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Protein Therapeutics

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Springer

Editors
Zuben E. Sauna
Division of Plasma Protein Therapeutics
Office of Tissues and Advanced Therapies
FDA | CBER | OTAT
Federal Research Center at White Oak
Silver Spring, Maryland, USA

Chava Kimchi-Sarfaty
Division of Plasma Protein Therapeutics
Office of Tissues and Advanced Therapies
FDA | CBER | OTAT
Federal Research Center at White Oak
Silver Spring, Maryland, USA

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Introduction

Chava Kimchi-Sarfaty, Aikaterini Alexaki, and Zuben E. Sauna

Protein therapeutics were first developed only a few decades ago but now dominate pharmaceutical sales. For example, protein therapeutics accounted for about 7% of revenues from the top ten best selling drugs in 2001 but generated 70% of revenues a decade later [1]. This class of drugs which represents a core component of modern pharmacotherapy thus includes some of the most expensive drugs on the market. Tables 1 and 2 provide an overview of the diversity of protein molecules used as therapeutics and the different platform technologies used.

Table 1 Number of recombinant therapeutics (not including antibodies) that are marketed or under development

	Cytokines	Hormones	Coagulation factors	Inhibitors	Enzymes	Status
Unmodified human protein	34	16	13	5	17	Marketed
	47	32	21	8	35	In pipeline
	6	7	3(1)	1(1)	2	Biosimilars
Pegylated protein	4	1	1	2	2	Marketed
				2	7	In pipeline
	1(1)					Biosimilars
Polyxen fusion protein						Marketed
	2	2				In pipeline
						Biosimilars

(continued)

C. Kimchi-Sarfaty • A. Alexaki • Z.E. Sauna
Division of Plasma Protein Therapeutics
Office of Tissues and Advanced Therapies
FDA | CBER | OTAT
Federal Research Center at White Oak
Silver Spring, Maryland, USA

Table 1 (continued)

	Cytokines	Hormones	Coagulation factors	Inhibitors	Enzymes	Status
Fc fusion protein			2	7	1	Marketed
				3		In pipeline
				(1)		Biosimilars
Albumin fusion protein	2	1	2			Marketed
				1		In pipeline
						Biosimilars
XTEN fusion protein						Marketed
		6	3		1	In pipeline
						Biosimilars

Numbers in parenthesis indicate biosimilars under development which have not yet been approved. Data were derived from Pharmaprojects (https://fda-pipeline.citeline.com)

Table 2 Number of therapeutic antibodies that are marketed or under development

	Marketed	In pipeline	Marketed biosimilars
Unconjugated antibody	52	118	1(19)
Pegylated antibodies	1	3	1
Toxin-conjugated antibodies	3	17	
Radio-immuno-conjugates	10	5	

Numbers in parenthesis indicate biosimilars under development which have not yet been approved. Data were derived from Pharmaprojects (https://fda-pipeline.citeline.com)

Proteins differ from small molecule drug both in terms of their characteristics (see below) and the manner in which they are manufactured. For example, a typical protein therapeutic is much larger, exhibits complex secondary and tertiary structures, and cannot be synthesized by chemical processes. As the products are synthesized by cells, complex extraction and purification processes are involved which could potentially introduce modifications in the protein. It is therefore not surprising that the manufacture of a typical protein therapeutic is a far more complex process than that of a small molecule drug [2]. A sense of just how much more complex manufacturing a protein therapeutic can be is illustrated by the information provided in Table 3. Compared to small molecule drugs, the number of batch records, product quality tests, critical process steps, and process data entries are all at least an order of magnitude higher for protein therapeutics.

Table 3 The complexity of manufacturing protein therapeutics compared to small molecule drugs

	Small molecule drugs	Protein therapeutics
Batch records	<10	>250
Product quality tests	<100	>2,000
Critical process steps	<100	>5,000
Process data entries	<4,000	>60,000

Several forces at play in recent years have added even more complexity to the drug-development and manufacturing processes for protein therapeutics. Experience with the first generation of native or wild-type proteins indicated that there was an unmet need for molecules with better clinical outcomes, improved patient convenience, or simplified and more reliable manufacturing processes [3]. Driven by scientific innovations and new technologies, a new generation(s) of bioengineered protein molecules, that seek to fulfill these needs, has entered the drug-development pipeline [4]. These advances have come at the same time as the manufacturing process and sourcing of materials has become more global. Together these changes have added additional challenges to the development, licensure, and manufacture of protein therapeutics. This book provides a high level view of what the specific challenges are and how they are being met.

Characteristics of Protein Therapeutics and How They Differ from Small Molecule Drugs

Characterization by analytical methods is generally considered to be a good predictor of the biological and clinical properties of small molecule drugs. This experience, for example, is the key reason why generic versions of these drugs can be rapidly and inexpensively developed and marketed. The same is not true for protein therapeutics due to the following distinctive characteristics.

Size: The most prominent difference between a small molecule drug and a protein therapeutic is the size; the latter being 100–1,000 times larger. Due to their size and complexity, currently, protein therapeutics cannot be synthesized by chemical processes and have to be manufactured in living cells (see the chapters, "Protein Production in Eukaryotic Cells" and "Production of Protein Therapeutics in the Quality by Design (QbD) Paradigm"). Thus cell characteristics such as choice of the cell line, species origin of the cell line, and culture conditions all affect the final product characteristics [5]. In addition the use of materials of biological origin increases the potential risk of the final product. Finally the large size of the product complicates drug delivery as well as storage and distribution. Proteins are also much more likely to elicit an immune response in patients which is an important safety issue (see the chapter "Immunogenicity Lessons Learned from the Clinical Development of Vatreptacog Alfa, a Recombinant Activated Factor VII Analog, in Hemophilia with Inhibitors") [6].

Structure: To possess biological activity, proteins have to adopt the correct three-dimensionally folded secondary, tertiary, and quaternary structures. Thus the development, regulation, and manufacture of protein therapeutics all require very sophisticated analytical techniques (see the chapter "Characterization of Therapeutic Proteins"). Analytical techniques are becoming increasingly sophisticated but this also means that more biophysical or biochemical changes that are not clinically relevant or are not a safety issue can be identified. Quality-by-Design (QbD) [7] offers a means of identifying and monitoring critical quality attributes (see chapter "Production of Protein Therapeutics in the Quality by Design (QbD) Paradigm").

Structure–Function relationship: In small molecules, it is often known that every atom of the molecule will play a role in defining the clinical profile of the compound; the structure–function relationship is usually unknown, or at best partially known, for proteins. Thus, the impact of differences in the molecular structure in most cases cannot be predicted [8]. This can result in safety issues late in the drug-development cycle (see the chapter "Immunogenicity Lessons Learned from the Clinical Development of Vatreptacog Alfa, a Recombinant Activated Factor VII Analog, in Hemophilia with Inhibitors" for an example).

Stability: Proteins are inherently unstable molecules, and may be altered structurally by heat, prolonged storage, denaturants, organic solvents, oxygen, pH changes, and other factors, which are all part of the manufacturing process [9]. This can be a consistent challenge and emerging strategies like QbD [2] can help to maintain consistency at a manufacturing facility and also when manufacturing facilities are moved or added (see the chapter "Production of Protein Therapeutics in the Quality by Design (QbD) Paradigm"). Proteins can be bioengineered [10] to enhance their utility as drugs. However, this can be a double-edged sword and results in unanticipated and undesirable outcomes (see the chapters "Immunogenicity Lessons Learned from the Clinical Development of Vatreptacog Alfa, a Recombinant Activated Factor VII Analog, in Hemophilia with Inhibitors" and "The Art of Gene Redesign and Recombinant Protein Production: Approaches and Perspectives").

Microheterogeneity: Proteins are modified both biologically by the producing cell (e.g., glycosylation, acylation, sulfatation, phosphorylation, and proteolysis) and by the process conditions (e.g., oxidation, deamidation, reaction with auxiliary substances, partial denaturation, and aggregation) [9]. Further heterogeneity may arise if the protein is intentionally modified, for example, by multi-site pegylation [11]. Thus, even highly purified proteins never consist of a single molecular entity but are mixtures of many closely related molecular species. This microheterogeneity can be substantial. It has been estimated that up to 10^8 different species exist in an immunoglobulin G molecule [2]. This inherent variability in the reference molecule itself makes the identification of clinically relevant variations extremely difficult. Thus the phrase, "the process is the product" [5, 8, 12] is often used in the context of the manufacture of protein therapeutics. The emphasis therefore has been on the genetic stability of the expression system and the reproducibility of the production process.

Protein Therapeutics and Immunogenicity

A significant concern unique to the development and licensure of protein therapeutics is the risk of developing anti-drug antibodies (ADAs). Such antibodies are rarely, if ever, a concern during the development of small molecule drugs. However, the development of ADAs against protein therapeutics can lead to adverse events and also make the biologic less effective for its intended use. Thus, immunogenicity assessments are now an integral part of the development, licensure, and use of this class of products [6]. Factors influencing immunogenicity against a

protein therapeutic are both patient- and product-related [13] and the latter are often associated with the manufacturing process. There are several examples where small changes in the protein or manufacturing processes have resulted in significant increase in immunogenicity [14, 15]. The chapter "Immunogenicity Lessons Learned from the Clinical Development of Vatreptacog Alfa, a Recombinant Activated Factor VII Analog, in Hemophilia with Inhibitors" provides a detailed case study where three amino acid substitutions in Factor VIIa resulted in the development of ADAs in >10% of patients in the phase 3 trial. In contrast the parent molecule which was not engineered has been used as a drug for almost two decades with no reports of ADAs, consequently the development of this bioengineered analog was discontinued [15].

Several factors influence the immunogenicity of a protein product. The presence of impurities such as host-cell impurities (host-cell proteins, DNA, lipids, viruses, or endotoxins), protein aggregates, or leachates can affect immunogenicity. Also of concern are sequence modifications made in protein products that are bioengineered to improve yields or product characteristics such as increased circulating half-life [3]. Methodology used for measuring immunogenicity includes immunoreactivity assays (radioimmunoassay, surface plasma resonance, or enzyme-based solid-phase immunoassay) or functional cell-based bioassays. It must be emphasized that despite considerable technological progress in non-clinical approaches the current state of the art does not permit immunogenicity assessments to be made in the absence of clinical trials. This again emphasizes the importance of developing manufacturing processes that are consistent and identify and monitor critical quality attributes.

Protein Therapeutics: Biosimilars

The use of proteins as therapeutics has revolutionized the treatment of many disease areas but these medications are some of the most expensive in the market place. As many biopharmaceutical products are poised to go off patent it has been recognized that replicating the highly successful generic model to contain the costs of these therapeutics is a desirable goal [7, 12]. However, the primary function of regulatory agencies is to ensure that patient safety is not compromised. Given the complexity of protein therapeutics, as well as of the manufacturing process it is unlikely that in the near term the development process for biosimilars can be abridged to quite the extent as that for classical generics [16, 17]. Significant challenges remain in developing analytical techniques to comprehensively characterize protein therapeutics. Moreover, unlike small molecule drugs protein therapeutics exhibit considerable micro-heterogeneity and thus development of more sensitive and accurate technological analytical tools alone may not be sufficient. It has been recognized that biosimilars are not generics (as the nomenclature implies) and will not be identical to the reference drug.

We do not address the thorny legal and scientific issues surrounding biosimilars. However, the difficult questions surrounding biosimilars arise due to the characteristics and complexities of protein molecules and these are adequately addressed in this volume.

Overview of This Book

In this book we have endeavored to provide a broad overview of developing and manufacturing therapeutic proteins. The individual chapters written by experts can be used as a source of information on specific topics. However, the book as a whole also provides a narrative that describes the art and science of developing a protein therapeutic in a rapidly globalizing marketplace. The book begins with the basics; Ram et al. describe the nuts and bolts of manufacturing a recombinant protein in eukaryotic cells. However, protein therapeutics are increasingly being manufactured in a global setting. This means that the same product could be manufactured at different locales; parts of the manufacturing process may be outsourced, etc. Managing the quality and consistency of a complex product in such a setting is critically important and extremely challenging. Rathore and Singh introduce the concept of QbD in the context of protein therapeutics. The importance of identifying the underlying relationship between the quality attributes of the product and clinical safety and efficacy is the ultimate goal of QbD and likely to play a critical role in maintaining product quality in an increasingly global market. Protein therapeutics require complex and sophisticated manufacturing processes; but the molecules themselves are also inherently complex. Struble et al. provide a comprehensive survey of the tools and strategies for the characterization of proteins. More importantly they discuss these in the context of the regulatory framework which is essential for translating a molecule with promise into a successful drug. The ability to engineer proteins permits the incorporation of characteristics sought after in a drug such as enhanced serum half-life, a better safety-efficacy profile, patient convenience, and delivery to target. However, these manipulations can also sometimes result in unintended consequences and termination of the drug-development process. Rather than an abstract discussion of this topic Lamberth et al. present a case study where a Factor VIIa analog with an improved safety-efficacy profile was discontinued from further development because of the identification of unwanted anti-drug antibodies in phase 3 trials. Finally, Komar provides an in-depth discussion of a single platform technology, namely codon optimization and discusses the potential consequences (both desirable and potentially hazardous) based on rapid, recent progress in basic sciences.

Disclaimer

Our contributions are an informal communication and represent our own best judgment. These comments do not bind or obligate FDA.

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