase protein production. These opportunities do come with risks but rapid advances in new technologies as well as the underlying science suggest that these risks can be managed.

**Competing interests**

The authors declare that they have no competing interests.

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