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Chapter 1

Monoclonal Antibody Therapeutics: The Need for Biopharmaceutical Reference Materials

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Therapeutic monoclonal antibodies (mAbs) harness the highly evolved specificity of adaptive immunity to fight disease. mAb-based therapeutics have grown exponentially with the advent of mammalian cell culture, process, and formulation technology. At the same time, state-of-the-art and emerging analytical and biophysical methodology provides very detailed process and product information. Although such a battery of methodology and wealth of information is critical to product understanding, the accuracy, precision, robustness, and suitability of such techniques are also of critical importance. Performance specifications have previously been set on a product-specific basis and continued suitability verified with trending and comparability to in-house product-specific reference standards. This mechanism is likely irreplaceable due to the highly individual yet heterogeneous nature of mAb therapeutics. However, a representative and widely available material, coupled with detailed historical data, would greatly supplement characterization efforts throughout the drug product lifecycle. To this end, a first-of-its kind

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qualitative and quantitative biopharmaceutical reference material to supplement drug substance/product characterization is described. The NISTmAb IgG1κ is intended to provide a well-characterized, longitudinally available test material that is expected to greatly facilitate development of originator and follow-on biologics for the foreseeable future.

Introduction

Significant advances in modern medicine are often directly intertwined with production of novel disease treatments. The documented use of herbal remedies for ailments dates back to 3,000 B.C., when ancient Egyptian and Chinese cultures used various plants for their healing properties (*1*). Therapeutic effects of herbal medicine are a result of bioactive chemical substances, many of which have been identified and synthetically manufactured as small molecule drugs. For example, acetylsalicylic acid (Aspirin®) is a synthetic derivative of willow tree extract identified to have fewer side effects and eventually became the first blockbuster drug (*2*). Since that time, small molecule drugs have been developed for many indications and will likely continue to play a significant role in healthcare.

In addition to serving as human and animal medicines, many naturally derived drug products confer a selective advantage to the host species. In the same manner as humans have utilized this natural selection from plants, fungi, and other natural materials to derive small molecule drugs, it is a logical step to harness animal-derived immune defenses to produce therapeutics. The human immune system is comprised of both innate and adaptive immunity (*3*, *4*). Innate immunity confers a rapid initial line of defense via recognition of evolutionarily conserved features from pathogenic bacteria, viruses, and other invading organisms. Innate immune responses include the epithelial layer, which serves to block pathogen entry; phagocytic cells such as neutrophils and macrophages that directly ingest and kill pathogens; and inflammatory responses (e.g., cytokines, chemokines) that assist in recruiting additional innate and/or adaptive immune responses (*3*, *4*).

The adaptive immune response centers around the ability of T-cells and B-cells to form a learned response against a specific target pathogen following initial sensitization (*4–6*). Although adaptive immunity is relatively slow (days or more) to respond to initial infection, it is unique in that it remembers specific pathogenic antigens and is able to mount a more rapid and specific protection against subsequent exposure. T-cells are adaptive immune cells that recognize peptide antigens. When an organism is initially infected, phagocytic cells or infected cells will process pathogen proteins into their constituent peptides through lysosomal degradation. Pathogen peptides can then be associated with a major histocompatibility complex (MHC) and presented to the extracellular surface. These MHC-associated peptides on the antigen-presenting cell surface are recognized by T-cells, which induce apoptosis of the infected cell and/or a chemotactic response that recruits additional adaptive and innate immune functions to aid in clearance of the infection (*5*).

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The second major adaptive immune response (humoral response) is reliant on B-cells expressing immunoglobulin (Ig or antibody) proteins (*4–6*). Antibodies specifically recognize regions of pathogens such as proteins, carbohydrates, or lipids that may be present on the invading organism (*4*). Immunoglobulins are divided into classes (isotypes) and subclasses based on their structure as described in more detail in the Mechanisms of Action chapter/Volume 1, Chapter 2. The different human isotypes (IgA, IgG, IgM, IgD, and IgE) each have a unique distribution and function in the adaptive immune response (*4*). All currently approved monoclonal antibody (mAb) therapeutics harness the immunological capability of the IgG-class antibody, which also happens to be the highest concentration Ig class in blood (*4*).

The naturally occurring humoral response begins with activation of a naive B-cell expressing an IgM antibody on its cell surface. Each individual B-cell produces an IgM on its cell surface that specifically targets a single antigenic site or epitope. When a circulating B-cell recognizes its particular antigen, the cell will proliferate memory and effector B-cells. Memory B-cells continue to express antigen-specific IgM, thereby conferring a long-lasting learned memory of the initial infection. Effector B-cells, on the other hand, undergo class switching and are induced to produce soluble IgG targeting the same epitope. Soluble IgG binds to circulating pathogen and leads to removal of the invading pathogen through effector-mediated functions such as complement-dependent cytotoxicity (CDC), antigen-dependent cellular cytotoxicity (ADCC), or direct clearance through Fc binding in appropriate organs (*4*). Antibodies and the humoral defense are very effective at fighting a wide range of diseases. This response mechanism can also be considered somewhat more simplistic because the antibody recognizes intact pathogen as opposed to a T-cell response via antigen-presenting cell (APC)-processed antigen. It is therefore no surprise that IgG proteins were targeted for their potential utility as therapeutics.

The first demonstration of IgG-related therapeutic efficacy dates back to 1890, when serum from rabbits immunized with tetanus toxin conferred immunity to naive animals (*7*). The first clinical use of whole human serum was in 1907 for the prevention of measles, and this treatment proved to be of great importance during the early 20th century (*8*). The Ig component of serum was quickly recognized for its role in adaptive immunity, and technology was developed to purify the Ig fraction for selective use as a therapeutic (*9*, *10*). Intramuscular injection of serum Ig was initially used; however, intravenous (IV) administration was soon recognized to result in fewer infections. The use of IV Ig therapies is now approved for a variety of indications, including primary humoral immunodeficiency, B-cell chronic lymphocytic leukemia, Kawasaki disease, and bone marrow transplantation (*11*).

The therapeutic benefits derived from IV Ig, as well as the typical humoral response in animals, are polyclonal in nature. In other words, an invading organism elicits a response from numerous B-cells, and IgGs of different epitopic specificity are produced. In 1975, Kohler and Milstein first described the *in vitro* production of mAbs with specificity for a single epitope using murine hybridoma technology (*12*) and were later awarded the Nobel prize. Production of a mAb with this technique involves first sensitizing a mouse with a human antigen. Murine B-cells

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are then extracted from the spleen and fused with immortalized myeloma cells (a cancerous plasma cell) to form a mAb-producing hybridoma. Tissue cultures or living mice can then be used to increase production of the mAb.

Due to the highly selective nature of a given mAb, mAbs of a given primary amino acid sequence can be thought of as unique entities. Therapeutic mAbs are, therefore, individually named, typically with both a trademarked name (trade name) as well as a nonproprietary name based on the accepted International Nonproprietary Names (INN) Programme (*13*, *14*). INN nomenclature consists of a sufficiently distinctive prefix, a series of substems, and a suffix in the form of "Prefix-SubstemA-SubstemB-suffix." The suffix "-mab" is common to all nonproprietary names. Substem A and substem B indicate the antigen target class and the species on which the immunoglobulin sequence is based, respectively, as described in Table 1.

Table 1. System for International Nonproprietary Naming of Monoclonal Antibody (mAb) Therapeutics*

Prefix	*Substem A*		*Substem B*		*Suffix*
The prefix must be a unique, distinctive name	-b(a)-	Bacterial	a	Rat	-mAb
	-c(i)-	Cardiovascular	axo	Rat-mouse	
	-f(u)-	Fungal	e	Hamster	
	-k(i)-	Interleukin	i	Primate	
	-l(i)-	Immunomodulating	o	Mouse	
	-n(e)-	Neural	u	Human	
	-s(o)-	Bone	xi	Chimeric	
	-tox(a)-	Toxin	xizu	Chimeric-humanized	
	-t(u)-	Tumor	zu	Humanized	
	-v(i)-	Viral			

* Substem A represents the classification of the mAbs antigenic specificity, and substem B represents the species upon which the primary amino acid sequence is based.

The first murine (-omab) hybridoma-produced mAb therapeutic was realized in 1986 with the U.S. Food and Drug Administration (FDA) market approval of Orthoclone® (muronomab) (*15*, *16*). Interest in mAb therapies rapidly grew due to their potential for a long half-life (as a result of catabolic recycling described in the Mechanisms of Action chapter/Volume 1, Chapter 2) and their unsurpassed specificity. However, extraction of therapeutic mAbs from mouse ascites fluid via hybridoma technology did not yield a large number of approved therapeutics due to the need for animal hosts as well as insufficient titers to support drug development (*17*). Their murine origin was also quickly identified to result in non-self recognition of idiotypic determinants by the human immune system as well as a less than optimal elucidation of effector functions (*18*, *19*).

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Recombinant DNA technology resolved many difficulties associated with the production of protein therapeutics using animal hosts for therapeutic expression. Production of protein therapeutics via recombinant DNA technology begins with a cloning vector (e.g., plasmid or viral DNA). The desired sequence encoding the protein therapeutic, a promoter, and a selection marker sequence is ligated with the vector to form appropriate recombinant DNA. Recombinant DNA can then be transfected, or inserted, into the host cell DNA of a suitable expression system containing the molecular machinery required for replication (*20–22*). Successfully transfected host cells are selected through growth in a medium requiring expression of metabolic-selectable markers or antibiotic-selectable markers for cell viability (*20*). Further clonal selection can also be undertaken to obtain a population optimized for characteristics such as cell line stability, product yield, and product quality (*20*). Through the years, there have been a number of advances in gene integration, as well as clonal selection, which have been recently reviewed (*22*, *23*). Selected cells contain incorporated DNA that encodes the product, as well as a promoter sequence capable of inducing high levels of transcription and, therefore, protein therapeutic production. The ability to insert "your favorite gene" also paved the way for introduction of sequences encoding for more human-like DNA.

Chimeric antibodies were the first recombinant therapeutics developed in an effort to reduce immunogenic responses and improve effector functions compared to fully murine mAbs (*24*, *25*). Chimeric antibodies (-ximab), first demonstrated in 1984, consist of a human constant region spliced with a fully murine variable region (*24*). The "self" Fc domain resulted in longer half-life and a higher propensity to elicit the Fc effector functions that are critical to certain modes of action, as described in the Mechanisms of Action chapter/Volume 1, Chapter 2). The first approved chimeric product was Reopro®, a chimeric monoclonal antibody antigen-binding fragment (Fab) for the prevention of ischemic complications during angioplasty (*25–27*). Despite potential for non-self immunogenic responses to the remaining murine component, numerous chimeric intact antibodies have also been approved, including Rituxan® and Erbitux® (anticancer agents), and Remicade® (an anti-inflammatory).

Recombinant DNA technology also opened the doorway to produce mAbs with even lower murine composition. These humanized mAbs (-zumab), retaining murine sequence in the complementarity-determining region (CDR) only, were first produced in 1986 (*28*). As with chimeric technology, approval of the first humanized mAb therapeutic followed approximately 10 years later (Zenapax® for transplant rejection). A large number of humanized mAb products have since been successfully marketed, including Synagis®, Herceptin®, Mylotarg®, Xolair®, and Avastin®.

Chimeric and humanized antibody therapeutics are often produced in murine-derived cells. NS0 and SP2/0 myeloma cell lines, derived from B-lymphocytes of mice, have become commonplace for therapeutic development because they can be adapted to produce sufficiently high IgG titers in bioreactor cultures (*29*). NS0 cells, for example, lack the ability to express sufficient levels of glutathione synthase (GS), an enzyme necessary for biosynthesis of the essential nutrient glutamine. High-titer cell lines can be selected through

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co-transfection with a GS gene in a glutamine-free medium (*30*). Additional murine cells, such as CHO cells (derived from epithelial cells of Chinese hamster ovaries), have also become commonplace for drug development. CHO cells have the ability to produce self-sustaining levels of GS. However, GS inhibitors can be used in cell cultures to select only cells co-transfected with additional GS activity (*30*). Additional selectable markers, such as dihydrofolate reductase, can also be used for selection of suitably transfected clones (*20*, *22*, *30*). CHO cells as production hosts have been well-received by the biopharmaceutical community due to their ability to grow at high cell density and amenability to serum-free media (*23*). CHO cells have also been known for their production of proteins with a preferable glycoprofile, as described in more detail in following chapters: Mechanisms of Action chapter/Volume 1, Chapter 2 and Glycosylation chapter/Volume 2, Chapter 4. However, CHO cells have recently been reported to be capable of producing some of the undesired foreign glycan epitopes that are commonly produced in murine myeloma-based cell lines and were originally thought to be absent in CHO (e.g., gal-α-gal) (*31*). Throughout many years of development, a high level of process knowledge associated with NS0, SP2/0, and CHO has been compiled and will likely result in their continued use as platform cell lines for mAb production.

Considering the potential for murine epitopic determinants to elicit immunogenic responses, it makes sense that the production of fully human mAbs (-umab) for therapeutic use would also be explored. Transgenic mouse strains expressing human variable domains, phage display, and human-derived cell lines all offer the potential for fully human mAb expression (*32*, *33*). Phage display, an *in vitro* technique that expresses and screens a library of antibody sequences, was the first technology to identify a fully human mAb for clinical use (*34*). The fully human construct for this mAb product (Humira®) was later transferred to a CHO cell expression system for commercial-scale production and licensed as a tumor necrosis factor (TNF) inhibitor useful for rheumatoid arthritis, Crohn's disease, and plaque psoriasis (*35*). Human-derived cell lines are a logical target for expression of therapeutics as they possess the biosynthetic pathways for human glycosylation and other post-translational modifications (PTMs), thereby minimizing the risks associated with anti-mAb immune responses. Fully human cell lines developed for biopharmaceutical production include the human embryonic kidney cell line (HEK 293) and its successors, as well as the Per.C6 cell line derived from human retinal cells (*23*). Per.C6 cell lines have been shown to offer several advantages, including very high titers and the ability to provide stable cell lines without selection agents (*29*). Per.C6 and HEK cell lines can be used for the expression of fully human mAbs (*36*, *37*). However, full-length mAbs from these expression systems (Per.C6 or HEK) have yet to gain market approval.

Mammalian cell culture using the aforementioned cell lines has clearly dominated production of mAb therapeutics, in large part due to their ability to produce human-like form and function. Product development with CHO, NS0, and SP2/0 cell lines will undoubtedly continue to contribute novel therapeutics. Fully human expression systems will also likely increase in popularity, and it should be noted that recent advances in microbial expression systems may soon

begin to play a role in this ever-expanding market (*38*). Since the initial inception of mAb therapeutics, a range of mammalian culture-derived mAb drug products have been approved by the FDA and are currently in use, as described in Table 2 (note that only full-length mAb and Fab therapeutics are listed) (*20*, *39–41*). Murine, chimeric, humanized, and fully human mAbs of IgG1, 2, and 4 subclasses are in current clinical use today and have revolutionized modern medicine.

A variety of mAb-related therapies, such as Fab, Fc-fusion proteins, and antibody-drug conjugates (ADCs), have also been developed using mammalian cell culture (*39*, *42*). Fab therapeutics are composed only of the antigen-binding subunit of the mAb and, therefore, do not have effector function capabilities (see the Mechanisms of Action chapter/Volume 1, Chapter 2). They also do not contain glycosylation and, therefore, have been expressed in bacterial cell culture (e.g., Lucentis®, approved for treatment of macular degeneration) or expressed as full-length mAbs and further truncated enzymatically (e.g., ReoPro®, approved for use as an antithrombotic agent) (*41*, *43*).

Fc fusion proteins and ADCs harness mAb biochemical activity as a means for improving the pharmaceutical properties of an attached active pharmaceutical ingredient (API) or peptide. Fc-fusion proteins utilize the FcRn recycling pathway to improve half-life and pharmacokinetic properties (*42*, *44*). Examples of approved Fc-fusion proteins include cytotoxic T-lymphocyte antigen (Orencia®) and TNF receptor type 2 (Enbrel®) for rheumatoid arthritis (*42*). Recently, there has been a great interest in Fc-fusion proteins with blood clotting factors to improve their half-life and reduce the frequency of injections for treatment of hematological disorders (*45*, *46*).

ADCs are comprised of small-molecule APIs conjugated to full-length mAbs. ADCs harness the antigen-binding affinity and specificity of the mAb to deliver an API (e.g., chemotherapeutic agent) to a specific physiological location (*42*, *47*). For example, Kadcyla® is a conjugate of trastuzimab and a microtubule antagonist. The mAb binds a target cancer cell expressing the selective HER-2 marker and provides localized drug targeting of an otherwise globally cytotoxic API (*47*).

A variety of additional mAb-based therapeutic strategies are also under development, including smaller single-chain fragment variable (scFv) antibodies, bi-specific antibodies with the ability to bind two separate epitopes, and multimer constructs of antigen-binding domains (*48–51*). Although scFcs, bi-specifics, and multimer constructs have yet to gain market approval in the United States, each of them is based upon critical recombinant mAb components and subject to the same production, regulatory, and characterization considerations described throughout this book.

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Table 2. FDA-Approved Monoclonal Antibody (mAb) and Antigen-Binding Fragment (Fab) Therapeutics as of July 2014 [*,†] (*20, 39–41*)

Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Orthoclone®K3®	Muromonab-CD3	Centocor Ortho Biotech (Johnson & Johnson)	CD3	Murine ascites	Murine IgG2a	1986	Transplantation rejection
ReoPro®	abciximab	Centocor Ortho Biotech (Janssen) and Eli Lilly	GPIIb/IIIa	SP2/0	Chimeric Fab	1994	High risk angioplasty
Zenapax®	daclizumab	Roche	CD25	NS0	Humanized IgG1	1997	Transplantation rejection
Herceptin®	trastuzumab	Genentech (Roche)	HER-2	CHO	Humanized IgG1κ	1998	Breast cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma
Remicade®	infliximab	Centocor Ortho Biotech (Janssen)	TNF-α	SP2/0	Chimeric IgG1κ	1998	Crohns disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis

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Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Simulect®	basiliximab	Novartis	CD25	SP2/0	Chimeric IgG1κ	1998	Transplantation rejection
Synagis®	palivizumab	MedImmune (AZ)	RSV F protein	NS0	Humanized IgG1κ	1998	Respiratory syncytial virus
Campath®	alemtuzumab	Millennium and Genzyme	CD52	CHO	Humanized IgG1κ	2001	B-cell chronic lymphocytic leukemia
Humira®	adalimumab	Abbott (Abbvie)	TNF-α	CHO	Human IgG1κ	2002	Rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, plaque psoriasis
Zevalin®	ibritumomab tiuxetan	Biogen Idec	CD20	CHO	Murine IgG1κ	2002	Non-Hodgkin's lymphoma
Bexxar®	tositumomab and iodine-131 tositumomab	Corixa and GSK	CD20	Hybridoma	Murine IgG2aλ	2003	Non-Hodgkin's lymphoma
Xolair®	omalizumab	Genentech (Roche) and Novartis	IgE	CHO	Humanized IgG1κ	2003	Asthma

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Table 2. (Continued). FDA-Approved Monoclonal Antibody (mAb) and Antigen-Binding Fragment (Fab) Therapeutics as of July 2014 [*,†] (*20*, *39–41*)

Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Avastin®	bevacizumab	Genentech (Roche)	VEGF	CHO	Humanized IgG1κ	2003	Metastatic colorectal cancer, non-small cell lung cancer, metastatic breast cancer, glioblastoma multiforme, metastatic renal cell carcinoma
Erbitux®	cetuximab	ImClone (Eli Lilly), Merck Serono and BMS	EGFR	SP2/0	Chimeric IgG1κ	2004	Head and neck cancer, colorectal cancer
Tysabri®	natalizumab	Biogen Idec and Elan	VLA-4	NS0	Humanized IgG4κ	2004	Multiple sclerosis (relapsing), Crohns disease
Lucentis®	ranibizumab	Genentech (Roche)	VEGF-A	E. Coli	Humanized Fab IgG1κ	2006	Macular degeneration and macular edema
Soliris®	eculizumab	Alexion Pharmaceutical	Complement C5	Myeloma	Humanized IgG2κ	2007	Paroxysmal nocturnal hemoglobinuria

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Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Cimzia®	certolizumab pegol	UCB	TNF-α	E. Coli	Humanized Fab IgG1κ	2008	Crohns disease, rheumatoid arthritis
Arzerra®	ofatumumab	Genmab and GSK	CD20	NS0	Human IgG1κ	2009	Chronic lymphocytic leukemia
Ilaris®	canakinumab	Novartis	IL-1β	SP2/0	Human IgG1κ	2009	Cryopyrin-associated periodic syndromes
Simponi®	golimumab	Centocor Ortho Biotech (Janssen)	TNF-α	SP2/0	human IgG1κ	2009	Rheumatoid arthritis, Psoriatic arthritis, ankylosing spondylitis
Stelara®	ustekinumab	Centocor Ortho Biotech (Janssen)	IL-12 , IL-23	SP2/0	Human IgG1κ	2009	Plaque psoriasis
Actemra®	tocilizumab	Chugai (Roche)	IL-6	CHO	Humanized IgG1κ	2010	Rheumatoid arthritis

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Table 2. (Continued). FDA-Approved Monoclonal Antibody (mAb) and Antigen-Binding Fragment (Fab) Therapeutics as of July 2014 [*,†] (*20*, *39–41*)

Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Prolia® and Xgeva®	denosumab	Amgen	RANKL	CHO	Human IgG2κ	2010	Postmenopausal osteoporosis, prevention of SREs in patients with bone metastases from solid tumors
Benlysta®	belimumab	HGS, GSK	BLyS	NS0	Human IgG1λ	2011	Systemic lupus erythematosus (SLE)
Yervoy®	ipilimumab	BMS	CTLA-4	CHO	Human IgG1κ	2011	Melanoma
Adcetris®	brentuximab	Seattle Genetics	CD30	CHO	Chimeric ADC IgG1κ	2011	Hodgkin lymphoma, systemic anaplastic large cell lymphoma
Perjeta®	pertuzumab	Genentech	HER2	CHO	Humanized IgG1κ	2012	HER2-positive metastatic breast cancer
Raxibacumab®	raxibacumab	HGS, GSK	PA of *B. anthracis* toxin	NS0	Human IgG1λ	2012	Anthrax exposure

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Trade Name	*Nonproprietary Name*	*Company*	*Target*[‡]	*Cell Line*	*Isotype*	*FDA Approval*	*Therapeutic Indications Approved by FDA*
Gazyva®	obinutuzumab	Genentech	CD20	CHO	Humanized IgG1	2013	Chronic lymphocytic leukemia
Kadcyla®	ado-trastuzumab emtansine	Genentech	HER2	CHO	Humanized IgG1 ADC	2013	HER2-positive metastatic breast cancer
Cyramza®	ramucirumab	Eli Lilly and Co.	VEGFR2	NS0	Human IgG1	2014	Gastric cancer

[*] Only approved full-length mAb and Fab therapeutics are included. [†] Sources: (*20*, *39–41*). [‡] CD (cluster of differentiation), GPIIb/IIa (glycoprotein IIb/IIa), HER-2 (human epidermal growth factor receptor 2), TNF (tumor necrosis factor), RSV F protein (respiratory syncytial virus), IgE (immunoglobulin E), VEGF (vascular endothelial growth factor), EGFR (epidermal growth factor receptor), VLA-4 (very late antigen), IL (interluekin), RANKL (receptor activator of nuclear factor kappa-B ligand), BLyS (B-lymphocyte stimulator), PA of *B. anthracis* (protective antigen of *Bacillis anthracis*), CTLA (cytotoxic-lymphocyte antigen), VEGFR2 (vascular endothelial growth factor receptor 2).

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Production of mAb Therapeutics

Current mAb biomanufacturing has evolved into a highly controlled process, as described in Figures 1 and 2. Each stage in the production process—raw materials, process conditions and control, purification, fill finish, and storage—can affect quality attributes of the product. The production process requires years of optimization and highly regulated control to result in a suitable drug product. At this point, it is useful to differentiate commonly used terminology associated with the drug development process (*52*). **Process-related impurities** refer to any unwanted material introduced as part of the manufacturing process. This may include impurities derived from the cell system itself (host cell proteins [HCPs] and DNA), cell culture media components, and impurities introduced during targeted purification strategies (e.g., column leachables, processing reagents). **Product-related impurities** are variants of the desired product (precursors, truncated products, or degradation products) that do not have the desired activity, efficacy, and/or safety. **Product-related substances**, on the other hand, are also variations of the targeted product; however, they fall within predefined specifications for activity, efficacy, and safety. A series of processing steps (upstream and downstream, described below) are undertaken to clear most unwanted process and product-related impurities to initially provide the bulk drug substance.

Bulk drug substance contains desired product as well as associated product-related substances and excipients/buffer components. The final stage in processing is formulation of the drug substance into a **drug product** suitable for clinical use. Formulation of the drug product may involve dilution to appropriate dosage and addition/removal of excipients into a pharmaceutical product for patient use. Drug product may have essentially the same identity and purity as drug substance other than it is in a format directly amenable for delivery to the patient. Therefore, although intended to be the same active ingredient, the storage conditions, shelf life, and degradation pathways may differ and should be thoroughly evaluated. In addition, specifications should be set for identity, purity (including any residual impurities), and potency, as described in ICH Q6B (*52*).

The development process is divided into upstream and downstream processing. Upstream process development involves cell line, media composition, and culture condition optimization to produce mAbs in sufficient quantity to support clinical and, subsequently, commercial production scale. A representative overview of an upstream production process is show in Figure 1, where the optimized cell line will undergo multiple rounds of expansion to first seed a small-scale bioreactor. These seed-scale expansion reactor cultures are transferred to one or more larger production reactors as necessary to produce sufficient levels of mAb. Early-stage purification steps, such as centrifugation and filtration, remove cellular debris and result in the clarified cell culture media (*53*, *54*). Stable cell line development through delayed apoptosis, regulatory RNA, transient gene expression, improved cell culture media, single-use bioreactors, and process analytical technology (PAT) represent a sampling of recent advances in state-of-the-art upstream processes (*20*, *22*, *54–58*).

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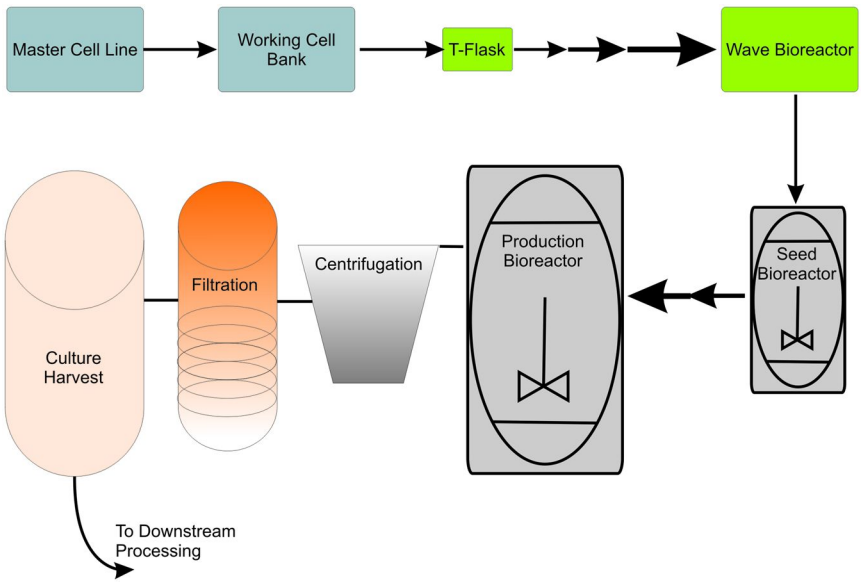

Figure 1. Representative upstream processing steps that may be used for monoclonal antibody production. (see color insert)

Following upstream processing, clarified cell culture media contains the desired mAb as well as other secreted HCPs, host cell DNA, media, feed components, and other potential process-related impurities. Downstream processing encompasses a variety of purification steps to selectively remove process-related impurities (Figure 2) (*59*). The first downstream processing stage is typically affinity enrichment of the IgG component of the culture media. This is most often achieved though protein A affinity chromatography, which selectively binds the Fc region. Although protein A is highly selective and can achieve purity greater than 90–98% (*60*, *61*), the inherent unit operation and limited binding capacity results in a relatively low throughput strategy (*62*). Optimization of ligand density, chromatographic support, immobilization strategy, and chromatographic parameters such as flow rate and buffer composition has been utilized to improve affinity enrichment capabilities (*63–65*) In addition, alternative ligands and non-chromatographic technologies have been explored to improve this initial capture step (*54*).

Despite the purity offered by protein A chromatography, residual impurities such as HCPs (Process Impurities chapter/Volume 2, Chapter 9 and LC-MS HCP chapter/Volume 3, Chapter 13) or adventitious agents (Adventitious chapter/Volume 3, Chapter 8) may remain after the initial capture step. HCPs may co-purify as adducts with the mAb of interest or as a result of nonspecific interaction and co-elution in the product fraction (*66*, *67*). Additional polishing chromatographic steps are often used such as cation exchange chromatography (CEX), anion exchange chromatography (AEX), and hydrophobic interaction chromatography (HIC) (*53*, *54*, *59*, *68*). A gel filtration step may also be present to remove aggregates during polishing chromatographic steps. The final stages

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of downstream process typically involved final filtration (nanometer-scale) and inactivation of potential viral contaminants, as well as ultrafiltration and/or dialysis to concentrate the product into its bulk drug substance form (*53*, *54*). Improvement in downstream processing is an ongoing area of research directed at achieving higher throughput purification to meet the demands of high-titer upstream production without sacrifice of drug substance purity. Many potential advances in chromatographic and non-chromatographic developments have recently been reviewed (*53*, *54*, *69–71*). Improvements in process-related technology continually are made as more sensitive and specific analytical technology for the detection and characterization of process-related impurities are developed. Emerging technologies for adventitious agent testing and HCP analysis are covered in detail throughout this book (Adventitious chapter/Volume 3, Chapter 8; Process Impurities chapter/Volume 2, Chapter 9; and LC-MS HCP chapter/Volume 3, Chapter 13). Genomics and proteomics have also bolstered the specificity of HCP identification and cell line-specific considerations (*72–74*), as described in chapters throughout this series (Genomics chapter/Volume 4 and Proteomics chapter/Volume 4).

Concurrent with the optimization of upstream and downstream processing to form a more pure and reproducible bulk drug substance, the material must undergo formulation development into a form suitable for direct clinical use. A variety of considerations go into drug product formulation, such as API concentration; dosage form (liquid vs. lyophilized); and selection of excipients and proposed storage conditions, including the container closure (e.g., vial, prefilled syringe). There is also increasing use of delivery devices such as auto-injectors and mini-dosers that allow for the delivery of high quantities of mAbs to patients.

The drug product matrix is of critical importance and ensures stability of the molecule throughout fill finish, transport, shelf life, and patient administration. Appropriate formulation minimizes chemical (e.g., proteolysis, disulfide scrambling, oxidation) and physical (e.g., denaturation, aggregation) instabilities and may include a variety of excipients such as carbohydrates, surfactants, polyols, and arginine or other amino acids (*75*). Assessment of the protein's stability begins early in the drug development lifecycle and often can be a determining factor in the developability (Developability chapter/Volume 2, Chapter 7) of a candidate mAb. A wide variety of analytical and biophysical techniques (Biophysical chapter/Volume 2, Chapter 6 and SMSLS chapter/Volume 3, Chapter 6) are used in such manufacturability studies.

The overall goal of process and formulation development is to produce a quality product suitable for its intended use. The quality of the drug substance or product is evaluated experimentally based on a variety of attributes determined to be critical to safety and efficacy (e.g., identity, potency, purity) (*52*). Critical quality attributes (CQAs) are physical, chemical, biological, or microbiological properties that must be maintained within a predefined limit, range, or distribution to ensure product quality (*76*). The identification of CQAs and evaluation of their level of criticality is a complex task that spans the totality of knowledge for a given process and product. A risk-based approach is taken to optimize and correlate all aspects of the production process for the severity of deviation from predefined specifications and the likelihood of each deviation. This combined approach of

CQA identification and correlation to process parameters followed by systematic process optimization is referred to as quality by design (QbD).

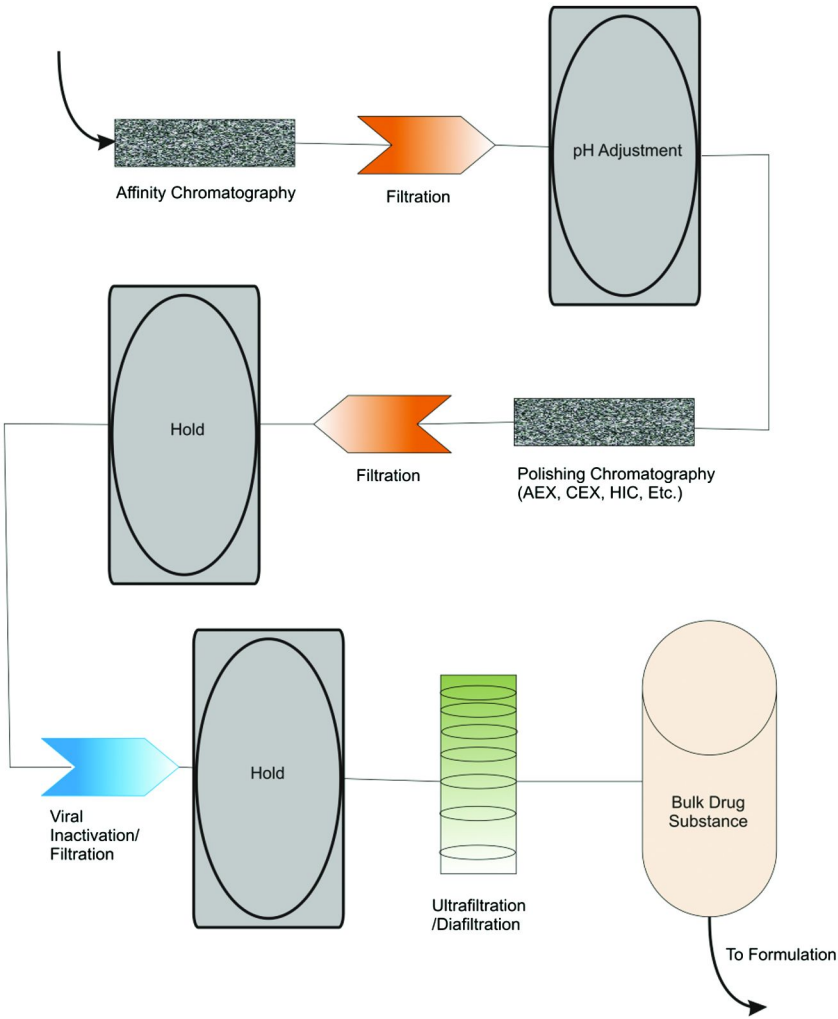

Figure 2. Representative downstream processing steps that may be used for monoclonal antibody production. Potential polishing chromatography steps include anion exchange chromatography (AEX), cation exchange chromatography (CEX), and hydrophobic interaction chromatography (HIC). (see color insert)

QbD is formally defined in ICH Q8(R2) as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk assessment" (*76*). ICH Q8(R2) gives a generalized guidance on how the

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concept of QbD can be incorporated into pharmaceutical development. In 2008, the chemistry, manufacturing, and controls (CMC) biotechnology working group organized a comprehensive, real-world case study to more comprehensively exemplify all aspects of QbD principles based on a representative humanized IgG1, A-mAb (*77*). The case study used a subset of quality attributes (e.g., aggregation, glycosylation) known from historical knowledge to span a range of criticality. A risk assessment was described to demonstrate how historical, analytical, biophysical, clinical, and nonclinical data were combined for evaluation of A-mAb CQAs. The A-mAb case study then went on to present an iterative risk assessment and optimization strategy for upstream and downstream processing, linking product quality to critical control parameters. A similar exercise was also applied to drug product formulation design, leveraging historical process platform and product class information in combination with risk assessment. Finally, cumulative risk assessment along with product and process knowledge were used to define a control space and strategy for the representative QAs and CQAs that were further assured throughout the lifecycle with product and process verification (see the QbD chapter/Volume 1, Chapter 5 for a more detailed discussion).

PAT is a very important component of a robust QbD approach to biomanufacturing. The concept of PAT utilizes process and product knowledge to incorporate measurements (on-line, in-line, at-line, or off-line) of raw and in-process materials to provide real-time information as to the control of the system and ensure product quality (*76*, *78*). PAT measurements are intended to correlate critical process parameters and resultant product CQA's. The complexity and variability associated with protein therapeutics, raw materials, and their production process make information-rich PAT a difficult task; however, significant advancements have been realized (*79*, *80*). Cell culture operations are widely monitored for biomass (yield), critical reagents (e.g., metabolites, nutrients), and medium conditions (pO_2, pH, and temperature) through a variety of image analysis (e.g., focused beam reflectance), spectroscopic (e.g., IR, Raman), electrochemical (e.g., pH, dielectric spectroscopy), and/or off-line analytical techniques (e.g., high-performance liquid chromatography [HPLC], nuclear magnetic resonance [NMR]) (*78*, *79*). PAT has also been applied to harvest unit operations, downstream processing, and formulation (*77*, *79*, *81*). The vast array of available PAT tools has also spurred a movement toward multivariate statistical models for these complex data sets (*82*). Although a complete discussion of PAT is outside the scope of this chapter, many reviews and the A-mAb case study present the correlation between QbD, PAT and process control, and resultant product quality (*58*, *79*, *80*, *83*).

The A-mAb case study is a good example of widespread industrial collaboration to harmonize thinking and significantly advance antibody production philosophy and applied science. Although every aspect of A-mAb will not be directly applicable to every future mAb product, widely available case studies on representative materials are critical to advancing the science of complex mAb development in concert with regulatory requirements and expectations. It is the hope that the NISTmAb IgG1κ, described throughout this book, can serve a purpose similar to that of the A-mAb study, in this case, focusing on evaluation

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of current and future analytical and biophysical technology for identification and characterization of mAb product attributes. The NISTmAb will provide a common material to serve as a fundamental measurand of mAb heterogeneity, as demonstrated throughout this book.

Despite stringent controls and highly regulated manufacturing processes, the biological origin of recombinant therapeutics produces a significant level of product heterogeneity. Product-related substances consist in part of a variety of PTMs (PTMs chapter/Volume 2, Chapter 3), sequence variants (Sequence Variant chapter/Volume 2, Chapter 2), and other modifications that can be identified using techniques discussed throughout this book. In addition, the final product must be free of adventitious agents and have acceptable limits of product- and process-related impurities. Identification and control of these process variables and their effects on product quality is of great importance early in product development of mAb products to reduce costly development choices and influence early process decisions. Ultimately, it is the attributes of drug substance and drug product that determine its fitness for an intended use. Product safety and efficacy are initially verified through preclinical and clinical trials, and quality must be ensured thereafter through stringent analytical testing to ensure consistency from batch to batch. Process performance and product quality are tracked and trended over time to ensure product consistency. Changes in the production process are critically evaluated for resultant comparability to previous lots or reference standards using a full battery of characterization methods (*84*). These physicochemical and biophysical analytical technologies are used to "define" the product, as described in Volume 2 of this series, and many of these methods will support the validation of quality testing for lot release and stability. To ensure consistent production, it is therefore essential to have a reference standard of the specific product for comparison. Note that throughout this chapter, the words "in-house reference standard" are intended to refer to a company-specific product and "reference standard" alone refers to a standard issued by the World Health Organization (WHO) or a pharmacopoeial registry to assist in ensuring identity, potency, and/or purity. The term "reference material" refers to national metrology materials with metrological traceability, as discussed below.

Product-Specific In-House Reference Standards

Ultimately, it is the responsibility of the manufacturer to ensure product consistency throughout its lifecycle, using appropriate analytical characterization and comparability to an in-house reference standard (*52*). The drug candidate development process, appropriate in-house reference standards, and analytical methods co-evolve throughout the product lifecycle. In the case of mAbs, there currently is not a repository of product-specific compendia standards (described below) as those provided for small molecule drugs. Therefore, current best practices require development of a product and manufacturer-specific in-house reference standard. In-house reference standard development evolves as the product moves through various stages of clinical development as described in Figure 3. The timeline described in Figure 3 is a general outline of a theoretical

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situation, and the actual timeline for qualification of in-house reference standards and analytical methods is highly depend on real-time process and product knowledge as well as incoming data from clinical trials.

An **in-house interim reference standard** is an appropriately characterized lot of production material set aside for quality control (QC) purposes during the *development stage*. The interim standard is often used as the product-specific reference standard for early technical development through Good Laboratory Practice-Toxicology (GLP-Tox) and early clinical studies. At this point, tentative process parameters and formulation for Phase 1 clinical trials have been defined, and a suite of analytical characterization methods to define product properties such as primary sequence, certain PTM modifications, charge and size isoforms, and potency are in place. These methods are used to **qualify the reference standard**, which refers to collection of sufficient physicochemical and biophysical characterization data such that the material can serve as a representative comparator for future lots and analytical method evaluation. During Phase 1 and Phase 2 trials, process changes may occur. The need to replace the interim standard will depend on the level of process change and/or detected changes to the product profile post-change. Qualification of new interim standards should be minimized to avoid product drift, but the decision must ensure the in-house reference standard is representative of the product to be used in the clinic so that it is fit for its intended purpose. When a significant process change is to be implemented that impacts relevant quality attributes, a batch of designated in-house reference standard should be simultaneously generated and characterized, the data from which can also be used as the basis for a comparability exercise (*84*).

At or near pivotal clinical trials, the overall upstream, downstream, and formulation scheme intended for commercial development will be in place. The entire suite of analytical characterization and QC (lot release and stability) methods should now be qualified and validated, respectively, as discussed in the following paragraph. A larger quantity of manufacturer's material must be selected from a batch that is representative of the commercial product for use in pivotal trials and post-commercialization. This batch is often split into two subsets for use as an **in-house primary reference** standard and the first lot of **in-house secondary (or working) reference standard**. The in-house primary standard is expected to be in quantities sufficient to be used throughout the product lifecycle for qualification/calibration of secondary standards. The **in-house secondary reference standard** is calibrated against the in-house primary reference standard and is used in QC testing of clinical material as well as marketed lots. Additional batches of secondary in-house reference standard may be made when supplies are exhausted and re-qualified against the in-house primary reference standard. Additional in-house primary reference standard may also be prepared if the initial batch is near exhaustion or changes in the reference profile are noted during regularly scheduled trending testing. However, qualification of new in-house primary reference standard should be avoided when possible to minimize potential drift.

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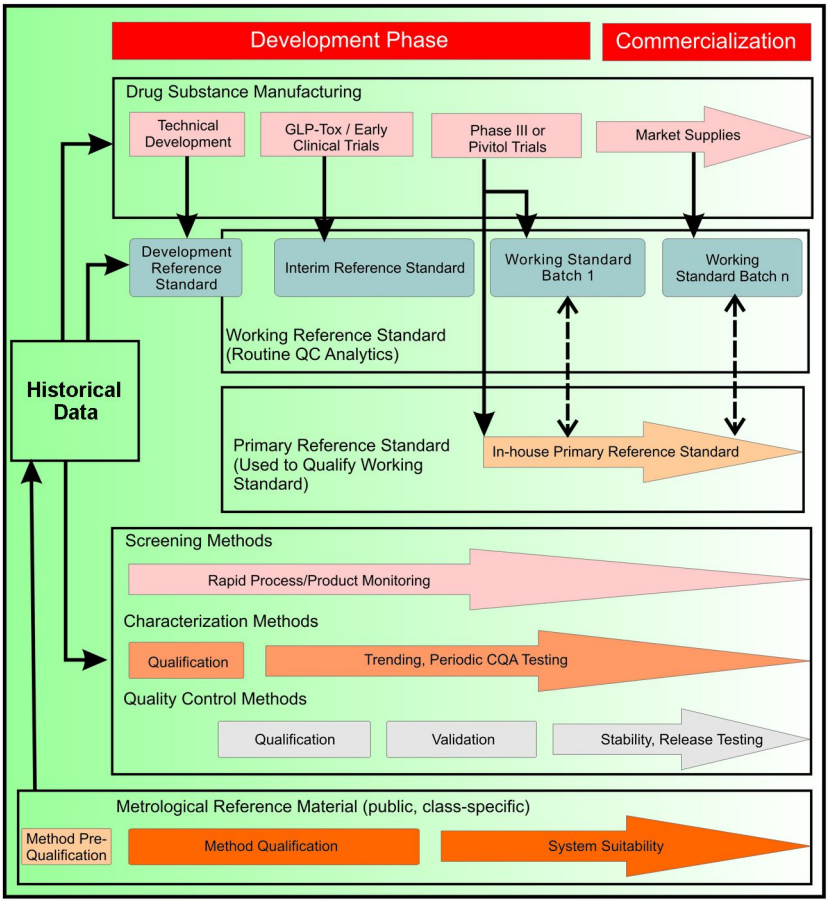

Figure 3. Representative monoclonal antibody lifecycle incorporating potential timelines for analytical method development, in-house reference standards, and potential supplementation with a metrological reference material. (see color insert)

Along with evolution of in-house reference standards and the drug development lifecycle, analytical methods for product testing also evolve in a manner appropriate to the current stage of development (*85*). **Method Qualification** refers to the use of an in-house standard along with challenge material (e.g., forced degraded material, known impurities) to test the ability of a method to provide information on the desired product attribute. For example, a method qualified for identity testing should be sufficient to differentiate the test subject from product-related impurities and other related molecules produced in that facility. **Method Validation** is a more in-depth verification of a proposed method's suitability for an intended purpose, as described in more detail in ICH Q2(R1) (*86*). Succinctly, validation consists of a method performance evaluation for accuracy, precision, specificity, detection limits, linearity, and range to provide a high degree of assurance that it is capable of consistently producing

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results within predetermined specifications for a given product. Analytical and biophysical methods may categorized as being informational (i.e. for research purposes), qualified, and/or validated, depending on their intended purpose and current role in a particular drug candidate's lifecycle.

Analytical and biophysical methods can be split into three categories that co-evolve throughout the product lifecycle. The first types of methods typically utilized during early product development are methods used to screen candidate molecules. These techniques assess for commonly known undesirable attributes such as a significant propensity to aggregate or high levels of product variability (e.g., in size, charge, or viscosity). *In vitro* immunogenicity and potency assays also play a significant role in determining viable candidates to move forward in development. In addition to these screening assays, promising candidates may be subjected to further, more detailed characterization.

Detailed characterization methods are used for high-level product understanding and often focus on specific product attributes of identity (primary sequence and higher order structure), and purity, which is evaluated based on intrinsic heterogeneity such as PTMs, sequence variants, size variants (aggregation), charge variants, and other characteristics. Product characterization methods have an important role as the product development cycle advances and may include mass spectrometry (e.g., for sequence determination, glycoprofiling), HPLC (along with fraction collection of size, charge, and sequence variants), and methods that focus on higher order structure (e.g., NMR, hydrogen-deuterium exchange [HDX], circular dichroism, differential scanning calorimetry). These methods are generally not validated, but must be qualified to a level shown to be fit for use when compared to routine lot release methods intended for QC. This is because they yield critical data on potential product changes during process optimization, such as changes in primary sequence; PTMs; biophysical parameters; and secondary, tertiary, and higher order structure. Qualified characterization methods are often used to supplement the application for licensure (in the elucidation of structure section in the application), demonstrate a high level of product knowledge, and verify that more robust QC methods are fit for their intended purpose. Qualified characterization methods are required for qualification of future lots of in-house working standards. Trending characterization data associated with sequential production lots is also critical to post-approval comparability exercises that may be necessary to justify process changes have not adversely impacted the product (*84*).

A more robust set of methods with defined precision and acceptance criteria (QC methods) must also be developed for ensuring the product quality and stability of future clinical and commercial lots. Both screening and characterization methods may eventually become QC methods, depending on their suitability for an intended purpose. Characterization methods are often used to assess and/or supplement intended QC methods because of their ability to accurately and precisely identify deviations from desired quality attributes. Qualification of QC methods begins during early toxicology studies and clinical trials. As manufacturing processes are scaled to levels required to support pivotal clinical trials, multiple lots of material are used to validate such assays for their intended purpose. Appropriate validation of an analytical method serves to confirm

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acceptance criteria and suitable performance, as defined in earlier qualification studies. Validated QC methods are used to assure consistent production of commercial lots. In-house working standards and controls are run alongside commercial material to ensure QC method conformance to expectations and, ultimately, a safe and effective product for clinical and commercial use.

Finally, robust operation of characterization and QC methods is balanced by continued evaluation of system performance. All components, including instrumentation, consumables, software, and analytical personnel, should be included in the system suitability space of a particular test method. Historically, system suitability has been established through use of an in-house reference standard. Conformance to expectation indicates proper operation of a test system. Although consistent operation of a test system that has been validated to be capable of identifying a particular product change is strong evidence for product consistency, when deviations from the expected outcome are observed, additional mechanisms are needed to differentiate product versus method-related factors. For this reason, external, non-product-specific standards or reference materials are necessary to challenge analytical operations and yield a secondary confirmation of system suitability.

Metrological Reference Material

Establishing appropriate public reference standards for pharmaceutical development is a collaborative effort involving drug manufacturers, regulatory agencies, and a variety of standards organizations with unique yet overlapping missions. The WHO coordinates the development of standards associated with health care throughout the United Nations system. The WHO provides international reference materials (International Biological Reference Preparations) useful for designating a baseline definition of unit activity in a particular assay (e.g., potency assay) (*87*). These materials are intended for calibration of national/regional activity reference standards (e.g., United States Pharmacopeia) and/or in-house reference standards with regards to product potency or biological activity. These potency standards represent the gold standard for activity. However, for new molecular entities and all currently approved mAbs, such standards are typically not available. In this case, qualification and definition of activity are based on a representative lot of an in-house reference standard, as described above. In the case of a follow-on biological program, the originator molecule must be purchased from market supplies and used to assess an in-house standard manufactured by the follow-on manufacturer.

In the United States, the U.S. Pharmacopoeial Convention (USP) publishes official compendia for pharmaceutical products, the content of which are often enforceable by the FDA (*88*). The USP was established with the mindset that drug substances and products are articles of commerce that must pass stringent quality standards to prevent distribution of adulterated or misbranded products, thereby ensuring safety and efficacy for clinical use (*89*). To this end, the USP publishes product-specific pharmacopoeia monographs, including methodology and appropriate calibrants to aid in assessing whether a product meets required

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specifications. Requirements for defining identity, purity, stability, and potency for small molecule drugs are well-established due to the definitive chemical structure of such molecules (*88*). It is the intent of the USP that every legally approved article (e.g., drug substance, drug product) should have a monograph and a USP Reference Standard, where appropriate. To this end, the USP works diligently with originator manufacturers to develop monographs and associated reference standards. Alternative sources such as potential generic suppliers may also be sought as sources, however, if no monograph and reference standard are under development 5 years prior to the expiration of an originator patent (*90*). This mechanism has worked well for small molecule drugs, for which compendial methods and standards are typically available at or near the time of patent expiry.

In the case of biologically derived medicines, their inherent complexity requires additional consideration for attributes such as identity, stability, product-related impurities, and process-related impurities. To date, no mAb monographs have been published in the legally enforceable USP compendium. However, a monograph for rituximab is available through the non-mandatory USP Medicines Compendium (*91*). Although not legally enforceable unless submitted as part of a regulatory filing, Medicines Compendium standards are approved through extensive USP Expert Committee evaluation and may be useful to establish an article's identity, strength, and purity. In addition to product-specific monographs, the USP publishes General Chapters (often with associated procedural standards) aimed at best practices for techniques that may be broadly applied to a variety of health care-related products, including the future inclusion of a recently created chapter on size, charge, and glycosylation testing for mAbs (*92*). Other country-based or regional pharmacopoeial agencies are also publishing standards related to mAb drug substances and products. The Indian Pharmacopoeia is in advanced stages of publishing monographs for rituximab drug substance and drug product for injection (*93*). The European Pharmacopoeia includes a general monograph titled *Monoclonal Antibodies for Human Use* (2031), which provides definitions and general provisions for production, testing, and labeling. It is likely that major pharmacopoeial agencies in Japan, China, Brazil, and other countries will follow suit.

During method development and in-house reference standard evolution of complex drug products such as mAbs, it makes sense that the best comparability standard is a representative lot of the specific drug substance or product itself (in-house primary and working standards). In some cases, biologic pharmacopoeial standards such as erythropoietin (EPO) and granulocyte colony stimulating factor (GCSF) are available from Pharmacopoeia. However, physicochemical and biophysical standards of this type are typically not available for biotherapeutics. Given the process-specific nature and high complexity of mAb products, it may be impossible for one national or international reference standard to cover all of the needs when testing a company-specific product. Therefore, multiple company-specific lots and often attribute-specific reference standards (e.g., certain degradation products derived thereof) will be required to ensure a method's performance for a particular biopharmaceutical product, and an in-house reference standard will be required to rigorously monitor product consistency. The necessity for method validation and guidance for such an endeavor has

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been stated by a variety of regulatory and standardization organizations (*52*, *86*, *94*), and many excellent reviews have recently been published (*95*, *96*). It has been noted that guidelines are subject to some level of user interpretation, which can lead to inadvertent risk if appropriate validation parameters are not considered (*96*). However, the interpretability of guidance documents is also an essential factor that allows consideration of the totality of evidence for a specific product. A widely available metrological reference material would provide a representative material to more precisely define a balance between harmonization and product-specific validation packages. In addition, appropriate protocols for method qualification during early- to mid-phase product development are not as harmonized or clearly defined as those for later phases of product development because most regulatory guidance documents are designed for commercialization of a product (*85*, *95*). Such a void in qualification and assessment of changing analytical test methods would, therefore, be supplemented by a widely available metrological reference material and reference data to supplement current in-house reference standard protocols.

National metrology institutes such as NIST are responsible for such metrological reference materials as one aspect of assuring measurement equivalence. These institutes are involved in a variety of activities, ranging from establishing the fundamental unit of time measurement to providing physical reference materials useful for calibrating property measurements such as mass. To achieve this mission, a national metrology institute may provide chemical and physical reference materials to its stakeholders to establish a route of traceability to fundamental measurement units and/or assess the quality of a measurement procedure. In the health care setting, reference materials are often used for calibration and/or harmonization of test methods and focus on the accuracy and reproducibility of measurement technology itself, as opposed to assessing a specific product's conformance to predefined specifications. Metrological reference materials such as the NISTmAb described here are, therefore, similar to procedural standards established in USP compendia, and are intended to compliment these activities by providing a widely representative and internationally traceable material for analytical method assessment.

The NIST Biomanufacturing Program is directed toward developing a suite of fundamental measurement science, reference materials, and reference data to enable more accurate and confident characterization of key attributes directly linked to product safety and efficacy. A critical metric in achieving these goals is the production of a widely available reference material useful for establishing instrument performance and variability in analytical test methods (*97*). Recombinant mAbs are the fastest growing class of biotherapeutics and are, therefore, an obvious candidate for such a material. A **NIST reference material (RM)** is a material that is sufficiently homogeneous and stable with reference to specified properties and has been established to be fit for its intended use in measurement or in examination of nominal properties. The topic of the current book is a candidate IgG1κ mAb RM for which detailed analytical and biophysical characterization will be presented. Property values of an RM are a best estimate of the true value provided by NIST where all known or suspected sources of bias may not have been fully investigated. NIST RMs

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meet the International Organization for Standardization (ISO) definition of a reference material, including homogeneity, stability, and suitability for use in a measurement process (*98*). A **NIST Standard Reference Material (SRM)** is a material accompanied by documentation issued by NIST that assigns one or more specified property values with associated uncertainties and traceability. Property values of an SRM are certified as being traceable to an accurate realization of the unit in which the property values are expressed and having suspected sources of bias that have been fully investigated or accounted for by NIST. NIST SRMs meet the ISO definition of a certified reference material (CRM) (*98*). Both NIST RMs and SRMs are issued under the NIST trademark and can be used for measurement quality assurance.

The subject IgG1κ discussed throughout this book is intended for development into an RM and/or SRM that is expected to be used by a variety of stakeholders, including the biopharmaceutical industry, instrument manufacturers, academia, regulatory authorities, and other standards organizations. The RM is intended for a variety of uses, including, but not necessarily limited to, system suitability tests, establishing method or instrument performance and variability, comparing changing analytical test methods, and assisting in method qualification. To properly serve as a quantitative and qualitative RM, a variety of physical and chemical characterization methods may be used to determine biomolecular composition and structure, purity, and stability, including, but not necessarily limited to, liquid chromatographic methods; mass spectrometry; NMR; and optical, X-ray, and other product characterization assays.

Information pertaining to chemical and physical attributes of the NISTmAb RM or SRM may be reported to customers as NIST Certified Values, NIST Reference Values, or NIST Informational Values, depending on the level of certainty associated with the particular test methods. Analytical data may also be made available in a variety of formats. including, but not necessarily limited to certification sheets delivered with the material, an SRM or RM website, Standard Reference Data software and/or databases, or published material in scientific journals and books such as the current series. Characterization efforts throughout this book utilized the candidate RM 8670 (lot 3F1b) of the NIST IgG1κ mAb (100 mg/mL or 10 mg/mL). The molecule was distributed throughout industry, academia, regulatory agencies, and NIST to gain initial product understanding and identification of its physicochemical and biophysical attributes. The intention was to evoke best practices in a collaborative effort toward characterization of a mAb. Simultaneously, additional NISTmAb material intended for public release as an RM and/or SRM was prepared from multiple homogenized production lots and is expected to be available shortly after publication of this series.

Potential Utility of the NISTmAb IgG1κ

The pursuit of a candidate NIST RM is based on a variety of factors that stem from industry input. The decision to pursue an IgG1κ mAb RM arose largely through discussions and iterative research with industry stakeholders over a period of 5 years. mAbs of a given class are highly homologous and, therefore,

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have similar characteristics for which platform technology can provide a wealth of information. Screening methodologies for class-specific attributes are commonly developed with this highly similar behavior and composition in mind. However, start-up companies may not have such historical expertise, and/or investigational compounds may be present in very short supply. One of the strengths of having an established RM of the IgG1κ class is to assist with development and optimization of such techniques for new molecular entities. The NISTmAb reference material is expected to fill this void as a representative material for method prequalification during early drug development, as depicted in Figure 3, and feed forward into class-specific historical knowledge. One could imagine a series of follow-on isotypes, allotypes, or other class-specific molecules to support development of a variety of therapeutic proteins.

Further method development, incorporation of novel analytical and biophysical techniques, or method transfer (internal or to a contract organization) also requires a high level of analyte knowledge to evaluate suitability. Instrument vendors and industry consumers alike often use company-specific mAbs with intellectual property concerns, commercially available mAbs that may not be well-characterized, or proteins not representative of the class for such a purpose. The use of a single available material will be convenient for users and instrument developers alike to evaluate the instrument or method performance of evolving technology. Certified concentration and extensive characterization data collected by multiple companies and/or institutions provided along with such a material will greatly facilitate determination of dynamic range, detection limits, linearity, and precision of new technology. Again, although the use of degraded material or other products produced in the same facility is required for challenging methods, the NISTmAb will provide an external control that can be widely utilized to evaluate purity or identity-indicating assays. The baseline comparator NISTmAb molecule will, therefore, facilitate implementation of new characterization and/or QC strategies.

In addition, the historical data available for direct comparison will assist regulators in evaluating the suitability of new techniques for use in originator product licensure applications. The inevitable submission for follow-on biologic licensure is an even more pressing issue due to the expected impact of increased analytics and reduced clinical trials. Every aspect from sample handling to instrument performance must be verified to ensure precise and accurate method readouts. Technology associated with a follow-on antibody submission may differ greatly from legacy methods utilized for the originator product. Regulatory officials and developers therefore would greatly benefit from a goalpost molecule that can differentiate method-related artifacts from those inherent to the product and/or claims of similarity from multiple follow-on submissions.

The entire biopharmaceutical design space depicted in Figure 3 relies heavily on historical knowledge, including previous discovery platforms, cell line and process knowledge, appropriate production and use of in-house reference standards, and the analytical and biophysical expertise required to characterize such standards. The metrological IgG1κ reference material is intended to provide a widely available test product that is not associated with product-specific intellectual property concerns. Historical data and widespread availability of

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such a material will be useful for a broad community assessment of current and emerging analytical technology and will establish a more robust framework for method qualification. Historical product knowledge associated with the RM may serve to feed forward into the drug development process, thereby allowing more informed selection of test methods appropriate for mAb products and supplementing the totality of evidence that a specific method is capable of producing results in accord with its intended purpose.

Concluding Remarks

The development of mAb therapeutics is an astounding story of how groundbreaking research can translate into viable lifesaving products. In less than 30 years, significant biochemical discoveries have now resulted in novel treatments for numerous indications that have had an invaluable impact on patients worldwide. Continued collaboration between academia, industry, and federal agencies (as evidenced by the current collaborative series) demonstrates that this trend in innovative mAb health care will continue for years to come. As of April 2014, there were 30 mAb therapeutics in Phase 3 clinical trials (*99*). The proven mAb therapeutic track record as a sustainable and necessary health care market warrants addition of metrological standards and establishment of best practices for characterization.

The metrological reference material will not replace in-house reference standards, but rather will supplement best practices historically used to ensure product quality. The current project represents two very important milestones in furthering development of monoclonal therapeutics. The NISTmAb will first be subjected to state-of-the-art characterization practices as determined through a large interagency collaborative effort, setting a benchmark for mAb characterization and a forward-looking presentation of next-generation analytical methods. Simultaneously, historical data is being generated on this reference material similar to what typically would be performed on a primary in-house reference standard. This material is beginning its journey through a mAb lifecycle, and will serve as a tangible, openly available substance to critically evaluate analytical questions related to product characterization, method development, and in-house reference standard programs. Although the establishment of a suitable reference material for complex mAbs comes with qualitative and quantitative analytical challenges that have not been faced previously, implementation will supplement the unrivaled commitment to biopharmaceutical quality demonstrated by analytical scientists to improve the safety and efficacy of biopharmaceuticals.

Disclaimer

Commercial equipment, instruments, and materials are identified in this paper to adequately exemplify the discussion and experimental procedure. Such identification does not imply recommendations or endorsements by NIST nor does it imply that the equipment, instruments, or materials are necessarily the best available for the purpose.

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