Part 1
General Aspects

1 An Overview of the Discovery and Development Process for Biologics

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1.1 INTRODUCTION

Biologics, also called biotherapeutics or biopharmaceuticals, are drug substances derived from living organisms or produced using biotechnology that are composed of biological entities such as proteins, peptides, nucleic acids, or cells [1]. They differ from small molecule (SM) drugs that are chemically synthesized and have low molecular weights. Some biologics, such as antibody–drug conjugates, consist of both a protein moiety and an SM component, both of which are required for the therapeutic action of the drug. Traditional biologics that have reached the market include vaccines and blood-derived factors. The advancement in modern biotechnology has brought forth new classes of biologics as exemplified by monoclonal antibodies (mAbs), Fc fusion proteins, recombinant proteins, and peptide drugs. Some early clinical success is now seen in several novel classes of biologics, which include antibody variants, novel protein scaffolds, RNA therapeutics, and cell-based therapies [2–5]. This chapter focuses on protein-based biologics, particularly mAbs because they represent the largest class of biologic drugs. By the end of 2011, the US Food and Drug Administration (FDA) had approved close to 40 mAbs and antibody variants as summarized in Table 1.1. Details on other forms of biologics such as vaccines and RNA drugs can be found in later chapters.

The first protein-based biologic drug, recombinant insulin Humulin, was approved in the United States in 1982 [6]. Since then the field of biologics grew steadily, with the biotechnology sector laying the foundation for both the drug discovery process and technology innovation. Around late 1990s, the pharmaceutical industry started to invest more in the development of biologics. This shift from a primary focus on SM drugs was largely due to patent expiration on these drugs and the concurrent fierce competition from generic SM drugs. In addition, the increasing difficulty to bring new drugs to the market because of tightened regulations and a lack of breakthroughs in the drug discovery process has also contributed to this shift.

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TABLE 1.1 List of Food and Drug Administration–Approved Antibody-Based Therapeutics Up to 2011 as Categorized by Types

Type of Ab-Based Therapeutics	Nonproprietary/ Trade Name	Ab Target or Fc Fusion Partner	Company
Human antibodies	Ipilimumab/Yervoy	CTLA4	Bristol-Myers Squibb
	Belimumab/Benlysta	B-lymphocyte stimulator	Human Genome Sciences
	Ustekinumab/Stelara	p40 subunit of IL-12 and IL-23	Johnson & Johnson
	Canakinumab/Ilaris	IL-1β	Novartis
	Denosumab/Prolia/Xgeva	RANKL	Amgen
	Ofatumumab/Arzerra	CD20	Genmab
	Golimumab/Simponi	TNF	Centocor
	Panitumumab/Vectibix	EGFR	Amgen
	Adalimumab/Humira	TNF	Abbott
Humanized antibodies	Tocilizumab/Actemra	IL-6R	Roche
	Eculizumab/Soliris	C5	Alexion
	Natalizumab/Tysabri	Alpha4 integrin	Biogen/Elan
	Bevacizumab/Avastin	VEGFa	Genentech
	Efalizumab/Raptiva	CD11a	Genentech
	Omalizumab/Xolair	Human IgE Fc	Genentech
	Alemtuzumab/ Campath-IH	CD52	Genzyme
	Trastuzumab/Herceptin	Her2	Genentech
	Palivizumab/Synagis	RSV protein F	MedImmune
	Daclizumab	CD25	Roche
Chimeric antibodies	Cetuximab/Erbitux	EGFR	Imclone
	Infliximab/Remicade	TNFα	Centocor
	Basiliximab/Simulect	CD25	Novartis
	Rituximab/Rituxan	CD20	IDEC
Murine antibody	Muromonab-CD3/Orthoclone OKT3	CD3	Janssen-Cilag
Fab fragment	Abciximab/Reopro	CD43	Centocor
	Ranibizumab/Lucentis	VEGFa	Genentech
	Certolizumab pegol/Cimzia	TNFα	UCB
Antibody conjugates	Brentuximab vedotin/Adcetris	CD30	Seattle Genetics
	Tositumomab-I131/Bexxar	CD20	GlaxoSmithKline
	Ibritumomab tiuxetan/Zevalin	CD20	IDEC
	Gemtuzumab ozogamicin/Mylotarg	CD33	Wyeth

TABLE 1.1 (*Continued*)

Type of Ab-Based Therapeutics	Nonproprietary/ Trade Name	Ab Target or Fc Fusion Partner	Company
Fc fusions	Afilibercept/Eylea	VEGFR1 and 2 ECD	Regeneron, Bayer
	Belatacept/Nulojix	CTLA4 ECD	Bristol-Myers Squibb
	Romiplostim/Nplate	Peptide thrombopoietin mimetic	Amgen
	Rilonacept/Arcalyst	IL-1R ECD	Regeneron
	Abatacept/Orencia	CTLA4 ECD	Bristol-Myers Squibb
	Alefacept/Amevive	LFA-3 ECD	Biogen IDEC
	Etanercept/Enbrel	TNFRII ECD	Wyeth/Amgen

Presently, the number of biologics on the market has reached more than 200, and the sales of biologics in 2009 reached $93 billion, with approximately one third of current pharmaceutical pipelines consisting of biologics [7]. Given that almost all of the large pharmaceutical companies have acquired infrastructures and committed resources to develop biologics, we will continue to see a robust growth in this sector in the coming years.

Compared with SM drugs, protein-based biologics have unique therapeutic features. A therapeutic protein usually exhibits exquisite specificity when binding to and modulating its molecular target, which often translates into low off-target toxicity and clinical safety. For example, therapeutic mAbs bind to their target molecules with affinities in the picomolar to low nanomolar range (e.g., [8]). Furthermore, the interaction occurs over a broad interface with multiple physical and chemical bonds formed between an antibody and its cognate antigen, resulting in an extraordinary binding specificity that allows the differentiation of binding partners that differ by as few as one amino acid or subtle conformational difference. On the contrary, the small size of an SM drug makes it prone to off-target binding to proteins other than its intended target, which may result in unacceptable levels of toxicities. A potentially short development cycle is another advantage for the development of biologics, particularly mAbs and recombinant proteins. A clinical candidate for mAb or recombinant protein can be generated and selected in as short as 3 to 5 years compared with typically 7 to 8 years for SMs.

Protein-based biologics have their own limitations. Presently, almost all protein-based drugs must be administered as intravenous or subcutaneous injections because oral delivery is not yet a viable route of administration. Furthermore, protein drugs do not readily penetrate cell membrane and blood–brain barrier (BBB) and therefore are limited to the modulation of peripherally located extracellular targets. The cost of goods to manufacture protein drugs is significantly higher than for SM drugs, which translates into a high drug price that exacerbates health management cost issues [9].

Based on these pros and cons associated with the development of biologics, presently the pharmaceutical industry strives to achieve a balanced portfolio consisting of both SM and biologic drugs. This chapter provides an overview of the discovery and development process for protein therapeutics with a primary focus on mAbs (Fig. 1.1). Additionally, the chapter summarizes the current status of the protein-based biologics field and discusses several future trends.

1.2 THE DISCOVERY PROCESS FOR MONOCLONAL ANTIBODIES

Monoclonal antibodies and mAb variants such as antibody–drug conjugates and Fc fusion proteins are a major class of biologics. This section describes in detail the discovery process for mAbs. Later chapters illustrate the process for several other forms of biologics such as vaccines and RNA therapeutics.

In mammals, antibodies are proteins found in the blood that are produced by B cells from the humoral immune system in defense of foreign organisms recognized by the host system. Also known as immunoglobulins, antibodies can be categorized into five classes or isotypes, namely IgM, IgD, IgG, IgE, and IgA (reviewed by Schroeder and Cavacini [10]). IgG is the predominant class in therapeutic antibodies. An IgG molecule consists of two heavy chains and two light chain interlinked by disulfide bonds (Fig. 1.2). Each chain has an N-terminal variable region (Fv) and a C-terminal constant region. The variable region of each pair of heavy and light chains has six hypervariable loops (three per chain) called

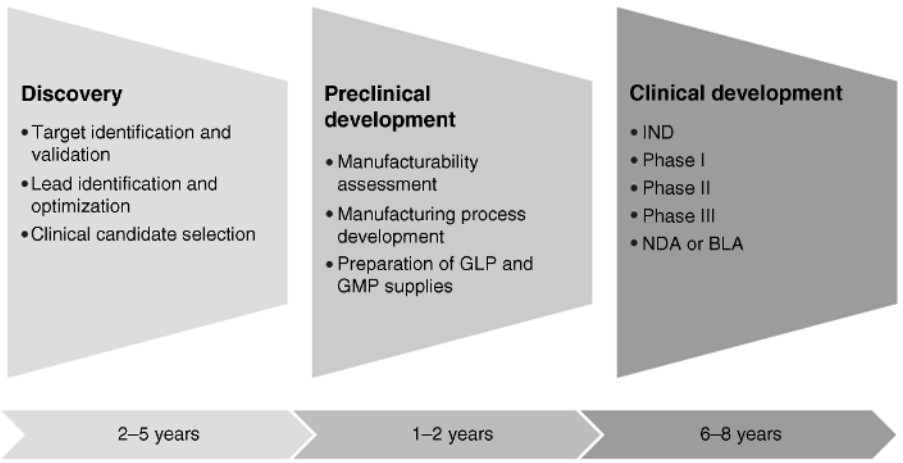

FIGURE 1.1 Major phases and general timelines of the drug development process for biologics. BLA: biologics license application; GLP: good laboratory practice; GMP: good manufacturing practices; IND: investigational new drug; NDA: new drug application.

complementarity determining regions (CDRs) that form the antigen binding region at the tip of the IgG molecule. The heavy and light chains in an Fv fragment can be joined via a linker using recombinant DNA technology and produced as a single-chain Fv (scFv) molecule, which is used in phage display technology as a method to generate therapeutic antibody candidates (see later discussion). Papain digestion of an IgG yields three components: two Fab fragments and an Fc fragment. A Fab molecule can be generated via molecular engineering into a therapeutic agent. Currently, three Fab-based antibody drugs are on the market: abciximab, ranibizumab, and certolizumab pegol (Table 1.1). The Fc fragment exhibits effector functions, namely the ability to engage immune system to kill antibody opsonized molecules. In addition, the binding of Fc to bacterial protein A and protein G has been applied to the purification of therapeutic antibodies at industrial manufacturing scales. Furthermore, Fc binds to the neonatal Fc receptor (FcRn) expressed on endothelial cells. Upon uptake by endothelial cells, FcRn recycles IgG molecules back into circulation, thus conferring a long *in vivo* half-time to IgG [11]. Via molecular engineering, the Fc fragment can be fused to another protein fragment such as a soluble factor or the extracellular domain of a cell surface receptor. The resulting Fc fusion proteins represent one type of antibody-based therapeutics (Table 1.1 and Fig. 1.2).

The advancement in molecular and cellular biology has transformed the isolation, molecular engineering, and production of recombinant mAbs into an industrial drug-making process. Compared with other protein drugs, mAbs demonstrate superior properties as therapeutic molecules. They typically exhibit exquisite specificity to their molecular targets and minimal off-targeting binding. The bivalency of each antibody molecule contributes to its extraordinarily high binding strength (avidity) as the summation of the affinity from each half of the molecule. It also confers a cross-linking function that can be applied to its therapeutic function. The effector functions associated with the Fc domain allow the molecule to effectively mobilize the immune system to attack and kill tumor cells when used to treat cancers. mAbs are highly stable proteins with natural resistance to biological and chemical degradation. They tend to be amenable to expression and purification at manufacturing scales. They typically exhibit long *in vivo* half-lives, which allow infrequent administration in patients. As a result of these multifaceted advantages associated with mAbs, the pharmaceutical industry has focused on the development of mAbs as a major class of biologic drugs.

In many pharmaceutical and biotechnology companies, the development process for therapeutic mAbs is well established and analogous to that for SM drugs. It can be generalized into four stages: target selection, screening preparation, lead selection and optimization, and clinical candidate selection. The discovery phase is followed by a preclinical development process and ultimately the clinical testing of the selected mAb candidate in human subjects. This section describes relevant research activities that take place in each of the four discovery stages leading to the selection of a clinical candidate protein. The major differences in the respective processes for developing biologics and SMs are discussed at the end of this section.

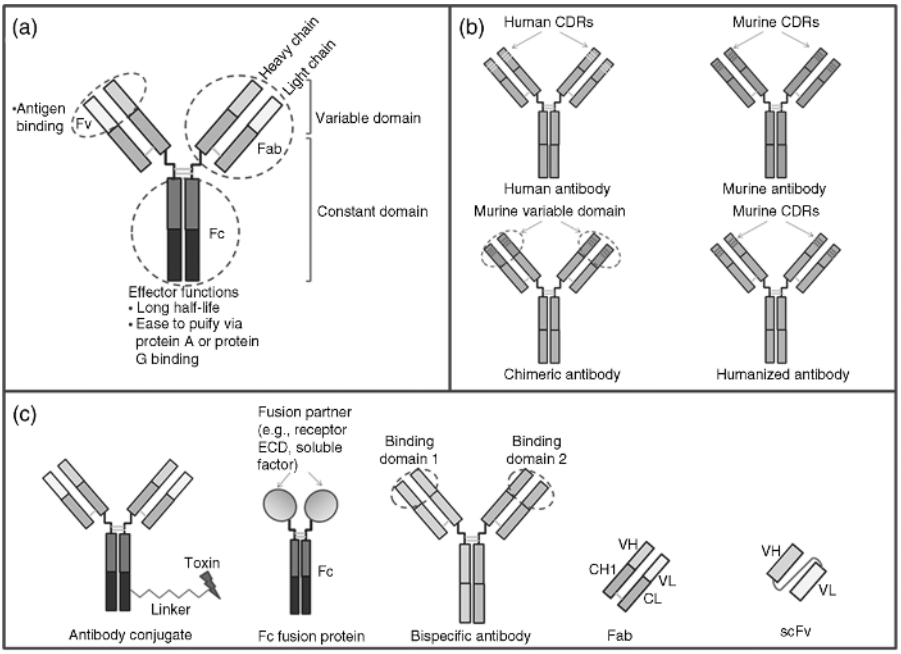

FIGURE 1.2 Major types and properties of therapeutic antibodies and antibody variants. (a) Depiction of a whole immunoglobulin molecule. The major domains and their associated functions are indicated. *Light lines* represent disulfide bonds. Fab: antigen binding fragment; Fc: crystallizable fragment; Fv: variable fragment. (b) Major forms of therapeutic antibodies. CDR: complementarity determining region. (c) Major forms of antibody variants. All but one (single-chain variable fragment [scFv]) drug is currently approved. The scFv is used in drug discovery to identify drug candidates.

1.2.1 Target Selection

A target is a biological entity in patients that can be specifically and effectively modulated by a drug to ameliorate or cure a pathological condition. Selecting a drug target is usually the first step in a drug development program, although there are exceptions to the rule when drug candidate screening is carried out using a functional readout without predefined targets. In a majority of such cases, the target is identified retrospectively after the functional candidates have been selected.

A novel target is often identified either during the studies of biological pathways underlying a disease or as a result of disease target identification efforts frequently using genomic technologies such as transcriptional profiling, proteomics, and genome-wide gene association studies. Usually a target must be "validated" before the initiation of a drug discovery program. *Target validation* refers to a process of collecting clinical and experimental data to predict a beneficial therapeutic outcome from a hypothesized modulation of a selected target. A typical target validation data package often includes some or all of the following elements: (1) the target is

aberrantly expressed in the disease tissue(s) (the polymorphism of the target gene in humans has a strong association with the disease); (2) in cellular and animal models, overexpression or deficiency of the target leads to a biological outcome consistent with the expected therapeutic outcome; and (3) pharmacologic modulation of the target gives rise to expected results in cellular and animal models. A drug discovery project may begin with some evidence that the target is tied to the pathogenesis of a disease, in which case target validation becomes a continuous endeavor carried out in parallel to the generation of therapeutic candidates.

1.2.2 Screening Preparation

With enough confidence that therapeutic modulation of a selected target can treat a pathological condition, screening preparation can be initiated. The goal of this drug discovery stage is to generate all the reagents, functional assays, and cellular and animal models to carry out a variety of functional screens in order to select the best therapeutic candidate for human testing. This process starts with a well-thought-out screening plan that defines the primary, secondary, and tertiary screening methods encompassing *in vitro* and cellular assays and animal studies. The plan should end with the selection of a clinical candidate. Included in the plan are estimated timelines for each step, "go" and "no-go" decision points, foreseeable challenges, and mitigation plans.

Screening reagents and assays are the "bricks and mortar" to a successful selection of a therapeutic candidate. The reagents include all the essential materials needed to develop and carry out the screening assays such as cDNA and expression vector for the target, purified target protein, and cells that express the target for testing candidate antibody functions. The screening assays allow the selection of mAb candidates based on target binding and biological activities.

For drug discovery projects, animal models are used to determine the efficacy, pharmacokinetics (PK), pharmacodynamics (PD), and safety of a drug candidate. The development of relevant animal models often takes months to years and should be initiated early in the drug discovery process.

1.2.3 Lead Selection and Optimization

Lead selection is a process in which a rigorous screening scheme is applied in order to identify a candidate that meets the defined drug-like criteria of functional and molecular properties. The leads from this process are early drug candidates and may not have all the drug-like properties such as minimal immunogenicity profile, favorable biochemical and biophysical properties, and optimal PK properties. These properties can be subsequently optimized using a variety of technologies and ultimately developed into a viable clinical candidate.

Three major technologies have been used to develop the mAb drugs currently on the market: hybridoma, phage display, and human transgenic mouse technologies.

The hybridoma technology is a classic mAb generation technology first developed by Kohler and Milstein in 1975 [12]. Using this method, rodents are immunized

with target proteins to induce a high-titer response indicative of the generation of high-affinity rodent antibodies. The splenocytes are subsequently harvested from responding animals and fused with myeloma cells to create clonal hybridoma cells capable of secreting antibody molecules. Each hybridoma clone generates a single IgG, which is the basis for the name of mAbs. The hybridoma clones can be perpetually expanded in cell culture to provide nondepletable sources of therapeutic candidate molecules. For clinical manufacturing, however, hybridoma culture is not well suited for an industry process because of the inefficiency of these cells producing high concentrations of antibodies. Instead, DNA sequences of the IgGs from hybridoma clones are determined, and recombinant mAbs are generated in industrial quantities for clinical testing. The ultimate therapeutic recombinant mAbs have often been modified from the original rodent hybridoma mAbs using antibody engineering methods such as humanization (see later discussion).

As demonstrated by its long history, hybridoma technology is a reliable method to generate high-affinity antibodies to many protein targets and is still widely used in industry to derive early mAb therapeutic candidates. The shortcomings of this method include a relatively long cycle time from immunization to the identification of desirable mAbs (3–4 months) and difficulties to generate antibodies to proteins that are toxic to or immunotolerated by the host animal.

In contrast to the utilization of an *in vivo* system central to the hybridoma technology, phage display is a purely *in vitro* system. As first demonstrated by McCafferty et al. [13], a scFv or the Fab fragment of an IgG can be displayed on the surface of a phage particle as a fusion protein to the gene 3 phage coat protein. Built upon modern recombinant DNA technology, phage display is an elegant technology to derive antibody fragments that bind to a protein of interest. A library constituting a vast number of diverse scFv or Fab sequences up to the order of 10^{11} is generated. The diversity of the scFv or Fab sequences is derived from either a natural human antibody repertoire isolated from immune tissues or a synthetic repertoire where recombinant DNA technology is used to generate sequence variations [14]. During the screening process, the phage-displayed scFv or Fab is allowed to bind to the target protein and is separated from the nonbinders. The sequence of the binding scFvs or Fabs can be determined from the bacterial clones producing these "functional" phage particles. The selected scFv candidates are genetically converted back to full IgG molecules that are functionally indistinguishable from natural IgGs.

Compared with the hybridoma technology, phage display technology offers a quick way to find functional antibody fragments in a matter of weeks. In addition, the *in vitro* experimental conditions are highly amenable to manipulations and can facilitate the isolation of antibodies to difficult targets that may not be easily generated via a hybridoma approach. However, phage display has been a proprietary technology and thus is less accessible to general use than hybridoma technology. This may change given that the patent on gene 3 fusion technology has expired in 2012. To date, the majority of approved mAbs have been derived from hybridoma technology with adalimumab and recently belimumab being the only phage-derived mAb on the market. It is believed that this technology is reaching its maturity and will give rise to many more approved mAb drugs in the coming years [15].

The human transgenic mouse technology is built upon the hybridoma technology to directly generate fully human mAbs in immunized transgenic mice that express human IgGs in place of murine IgGs. The best known human transgenic mouse technologies are Medarex's HuMab-Mouse and Abgenix's XenoMouse. New strains of human transgenic mice will continue to be generated. Five therapeutic mAbs derived from this technology have gained FDA approval (Table 1.1). Despite the success of using this technology to bring forth marketed therapeutic mAbs, its proprietary nature limits general access and wide usage.

After a mAb lead is identified, it often needs to be optimized before being selected as a therapeutic candidate. Humanization has become widely used to maximize the content of the human sequence in a mAb of rodent origin to greater than 95% (reviewed by Almagro and Fransson [16]). Compared with the parental rodent mAb, the "humanized" mAb has significantly reduced "foreignness" and thus an improved safety profile in relation to the immunogenicity of the molecule (see later discussion). The process in general takes 3 to 6 months with a near 100% success rate to yield a humanized molecule with activity comparable to the parental rodent mAb.

Affinity maturation is another widely used antibody optimization method used to significantly increase the antigen binding affinity of the parental antibody [17]. Either selected CDR sequences or the entire variable domains of the parent antibody are randomized to create a library of mutant antibody molecules, from which those with improved antigen binding affinity are selected. Two widely used enabling technologies for affinity maturation are phage display and ribosomal display. An increase in binding affinity in the range of 10- to 100-fold can be achieved from this optimization process.

Fc engineering is a technology aimed at endowing optimal effector functions and half-life extension to the parental mAb. The effector function of a mAb often plays a role in its therapeutic function such as the killing of cancer cells via antibody-dependent cellular cytotoxicity (ADCC) or antibody-mediated complement-dependent cytotoxicity (CDC). In other cases such as targeting receptors on normal cells, it is beneficial to reduce or abrogate the effector function of a mAb to avoid deleterious effects resulting from potential ADCC or CDC. Mutations in the Fc that up-or down-regulate the effector functions of an IgG have been vigorously studied and presently applied to candidate molecules in clinical testing [18]. Additionally, Fc mutations that increase IgG interaction with FcRn, a receptor on endothelial cells that helps recycle a bound IgG back into circulation, have been shown to significantly improve the serum half-life of an IgG [19].

1.2.4 Selection of a Clinical Candidate

The selection of a clinical candidate is a process in which a lead mAb has been thoroughly evaluated for its biological activities and molecular characteristics. The biological activities refer to desirable biological functions as characterized in a combination of *in vitro* systems, cellular systems, and animal models. Based on these characterizations, a clinical candidate molecule is deemed to have met predefined efficacy and safety criteria for clinical testing in human subjects. The molecular

characteristics refer to the intrinsic behavior of a candidate molecule that must be amenable for manufacturing as well as delivering into human patients. The molecule must demonstrate acceptable levels of expression in a manufacturing production system, display favorable physiochemical characteristics, and exhibit desirable solubility in a formulation used for clinical administration. Other considerations for clinical candidate selection that need to be taken into account include commercial assessment, competitive landscape, and intellectual properties. After a molecule is selected as a clinical candidate, it triggers a number of preclinical development activities, leading to investigational new drug (IND) filing and the commencement of clinical studies. It is not unusual that a candidate selected from early stages of discovery process fails candidate selection because of various reasons associated with the criteria described.

1.2.5 Key Differences in the Discovery Process for Monoclonal Antibodies Versus Small Molecule Drugs

Although the general concept and overall drug discovery process from selecting a target to nominating a clinical candidate are similar for mAbs and SMs, there are some key differences. In general, the discovery cycle time for mAb drugs can be significantly shorter than that for SMs. Therapeutic mAb discovery is becoming a platform process that allows rapid identification, characterization, and production of lead candidates. Whereas it is very challenging to optimize an initial SM "hit" to turn it into a drug candidate amenable for human testing, many drug-like properties of mAbs are intrinsic to this class of molecule. This section discusses several specific areas that illustrate key differences in this process for the two different classes of drugs.

1.2.5.1 Therapeutic Targets Protein therapeutics including mAbs are best suited for modulating molecular targets expressed on the surface and outside of the cells. Proteins lack intrinsic abilities to penetrate cell membranes to reach intracellular targets. SMs can access targets localized either inside or outside of a cell. In addition to the cellular location of a target, physiological barriers such as the BBB in the central nervous system (CNS) can greatly limit the passage of large molecules such as mAbs from the circulation into the brain.

The mode of target modulation can be different for mAb and SM drugs. Being structurally small, an SM drug typically modulates a target by docking onto a small pocket in a protein such as an adenosine triphosphate (ATP) binding site of a kinase and functionally altering the target molecule. It is difficult for SM drugs to disrupt protein–protein interactions that take place over large binding interfaces, a task that can be easily achieved by mAbs.

1.2.5.2 Screening Scale The screening scales are significantly different for the two classes of drugs during the lead discovery phase. Whereas screening tens to hundreds of thousands of chemical compounds is a routine practice for a SM project, the screening scale is much smaller for mAbs, which is typically in the range

of thousands of test molecules. For mAbs, a build-in enrichment process before the screening step facilitates a reduction in the screening scale. For hybridoma antibody generation, the immunized rodents are an *in vivo* selection system that filters out nonspecific antibodies from the naïve antibody repertoire and enriches for high-affinity and antigen-specific antibodies. For phage antibody generation, the initial *in vitro* selection process enriches populations of phage particles that display target-binding antibody fragments. In both processes, the screening is carried out primarily to deconvolute single clone binding activities from an enriched binder pool. SM lead generation lacks this selection and enrichment process and generally relies on large-scale screening to derive candidate molecules.

1.2.5.3 Need for Surrogate Molecule Surrogate antibodies are pertinent to many mAb drug discovery projects. Because of its exquisite specificity, a mAb developed to target a human protein in many cases fails to bind to its orthologous proteins in other animal species. The evaluation of a drug candidate in animals for efficacy, safety, and PK and PD, however, is important, and these are required preclinical studies. Therefore, in many cases, in parallel to the generation of the therapeutic candidate, a surrogate antibody is also created that is functionally equivalent to the therapeutic candidate and binds the target ortholog expressed in the appropriate animal species used for drug discovery models, most often mouse. It is noteworthy that SMs are not entirely excluded from the issue of lacking species cross-reactivity. However, the surrogate approach is not a routine practice during the discovery process of an SM drug.

1.2.5.4 Drug Properties Lipinski's rule of five is a general set of rules to evaluate drug-like molecular properties of a SM compound [20]. Similarly, there are emerging rules to describe the drug-like physicochemical properties for protein drug candidates. Given their distinctive molecular compositions, SMs and mAbs each have uniquely defined drug-like properties. For SMs, these properties are associated with the presence of H-bond donors and acceptors, molecular weight, and partition coefficient; for mAbs, they are based on expression levels, aggregation propensity, stability, solubility, and posttranslational modifications.

The two classes of drugs also bear different intrinsic toxicity risks. A SM is prone to bind to unintended molecular targets in humans and elicit off-target toxicity, which may not be manifested in an *in vitro* screen or animal toxicity models. On the contrary, mAbs in general exhibit low off-target toxicity because of highly specific binding to their molecular targets.

Immunogenicity is a unique safety concern for protein therapeutics, including mAbs. The immune system of a patient who receives a protein drug can recognize it as a foreign entity and react with an antidrug response. The consequence of such immunogenicity response ranges from negligible or mild effects to severe anaphylactic response [21]. Presently, there are few methods at the preclinical stages to accurately predict the immunogenicity of a clinical protein drug candidate, albeit that a number of practices are used to reduce the likelihood of immunogenicity, which include the prediction and elimination of T-cell epitopes in the mAb molecule and the

utilization of *ex vivo* assays to monitor the potential of a mAb to activate T cells [22]. A vigorous determination of antidrug antibody response is mandated to monitor the immunogenicity of a protein drug during clinical development.

1.3 MANUFACTURING PROCESS DEVELOPMENT FOR BIOLOGICS

1.3.1 Introduction

After a candidate with appropriate efficacy and safety has been identified, the research focus shifts to process development and manufacture of clinical trial materials. A robust manufacturing process is critical to ensure that appropriate quality is reproducibly achieved in the packaged clinical supplies. Compared with SMs, biotherapeutics are large, complex molecules that fold to incorporate specific tertiary structure and are subject to a variety of posttranslational modifications. As such, a biotherapeutic drug is a complex mixture of species. Furthermore, the specific composition of the mixture is profoundly affected by the manufacturing process as well as handling and storage conditions. Therefore, to reproducibly control the specific composition of the mixture and the quality of the resulting clinical supplies, it is necessary to understand the critical processing parameters during manufacture and handling and storage conditions and how they ultimately impact product quality.

1.3.2 Early Assessment of Discovery Candidate(s)

During the transition from the discovery research phase to the clinical development phase, a candidate or several candidates are typically assessed for suitability for manufacturing process development. mAbs are a special class of biologics that share common structural features that make them well suited to platform production and purification. Platform production involves defining a set of conditions for production that are suitable for most antibodies, albeit the platform may not be optimal for any given candidate. Antibodies are typically assessed for fit in a defined platform for process development and production. This usually involves an assessment of expression in mammalian cell lines or titer based on data available from the discovery process, which may include data from transient expression or pools derived from stable transfection into a Chinese hamster ovary (CHO) host cell line, for example. In transient HEK-293 systems, titers (i.e., expression levels) of less than 50 mg/l may present a potential concern and would likely require close monitoring during development to ensure acceptable expression titers are achieved in mammalian cell lines resulting from stable transfection.

Evaluation of a candidate's propensity to aggregate and to undergo degradation in a preferred formulation or set of formulations is an important part of the early assessment process. Aggregation can occur during all phases of production, and controlling the levels of aggregate in the final product can be challenging. In addition to aggregation, significant degradation pathways such as oxidation,

deamidation, isomerization, and peptide bond cleavage are also evaluated early, typically at multiple temperatures. Often accelerated stability studies are carried out under more extreme conditions to understand the major degradation pathways for a specific candidate or set of candidates. It is important to recognize that because different degradation pathways may be accelerated at different rates, these studies need to be analyzed carefully and may not represent the distribution or even the specific composition of the various impurities under standard conditions [23,24].

The early assessment of candidates is largely intended to identify those that may present significant challenges during development. If multiple candidates are being considered for development, the selection can be based on a panel of data including, but not limited to, efficacy, tolerability, and stability. Early formulation studies can help to inform the selection decision. If a candidate shows particularly poor stability during the early assessment, it can be a significant or determining factor in candidate selection. It is also important to understand that although an early assessment can highlight potential challenges for a specific candidate, it does not replace subsequent development work; rather, it is the foundation for the preclinical development that follows.

1.3.3 Bioprocess Development and Manufacture

After a development candidate has been identified, cell line development begins with transfection of a suitable expression vector into a host cell line. Mammalian expression systems predominate for the large-scale production of antibodies because they can perform complex posttranslational modifications that are important for correct protein folding, stability, multimer formation, and secretion into the medium [25]. CHO cell lines are some of the more commonly used host cell lines for antibody production and use selectable markers based on dihydroxy folate reductase (dhfr) and glutamine synthetase (GS) that are available in suitable stable expression vectors for these cell lines [26,27]. Cell line stability is another key consideration during development. Typically, a good production cell line will be stable for 70 to 100 generations. The primary goal is to create a stable cell line with the appropriate growth properties and a high specific productivity (Q_p). The selection process is carried out over progressively increasing scales from microtiter plates to small bioreactors. During the selection process, the material that is produced is used to develop or confirm a suitable formulation and downstream processing methods and to evaluate analytical platform methods. The availability of this material and material produced during subsequent cell culture process development for parallel analytical and pharmaceutics development is critical for an efficient and integrated strategy for production of clinical trial material. After a clone is selected, a master cell bank (MCB) is prepared. The MCB, or a working cell bank (WCB) prepared from the MCB, is used in the scale-up and production of material for enabling regulatory toxicology studies and clinical trials. It is worth noting that the MCB is prepared under good manufacturing practices (GMP) conditions and is used to generate all the antibody supplies for the lifetime of the product if the candidate is successful. Thus, the preparation of an MCB represents the earliest component of the commercial process.

For mAbs, platform cell culture process development presents a number of challenges, including the need to adequately control for protein misfolding, aggregation, oxidation, deamidation, proteolysis, and glycosylation variants. Each of these product-related impurities should be monitored and controlled under the platform conditions. In some cases, the platform process may need to be modified to address candidate specific issues.

Scaling the process involves thawing vials from an MCB or WCB. This initial inoculum is expanded in shake flasks and small bioreactors and is then transferred to progressively larger seed reactors before transfer of the cell mass to the production bioreactor. There are two growth phases during fermentation. During the initial growth phase, the primary objective is to increase the viable cell mass; during the protein production phase, cell growth slows, and antibody expression and secretion ensue. A typical mAb cycle time in the production bioreactor is 10 to 12 days.

The common structural features of antibodies make them well suited to platform purification. Most, if not all, large-scale purification schemes incorporate protein A or MabSelect chromatography as the first downstream purification step. The protein A–immobilized ligand binds to the Fc region of antibodies while host cell proteins (HCPs), DNA, and other process-related impurities flow through in the mobile phase. Typically, protein A chromatography affords product in greater than 90% purity after elution from the column at low pH [28]. Ion exchange chromatography is commonly used to further purify the antibody product from HCPs, DNA, and other process- and product-related impurities. Additional filters or chromatography steps can be used to address candidate-specific issues.

Because antibodies are produced in mammalian cell culture, which can harbor viruses harmful to humans, two orthogonal methods to remove viral particles are typically incorporated in the downstream process in addition to the chromatography steps. These generally include a low pH viral inactivation step followed by viral filtration.

For first in human (FIH) clinical trials, it is generally acceptable to demonstrate clearance of two representative viruses, assayed once using new resin; however, because subsequent trials in Europe would fall under the 2008 European Medicines Agency (EMA) guidelines and because viral clearance studies are expensive and time consuming, more and more companies are choosing to comply with the more recent EMA guidelines in US trials even at early stages of development. Viral clearance validation for phase 3 and biologic license application (BLA) filing is much more comprehensive, including, but not limited to, demonstrating clearance for a minimum panel of four representative viruses, assayed in duplicate using new and recycled resins [29].

The last downstream step is commonly ultrafiltration/diafiltration (UF/DF) to reduce the volume, increase the protein concentration to an appropriate level for formulation, and incorporate selected stabilizing excipients to facilitate storage and preparation of the fully formulated drug product. Production of clinical-grade material involves execution of multistep processes, and often multiple facilities are required to support manufacture of the drug substance, the drug product, and the required analytical testing. To simplify logistics, it is often desirable to develop and

define hold conditions for downstream process intermediates and storage conditions for the drug substance. The downstream process for production of drug substance is designed to eliminate or reduce impurities and degradation products within acceptable limits and to minimize formation during processing to afford a drug substance with the appropriate quality.

1.3.4 Formulation Process Development and Manufacture

All proteins are subject to various degradation pathways. Degradation can occur during the process to prepare the drug substance or drug product and during their storage in the liquid, frozen, or solid state. An understanding of the various degradation pathways is critical not only to minimize and remove impurities during production but also to maintain the appropriate quality attributes of the final drug product during and after production and during shipping and storage.

Degradation of proteins can be generally categorized as either physical or chemical. Physical degradation includes surface adsorption, denaturation, and aggregation. Examples of chemical degradation include deamidation, oxidation, isomerization, fragmentation, and cross-linking.

As the demand for subcutaneous and intramuscular injection routes of administration to support patient focused delivery approaches increases, the ability to formulate proteins at increasingly higher concentrations has become a major focus in the pharmaceutical industry. These high concentrations present unique challenges, including the possibility for aggregation, precipitation, and high-viscosity solutions that can be difficult to administer to patients.

Although antibodies share a common framework, many of their degradation pathways are associated with specific primary sequences or the unique antigen-binding regions (i.e., CDRs). Additionally, the rates of various degradation pathways for a single antibody differ under a specific set of conditions, including pH, temperature, and concentration, as well as processing and handling conditions. Therefore, determining the optimal formulation and storage conditions can be very candidate specific. Most platform strategies strive to define a formulation or set of formulations that are suitable for the majority of candidates but not optimized for any specific candidate. This approach has been quite successful for antibodies but does not eliminate the need to understand the degradation pathways for a specific candidate and document that the selected formulation(s) are suitable for that antibody. This is particularly important for antibody drug candidates that ultimately become successful commercial products because the dose and dosage form generally evolve as development progresses from FIH studies to regulatory approval and launch.

1.3.5 Analytical Method Development and Manufacture

In the previous sections, a common theme is the importance of understanding how the production process, formulation, handling, and storage conditions affect the critical quality attributes of biological drug candidates. Key to this understanding is the ability to monitor and measure the process- and product-related impurities

observed during development and production and relate them to the efficacy and stability of a given candidate. A variety of methods can be used to identify, monitor, and quantify the various process- and product-related impurities.

Of the various process-related impurities, HCPs, DNA, and endotoxins are of primary concern because they can have negative side effects on patients. Endotoxins, or pyrogenic lipopolysaccharides (LPS), are derived from Gram-negative bacteria such as *Escherichia coli*. In mammalian production hosts, endotoxins can be introduced into a process via raw materials [30,31]. DNA can be extracted and quantified by standard techniques, including quantitative polymerase chain reaction (qPCR). Additionally, a number of host cell–specific enzyme-linked immunosorbent assay– (ELISA-) based assays to quantify HCP and DNA are also available [32].

Product-related impurities are equally important to monitor, and a variety of analytical methods have been used to quantify the physical and chemical degradation pathways of proteins. Aggregation is one of the more significant types of physical degradation. A variety of methods for detection and quantification can be used, depending on the size and type of aggregates. Size exclusion chromatography–high-performance liquid chromatography (SEC-HPLC) supplemented with appropriate orthogonal methods is a commonly used analytical strategy. Analytical methods to sufficiently characterize glycosylation patterns are extremely important for antibodies. Although heterogeneity in glycosylation patterns is not unusual, proper glycosylation and distribution of variants may be critical for proper antibody function [24]. Oxidation, deamidation, and fragmentation are some of the more common chemical degradation pathways observed for antibodies. Fragmentation can be detected using methods that separate intact and fragmented molecules based on size. Deamidation is the primary source of charge heterogeneity, and initial identification may involve detection of differences in charge distribution by isoelectric focusing (IEF) or high-performance cation exchange chromatography [33]. Similar methods can be used to assess oxidative degradation.

The methods described can be used for most protein therapeutics, and many are amenable to platform development for antibodies. Platform analytical methods are designed to detect and quantify the expected degradation pathways for antibodies, although the impurity profile for any specific antibody tends to be unique. Ultimately, however, for each candidate, it is necessary to develop candidate-specific identity and bioassays. Often simple binding ELISA-based (or BIACore) bioassays are initially developed for early clinical development. It is necessary, however, to develop a cell-based or functional bioassay for later stage clinical trials.

1.3.6 Project Management and Critical Path to the Clinic

Production of clinical supplies requires close collaboration among multiple scientific lines. Often multiple teams, each with a different focus, are working in an integrated and iterative way during research, process development, and manufacture with multiple hand-offs between these teams. More often than not, research, process development, and manufacture occur over multiple locations and long periods of time, during which personnel move in and out of various roles on the teams.

Furthermore, outsourcing some or all of the process development or manufacturing to contract manufacturing organizations (CMOs) is becoming increasingly common, which brings with it additional complexity. The outsourced work typically requires a technology transfer and some level of oversight personnel to facilitate and act as technical consultants. The lead times to identify a CMO, conduct a quality audit, negotiate a contract, and schedule a slot for the work can cause delays if appropriate planning is not in place. Moreover, managing the contract and the payment schedule can require additional nontechnical oversight. Although the pharmaceutical industry's focus on speed to the clinic in recent years has fueled the implementation of platform process development for antibodies, the technical and business complexities have necessitated careful planning and project management to facilitate and streamline platform antibody production across scientific lines and to ensure there is an appropriate balance between speed, cost, and quality.

In an accelerated development strategy, the availability of representative protein generally defines the critical path to the clinic: first the material from cell culture development to enable the parallel development of the downstream purification process, analytical methods, and a suitable formulation; then the good laboratory practice (GLP) material for a regulatory toxicology study; and finally, packaged GMP supplies with supporting stability studies to enable clinical dosing. Because GMP supplies can be prepared while the regulatory toxicology is underway, from a production perspective, it is the process development and manufacture of GLP material for regulatory toxicology that is on the critical path to the clinic.

1.4 REGULATORY REVIEW AND APPROVAL FOR BIOLOGICS

1.4.1 US Drug Law

The basis of US drug law is the Federal Food, Drug, and Cosmetic Act (FD&C Act) of 1938. Its passage by the US Congress required new drugs (prescription and over-the-counter drugs, medical devices, foods, and cosmetics) to be shown safe and effective before marketing and ushered in the modern area of drug development. In 1944, the Public Health Service Act (PHS Act) was subsequently passed to provide the pathway to regulate the licensure of biologic products such as vaccines, blood products, allergenic products, and other products such as mAbs. The significance of this is that for marketing of a new product, a New Drug Application (NDA) is used for SM drugs subject to the drug approval provisions of the FD&C Act, and a biologics license application (BLA) is required for biological products subject to licensure under the PHS Act. The Prescription Drug User Fee Act (PDUFA) required drug and biologics manufacturers to pay fees for product applications and supplements, and other services.

1.4.1.1 Food and Drug Administration Modernization Act of 1997 The Food and Drug Administration Modernization Act (FDAMA) reauthorized PDUFA and, at the time, mandated the most wide-ranging reforms in FDA practices since 1938.

Provisions include measures to regulate advertising of unapproved uses of approved drugs, to increase patient access to experimental drugs, and to accelerate review of important new medications. In addition, the law provided for an expanded database on clinical trials accessible by patients.

1.4.1.2 Best Pharmaceuticals for Children Act of 2002 The Best Pharmaceuticals for Children Act (BPCA) continued the exclusivity provisions for pediatric drugs as mandated under the FDAMA, in which market exclusivity of a drug is extended by 6 months, and in exchange, the manufacturer carries out studies of the effects of drugs when taken by children.

1.4.1.3 Pediatric Research Equity Act of 2003 The FDA is given clear authority under the Pediatric Research Equity Act (PREA) to require sponsors to conduct clinical research into pediatric applications for new drugs and biological products.

1.4.1.4 Food and Drug Administration Amendments Act (FDAAA) of 2007 Among other things, the FDAAA reauthorized PDUFA, PREA, and the BPCA. Other major topics covered within this wide-ranging legislation are new FDA authorities for requiring label changes and postmarketing studies, new and expanded requirements for registration of clinical trials and disclosure of results, formation of the Reagan-Udall Institute in support of the FDA's Critical Path initiative, new conflict of interest rules for the FDA Advisory Committee members, and new rules on citizen petitions.

Another landmark act was the Federal Register Act of 1935, which established the basic legal structure of the US regulatory system. The Administrative Procedure Act of 1946 added procedural requirements to ensure due process and public participation. As a result of the Federal Register Act, the daily *Federal Register* provides a single, uniform publication for executive agency rules, notices, and presidential documents, thus providing an official notice of a document's existence, its contents, and its legal effect. From this came the Code of Federal Regulations (CFR). It is a codification of rules published in the *Federal Register* by the executive departments and agencies of the federal government.

1.4.2 Food and Drug Administration Overview

The FDA is a consumer protection agency and falls within the executive branch of the US government under the Department of Health and Human Services (DHHS). The FDA has a broad range of responsibilities, including regulation of drugs, biologics, medical devices, animal drugs, food, and cosmetics. The FDA's major activities are: (1) reviewing new products; (2) ensuring safe drug manufacturing and handling; (3) monitoring for new risks, standards, and regulations (4) research; and (5) enforcing actions to protect public health. There are five FDA centers with the Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER) responsible for providing regulatory guidance for new drug products while ensuring that marketed products are safe and effective.

The CBER reviews products according to type (e.g., vaccines), and the CDER reviews products by area of medicine (e.g., oncology). The CDER does review certain biologic agents, proteins intended for therapeutic use that are extracted from animals or microorganisms. These include most of the recombinant proteins such as mAbs, cytokines, and growth factors as well as the more traditional pharmaceuticals. The other biologic products, including blood and blood components, cellular and gene therapy products, vaccines, antitoxins, allergenic extracts, and venoms, are reviewed by the CBER.

1.4.3 Drug Development in the United States

The major phases of the drug development process in the United States are discovery or preclinical testing, phase 1 FIH studies (typically a single ascending dose and multiple ascending dose study), a phase 2 proof of mechanism study, and phase 3 pivotal registration studies in the intended patient population followed by market approval with potential phase IV postmarketing commitments. In the United States, it takes anywhere from 10 to 15 years for an experimental drug to move from the laboratory to the patient. Approximately only one in 5000 synthetic SM pharmaceuticals proceed from discovery to approval.

To get approval in the United States to begin clinical testing, the sponsor must submit an IND application to the FDA. The contents of the IND provide the data and rationale to support proceeding with human clinical testing of the drug. The IND application must contain information in three broad areas. The first is animal pharmacology and toxicology studies that permit an assessment as to whether the product is reasonably safe for initial testing in humans. The second is manufacturing information pertaining to the composition, manufacture, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company can adequately produce and supply consistent batches of the drug. Finally, the clinical development plan is required with detailed protocols for the proposed clinical studies to allow an assessment of whether the initial-phase trials will expose subjects to unnecessary risks. Also, information should be provided on the qualifications of clinical investigators as well as commitments to obtain informed consent from the research subjects, to obtain review of the study by an institutional review board (IRB) and to adhere to the IND regulations.

In general, an NDA or BLA is filed at the completion of all three clinical trial phases (with the exception of accelerated approval). It contains all of the scientific information (studies in animals and humans) and addresses the safety and efficacy of the drug. The goals of a NDA or BLA are to provide sufficient information to permit the FDA reviewer to reach the following key decisions: (1) that the drug is safe and effective in its proposed use(s); (2) the benefits of the drug outweigh the risks; (3) the proposed labeling (package insert) is appropriate for what it should contain; and (4) methods used in manufacturing the drug and the controls used to maintain the drug's quality are adequate to preserve the drug's identity, strength, quality, and purity.

After the FDA receives a NDA or BLA, the review period is defined by the PDUFA, and the FDA has 60 days to accept or refuse to file. The FDA begins the review by assembling a review team. Based on requirements or to gain expert input, the FDA may also seek the advice of an advisory committee. Its role is to provide independent advice that contributes to the quality of the agency's regulatory decision making and lends credibility to the product review process. The ultimate decision to approve is based on whether the drug's benefits outweigh the potential risks. After a recommendation (e.g., approved or complete response) on the application is reached by the reviewers and their supervisors, the director of the applicable Drug Review Division or office evaluates the decision. After the product is approved, the product can be legally marketed in the United States starting on the date of approval.

1.4.4 US Generic and Biosimilar Legislation

After a product is marketed, it has a finite time of exclusivity before a generic competitor can enter the marketplace. This time frame is dictated by the type and quality of approved patents associated with the innovator product. This patent protection provides the innovator drug maker time to recoup its development costs associated with bringing the therapy to market and thus also provides incentive for continual new drug development. The current system of generic drug approval was established by the Drug Price Competition and Patent Term Restoration Act of 1984 (also known as the Hatch-Waxman Act) [34]. This act amended the FD&C Act and provided two abbreviated pathways for the approval of generic drugs, including natural source products and recombinant proteins.

Section 505(b)(2) of the Hatch-Waxman Act eliminated the necessity of the generic competitor to duplicate all of the preclinical and clinical studies performed by the innovator for drug approval. This allowed the direct comparison of the generic drug with the innovator product already approved for the same indication while using abbreviated clinical trials of 3 to 6 months in duration to demonstrate comparability. The second abbreviated pathway as outlined in Section 505(j) sets forth the process by which the producer of a generic drug that is identical to a previously approved innovator product can file an Abbreviated New Drug Application (ANDA) to seek FDA approval. An ANDA allows the applicant to rely on the FDA's previous finding of safety and efficacy for the already approved drug; the FDA can request only bioavailability studies as supportive data. These allowances for an ANDA application and ultimate approval were tied to a couple of assumptions. The first assumption was that the generic drugs would be exactly the same as the innovator drug. The FDA instituted a policy that the bioavailability of the generic drug needs to be within $\pm 20\%$ of active ingredient over a period of time compared with the innovator product. For drugs with a very narrow therapeutic range, this may be problematic. The second assumption was that bioequivalence data were accurate surrogates to an innovator drug's safety and efficacy results.

The Hatch-Waxman Act does provide the innovator a period of exclusivity, so-called "data exclusivity," of up to 5 years upon approval before a generic version can enter the market. However, the act is also intended to provide a mechanism for

generic competitors to challenge the innovator's patents and provide the first in time challenger with 180 days of generic market exclusivity. To file an ANDA, the competitor must make one of four certifications: (1) the drug has not been patented, (2) the patent has expired, (3) the generic will not go on the market until the patent expires, or (4) the generic will not infringe on the patent or the patent is invalid.

In March 2010, as a part of the Patient Protection and Affordable Care Act (PPACA), or health care reform, Congress passed the Biologics Price Competition and Innovation Act (BPCI Act) of 2009, which amends Section 351 of the Public Health Service Act, creating a statutory pathway for FDA approval of follow-on biologics as "biosimilar" to or "interchangeable" with the innovator marketed biologic products [35]. The biosimilar product must have the same presumed mechanism of action, route of administration, dosage form, and potency as the innovator product. It may only be reviewed and approved for indications for which the FDA already has approved the innovator product.

The necessity for the BPCI Act is because although Section 505(b)(2) of the FD&C Act allows for the approval of generic drugs involving natural source products and recombinant proteins, the complexity of the large biopharmaceuticals makes it difficult to demonstrate that a biosimilar product is structurally identical to an already approved biopharmaceutical. Unlike SMs, for which analytical methods are adequate to ensure the sameness of a generic product to the innovator product, the size and complexity of the molecular structure of biologics preclude any exact structural comparison between the innovator and the biosimilar product. Moreover, the manufacture of recombinant proteins in living cells can result in subtle differences with respect to glycosylation and other posttranslational modifications. Even nonrecombinant proteins that are purified from their natural sources can exhibit different posttranslational modifications and have alterations in their amino acid sequences. Because the correlation between the efficacy and safety of a biopharmaceutical and its structural characteristics is rarely established, it is uncertain how these slight modifications may impact its effectiveness. In light of these factors, a follow-on biologic cannot be expected to replicate the innovator product precisely; therefore, these molecules are referred to as *biosimilars*. A biosimilar product is defined as one that is "highly similar" to the reference product notwithstanding minor differences in clinically inactive components and for which there are no clinically meaningful differences between the biological product and the reference product in terms of safety, purity, and potency of the product [36].

Under the BPCI Act, biosimilars also have the opportunity to meet a higher standard of similarity to a reference product, "interchangeability," reflecting an FDA assessment that pharmacists can make substitutions between biologics without the prescriber's intervention. A biologic will be considered interchangeable with a reference product if the developer demonstrates that it can be expected to produce the same clinical result in any given patient and that the risk associated with alternating or switching between the two products is not greater than that involved in continuing to use the reference product.

In response to the BPCI Act, the FDA has recently issued draft guidelines to define the approval process and requirements for biosimilars [37–39]. In these draft

guidelines, the FDA is advocating a stepwise approach to demonstrate biosimilarity. The first step relies on extensive comparative analytical data to the reference product to set the stage for the clinical development requirements. The more robust the analytical data demonstrating comparability to the reference product, the potential for a more targeted, streamlined clinical development program. The next step would involve consideration as to the toxicity studies that should be conducted to support biosimilarity. To avoid large head-to-head phase 3 clinical trials, the FDA is putting an emphasis on immunogenicity and PK and PD studies to mitigate this "residual uncertainty" of the biosimilarity of the product. At a minimum, the FDA anticipates that at least one clinical study to assess immunogenicity will likely be required. In addition, a clinical trial will also be required for a biosimilar to be considered interchangeable with the reference product. In addition, postmarketing surveillance may also be a key point of emphasis for any biosimilars application.

1.5 BIOLOGICS: THE PAST, THE PRESENT, AND THE FUTURE

With the ongoing shift in the pharmaceutical industry from an SM-centric to a balanced portfolio with equal shares of SMs and biologics, the development of biological drugs has gained a great deal of momentum as reflected by a large number of biologics in preclinical and clinical pipelines. Most major pharmaceutical companies have now implemented sophisticated processes and infrastructures to develop biologics, particularly mAbs and other protein therapeutics.

Cutting-edge technologies have significantly impacted the field of biologics discovery and development. At the molecular level, considerable protein engineering efforts have led to the development of novel molecular scaffolds, optimized molecular functions, and enhanced drug properties. At the process level, novel technologies have resulted in an increase in screening throughput, innovative methods to generate antibody candidates, reduced cycle time of early drug discovery process, and new capabilities to tackle challenging issues.

This section discusses several major trends in the discovery and development of biologics with a focus on mAb therapeutics.

1.5.1 Biosimilars

By McCamish and Woollett's definition, a biosimilar is a "follow-on biologic that meets extremely high standards for comparability or similarity to the originator biologic drug that is approved for use in the same indication" [40]. Having suffered from severe loss of profits immediately after a brand-name drug comes off patent, the industry has taken a drastically different approach with generic biologics, or biosimilars. Large pharmaceutical manufacturers in addition to generic drug makers have both aggressively entered the play field. Led by Merck, pharmaceutical giants Eli Lilly, Pfizer, and several others have publicly announced their endeavor in the making of biosimilars and are actively developing pipeline portfolios. Since the approval of the first biosimilar omnitrope (recombinant human growth hormone;

Sandoz) in 2006 in Europe, a number of biosimilars have now been approved by EMEA, including two biosimilar somatropins, five biosimilar rHuEPOs (recombinant human erythropoietins), and four biosimilar filgrastims [41].

The regulatory path for approving biosimilars is emerging. The EMA has led the way and issued a series of guidelines to regulate the approval of biosimilars in Europe. In the United States, FDA has started to define the approval processes and requirements (see earlier discussion). In contrast to the approval of generic SM drugs, the regulations for biosimilars are considerably more sophisticated because of the complex nature of biologics. In addition to the demonstration of composition equivalency, for a biosimilar, the proof for biological equivalency is required. As a result, the cost for developing biosimilars is considerably higher than that for generic SMs, and their market share relative to the brand name counterpart could fall in the 30% range as opposed to 70 to 90% for generic SMs (http://www.gabionline.net/Biosimilars/Research/How-profitable-will-biosimilars-be). In the coming years, we will learn much more about the regulations for biosimilar approval, the safety profiles of marketed biosimilars, the market shares relative to their brand-name counterparts, and the competitive landscape composed of both large pharmaceutical and generic drug makers.

1.5.2 Novel Scaffolds

One actively explored area in protein therapeutic discovery is the development of novel scaffolds that demonstrate unique and potentially superior features as compared with the established scaffolds best represented by mAbs. Novel molecular scaffolds from fibronectin-based scaffold to camelid nanobodies have been incepted first in academia more than a decade ago [42,43]. A myriad of biotechnology companies have devoted their efforts to transforming novel scaffolds into drug discovery platforms. A key challenge is to endow these novel scaffolds with drug-like attributes comparable to the well-established mAb-based scaffold. Although molecules derived from many novel scaffolds exhibit extraordinarily high target binding affinity and specificity, it is not an easy task for these molecules to achieve long *in vivo* half-lives, low immunogenicity, and good manufacturability, which are the drug-like features associated with mAbs that make them such a successful class of therapeutics. Despite these challenges, the biologics field has not been deterred to invest in the development of novel scaffolds [44]. Over the next 5 to 10 years the industry may see the maturation of some of these novel scaffold-based drugs coming onto the market. It is likely that mAbs will remain a formidable mainstream scaffold with novel scaffold-based proteins fulfilling niche applications.

Antibody–drug conjugates (ADCs) are becoming a promising new class of antibody variants for cancer treatment. An ADC is a therapeutic molecule consisting of an antibody conjugated to a toxin. The antibody functions as a targeting vehicle via its specific binding to a tumor cell surface antigen and brings the toxin to the targeted cancer cell. After the ADC is internalized by the targeted cancer cell, the toxin becomes dissociated from the antibody and subsequently leads to cell killing [45]. With the recent FDA approval of the first ADC brentuximab vedotin in 2011 and

promising data on Genentech's T-DM1 in late-stage development, there is a lot of excitement in the field to pursue this class of antibody-based therapeutics to effectively kill both solid tumor and hematologic cancer cells [46,47].

Although presently still in development, combinatorial biologics therapy may become an important treatment strategy in the future. Human diseases are generally complex in nature, and intervention of more than one molecular target and pathway is often required for successful treatment. For example, most cancers result from mutations in a large number of genes that cause dysregulation in both growth-inhibitory as well as growth-promoting pathways. In the clinic, it is already a common practice to treat patients with combinatorial therapies, and many cancer trials testing a novel biologic drug are done in combination with anticancer SM drugs. For many inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, inhibiting one cytokine may not put a potent brake on a raging disease triggered by a multicytokine storm and simultaneous inhibition of different cytokines and their receptors may be required to manifest clinical efficacy.

Currently, there are two main approaches for combinatorial protein therapies. A multifunctional antibody (often referred to as bispecific antibody) combines two antigen-binding domains in a single molecule capable of simultaneously binding to and modulating the functions of two different molecular targets. Currently, a "trifunctional" bispecific antibody, catumaxomab, is a drug marketed in Europe for the treatment of malignant ascites. The "trifunction" refers to the simultaneous binding to EpCAM expressed on tumor cells, CD3 expressed on T cells, and Fc binding to FcγR on accessory immune cells [48]. The challenge for developing a multifunctional antibody arises from the difficulty to manufacture such an extensively engineered molecule, which often exhibits issues such as low expression levels, insolubility at high concentration, and high propensity to aggregation.

The second approach for combinatorial protein therapy is to apply a cocktail of two or more mAbs. Symphogen is one of the pioneers in this approach and has several therapeutic antibody combinations in the pipeline. The most complex one, rozrolimupab for the treatment of idiopathic thrombocytopenic purpura, consists of 25 IgG1 antibodies in one mixture [49]. A key challenge of developing therapeutic antibody mixtures is an unclear regulatory path. In theory, both the individual components and the combination in the antibody cocktail need to be proven safe and efficacious in clinical trials. This scenario would require large, complex, and costly clinical trials and could be cost prohibitive.

Even with many formidable obstacles, progress continues to be made developing combinatorial biologics. We expect this field to overcome some of these hurdles in the coming years to deliver novel antibody-based therapeutics and treatment methods into the clinic.

1.5.3 Drug Delivery

The delivery of protein-based biologics is an actively investigated area in the pharmaceutical industry. Unlike SM drugs, which are primarily delivered orally as tablets or capsules, the delivery of protein-based biologics is largely limited to

parenteral administration. Nasal and local delivery methods have also been applied in some cases. However, oral delivery remains largely infeasible and represents the holy grail of the field. Many efforts are under way, including novel nanoparticle formulation and the development of small and highly stable scaffolds that may survive trafficking through the digestive tracks to reach circulation. However, it will likely take years before oral delivery of protein drugs becomes a reality.

Another delivery challenge is for biologics to cross BBB to reach therapeutic targets that reside in the CNS. A number of biotechnology companies are applying novel technologies to this area using both targeted delivery and nanoparticle-based method to deliver biologics across BBB. So far the success has been limited.

1.5.4 Immunogenicity

Because of the complex multifactorial causes of immunogenic response in patients to protein drugs, it is extremely difficult to accurately predict the immunogenicity of a therapeutic candidate. It is possible, however, to assess the potential immunogenicity risks associated with a candidate molecule during preclinical development and devise clinical plans that monitor and manage the antidrug response in human patients. Because this is a critical safety aspect of biologic drugs, the field will strive to unravel the immunologic basis of immunogenicity at the molecular, cellular, and physiological levels, as well as design and implement new testing methods to accurately measure the clinical antidrug response.

1.5.5 Streamline the Drug Discovery and Development Process

Cutting-edge technologies are constantly applied to streamline both early drug discovery and manufacturing processes for the development of biologics. In discovery, automation is routinely implemented at various stages of the workflow to reduce manual labor and increase the throughput of various screening steps. In manufacturing, there is a strong trend toward switching from the traditional permanent bioreactors to disposable bioreactors. Meanwhile, production of extraordinarily high levels of therapeutic proteins using optimized protein expression systems has become a reality. These technology innovations will ultimately lead to a significant cost reduction in making biologics, ultimately improving the commercial viability for this class of drugs.

1.6 CONCLUSION

An industrial focus on the development of biologic drugs, including both new therapeutic entities and biosimilars, will continue for years. Currently, a sophisticated process built on the foundation of cutting-edge technologies has been put in place to support biologic drug development. Technological advancement will continue to make a major impact to this field, shaping both the process and the therapeutic entity. There are similarities as well as differences in the development of SM and biologic drugs. A

balanced portfolio with an equal share of both SM drugs and biologics is becoming a typical business strategy for large pharmaceutical companies.

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