

## Chapter 1

# Current Immune Aspects of Biologics and Nanodrugs: An Overview

**Raj Bawa, MS, PhD**

*Patent Law Department, Bawa Biotech LLC, Ashburn, Virginia, USA*

*The Pharmaceutical Research Institute,*

*Albany College of Pharmacy and Health Sciences, Albany, New York, USA*

*Department of Biological Sciences,*

*Rensselaer Polytechnic Institute, Troy, New York, USA*

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*Immune Aspects of Biopharmaceuticals and Nanomedicines*

Edited by Raj Bawa, János Szebeni, Thomas J. Webster, and Gerald F. Audette

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ISBN 978-981-4774-52-9 (Hardcover), 978-0-203-73153-6 (eBook)

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*Keywords:* biotherapeutics, biologics, biologicals, biological products, biopharmaceuticals, biomolecular drugs, protein products, nanomedicine, nanodrugs, nanoparticulate drug formulations, nanopharmaceuticals, nanotechnology, nanomaterial, nanoscale, patents, commercialization, research and development (R&D), US Food and Drug Administration (FDA), European Medicines Agency (EMA), US Patent and Trademark Office (PTO), drug delivery systems (DDS), site-specific delivery, nanoparticles (NPs), protein aggregation, small-molecule drug, New Chemical Entities (NCEs), New Biological Entities (NBEs), New Drug Application (NDA), Biologic License Application (BLA), Bayh–Dole Act, Hatch–Waxman Act, Biologics Price Competition and Innovation Act (BPCI Act), immunotoxic effects, complement activation, immunogenicity, antidrug antibodies (ADAs), antibody–drug conjugates (ADCs), adverse drug reaction (ADR), conjugated proteins, functionalized antibodies, Federal Food, Drug, and Cosmetic Act (FD&C Act), target mediated drug disposition (TMDD), pharmacodynamic (PD), Public Health Service (PHS) Act, pharmacokinetics (PK), Humira<sup>®</sup>, protein aggregates, active pharmaceutical ingredient (API), hypersensitivity reactions (HSR), anaphylactoid reactions, complement activation-related pseudoallergy (CARPA), Doxil<sup>®</sup>, Ambisome<sup>®</sup>, DaunoXome<sup>®</sup>, Abelcet<sup>®</sup>, Visudyne<sup>®</sup>, Cremophor EL, PEGylated proteins, monoclonal antibodies (mABs), Humulin<sup>®</sup>, PEGylated liposomes, accelerated blood clearance (ABC), reticuloendothelial system (RES), immune complexes (ICs), biosimilar, generic drugs, bioequivalent, interchangeable product, nanosimilars, nonbiologic complex drug (NBCD), NBCD similar, glatiramer acetate, Copaxone<sup>®</sup>, immunomodulator, clinical trials, immunopharmacology, immunomodulatory effects, iTope<sup>™</sup>, TCED<sup>™</sup>, EpiBase<sup>®</sup>, EpiMatrix<sup>™</sup>, EpiScreen<sup>™</sup>, immunogenic epitopes, artificial intelligence (AI), single-cell genomics, user fees, druggable genome, cryo-electromicroscopy (cryo-EM), epitope mapping analysis, bench-to-bedside, translation, drug-like molecule, CRISPR-Cas9

## 1.1 Introduction

A wave of “newer” therapeutics is sweeping the drug world. Specifically, there is a rapid introduction of two somewhat distinct yet overlapping classes of drugs into the pharmaceutical landscape: (1) biologics<sup>1</sup> and (2) nanodrugs.<sup>2</sup> Biologics have already entered an era of rapid growth due to their wider applications, and in the near future they will replace many existing organic based small-molecule drugs. According to one drug analysis firm, biologics have grown from 11% of the total global drug market in 2002 to around 20% in 2017.<sup>3</sup> On the other hand, nanodrugs have sputtered along a somewhat different trajectory with greater challenges to their translation [1–3]. I estimate that since the approval of the first recombinant biologic (recombinant human insulin, in 1982), there are over 225+ marketed biologics and at least 75 nanodrugs for various clinical applications approved by various regulatory agencies.<sup>4</sup> According to the Pharmaceutical Research and Manufacturers of America (PhRMA) website, as of 2013, there are over 900 biologic medicines and vaccines in development. I estimate that hundreds of companies globally are engaged in nanomedicine research and development (R&D), the clear majority of these have continued to be startups or small- to medium-sized enterprises rather than big pharma. Despite immature regulatory mechanisms, follow-on versions of these two drug classes, namely biosimilars and nanosimilars, respectively, have also started to trickle into the marketplace.

According to the US Food and Drug Administration (FDA), the products it regulates represent around 20% of all products sold in the United States, representing more than \$2.4 trillion. The FDA regulates products according to specific categories: food, dietary supplements, cosmetics, drugs, biologics, medical devices, veterinary products, and tobacco. The Center for Biologics

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<sup>1</sup>Analogous terms include biotherapeutics, biologicals, biological products, biopharmaceuticals, biomolecular drugs, therapeutic protein product (TPP), and protein products.

<sup>2</sup>Analogous terms include nanomedicines, nanoparticulate drug formulations, and nanopharmaceuticals.

<sup>3</sup>Data from the IMS Institute for Healthcare Informatics.

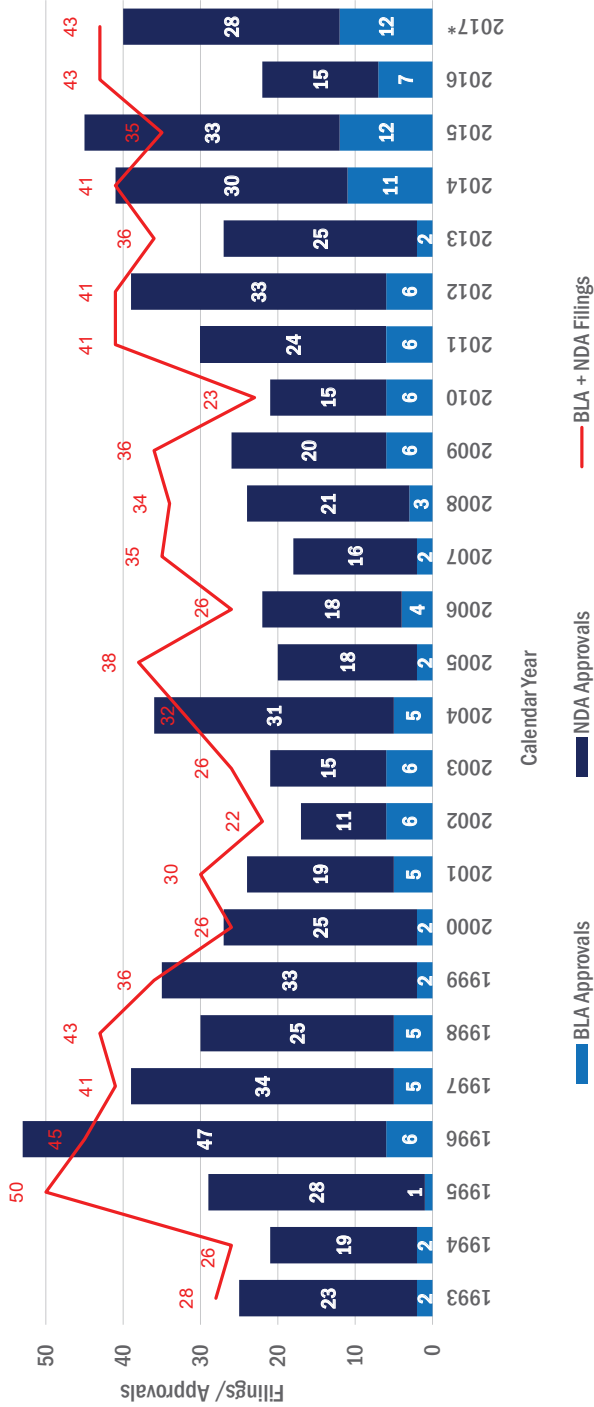
<sup>4</sup>My estimate for nanodrugs is based on my broader definition of a nanodrug that appears in Section 1.3.

Evaluation and Research (CBER) regulates what are often referred to as traditional biologics, such as vaccines, blood and blood products, allergenic extracts, and certain devices and test kits. CBER also regulates gene therapy products, cellular therapy products, human tissue used in transplantation, and the tissue used in xenotransplantation—the transplantation of nonhuman cells, tissues, or organs into a human. On the other hand, the Center for Drug Evaluation and Research (CDER) regulates branded and generic drugs, over-the-counter (OTC) drugs, and most therapeutic biologics (Fig. 1.1a). Food, dietary supplements, and cosmetics fall under the jurisdiction of the Center for Food Safety and Nutrition (CFSAN). Since dietary supplements are intended to supplement the diet, they are classified under the “umbrella” of foods and do not require premarket authorization from the FDA. Cosmetics containing sunscreen components are regulated as drugs. In these cases, the products must be labeled as OTC drugs and meet OTC drug requirements. Tobacco products are subject to a unique regulatory framework as they only pose risks without providing any health benefits. They are regulated by the Center for Tobacco Products (CTP). Medical devices are regulated by the Center for Devices and Radiological Health (CDRH), and veterinary products by the Center for Veterinary Medicine (CVM). Drugs that have high potential for abuse with no accepted medical use are illegal and cannot be imported, manufactured, distributed, possessed, or used. The Drug Enforcement Administration (DEA) is the US agency tasked with overseeing these dangerous products and enforcing the controlled substances laws. The Office of Combination Products (OCP) has authority over the regulatory life cycle of combination products. Combination products are therapeutic and diagnostic products that combine drugs, devices, and/or biological products. As technological advances continue to merge product types and blur the historical lines of separation between various FDA centers, I expect that more products in the near future will fall into the category of combination products. Naturally, this will present unique regulatory, policy, and review management challenges.

The main law that governs various products in the United States is the Federal Food, Drug, and Cosmetic Act (FD&C Act). It was established in 1938 and has been amended numerous times since. The laws are passed as Acts of Congress and organized/codified into United States Code (USC). Of the 53 titles

in the USC, title 21 corresponds to the FD&C Act. To operationalize the law for enforcement, federal agencies, including the FDA, are authorized to create regulations. The Code of Federal Regulations (CFR) details how the law will be enforced. The CFR is divided into 50 titles according to subject matter. Therefore, there are three types of references for regulatory compliance: FD&C Act, 21USC, and 21CFR. The FD&C Act provides definitions for the different product categories along with allowable claims. For example, drugs, biologics, and medical devices can make therapeutic claims like “treatment of a particular disease” or “reduction of symptoms associated with a particular disease.” Therapeutic claims also include implied statements like “relieves nausea” or “relieves congestion.” It is illegal for nonmedical products like pharma-cosmetics, dietary supplements, and cosmetics to make therapeutic claims. Even if a product lacks any therapeutic ingredient, its intended use may cause it to be categorized as a drug.

This chapter focuses on those biologics, biotechnology products, nanomedicines, nanodrug products, and nanomaterials that are used for medicinal purposes in humans. Many biologics (e.g., monoclonal antibodies or drug-protein conjugates) are of nanoscale and hence can also be considered to be nanodrugs. Conversely, many nanodrugs are biologics according to standard definitions (Sections 1.2 and 1.3). For example, Copaxone<sup>®</sup> (Section 1.7) is a biologic (Section 1.2) but also falls within the definition of a nanodrug (Section 1.3). Many terms used here are definitions that come from specific regulations or compendia. The terms “product,” “drug formulation,” “therapeutic product,” or “medicinal product” will be used in the manner the FDA defines a “drug,” encompassing pharmaceutical drugs, biologics, and nanomedicines in the context of describing the final “drug product.” Some of the terms will be used synonymously. For example, biotherapeutics, protein drugs, biologicals, biological products, and biologics are equivalent terms.<sup>1</sup> Similarly, nanomedicines, nanodrugs, nanopharmaceuticals, nanoparticulate drug formulations, and nanotherapeutics are the same.<sup>2</sup> Branded drugs are referred to as “pioneer,” “originator,” “branded,” or “reference” drugs. Small-molecule drugs approved by the FDA are known as New Chemical Entities (NCEs) while approved biologics are referred to as New Biological Entities (NBEs) (Fig. 1.1a, Table 1.1, and Box 1.1). As a result, a new drug application for an



\*The data in this figure for 2017 reflect 43 drug filings and are accurate as of November 30, 2017. However, the final count for 2017 (as of December 31, 2017) is 46.

**Figure 1.1a Drug filings and approvals by the FDA's Center for Drug Evaluation and Research (CDER) since 1993.** Overall, new drugs approved by the FDA have risen in recent years. Strong numbers were posted in 2017, rebounding from a steep drop in 2016. In fact, the 2017 bounty of 46 new drugs was only second to the peak of 59 drugs approved in 1996. Emphasizing its focus on orphan drugs, the FDA approved 18 drugs with orphan designation, 7 potential blockbuster candidates, 12 cancer drugs, and 17 products with breakthrough designation.

CDER approves novel drugs, either as New Molecular Entities (NMEs) under New Drug Applications (NDAs), or as New Biological Entities (NBEs) under Biologics License Applications (BLAs). In recent years, many products were known more for their breathtaking price tags and rapidity of approval rather than innovation. The FDA defines “novel drugs” as innovative products that serve previously unmet medical needs or otherwise significantly help to advance patient treatments and the active ingredient or ingredients in a novel drug have never before been approved in the United States.

In rare instances, it may be necessary for the FDA to change a drug’s NME designation or the status of its application as a new BLA. This applies to all references to NMEs/BLAs in this figure. For instance, new information may become available which could lead to a reconsideration of the original designation or status. Note that approvals by the Center for Biologics Evaluation and Research (CBER) are excluded from this figure. Since applications are received and filed throughout a calendar year, the filed applications in a given calendar year do not necessarily correspond to approvals in the same calendar year. Certain applications are within their 60-day filing review period and may not be filed upon completion of the review. Original BLAs that do not contain a new active ingredient are excluded.

Data courtesy of the FDA and various drug companies. For more information about the approved drugs in 2017 and for their complete risk information, refer to the drug approval letters and FDA-approved labeling at [Drugs@FDA](mailto:Drugs@FDA). Also see CDER’s Novel Drug Approvals for 2017 on the FDA website for the approval dates, nonproprietary names and what each drug is used for: <https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm537040.htm>.

Figure is courtesy of the FDA while the legend is by the author.

Table 1.1 Properties of biologics versus small-molecule drugs

Property	Biologics	Small-Molecule Drugs
<b>Size and MW</b>	generally large and high MW; MW >700 Da; complex structure	generally small and low; MW <700 Da; simple and defined structure
<b>Manufacturing</b>	numerous critical process steps; highly susceptible to slight alterations in production process; lengthy and complex purification; great possibility of contamination and detection/removal often impossible	fewer critical process steps; not affected by slight alterations in production process; easy to purify; contamination can generally be avoided and detection/removal easy
<b>Composition</b>	protein-based; amino acids; heterogeneous mixture that may include variants; may involve post-translational modifications	chemical-based; synthetic organic compound(s); homogenous drug substance (single entity)
<b>Origin</b>	isolated from living cells or recombinantly produced	chemical synthesis
<b>Toxicity</b>	more consistent with exaggerated pharmacology than off-target toxicity; much greater contact surface area for binding allows access to a much wider range of protein targets as well as a more specific binding interaction, decreasing the potential for off-target effects	drug product or metabolites that are generated can be toxic; target binding results in the small-molecule drug being nearly completely buried within a hydrophobic pocket of the protein target to maximize hydrophobic contact plus create a more stable complex, thereby effectively limiting targets to those that possess solvent accessible pockets



Property	Biologics	Small-Molecule Drugs
<b>Dosing Frequency</b>	increased blood circulation time can allow far less frequent dosing	greater dosing frequency
<b>Half-Life</b>	variable; longer half-life (hours, days, weeks)	variable; mostly shorter half-life (hours to days)
<b>Clearance</b>	slow	rapid
<b>Pharmacokinetic (PK) and Distribution</b>	target can affect PK behavior (TMDD); larger molecule(s) and hence reach blood via lymphatics; subject to proteolysis during interstitial and lymphatic transit; distribution generally limited to plasma and/or extracellular fluid	mostly linear PK; nonlinearity from saturation of metabolic pathways; rapid entry into systemic circulation via capillaries; distributed to any combination of organ/tissue
<b>Cost</b>	high, often extremely high	generally low
<b>Drug-Drug Interaction (DDI)</b>	rare or few examples, mostly pharmacodynamic (PD)-related	possible and many examples; metabolic and/or PD related
<b>Off-target Action</b>	rare; mostly “on-target” effects	often “off-target” effects
<b>Mode of Action</b>	regulatory or enzyme activity to replace/augment cell action; may target cell surface to induce action; binding to cell-surface receptors and other markers specifically associated with or overexpressed; limited to extracellular and cell surface interactions	antagonistic/agonistic activity on intracellular and extracellular targets
<b>Storage and Handling Risk</b>	variable; sensitive to environmental conditions (heat and shear)	relatively stable

(Continued)

Table 1.1 (Continued)

Property	Biologics	Small-Molecule Drugs
<b>Contamination Risk</b>	high	low
<b>Structure</b>	may or may not be precisely elucidated or known; inherent variability due to complex manufacturing	precisely defined structure (or structures, e.g., racemic mixtures)
<b>Delivery</b>	generally parenteral (e.g., IV and SC)	various routes; generally oral
<b>Dispensed By</b>	physicians (often specialists) or hospitals	general practitioner or retail pharmacies
<b>Duration of Action</b>	long; days to weeks	short; hours
<b>Characterization</b>	less easily characterized; cannot always be fully characterized	can be fully characterized
<b>Immunogenicity</b>	low to high; usually antigenic and hence potential exists	often non-antigenic and hence low to none
<b>Toxicity</b>	receptor-mediated toxicity	specific toxicity
<b>FDA Approval</b>	licensed under the provisions of both the FD&C Act and the PHS Act (for exceptions see Box 1.1); biologics approved by the FDA are referred to as New Biological Entities (NBEs); a new drug application for an NBE is called a Biologic License Application (BLA) (see Fig. 1.1a)	licensed under the FD&C Act; small-molecule drugs approved by the FDA are known as New Molecular Entities (NMEs); a new drug application for an NCE is known as a New Drug Application (NDA) (see Fig. 1.1a)

Property	Biologics	Small-Molecule Drugs
<b>Compilation</b>	<i>Purple Book</i> published by the FDA lists biologics, their biosimilars and interchangeable generic equivalents	<i>Orange Book</i> published by the FDA lists drugs and their generic equivalents
<b>Follow-on Versions</b>	biosimilars (see Section 1.6); high barriers to entry; follow-ons will not be identical to the reference innovator product; preclinical and clinical (i.e., safety/efficacy) studies are needed to demonstrate comparability	generics (see Section 1.6); preclinical analytical methods can be used to validate and demonstrate comparability; full clinical studies not needed; follow-ons have identical API(s), strength, dosage form, route, and purity
<b>Patent Issues</b>	patent prosecution and litigation are often more complex; patents and legal exclusivities may delay the FDA approval of applications for biosimilars	patent prosecution and litigation generally less complex; patents and legal exclusivities may delay the FDA approval of applications for generics
<b>Selectivity</b>	high species selectivity (affinity/potency)	generally low species selectivity
<b>Targets</b>	multiple target binding	mostly a single or few targets

*Abbreviations:* BLA, Biologic License Application; Da, Daltons; DDI, drug–drug interaction; FD&C Act, Federal Food, Drug, and Cosmetic Act; IV, intravenous; MW, molecular weight; NBE, New Biological Entity; NME, New Molecular Entity; NDA, New Drug Application; TMDD, target mediated drug disposition; PD, pharmacodynamic; PHS Act, Public Health Service Act; PK, pharmacokinetic; SC, subcutaneous; API, active pharmaceutical ingredient. Copyright 2018 Raj Bawa. All rights reserved.

NCE is known as a New Drug Application (NDA) while a new drug application for an NBE is known as a Biologic License Application (BLA). Note that prior to the 1980s, there were very few marketed biologics, so the very term “pharmaceutical” or “drug” implied a small-molecule drug. Although biologics are subject to federal regulation under the Public Health Service (PHS) Act, they also meet the definition of “drugs” and are considered a subset of drugs. Hence, biologics are regulated under the provisions of both PHS Act and FD&C Act. Table 1.2 shows the different regulatory routes for therapeutic products.

**Table 1.2 FDA regulatory routes for therapeutic products**

	Medical Devices	Drugs	Biologics
<b>FDA Center Jurisdiction</b>	CDRH	CDER	CBER/CDER
<b>Regulatory Route(s)</b>	510(k) waived 510(k) notification PMA	OTC ANDA NDA	BLA
<b>Clinical Trial Initiation</b>	IDE	IND	IND

*Abbreviations:* CBER, Center for Biologics Evaluation and Research; CDER, Center for Drug Evaluation and Research; CDRH, Center for Devices and Radiological Health; NDA, New Drug Application; BLA, Biologic License Application; OTC, over-the-counter; ANDA, Abbreviated New Drug Application; PMA, Premarket Approval Application; IND, Investigational New Drug; IDE, Investigational Device Exemption. Copyright 2018 Raj Bawa. All rights reserved.

As the boundaries between big pharma and biotech companies have further blurred, big pharma has adapted its operational strategy, employing outside collaborations with respect to research, technology, workforce, and marketing. Obviously, big pharma’s evolving role has resulted partly from the “biotech boom” and the “genomics boom,” where enormous advances resulted from molecular biology and DNA technology, but also from advances in information and computer technology. In addition, two important pieces of legislation in the 1980s have had a major impact on the drug industry in the United States. The first was the Bayh–Dole (or Patent and Trademark Law Amendments) Act of 1980, which allowed universities, hospitals, nonprofit organizations and small businesses to patent and retain ownership arising from

federally funded research [4]. The second was the Hatch–Waxman (or Drug Price Competition and Patent Term Restoration) Act of 1984, which established abbreviated pathways for the approval of small-molecule drug products [5]. It set up the modern system of generic drug regulations in the United States by amending the FD&C Act. Section 505(j) of the Hatch–Waxman Act, codified as 21 USC § 355(j), outlines the process for pharmaceutical manufacturers to file an Abbreviated New Drug Application (ANDA) for approval of a generic drug by the FDA.

In addition to the Bayh–Dole Act and Hatch–Waxman Act, the more recent Biologics Price Competition and Innovation Act of 2009 (BPCI Act), which is included in the Patient Protection and Affordable Care Act signed into law by President Obama in 2010, pertains specifically to biologics. This Act created an abbreviated approval pathway for biologics proven to be “highly similar” (biosimilar) to or “interchangeable” with an FDA-licensed reference biologic product [6]. In concept, the goal of the BPCI Act is similar to the Hatch–Waxman Act.

The prohibitive costs of most biologics and some small-molecule drugs has led to increased scrutiny in understanding the US government’s role in the development of costly novel drug products. For example, for almost all of the biosimilars approved by the FDA so far, the associated brand-name drug (among the top-selling drugs in the world) was originally formulated by scientists at public-sector research institutions. Hence, like most US tax payers, I question the logic behind allowing sky-rocketing drug prices, especially for branded biologics. Should there be more robust governmental controls on this front? Should the US taxpayer have significant leverage to affect the process? Based on two recent US Court of Appeals for the Federal Circuit (CAFC) decisions and imperfections in the BPCI Act itself, some argue that the law impairs the potential for a flourishing generic market for biologics [7a]. Moreover, since around 90% of the global biosimilar sales come from the European Union (EU), compared to just 2% from the United States, some have questioned whether the US biosimilar industry is falling behind [7b]. The global biosimilars market in 2017 was \$4.49 billion and is expected to grow with a compound annual growth rate (CAGR) of 31.7% to \$23.63 billion by 2023.<sup>5</sup> Biosimilars are discussed in Section 1.6. Figures 1.1b and 1.1c represent the FDA drug approval process.

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<sup>5</sup>Data from MarketsandMarkets.com



Figure 1.1b Drug sponsor's discovery and screening phase (preclinical).

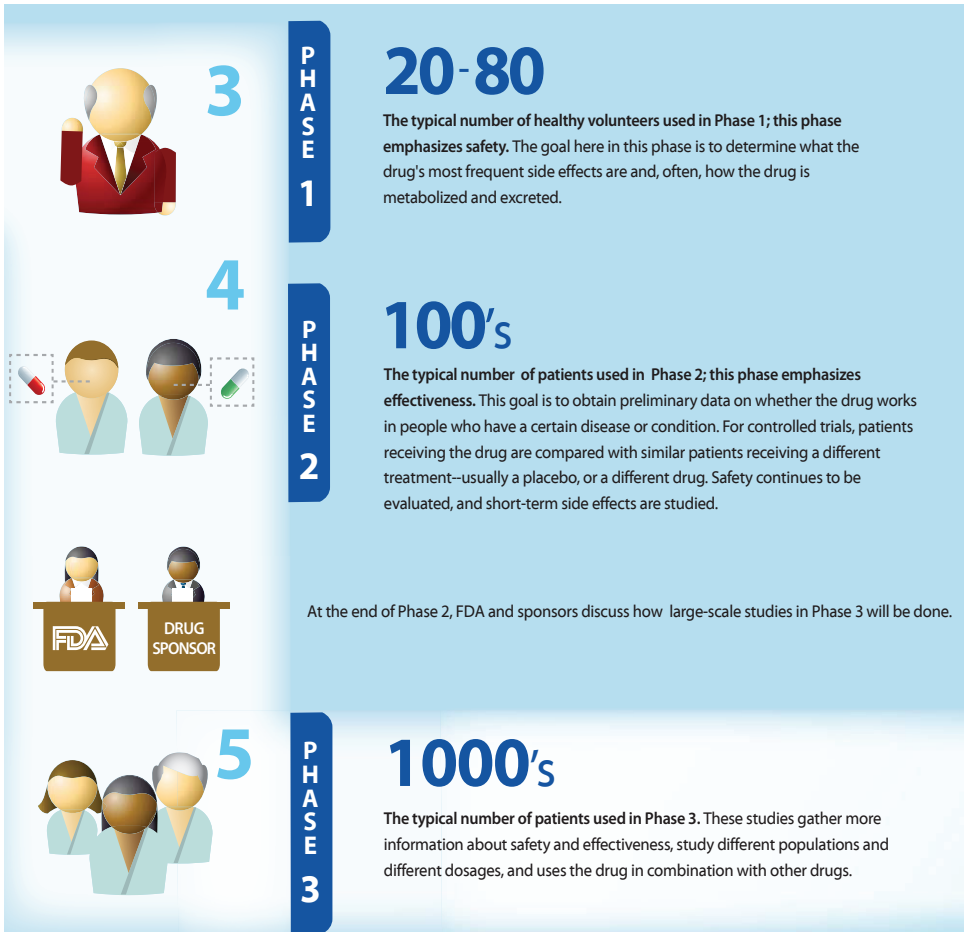


Figure 1.1c Drug sponsor's clinical studies/trials.

**Box 1.1 The FDA's view of biological products**

(courtesy of the FDA, with modifications by the author)

**1. What is a biological product?**

Biological products, like other drugs, are used for the treatment, prevention, or cure of disease in humans. In contrast to chemically synthesized small-molecular-weight drugs, which have a well-defined structure and can be thoroughly characterized, biological products are generally derived from living material—human, animal, or microorganism—are complex in structure, and thus are usually not fully characterized. Section 351 of the *Public Health Service (PHS) Act* defines a biological product as a “virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, ... applicable to the prevention, treatment, or cure of a disease or condition of human beings.” FDA regulations and policies have established that biological products include blood-derived products, vaccines, *in vivo* diagnostic allergenic products, immunoglobulin products, products containing cells or microorganisms, and most protein products. Biological products subject to the *PHS Act* also meet the definition of *drugs* under the *Federal Food, Drug and Cosmetic Act (FDC Act)*. Note that hormones such as insulin, glucagon, and human growth hormone are regulated as drugs under the *FDC Act*, not biological products under the *PHS Act*.

**2. What Center has the regulatory responsibility for therapeutic biological products?**

Both the FDA's Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER) have regulatory responsibility for therapeutic biological products, including premarket review and oversight. The categories of therapeutic biological products regulated by CDER (under the *FDC Act* and/or the *PHS Act*, as appropriate) are the following:

- Monoclonal antibodies for *in vivo* use.



- Most proteins intended for therapeutic use, including cytokines (e.g., interferons), enzymes (e.g., thrombolytics), and other novel proteins, except for those that are specifically assigned to the Center for Biologics Evaluation and Research (CBER) (e.g., vaccines and blood products). This category includes therapeutic proteins derived from plants, animals, humans, or microorganisms, and recombinant versions of these products. Exceptions to this rule are coagulation factors (both recombinant and human plasma derived).
- Immunomodulators (non-vaccine and non-allergenic products intended to treat disease by inhibiting or down-regulating a pre-existing, pathological immune response).
- Growth factors, cytokines, and monoclonal antibodies intended to mobilize, stimulate, decrease, or otherwise alter the production of hematopoietic cells *in vivo*.

### **3. Are the biologic development requirements different from the requirements for a new drug product?**

Biological products are a subset of drugs; therefore, both are regulated under provisions of the *FDC Act*. However, only biological products are licensed under section 351 of the *PHS Act*. (As previously noted, some therapeutic protein products are approved under section 505 of the *FDC Act*, not under the *PHS Act*.) Following initial laboratory and animal testing that shows that investigational use in humans is reasonably safe, biological products (like other drugs) can be studied in clinical trials in humans under an investigational new drug application (IND) in accordance with the regulations at *21 CFR 312*. If the data generated by the studies demonstrate that the product is safe and effective for its intended use, the data are submitted as part of a marketing application. Whereas a new drug application (NDA) is used for drugs subject to the drug approval provisions of the *FDC Act*, a biologics license application (BLA) is required for biological products subject to licensure under the *PHS Act*. FDA form 356h is used for both NDA and BLA submissions.

FDA approval to market a biologic is granted by issuance of a biologics license (see Fig. 1.1a).

#### **4. What are the requirements for licensing a biologic?**

Issuance of a biologics license is a determination that the product, the manufacturing process, and the manufacturing facilities meet applicable requirements to ensure the continued safety, purity, and potency of the product. Among other things, safety and purity assessments must consider the storage and testing of cell substrates that are often used to manufacture biologics. A potency assay is required due to the complexity and heterogeneity of biologics. The regulations regarding BLAs for therapeutic biological products include *21 CFR parts 600, 601, and 610*.

#### **5. What does safety mean?**

The word safety means the relative freedom from harmful effects, direct or indirect, when a product is prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

#### **6. What is purity?**

Purity means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances.

#### **7. What is potency?**

The word potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests, to yield a given result.

#### **8. Does FDA issue license certificates upon approval of a BLA?**

Approval to market a biologic is granted by issuance of a biologics license (including US license number) as part of the approval

letter. The FDA does not issue a license certificate. The US License number must appear on the product labeling.

### **9. Why are biologics regulated under the *PHS Act*?**

As mentioned above, biologics are subject to provisions of both the *FD&C Act* and the *PHS Act*. Because of the complexity of manufacturing and characterizing a biologic, the *PHS Act* emphasizes the importance of appropriate manufacturing control for products. The *PHS Act* provides for a system of controls over all aspects of the manufacturing process. In some cases, manufacturing changes could result in changes to the biological molecule that might not be detected by standard chemical and molecular biology characterization techniques yet could profoundly alter the safety or efficacy profile. Therefore, changes in the manufacturing process, equipment, or facilities may require additional clinical studies to demonstrate the product's continued safety, identity, purity, and potency. The *PHS Act* also provides authority to immediately suspend licenses in situations where there exists a danger to public health.

### **10. How is the manufacturing process for a biological product usually different from the process for drugs?**

Because, in many cases, there is limited ability to identify the identity of the clinically active component(s) of a complex biological product, such products are often defined by their manufacturing processes. Changes in the manufacturing process, equipment, or facilities could result in changes in the biological product itself and sometimes require additional clinical studies to demonstrate the product's safety, identity, purity, and potency. Traditional drug products usually consist of pure chemical substances that are easily analyzed after manufacture. Since there is a significant difference in how biological products are made, the production is monitored by the agency from the initial stages to make sure the final product turns out as expected.

### **11. What is comparability testing of biologics?**

A sponsor may be able to demonstrate product comparability between a biological product made after a manufacturing change and a product made before implementation of the change through different types of analytical and functional testing without additional clinical studies. The agency may determine that the two products are comparable if the results of the comparability testing demonstrate that the manufacturing change does not affect safety, identity, purity, or potency. For more information, see Chapter 17, titled “Immunogenicity Assessment for Therapeutic Protein Products” (FDA), and Chapter 18, titled “Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products” (FDA).

### **12. Where can I find additional information about therapeutic biologics?**

There are several guidances that may be helpful:

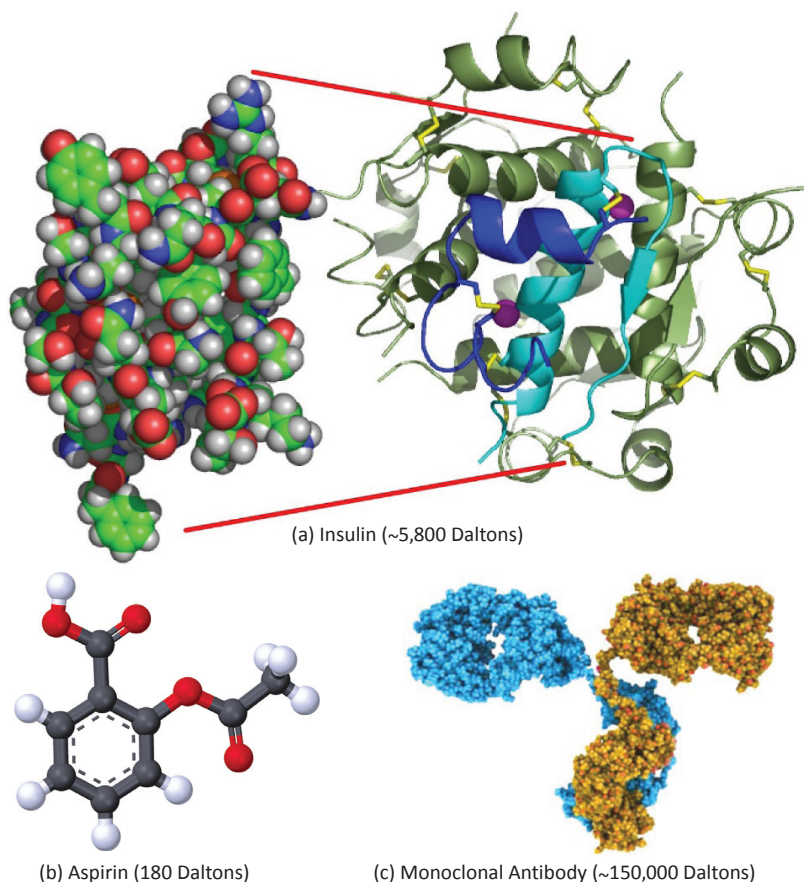
- “Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products” (PDF-33 KB) (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM124805.pdf>)
- “Content and Format of INDs for Phase I Studies of Drugs Including Well Characterized, Therapeutic, Biotechnology-Derived Products” (PDF-42 KB) (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071597.pdf>)
- “Providing Clinical Effectiveness of Human Drugs and Biological Products” (<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>)
- “Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use” (PDF-140 KB) (<http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/UCM153182.pdf>)

### ***Pharmaceutical versus Biotechnology Companies***

The demarcations between pharmaceutical and biotechnology (and between branded and generic) companies are no longer that clear. For example, Genentech (owned by Roche) and Medimmune (owned by AstraZeneca), although operate independently, are technically part of big pharma. Many biotechs are developing therapeutics that are traditional small-molecule drugs rather than biotech products. Conversely, big pharma is developing biotech products along with traditional small molecules. Often, branded companies are developing generics and *vice versa*. Currently, there is a symbiotic relationship between all these diverse players. For example, big pharma (which is well versed in clinical trials and commercialization) often turns to biotech companies (that are generally low on funds, lack a robust sales force or lack regulatory expertise) to license compounds or to develop platform technologies with the promise to yield multiple molecules.

## **1.2 Biologics versus Small-Molecule Drugs**

Biologics are a distinct regulatory category of drugs that differ from conventional small-molecule drugs by their manufacturing processes (i.e., biological sources vs. chemical/synthetic manufacturing). They are biologically derived from microorganisms (generally engineered) or cells (often mammalian, including human). In other words, biologics are drugs produced via modern molecular biological methods, and they are distinguished from traditional biological products that are directly extracted from natural biological sources (such as proteins derived from plasma or plants). Biologics include a diverse range of therapeutics, including blockbuster monoclonal antibodies (mAbs) (e.g., Avastin<sup>®</sup> (bevacizumab) and Humira<sup>®</sup> (adalimumab)), Fc fusion proteins, anticoagulants, blood factors, hormones, cytokines, growth factors, engineered protein scaffolds, and cell-based gene therapies (e.g., chimeric antigen receptor T-cell therapy (CAR-T)) to treat various diseases—cancers, rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), hemophilia, anemia, etc. Most biologics are large, complex molecules as compared to small-molecule drugs (Fig. 1.2) and are often more difficult to characterize than small-molecule drugs (Table 1.1).



**Figure 1.2 Comparing biologics to small-molecule drugs.** The molecular model of two biologics (insulin and monoclonal antibody) and the molecular structure of a small-molecule drug (acetylsalicylic acid or aspirin) are shown to demonstrate the differences in size and molecular complexity associated with these two overlapping drug classes. The molecular weight (MW) of insulin is ~5,800 Daltons and that of a monoclonal antibody is ~150,000 Daltons. The MW of aspirin is 180 Daltons. Structures shown are not to scale. (a) The left side is a space-filling model of the insulin monomer, believed to be biologically active. Carbon atoms are shown in green, hydrogen in gray, oxygen in red, and nitrogen in blue. On the right side is a ribbon diagram of the insulin hexamer, believed to be the stored form. A monomer unit is highlighted with the A chain in blue and the B chain in cyan. Yellow denotes disulfide bonds, and magenta spheres are zinc ions (courtesy of Wikipedia). (b) Ball-and-stick model of the aspirin molecule. (c) X-ray crystallographic structure of a monoclonal antibody shown as a space-filling model (courtesy of the FDA).

The FDA's statutory definition of a "biological product" is listed in Box 1.1.<sup>6</sup> This definition has important regulatory and commercial ramifications as it determines which regulatory pathway governs the approval/licensure of an innovator product and any subsequent follow-on competitor products (i.e., biosimilars, see Section 1.6) that seek to rely on that product's approval. Note that some protein drug products (hormones such as insulin and human growth hormone) are regulated by the FDA as drugs under the FD&C Act, not biological products under the PHS Act. In fact, when human insulin (Humulin<sup>®</sup>) was approved as the world's first recombinant protein therapeutic in 1982, it was approved under the FD&C Act. This bizarre dichotomy continues today, with some proteins licensed under the PHS Act and some approved under the FD&C Act. Thankfully, this mess is set to clear up in March 2020, when an approved application for a biological product under section 505 of the FD&C Act "shall be deemed to be a license for the biological product under section 351 of the PHS Act."<sup>7</sup>

### ***Growth of Biologics: Technological Drivers***

Advances built on two seminal technologies (recombinant DNA technology and hybridoma technology) have been the driving forces behind the expansion of biologics. Specifically, the development of recombinant DNA technology in the 1970s revolutionized the production of biologics. In 1982, human insulin (brand name Humulin<sup>®</sup> and manufactured by Genentech in partnership with Eli Lilly) was the first recombinant protein therapeutic approved by the FDA. Since Humulin<sup>®</sup> was fully human and produced via genetically engineered *Escherichia coli*, issues with immunogenicity were minimized. In the 1980s, modified biologics joined recombinant versions of natural proteins as a major new class of biologics. In 1975, Köhler and Milstein's hybridoma technology established a continuous immortal culture of cells secreting an antibody of predefined specificity (monoclonal antibody (mAb)) by fusing antibody-producing B cells with myeloma cells.

<sup>6</sup>In December 2017, the FDA formally announces rulemaking to amend the definition of a biologic to conform to the statutory definition (21 U.S.C. 262) adopted in the Biologics Price Competition and Innovation Act of 2009. See: Definition of the Term "Biological Product." Available at: <https://www.reginfo.gov/public/do/eAgendaViewRule?pubId=201710&RIN=0910-AH57> (accessed on May 1, 2018).

<sup>7</sup>Federal Register (2016). Notices, vol. 81, no. 49, p. 13373, Docket No. FDA-2015-D-4750.

Below appears a well-accepted definition of a biologic [8]:

*“A biopharmaceutical is a protein or nucleic acid-based pharmaceutical substance used for therapeutic or in vivo diagnostic purposes, which is produced by means other than direct extraction from a native (non-engineered) biological source.”*

Since most biologics are very complex molecules and cannot be fully characterized by existing scientific technologies, they are often characterized via their manufacturing processes. However, due to their structural complexity, the manufacturing processes are also often complex, very sensitive, and proprietary. In fact, minor variations in temperature or other production factors can profoundly change the final biologic drug product. Naturally, this can affect product performance and patient safety. Hence, even minor alterations in the manufacturing process or facility may require clinical studies to demonstrate safety (including immune-related), purity, and potency of the synthesized biologic. According to the FDA [9], “[t]he nature of biological products, including the inherent variations that can result from the manufacturing process, can present challenges in characterizing and manufacturing these products that often do not exist in the development of small-molecule drugs. Slight differences between manufactured lots of the same biological product (i.e., acceptable within-product variations) are normal and expected within the manufacturing process.”

### **1.3 What Are Nanodrugs?**

Optimists tout nanotechnology as an enabling technology, a sort of next industrial revolution that could enhance the wealth and health of nations. They promise that many areas within nanomedicine (nanoscale drug delivery systems, theranostics, imaging, etc.) will soon be a healthcare game-changer by offering patients access to personalized or precision medicine. Pessimists, on the other hand, take a cautionary position, preaching instead a go-slow approach and pointing to lack of sufficient scientific data on health risks, general failure on the part of regulatory agencies to provide clearer guidelines and issuance of patents of dubious scope by patent offices. As usual, the reality is somewhere between



such extremes. Whatever your stance, nanomedicine has already permeated virtually every sector of the global economy. It continues to evolve and play a pivotal role in various industry segments, spurring new directions in research, product development, and translational efforts [1–3].

### ***Nano Frontiers: Dreams, Hype and Reality***

The rush to celebrate “eureka” moments is overshadowing the research enterprise. Some blame the current pervasive culture of science that focuses on rewarding eye-catching and positive findings. Others point to an increased emphasis on making provocative statements rather than presenting technical details or reporting basic elements of experimental design. “Fantastical claiming” is nothing new to academia and start-ups where exaggerated basic research developments are often touted as revolutionary and translatable advances. Claims of early-stage discoveries are highlighted as confirmation of downstream novel products and applications to come. Even distinguished professors at reputable universities are guilty of such hype. In this context, nano’s potential benefits are also often overstated or inferred to be very close to application when clear bottlenecks to commercial translation persist.

In the nanoworld, many have desperately and without scientific basis thrown around the “nano” prefix to suit their selfish purpose, whether it is to obtain research funds, gain patent approval, raise venture capital, run for public office, or seek publication of a manuscript. Sadly, many fall prey to such outrageous hype and are even willing to provide venture funds. An extreme example of this is the recent Theranos case where the blood-testing company concocted fantastical claims of doing hundreds of tests from a single drop of human blood and raised billions in the process (market valuation of \$9 billion). See: Carreyrou, J. (2018). *Bad Blood: Secrets and Lies in a Silicon Valley Startup*, Alfred A. Knopf, New York. There are also a few cautionary tales from the world of nanomedicine. Consider, for example, the recent demise and bankruptcy of BIND Therapeutics Inc. See: WTF happened to bind therapeutics? Available at: <https://www.nanalyze.com/2017/08/wtf-happened-bind-therapeutics/> (accessed on August 5, 2018).

Obviously, the Holy Grail of any drug delivery system, whether it is nanoscale or not, is to deliver to a patient the correct dose of an active agent to a specific disease or tissue site while simultaneously minimizing toxic side effects and optimizing therapeutic benefit. This is mostly unachievable via conventional small-molecule formulations and drug delivery systems. However, the potential to do so may be greater now via nanodrugs. The prototype of targeted drug delivery can be traced back to the concept of a “magic bullet” that was postulated by Nobel laureate Paul Ehrlich in 1908 (*magische Kugel*, his term for an ideal therapeutic agent) wherein a drug could selectively target a pathogenic organism or diseased tissue while leaving healthy cells unharmed [10]. Half a century later, this concept of the magic bullet was realized by the development of antibody–drug conjugates (ADCs) when in 1958 methotrexate was linked to an antibody targeting leukemia cells wherein the antibody component provided specificity for a target antigen and the active agent portion conferred cytotoxicity. (Technically, ADCs are nanodrugs.) Half a century since ADCs, various classes of nanoscale drug delivery systems are in early development though first-generation nanodrugs have been commercialized (Fig. 1.3). However, the arrival of revolutionary nanodrugs are just promises at this stage. There are many second- and third-generation nanodrugs at various stages of R&D (Fig. 1.3). Obviously, advanced nanodrugs will be (i) those that can specifically deliver active agents to target tissue, specific cells or even organelles (site-specific drug delivery); or (ii) offer simultaneous controlled delivery of active agents with concurrent real-time imaging (theranostic drug delivery).


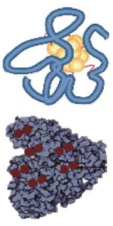

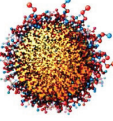
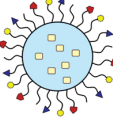
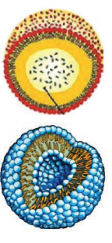
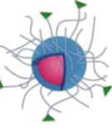

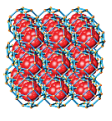
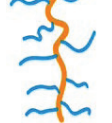

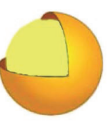
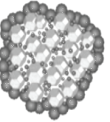
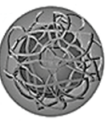
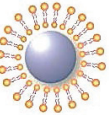
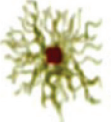
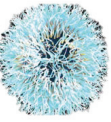

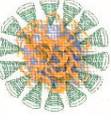
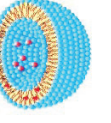
Data obtained from industry and the FDA show that most of the approved or pending nanodrugs are oncology-related and based on protein–polymer conjugates or liposomes. The first FDA-approved nanodrug was Doxil<sup>®</sup> (doxorubicin hydrochloride liposome injection) in 1995 while AmBisome<sup>®</sup> (amphotericin B liposome injection) was the first one approved by the EMA in 1997. The first protein-based nanodrug to receive regulatory approval was albumin-bound paclitaxel (Abraxane<sup>®</sup>), approved by the FDA in 2005. However, note that a nanoparticulate iron oxide intravenous solution that was marketed in the 1960s and


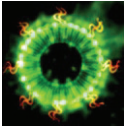



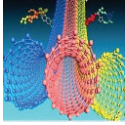
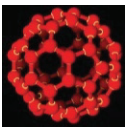

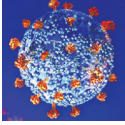
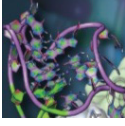
certain nanoliposomal products that were approved in the 1950s should, in fact, be considered true first-generation nanodrugs. Polymer–drug conjugates (with a short peptide spacer between the two that prolonged release) were also prepared back in the 1950s, when a polyvinylpyrrolidone–mescaline conjugate was produced.

### ***Nanodrugs: Relabeling of Earlier Terms?***

“The new concept of nanomedicine arose from merging nanoscience and nanotechnology with medicine. Pharmaceutical scientists quickly adopted nanoscience terminology, thus “creating” “nanopharmaceuticals.” Moreover, just using the term “nano” intuitively implied state-of-the-art research and became very fashionable within the pharmaceutical science community. Colloidal systems reemerged as nanosystems. Colloidal gold, a traditional alchemical preparation, was turned into a suspension of gold nanoparticles, and colloidal drug-delivery systems became nanodrug delivery systems. The exploration of colloidal systems, i.e., systems containing nanometer sized components, for biomedical research was, however, launched already more than 50 years ago and efforts to explore colloidal (nano) particles for drug delivery date back about 40 years. For example, efforts to reduce the cardiotoxicity of anthracyclines via encapsulation into nanosized phospholipid vesicles (liposomes) began at the end of the 1970s. During the 1980s, three liposome-dedicated US start-up companies (Vestar in Pasadena, CA, USA, The Liposome Company in Princeton, NJ, USA, and Liposome Technology Inc., in Menlo Park, CA, USA) were competing with each other in developing three different liposomal anthracycline formulations. Liposome technology research culminated in 1995 in the US Food and Drug Administration (FDA) approval of Doxil®, “the first FDA-approved nanodrug”. Notwithstanding, it should be noted that in the liposome literature the term “nano” was essentially absent until the year 2000.”

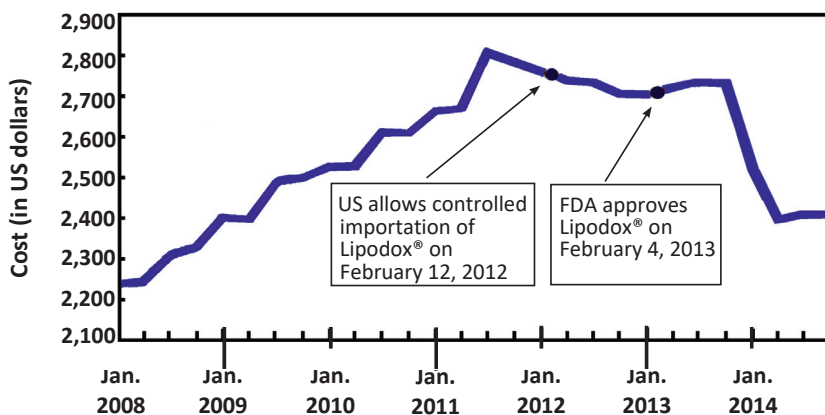
*Source:* Weissig, V., Pettinger, T. K., Murdock, N. (2014). Nanopharmaceuticals (part 1): Products on the market. *Int. J. Nanomed.*, **9**, 4357–4373.

 <p><b>Solid Nanoparticles</b></p>	 <p><b>Drug-Polymer Conjugate</b></p>	 <p><b>Polymeric Micelle</b></p>	 <p><b>Functionalized Gold NP</b></p>	 <p><b>Biomimetic NP</b></p>
 <p><b>Ligand-Functionalized Nanoliposomes</b></p>	 <p><b>Ultrasmall Silica Nanoparticle (C Dot)</b></p>	 <p><b>Drug-Encapsulated Dendrimer</b></p>	 <p><b>Drug-Loaded NMOFs</b></p>	 <p><b>Polymer-Polypeptide Conjugate</b></p>
 <p><b>GRAS-Stabilized Nanocrystal</b></p>	 <p><b>Silica Gold Nanoshell</b></p>	 <p><b>Functionalized Nanodiamonds</b></p>	 <p><b>Polymeric Nanoparticle</b></p>	 <p><b>Phospholipid-Coated Magnetic NP</b></p>
 <p><b>Dextran-Coated USPION</b></p>	 <p><b>Self-Assembling Peptides</b></p>	 <p><b>Drug-Loaded Bioinspired Bile Micelle</b></p>	 <p><b>Drug-Loaded Chitosan-PEG Coated Nanogel</b></p>	 <p><b>Drug-Loaded Nanoliposome</b></p>

 <p><b>Nanonized API</b></p>	 <p><b>Peptide-siRNA Liposome</b></p>	 <p><b>Stealth Pegylated Nanoliposome</b></p>	 <p><b>Functionalized Drug Magnetic NP</b></p>	 <p><b>Nanogel</b></p>
 <p><b>Peptide f-CNTs</b></p>	 <p><b>C<sub>60</sub> Buckminsterfullerene</b></p>	 <p><b>Enzyme Nanocarrier</b></p>	 <p><b>Functionalized Solid NP</b></p>	 <p><b>Aptamers</b></p>

**Figure 1.3 Nanoscale Drug delivery system platforms (nanodrug products).** Schematic representation of selected engineered nanoparticles (NPs) used in drug delivery that are either approved by regulatory bodies, are in preclinical development or are in clinical trials. In most cases shown above, they are considered as first or second generation multifunctional engineered NPs, ranging in average diameter from one nanometer (1 nm) to a micron (1000 nm). Active bio-targeting of the NP is often achieved via conjugating ligands or functional groups (antibodies, peptides, aptamers, folate, hyaluronic acid). These molecules are tagged to the NP surface with or without spacers/linkers such as PEG. Many nanodrugs depicted above (e.g., metal-based NPs, f-CNTs, NMOFs, etc.), although extensively advertised for drug delivery, will pose enormous drug approval and commercialization challenges and will not appear in the clinic this century. Non-engineered antibodies, biological motors (e.g., sperm), engineered nanomotors, and naturally occurring NPs (natural protein nanotubes) are specifically excluded here. Antibody-drug conjugates (ADCs) are encompassed by the cartoons labeled “Polymer-Polypeptide Conjugate” or “Drug-Polymer Conjugate.” Therapeutic monoclonal antibodies (TMABs), polymer-polypeptide conjugates, and aptamers shown above are classic biologics but they are also nanodrugs as they fall within the widely accepted standard definition of nanodrugs. The list of NPs depicted here is not meant to be exhaustive, the illustrations do not reflect precise three-dimensional shape or configuration, and the NPs are not drawn to scale. *Abbreviations:* NPs, nanoparticles; PEG, polyethylene glycol; GRAS, Generally Recognized As Safe; C dot, Cornell dot; API, active pharmaceutical ingredient; ADCs, antibody-drug conjugates; NMOFs, nanoscale metal organic frameworks; f-CNTs, functionalized carbon nanotubes; siRNA, small interfering ribonucleic acid; USPIO, ultrasmall superparamagnetic iron oxide nanoparticle. Copyright 2018 Raj Bawa. All rights reserved.

In 2011, drug shortages were such a pressing issue in the United States that an executive order from the President was issued directing the FDA to streamline the approval process for new therapeutics that could fill the voids. One of the major drugs whose supply was deficient in the United States was Doxil<sup>®</sup>, and to curb this shortage, the FDA on February 21, 2012, authorized the temporary importation of Lipodox<sup>®</sup> (doxorubicin hydrochloride liposome injection, Sun Pharmaceutical Industries Ltd., India), a generic version of Doxil<sup>®</sup>. Following this, the FDA evaluated and approved Lipodox<sup>®</sup> within a year on February 4, 2013, in roughly one-third of the time it takes for an average generic to receive premarket regulatory approval. Hence, Lipodox<sup>®</sup> became the first generic nanodrug (i.e., nanosimilar) approved in the United States. Obviously, this helped alleviate the Doxil<sup>®</sup> shortage and reduced the cost of care (Fig. 1.4). However, a recent study [11] concluded that *“the data available from this study and in the peer-reviewed literature are compelling suggesting that Lipodox for treatment of recurrent ovarian cancer does not appear to have equal efficacy compared to Doxil. It raises many concerns how to balance the challenges of drug shortages with maintaining the standards for drug approval. A prospective clinical study to compare the two products is warranted before Lipodox can be deemed equivalent substitution for Doxil.”*



**Figure 1.4** Cost for treatment of AIDS-related Kaposi sarcoma (KS) from January 2008 to September 2014.

### ***What Is Nanotechnology?***

My definition of nanotechnology omits any strict size limitation. See: Bawa, R. (2007). Patents and nanomedicine. *Nanomedicine (London)*, 2(3), 351–374: “*The design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property.*”

This flexible definition has four key features: (i) It recognizes that the properties and performance of the synthetic, engineered “*structures, devices, and systems*” are inherently rooted in their nanoscale dimensions. The definition focuses on the unique physiological behavior of these “*structures, devices, and systems*” that is occurring at the nanoscale; it does not focus on any shape, aspect ratio, specific size or dimension; (ii) it focuses on “*technology*” that has commercial potential, not “*nanoscience*” or “*basic R&D*” conducted in a lab setting; (iii) the “*structures, devices, and systems*” that result from or incorporate nano must be “*novel/superior*” compared to their bulk/conventional counterparts; and (iv) the concept of “*controlled manipulation*” (as compared to “*self-assembly*”) is critical.

The prefix “*nano*” in the SI measurement system denotes  $10^{-9}$  or one-billionth. There is no firm consensus over whether the prefix “*nano*” is Greek or Latin. While the term “*nano*” is often linked to the Greek word for “*dwarf*,” the ancient Greek word for “*dwarf*” is spelled “*nanno*” (with a double “*n*”) while the Latin word for dwarf is “*nanus*” (with a single “*n*”). While a nanometer refers to one-billionth of a meter in size ( $10^{-9}$  m = 1 nm), a nanosecond refers to one billionth of a second ( $10^{-9}$  s = 1 ns), a nanoliter refers to one billionth of a liter ( $10^{-9}$  l = 1 nl) and a nanogram refers to one billionth of a gram ( $10^{-9}$  g = 1 ng). The diameter of an atom ranges from about 0.1–0.5 nm, a DNA molecule about 2–3 nm, and a gold atom about 1/3rd of a nm.

Given that this specific example of generic approval is a problem, I believe that while the development of generics is important to facilitate patient access to vital medications at a



reasonable price, generic approvals should be science-based, data-driven, and reported transparently. Another example of the issuance of generics is discussed in detail in Section 1.7.

There is no formal or internationally accepted definition for anything “nano.” In this regard, a harmonized definition and nomenclature is urgently needed. For example, there is no standard definition for a nanodrug. The following is my definition for a nanodrug [12]:

*“A nanodrug is: (1) a formulation, often colloidal, containing therapeutic particles (nanoparticles) ranging in size from 1–1,000 nm; and (2) either (a) the carrier(s) is/are the therapeutic (i.e., a conventional therapeutic agent is absent), or (b) the therapeutic is directly coupled (functionalized, solubilized, entrapped, coated, etc.) to a carrier.”*

Nanodrugs cannot merely be defined by their size. Nanodrugs may have unique properties (nanocharacter) that can be beneficial for various clinical applications but there is no specific size range or dimensional limit to which superior properties are confined to. In fact, size limitation below 100 nm, frequently recited in journals and talks as well as touted as the definition of anything “nano” by US federal agencies like the National Nanotechnology Initiative (NNI), cannot serve as an arbitrary basis of novel properties of nanodrugs [12]. For instance, such a bizarre definition falls on its face in all these scenarios: Larger materials may contain nanostructures with size-specific properties; nanomaterials may be employed during a manufacturing step but not found in the final finished product; or nanoparticles may aggregate/dissociate in a dynamic equilibrium state and therefore the formulation may contain a mixed population of size ranges. *In conclusion, viable sui generis definition of nanodrugs having a bright-line size limit below 100 nm has no scientific or legal basis.*

## 1.4 Are Biologics and Nanodrugs Adversely Immunogenic?

Almost all small-molecule drug-induced allergic reactions may be easily classified into one of four classic Gell and Coombs



hypersensitivity categories.<sup>8</sup> However, many others with an immunologic component, including biologics and nanodrugs, are difficult to classify in such a manner because of a lack of mechanistic information [13]. Adverse clinical events (sometimes referred to as Adverse Drug Reactions or ADRs) can not only occur due to primary factors such as off-target toxicity or exaggerated pharmacologic effects, but also due to secondary drug effects such as immune reactions to the drug product. While approximately 80% of human adverse drug reactions are directly related to an effect of the drug or a metabolite, around 6–10% are immune-mediated and unpredictable [13]. One study showed that 10–20% of the medicinal products removed from clinical practice between 1969 and 2002 were withdrawn due to immunotoxic effects [14]. Some claim that the actual number of serious adverse events like hospitalizations and death from FDA-approved drugs, vaccines, and medical devices is grossly underreported by the FDA [15].<sup>9</sup> Suspected ADRs can be reported to the FDA at 1-800-FDA-1088 or [www.fda.gov/medwatch](http://www.fda.gov/medwatch).

Immune-mediated side effects of small molecules are unpredictable. Most small molecules that have a MW <1 kDa do not elicit an immune response in their native state, becoming immunogenic only when they act as a hapten, bind covalently to high-molecular-weight proteins, and undergo antigen processing and presentation. On the other hand, “newer” larger molecule drugs can be inherently immunogenic. For example, protein-based biologics and nanodrugs can be digested and processed for presentation by antigen-presenting cells (APCs); this can sometimes cause ADRs. The very untested nature of these therapeutics that make them so revolutionary in some respects also makes them problematic and potentially dangerous. For example, major benefits touted for nanodrugs—a reduction in unwanted side effects, increased specificity, fewer off-target effects, generation of fewer harmful metabolites, slower clearance from the body, longer

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<sup>8</sup>Gell and Coombs developed their widely accepted classification of hypersensitivity reactions in the context of deleterious connotation. See: Gell, P. G. H., Coombs, R. R. A. (1963). The classification of allergic reactions underlying disease. In: Coombs, R. R. A., Gell, P. G. H., eds., *Clinical Aspects of Immunology*, Blackwell Science.

<sup>9</sup>Also, see the FDA Death Meter: <http://www.anh-usa.org/microsite-subpage/fda-death-meter/>.

### ***What Is Immunogenicity?***

Immunogenicity is the ability of an antigen or epitope to provoke an immune response, i.e., to induce a humoral and/or cell-mediated immune response. Put differently, it is the propensity of a therapeutic (e.g., biologic or nanodrug) to generate immune responses to itself and to related products. These responses can either (i) induce immunologically related nonclinical effect(s); (ii) provide beneficial or protective effect(s); or (iii) result in adverse clinical events (Adverse Drug Reactions or ADRs). Immunogenicity can be one of two types: (a) wanted or (b) unwanted. Wanted immunogenicity is typically related to vaccines where the injection of the vaccine (the antigen) stimulates an immune response against a pathogen. On the other hand, unwanted immune responses are adverse events (i.e., ADRs). The meaning of immunogenicity in this chapter is the latter, namely, an adverse immune response to the therapeutic. The detection of antidrug antibodies (ADAs) (Section 1.4.1(a)) has generally been equated as a measure of immunogenicity. ADAs may neutralize a therapeutic and inhibit its efficacy or cross-react to endogenous counterparts, leading to loss of physiological function. An example of unwanted immunogenicity is the generation of neutralizing antibodies against recombinant erythropoietin (EPO) in patients receiving EPO for chronic kidney disease (CKD) and resulting pure red cell aplasia (PRCA)-related anemia due to the neutralization of the endogenous EPO. Immunogenicity associated with protein drugs was first observed more than a century ago in 30% of diphtheria patients treated with antitoxin administered in whole horse serum. See: Weaver, G. H. (1900). Serum disease. *Arch. Intern. Med. (Chic.)*, **5**, 485–513.

duration of effect, reduced intrinsic toxicity, etc.—do not guarantee the absence of adverse immune side effects: *An inherent risk of introducing these drug products into the human body is the potential to provoke an unwanted immune response.* Thus, managing their immunogenicity profile is critical during drug R&D and later. Studies have shown that these drug products can interact with various components of the immune system to various

immunological endpoints, interactions that are fast, complex, and poorly understood. These interactions with the immune system play a leading role in the intensity and extent of side effects occurring simultaneously with their therapeutic efficacy. In fact, when compared to conventional small-molecule drugs, both biologics and nanodrugs have biological and synthetic entities of a size, shape, reactivity, and structure that are *often* recognized by the human immune system, *sometimes* in an adverse manner. This can obviously negatively affect their effectiveness and safety, and thereby, limit their therapeutic application. This also poses challenges for regulatory agencies and patent offices, all serving as bottlenecks to effective translation of these therapeutics.

Multiple risk factors influencing the immunogenicity of biologics and nanodrugs include patient-, clinical use-, manufacturing-, and product-related factors (Fig. 1.5). Some of the ADRs include complement activation, tissue inflammation, leucocyte hypersensitivity, and formation of antibodies associated with clinical conditions. However, detailed mechanisms and causal linkages between various risk factors and immunogenicity induction onset as shown in Fig. 1.5 have yet to be fully elucidated. This is primarily due to the limited amount of data from mechanistic studies, a lack of multi-factorial analysis and a lack of standard immunogenicity assessment methods.

### 1.4.1 Immune Aspects of Biologics

#### (a) Antidrug Antibodies (ADAs) and Immune Complexes (ICs)

Early developers of biologics assumed that as many of these drugs were based on human genes, the human immune system would not treat them as foreign and not produce antibodies. However, this optimistic view has turned into alarm as some biologics can elicit a vigorous immune response that *may* sometimes neutralize, block or destroy the administered biologic. Also, most biologics are engineered to enable dual or multiple binding sites (via conjugated proteins, functionalized antibodies, etc.) — all of which could result in them being recognized as foreign and therefore immunogenic. In most cases, immunogenicity manifests itself

**DRUG PRODUCT**

origin  
 formulation, handling  
 aggregation/degradation of excipient(s) and/or active(s)  
 drug conjugates  
 mode of action/nature<sup>1</sup>  
 molecular structural differences from native active  
 proportion of “non-self” protein sequences/epitopes<sup>2</sup>  
 presence of foreign proteins  
 misfolding related to oxidation/deamidation  
 glycosylation patterns in proteins, protein mutations  
 nanoscale dimensions/nanoparticle size<sup>3</sup>  
 surface functionality, surface charge  
 protein size<sup>4</sup>  
 topology, shape, geometry, protein conformation

**MANUFACTURING**

production protocol variations  
 denaturation and/or alteration of structure  
 chemical modifications<sup>5</sup>  
 post translational modifications of proteins  
 impurities, contaminants, degradants, fragments<sup>6</sup>  
 aggregates, agglomerates<sup>7</sup>  
 leachables from containers<sup>8</sup>

**CLINICAL USE**

dose level  
 mechanism of action  
 dosing regimen (procedure, concentration)  
 delivery route<sup>9</sup>  
 frequency of administration<sup>10</sup>  
 duration of treatment<sup>11</sup>  
 use of DEHP or other plasticizers in plastic components<sup>12</sup>

**PATIENT**

patient genetics, predisposition, genetic deficiency<sup>13</sup>  
 age<sup>14</sup>  
 immunocompetency<sup>15</sup>  
 preexisting antibodies and CD4+T cells reactive to drug<sup>16</sup>  
 extended drug residence time<sup>17</sup>  
 presence of chronic conditions  
 disease state being treated, concurrent illness  
 prior exposure to related or cross-reacting drug products  
*in vivo* modifications of endogenous proteins  
 interruptions in therapy  
 concomitant therapies<sup>18</sup>  
 binding to specific cell surface versus soluble targets  
 and/or determinants  
 “superagonist” formation by cross-linking with ADAs

- <sup>1</sup>immunomodulatory versus immunosuppressive, or agonist versus antagonist
- <sup>2</sup>proportion of endogenous versus non-endogenous protein sequences; monoclonal antibody-based therapeutics have low immunogenicity
- <sup>3</sup>a high surface area to volume ratio when compared to their corresponding bulk counterpart
- <sup>4</sup>immunogenicity increases with size
- <sup>5</sup>oxidation, deamidation, isomerization has varying effects
- <sup>6</sup>host cell proteins, DNA and excipients from formulations are highly immunogenic
- <sup>7</sup>unique conformational epitopes may be present
- <sup>8</sup>introduction or exposure of new epitopes
- <sup>9</sup>immunogenicity order: inhalation > subcutaneous > intraperitoneal > intramuscular > intravenous
- <sup>10</sup>repeat administration increases immunogenicity
- <sup>11</sup>prolonged exposure increases immunogenicity
- <sup>12</sup>di-(2-ethylhexyl) phthalate (DEHP) is a manufactured chemical that is commonly added to plastics to make them flexible
- <sup>13</sup>certain MHC alleles, polymorphisms in cytokine genes, autoimmune or proinflammatory predisposition has a higher immunogenicity risk
- <sup>14</sup>pediatric versus adult immune system
- <sup>15</sup>if the patient is immunosuppressed, then may be more immunotolerant
- <sup>16</sup>examples include cross-reacting auto-antibodies, preexisting anti-PEG antibodies
- <sup>17</sup>at a specific site of action, within specific targeted tissue or in systemic circulation
- <sup>18</sup>co-medicated immunosuppressive drugs (e.g., methotrexate or steroids) reduce immunogenicity

**Figure 1.5 Key risk factors contributing to adverse immunogenicity of biologics and nanodrugs.** Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; ADAs, anti-drug antibodies; CD4<sup>+</sup>T cell, cluster of differentiation 4 T cell; MHC, major histocompatibility complex. Copyright 2018 Raj Bawa. All rights reserved.

as the generation of polyclonal neutralizing and non-neutralizing antidrug antibodies (ADAs) directed against the biologic, rendering it less effective. The detailed mechanisms leading to ADA formation are still not fully understood and characterized. Examples of ADA formation against biologics observed in clinical practice include the treatment of Crohn's disease and rheumatoid arthritis (RA) with anti-TNF adalimumab (Humira®), hemophilia A treatment with recombinant Factor VIII and multiple sclerosis (MS) patients receiving interferon-beta therapy, although the incidence rate of ADA varies among studies, even while using the same drug. Some studies have shown that Humira® does not work in ~20% of patients (the extensive warning list for Humira® includes "immune reactions, including a lupus-like syndrome"). Similarly, in 2016, Pfizer had to pull a promising anticholesterol biologic (bococizumab) after testing it in more than 25,000 persons. In six trials, ~50% of those who received the formulation developed ADAs, spelling doom for the drug candidate. According to Pfizer, this potential biologic was "not likely to provide value to patients, physicians or shareholders" [16]. In 2016, the Netherlands Cancer Institute reported that >50% of the anticancer biologics in 81 clinical trials worldwide were generating ADAs, although they could not confirm that this always negatively affected the drug candidate being tested.

Specifically, ADAs *may* (i) neutralize the activity of the biologic drug product, (ii) reduce half-life by enhancing clearance, (iii) result in allergic reactions, (iv) alter the drug's pharmacokinetic profile, (v) abrogate the pharmacological activity of the drug, and/or (vi) cross-react with endogenous counterparts to result in "autoimmune-like" reactions. Furthermore, antibody responses can potentially affect the interpretation of toxicology studies. As indicated earlier, such effects are much less frequently observed with conventional small-molecule drug products (Table 1.1).

Most biologics are administered to patients as repeated doses. This can elicit ADAs that can form antidrug immune complexes (ICs) with the biologic, which in turn can drive more ADA formation. In general, formation of ICs is a normal immunologic process. For example, binding of antibodies to their respective antigens forms ICs. Most formed ICs, even those that develop

due to ADAs, are small and cleared from circulation. It is only when systems to clear or degrade ICs are impaired, clinical or immune consequences may be observed. The role of ADAs is relevant to a discussion of ICs [17]: *“ADAs can be elicited in vivo to a therapeutic and their detection has generally been equated as a measure of immunogenicity. The detection, reporting, and characterization of the ADA are done in a tiered manner after careful consideration of immunogenic risk factors. Most adverse effects consequential to ADA formation, such as pharmacological abrogation, impact on therapeutic exposure, or hypersensitivity reactions, are a consequence of formation of immune complexes (ICs) between the ADA and therapeutic protein. Their levels, kinetics of interaction, size, polyclonal diversity, distribution, and Fc-mediated physiological effects can be potentially translated to clinically observable adverse effects. This leads to the paradigm of immunogenicity where therapeutic exposure leads to ADA generation that in turn forms ICs that mediate adverse effects related to immunogenicity. While the detection of such therapeutic specific IC from in vivo samples has remained analytically challenging, there are other biomarkers that mediate the interplay of the innate and adaptive immune responses and are potentially amenable to analysis. Such markers can reflect either the formation or the downstream effects of ICs... Clinical consequences of ADA make a compelling case for early IC formation that is an important consideration whether or not a long-lasting, pharmacologically meaningful ADA response will form. With the advent of personalized treatment, there will be a greater need to monitor underlying differences between individuals who are reactive to a therapeutic and how they impact either their response to treatment or their manifestation of any immunological adverse effects. Clinical decisions in routine practice rarely make use of information on the patient’s immune response to a therapeutic as a basis to understand poor therapeutic response or an unexpected adverse effect; to some extent, this has been due to limitations to identify the right dose of the drug required to neutralize the target in the presence of ADA, challenges in ascertaining total amount of ADA, and a general lack of immunogenicity assessments in patients to investigate failure of response after a drug has been approved for market.”*

### ***Immunotoxicology of Biological Response Modifiers***

“[F]or human biopharmaceuticals, the immune system is often the intended target of the therapy and the immunotoxicity observed may be exaggerated pharmacology. The intended effects of biotherapeutics on the immune system can be classified as immunopharmacology or as immunomodulatory effects. Adverse events can result from the intended immunomodulatory mechanism of action. For example, excessive downregulation of the immune system can result in recrudescence of a previously inactive virus. Immunotoxicity, on the other hand, refers to adverse immune effects that occur with products that are not targeting the immune system or with unintended effects on the immune system. These effects include inflammatory reaction at the injection site and autoimmunity due to altered expression of surface antigens. Although immunogenicity is an immune response of the animal to a foreign protein, it is not viewed as immunotoxicity *per se*.”

*Source:* Bussiere, J. L. (2016). Immunotoxicology of biological response modifiers. *Reference Module in Biomedical Sciences*. Elsevier.

The immunogenicity risk profile of a biologic is characterized by measurement of ADA levels in patients and correlation with therapeutic outcomes. An immune response to a biologic can occur in animal species, in clinical trial subjects or in patients. This is well recognized by regulatory agencies and hence it is mandatory to test immunogenicity of biologics in clinical trials as well as to monitor patients after drug approval. This minimizes an unnecessary safety risk for the patient while saving time, resources and effort. It is imperative that drug and biotechnology companies develop both novel tools as well as improve upon existing ADA-testing technologies to look for ADAs before and during clinical trials of biologics. In fact, multiple assay formats, technology platforms and sample preparation protocols are available to measure ADA responses including the enzyme-linked immunosorbent assay (ELISA), pH-shift anti-idiotypic antigen-binding test (PIA), surface plasmon resonance (SPR), radioimmunoassay (RIA), electrochemiluminescence assay (ECLA), and homogenous mobility shift assay (HMSA). Obviously, the



incidence of such reactions and their action on drug efficacy and patient safety must be transparently and promptly reported.

### **(b) Species Origin of Biologics**

The species origin of biologics has been identified as a significant factor in determining immunogenicity. For example, nonhuman proteins tend to elicit a prolonged and more pronounced immune responses than biologics developed from human or humanized molecules (Fig. 1.5). This may be because of amino acid sequence and glycosylation differences in the proteins such that the immune system sees them as self versus non-self [18]. Glycosylated proteins are generally less immunogenic than nonglycosylated proteins, possibly due to fewer exposed antigenic sites on the protein's tertiary structure. The greater the structural and amino acid sequence homology of the biologic with native human protein(s), the lesser the immunogenic potential. However, induction of antibody responses has been observed with biological products that are identical or nearly identical to native human proteins. This shows that other factors (Fig. 1.5) may be involved.

### **(c) Aggregation of Biologics**

Another issue with some biologics is that they show a concentration-dependent propensity for self-association, which often leads to the formation of aggregates that range in size from nanometers (oligomers) to microns (subvisible and visible particles). Aggregation<sup>10</sup> can occur throughout the life cycle of a biologic product: during upstream and downstream processing, during shipping, shelf-storage, and during handling in the clinic. The presence of aggregates in biologic drug products can induce adverse immune responses in patients that may affect drug safety and efficacy, cause infusion reactions, cytokine release syndrome, anaphylaxis, or even death [19, 20]. Hence, just like ADAs and ICs discussed above, aggregates are of concern to manufacturers, clinicians, patients, and regulatory agencies. Aggregation of biologics is a challenging phenomenon to mitigate due to knowledge gaps of the molecular mechanisms underlying aggregation as well as a lack of standard and reliable aggregation prediction tools.

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<sup>10</sup>Many diseases are characterized by protein aggregation *in vivo*, including Alzheimer's disease, prion disorders, amyotrophic lateral sclerosis (ALS), Huntington's disease and Parkinson's disease.

However, in recent years, regulators and drug industry experts have spearheaded development of novel techniques to detect and characterize aggregates, increase research into the role of protein aggregates of all sizes in immunogenicity, aid in revising pharmacopoeia monographs to improve subvisible particle testing, and clarify terminology like “practically” or “essentially free of particles.”

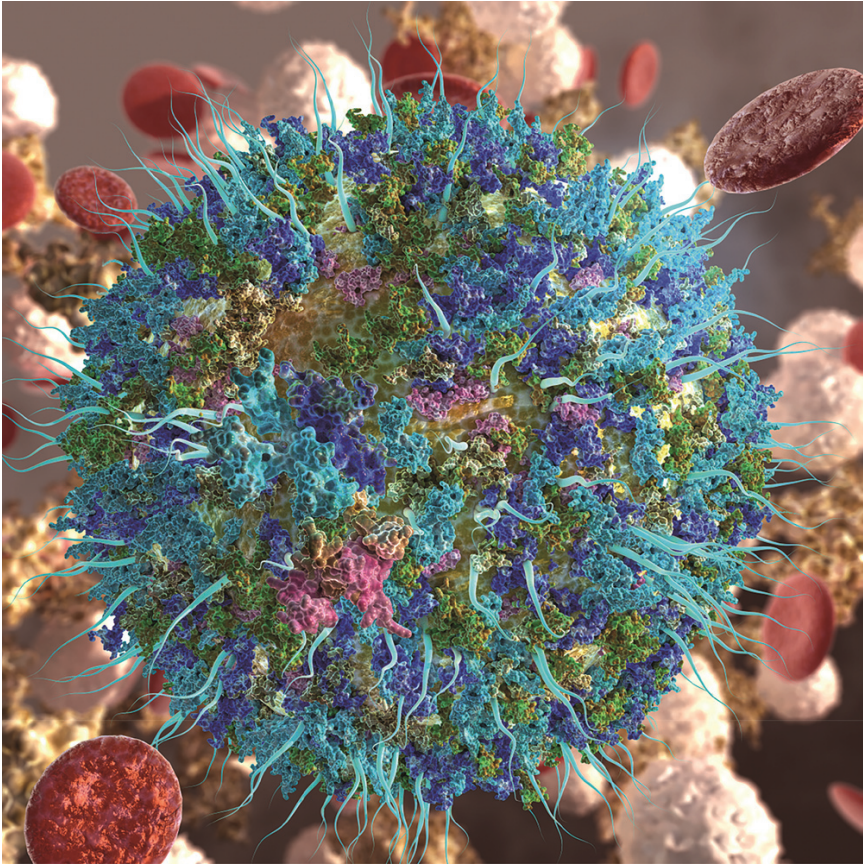
### 1.4.2 Immune Aspects of Nanodrugs

The clinical application of nanodrugs and nanocarriers is dogged by safety and toxicity concerns, especially about their long-term use. As discussed earlier (Fig. 1.5), immunogenicity of nanodrugs may result from a unique combination of physicochemical properties, such as shape, size, surface charge, porosity, reactivity, and composition. Many nanodrugs are engineered to break tissue physiological barriers for entry and to escape immune surveillance, thereby persisting in body fluids and delivering their active pharmaceutical ingredients (APIs). However, this persistence in the body may trigger immune responses.

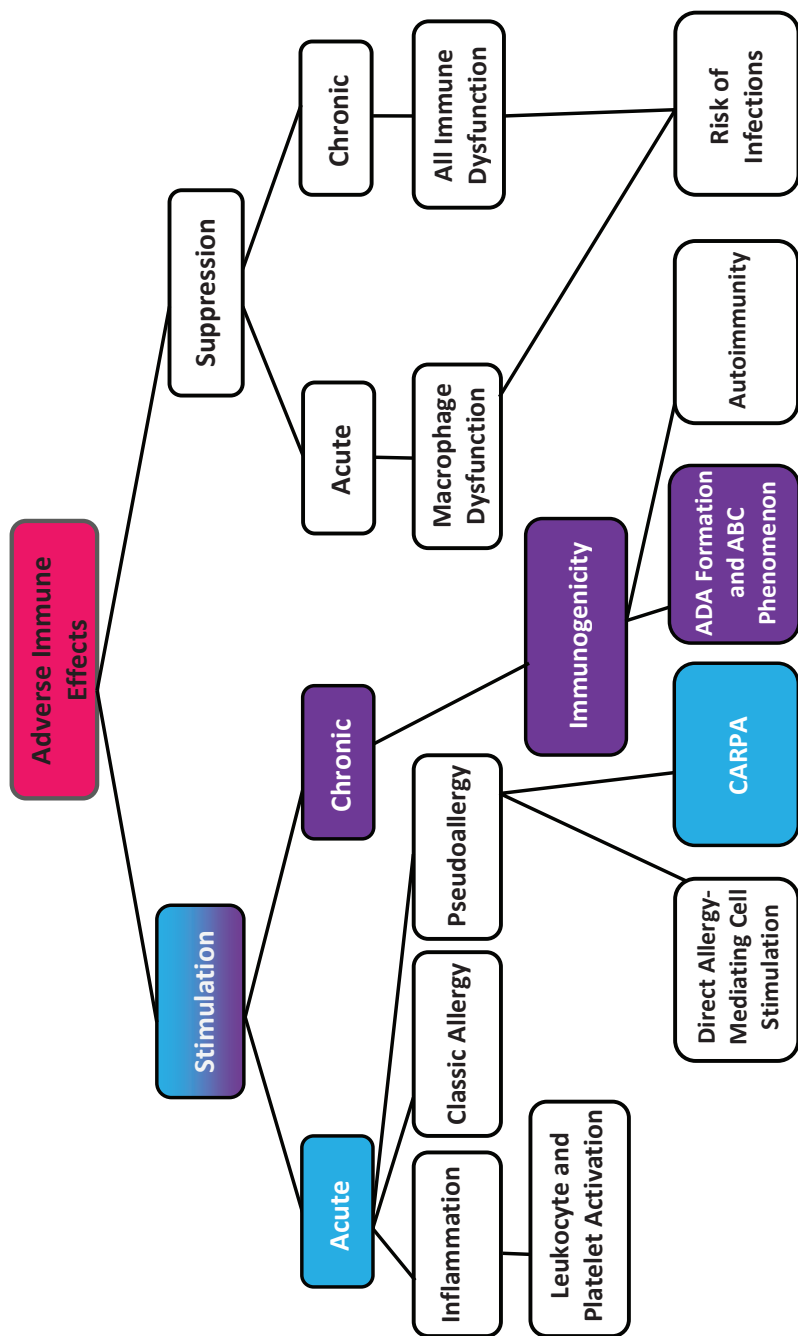
**(a)** A well-studied but poorly understood immune issue with nanodrugs is the formation of the so-called “protein corona” (Fig. 1.6) at the interface between nanodrugs and blood (bio-nano interface). Protein corona refers to the adsorption of proteins onto the nanodrug surface, thereby reducing their stability and facilitating their rapid *in vivo* clearance. Obviously, this phenomenon has important implications on immune safety, biocompatibility, and the use of nanodrugs in medicine [21, 22]. This formation of protein corona may be one factor that has contributed to the inefficient accumulation of nanodrugs (<10% accumulation [23]) in diseased tissues despite the oft-highlighted advantages of “targeted” nanodrug delivery. However, this area of research has suffered from a mechanistic understanding of the bio-nano interface.

**(b)** Since the surface area-to-volume ratio is very high at the nanoscale [12], the surface properties of nanodrugs dictate their interactions with the bioenvironment. This enormous surface area of nanoparticles can in turn cause increased biological activity, including immunogenicity. Adverse effects can lead to

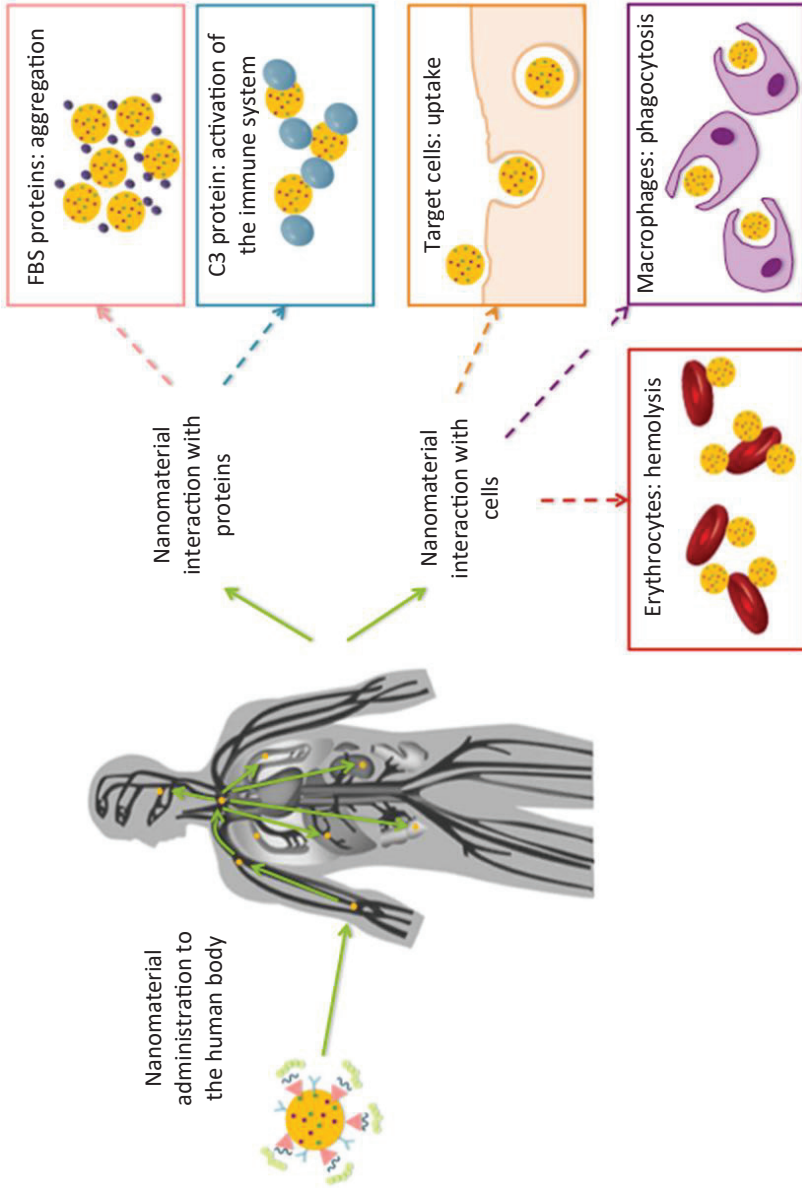
either suppressed or stimulated immune functions and they can involve various blood and immune cells (Fig. 1.7). Evaluation of the interaction of nanodrugs with blood components (Fig. 1.8) is, therefore, critical as most administered nanodrugs will end up being distributed by the bloodstream [24]. Hence, experimental techniques for the analysis of nanodrug interaction with biological components are critical [24].



**Figure 1.6 Protein Corona.** A nanoparticle gains a new biological identity upon its dynamic interactions with biological fluids, giving rise to the protein corona (shown as adsorbed green, blue, and cyan globules), which consequently influences drug delivery and targeting of the functionalized nanoparticle (illustrated as aqua blue fibrils). Reproduced with permission from [22]. Copyright 2017 American Chemical Society.



**Figure 1.7** Adverse immune effects of nanodrugs, classified according to their impact and time course. Abbreviations: ABC, accelerated blood clearance; ADA, antidrug antibody; CARPA, complement activation-related pseudoallergy.



**Figure 1.8 Schematic representation of possible interactions of some nanosystems with biological components, namely cells and proteins.** Courtesy of Dr. Cristina Fornaguera, Sagetis-Biotech, Barcelona, Spain.

(c) Often, intravenously administered therapeutics (certain nanodrugs, biologics, NBCDs, etc.) prime the immune system, leading to adverse reactions and/or the loss of efficacy of the drug product. It is now well established that these therapeutics may provoke “hypersensitivity reactions” (HSRs), also known as “infusion” or “anaphylactoid” reactions. Due to the association of complement activation with many of these adverse reactions, the term “complement activation-related pseudoallergy” (CARPA) was coined in the late 1990s [25–27] (Tables 1.3 and 1.4). CARPA was based on pig studies involving intravenously administered liposomes; the model is now known as the “porcine CARPA model” (Fig. 1.9).

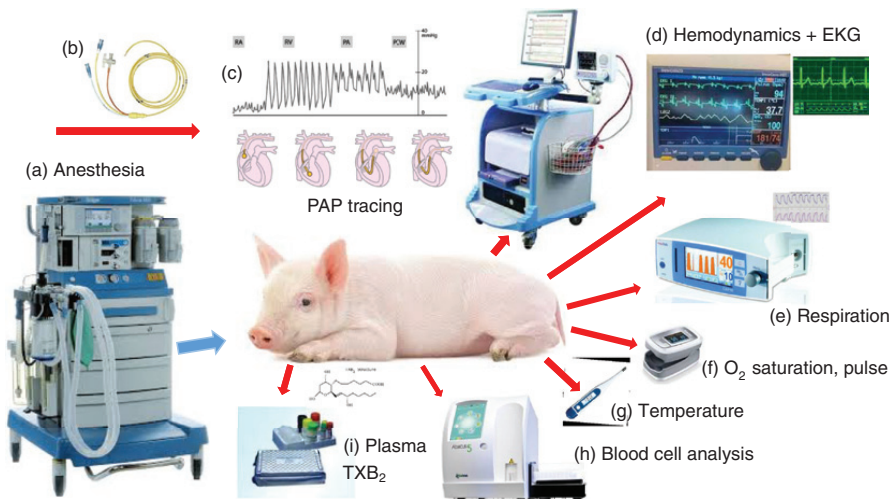
These hypersensitivity reactions typically occur directly at first exposure to the drug without prior sensitization, and the symptoms usually lessen and/or disappear upon later treatment. The rapidly arising symptoms, namely, shortness of breath, facial redness and swelling, chest pain, back pain, flashing, rash, chills, panic, and fever are also typical of acute or Type 1 hypersensitivity. However, a role of IgE has not been implicated in most of these reactions. Therefore, these HSRs are labeled as “pseudoallergic” or “nonspecific hypersensitivity.” Nanodrugs causing CARPA (Table 1.3) include radio-contrast media, liposomal drugs (Doxil<sup>®</sup>, Ambisome<sup>®</sup>, DaunoXome<sup>®</sup>, Abelcet<sup>®</sup>, Visudyne<sup>®</sup>), nanoparticulate iron, micellar solvents (Cremophor EL, the vehicle of Taxol<sup>®</sup>), PEGylated proteins, and monoclonal antibodies (mABs).<sup>11</sup> Drug products other than nanodrugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, and morphine can also trigger CARPA (Table 1.3). Now, CARPA is a well-established cornerstone of pharmacotherapy and liposomal chemotherapy. The clinical relevance of CARPA is highlighted by notes in industry guidances issued by the FDA and the EMA. The FDA recommends detection of complement activation by-products in animals showing signs of anaphylaxis [28a], while the EMA refers to CARPA tests as

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<sup>11</sup>Monoclonal antibodies (mABs) (Fig. 1.2) are the largest group of biologics. They include in their names the type of target (immune system, renal system, cancer, cardiovascular system, bone) and their origin (chimeric, humanized, human). Classification for different biologics includes the prefix of the name (generally provided by the pharmaceutical company), and the suffix defines the type of biologic, namely, a monoclonal antibody (mab), a soluble receptor (cept), or a kinase inhibitor (inib).



a potentially useful preclinical safety test for liposomal drug R&D [28b]. The World Health Organization (WHO) also emphasizes evaluating complement binding and activation for biologics [29]: *“Unless otherwise justified, the ability for complement-binding and activation, and/or other effector functions, should be evaluated even if the intended biological activity does not require such functions.”* The FDA has approved a few drugs for inhibiting various complement proteins, while many others are in preclinical and clinical stages of drug development (Table 1.4).



**Figure 1.9 Instruments and parameters measured in the porcine CARPA model.** (a) anesthesia machine; (b) Swan-Ganz catheter; (c) blood pressure wave forms directing the passage of the tip of the Swan-Ganz catheter via the right atrium (RA), right ventricle (RV) and pulmonary artery (PA) until being wedged into the pulmonary capillary bed; (d) computerized multiple parameter hemodynamic monitoring system tracing the systemic and pulmonary pressures, heart rate, and the EKG; (e) capnograph connected to the tracheal tube to measure respiratory rate (RR), etCO<sub>2</sub> and inCO<sub>2</sub>; (f) pulse oximeter (fixed on the tail) measures O<sub>2</sub> saturation in blood and pulse rate; (g) temperature is measured with a thermometer placed in the rectum; (h) veterinary hematology analyzer measuring all blood cell counts and WBC differential; (i) enzyme-linked immunosorbent assay (ELISA) for measuring biomarkers of allergic/inflammatory reactions, e.g., TXB<sub>2</sub>, histamine, leukotrienes, adenosine, tryptase, PAF and C3 levels, etc. Courtesy of Dr. János Szebeni, Semmelweis University School of Medicine, Hungary.

Table 1.3 Drugs linked to CARPA.

Liposomal Drugs	Micelle-Solubilized Drugs	Antibodies	Pegylated Proteins	Contrast Media	Enzymes/Proteins/Peptides	Miscellaneous
Abelcet	Cyclosporine	Avastin	Adagen	Diatrizoate	Abbokinase	ACE inhibitors
AmBisome	Elitec	Campath	Neulasta	Iodipamide	ACH	AR blockers
Amphotec/ Amphocyl	Etoposide	Erbtux	Oncaspar, Pegaspargas	Iodixanol	Actimmune	Aspirin
DaunoXome	Fasturec	Herceptin		Iohexol	Activase	Candidas
Doxil, Caelyx	Taxol	Infliximab		Iopamidol	Aldurazyme	Copaxone
Myocet	Taxotere	Muronomab		Iopromide	Avonex	Corticosteroids
Visudyne	Vumon	Mylotarg		Iothalamate	Fasturtec	Cyclofloxacin
		Remicade		Ioversol	Neulasta	Eloxatin
		Rituxan		Ioxaglate	Neupogen	Intralipid
		Vectibix		Ioxilan	Plenaxis	Opiates
		Xolair		Magnevist	Protamine	Orencia
				Metrizamide	Urokinase	Salicylates
				SonoVue	Zevalin	Vancomycin



**Table 1.4 FDA-approved complement inhibitors and new drugs under development**

Company/ Reference	Drug/Class	Target	Structure/ Derivation	Route	Biochemical Data	Stage
Alexion	Eculizumab	C5	Humanized monoclonal antibody	Intravenous	$K_d = 120$ pM	FDA approved for PNH and aHUS
ViroPharma, CSC Behring	C1 esterase inhibitor: plasma derived	C1 esterase	Human protein	Intravenous		FDA approved for hereditary angioedema
Pharming-Salix	C1 esterase inhibitor: recombinant	C1 esterase	Protein analogue, produced in rabbits	Intravenous		FDA approved for hereditary angioedema
Alexion (Tallgen)	TT30	C3 convertase	Factor H-CR2 fusion	Intravenous/ Subcutaneous	$IC_{50} = 0.5$ $\mu$ m	Human studies
Norvartis	LFG 316	C5	mAb	Intravenous/ Intravitreal		Human studies
Amyndas, Apellis, Potentia	Compstatin analogues	C3/C3b	Cyclic peptide/ Phage display	Intravitreal, Subcutaneous, Inhaled	$IC_{50} = 62$ nM, $K_d$ for C3b = 2.3 nM	Human studies

Company/Reference	Drug/Class	Target	Structure/ Derivation	Route	Biochemical Data	Stage
Volution Akari	Coversin	C5	Peptide/tick saliva	Subcutaneous	Maximal inhibition at 10 µg/mL	Human studies
Achillion	Small molecule	Complement factor D	X-ray crystallography	Oral	$K_d < 1$ nm, $IC_{50} = 17$ nm (protease inhibition)	Preclinical studies
Amyndas	Mini factor H	C3 convertase	Derived from factor H		$IC_{50} = 0.22$ µM (for C3 deposition)	Preclinical studies
Alnylam	ALN-CC5	C5 RNA	RNAi conjugate	Subcutaneous		Preclinical studies
Lindofer et al.	3E7/H17	C3b	mAb		100% blockage of lysis at 1 µM	Preclinical studies
Ra Pharmaceuticals	Several	C5	Cyclic peptide	Subcutaneous	$K_d = 2.6$ nM, $IC_{50} = 8.1$ nm (% RBC lysis)	Preclinical studies

Source: Reprinted with permission from [20]. Copyright (2018) Elsevier.

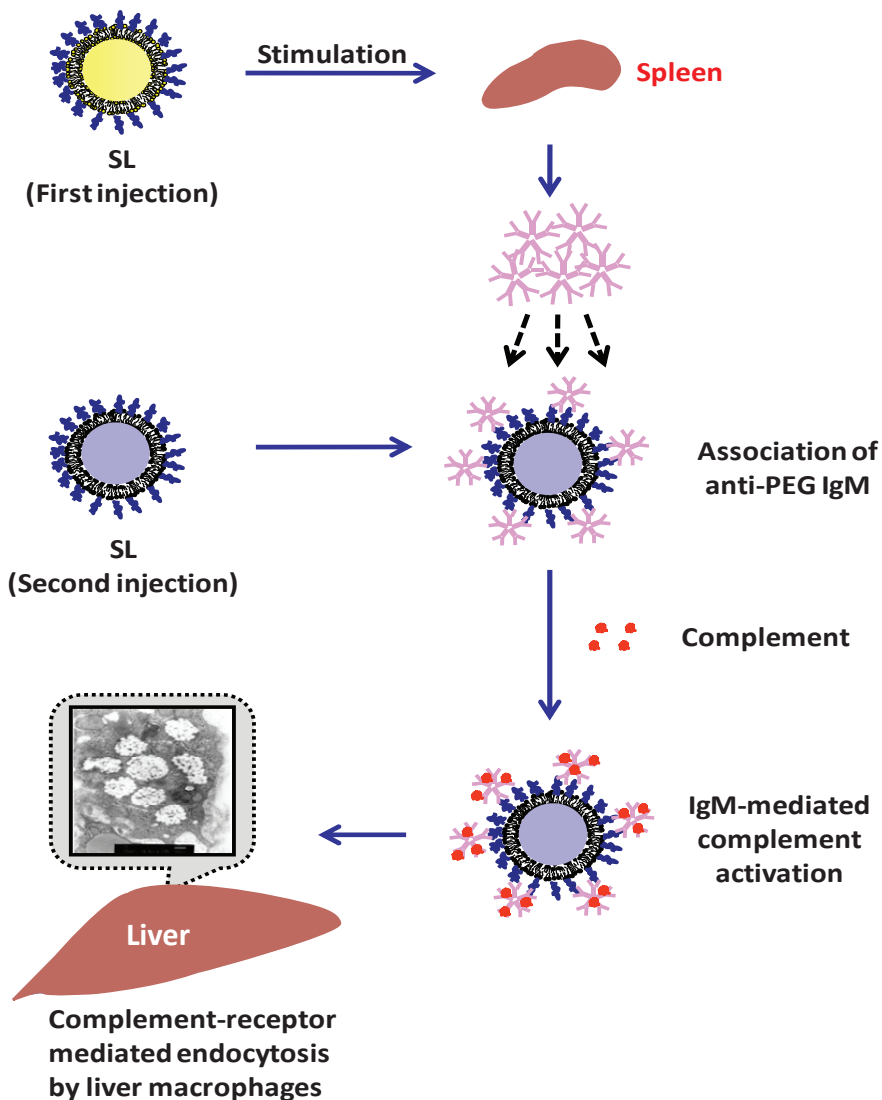
Abbreviations: aHUS, atypical hemolytic uremic syndrome;  $IC_{50}$ , half maximal inhibitory concentration;  $K_d$ , dissociation constant; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell.

**(d)** Another immunologic issue specific to PEGylated liposomes is referred to as the accelerated blood clearance (ABC) phenomenon [30–31] (Fig. 1.10). Liposomes are the most widely used nanodrugs and PEGylation is a common strategy involved in designing stealth liposomes to shield them from reticuloendothelial system (RES) uptake. However, a repeated-dose injection of PEGylated liposomes affects their clearance rate and bioavailability. The delivery of the first dose of PEGylated liposomes (“priming the system”) accelerates subsequent dose elimination as compared to the initial dose, mainly mediated through specific anti-PEG IgM. This finding is clinically significant as well as concerning if PEGylated liposome therapy is involved because it decreases the therapeutic efficacy upon repeated administration. Therefore, repeated-dosage PK studies are critical to prevent immunogenicity of PEGylated liposome drug products without hampering their efficacy or safety. Table 1.5 lists some PEGylated nanodrugs that have adverse immune effects.

## 1.5 Immunogenicity Assessment of Biologics and Nanodrugs

There is a crucial need to evaluate, assay, and devise strategies to overcome adverse immunogenicity aspects of both biologics and nanodrugs. Not all biologics and nanodrugs are created equal. Given this scientific fact, the risks for immunogenicity should be assessed on a case-by-case basis. Contrary to widely held belief, few ADAs elicit any clinically relevant issues. In fact, while some biologics, particularly glycoproteins, cause the body to produce ADAs, the safety and efficacy of most is unaffected during clinical use. Similarly, the diversity of nanodrugs makes it impossible to extrapolate or generalize the immunologic findings from one class of nanodrugs to another. Nevertheless, the degree of risk for eliciting immune responses from biologics and nanodrugs is considered a major issue during drug R&D and use in patients. Any biologic or nanodrug can *potentially* exert an immunogenic effect depending on a patient’s immunologic status, prior history, route/dose/frequency of delivery and unique characteristics of the administered therapeutic product (Fig. 1.5).

Therefore, regulatory agencies, particularly the FDA and the EMA, recommend that drug developers employ a risk-based approach for immunogenicity evaluation and reduction of adverse immune events related to the administration of these therapeutics.



**Figure 1.10 Mechanism of the ABC phenomenon.** Courtesy of Dr. Tatsuhiro Ishida, Tokushima University, Japan.

Table 1.5 PEGylated nanodrugs with documented adverse immune effects

Generic Name	Trade Name	Api or Vehicle	Company
PEGylated liposomal doxorubicin	Doxil <sup>®</sup> /Caelyx <sup>®</sup>	Liposome	ALZA/Janssen
Pegaspargase	Oncaspar <sup>®</sup>	Enzyme: asparaginase	Enzon
Pegfilgrastim	Neulasta <sup>®</sup>	Protein (GCSF)	Amgen
Pegaptanib	Macugen <sup>®</sup>	Aptamer (anti-VEGF)	Eye Tech/Pfizer
Mono-mPEG-epoetin-β	Mircera <sup>®</sup>	Protein (EPO)	Hoffmann-LaRoche
Certolizumab pegol	Cimzia <sup>®</sup>	Fab of anti-TNF mAb	UCB, Inc., Smyrna
<b>Withdrawn from the market</b>			
Pegvisomant	Somavert <sup>®</sup>	Peptid (somatotropin antagonist)	Pfizer
Pegloticase	Krystexxa <sup>®</sup>	Enzyme: Urate oxidase	Horizon Pharma
Peginesatide	Omontys <sup>®</sup> /Hematide <sup>®</sup>	Peptide (EPO-mimetic)	Affymax/Takeda
Pegnivacogin+Anivamersen	Revolixys kit	F-Ixα blocker RNA aptamer + reverse agent	Regado/Tobira

These must be carefully evaluated at the earliest stages of drug formulation/development as well as throughout the product lifecycle, including phase IV. Biologic drug products containing a nonbiologic component or nanomaterial component are on the rise and may have different immunogenic properties compared with those that contain the biologic alone. Consequently, it is also important that immunogenicity aspects and risks of these biologics be assessed with a focus on whether the nonbiologic component or nanomaterial component possesses adjuvant properties. Also, immunogenic potential of drug carriers and other adjuvants cannot be overlooked either as these drug components may exhibit inherent immunologic activity unrelated to the loaded API.

Immunogenicity could be measured by experimental approaches or predicted via mathematical models and *in vitro/in vivo/in silico* assays. Therefore, few tools have been developed to access potential immunogenicity of biologics and nanodrugs (Table 1.6). The key methods for preclinical measurement of immunogenicity use *in silico*, *in vitro*, and *in vivo* models to predict CD4+ T cell responses as well as conventional mouse models, immune-tolerant transgenic mice, HLA-immune-tolerant transgenic mice, and nonhuman primate models (Table 1.6).

The immunogenicity for biologics has been primarily assessed by monitoring the presence and amount (titer) of ADA responses and *in vitro* neutralizing ability of ADA following biologic administration. Such assessment strategies are often driven by indication-specific, product-specific or risk assessment/performance-based goals.

On the other hand, the immunogenicity assessment of nanodrugs is less well developed. There are very few detailed regulatory guidance documents specifically dedicated to evaluating immunogenicity. In fact, immunogenicity or immunotoxicity assessment of nanodrugs is often performed based on existing guidelines for conventional therapeutic drug products. However, due to various unique properties of nanodrugs as compared to conventional therapeutic drug products, the currently prescribed set of tests or assays may provide insufficient information for an adequate evaluation of potential immunogenicity or immunotoxicity of nanodrugs.

**Table 1.6 Standard industry immunogenicity prediction tools and models**

<i>In silico</i>	<i>In vitro</i>	<i>In vivo</i>
iTope™	EpiScreen™— <i>Ex vivo</i> assessment of immunogenicity	conventional mouse models
TCED™		
Epibase®	➤ EpiScreen™ time course T cell assay	immune-tolerant transgenic mice
EpiMatrix™	➤ EpiScreen™ DC:T cell assay	HLA-immune-tolerant transgenic mice
	➤ EpiScreen™ T Cell Epitope Mapping	nonhuman primate models
	➤ EpiScreen™ MAPPS—MHC Class II—Associated Peptide Proteomics	
	Epibase®	
	REVEAL®	

*Abbreviations:* DCs, dendritic cells; MHC, Major Histocompatibility Complex; MAPPS, MHC Class II Associated Peptide Proteomics; TCED™, T Cell Epitope Database; HLA, human leukocyte antigen.

*Note:* Although these tests are widely used for biologic immunogenicity prediction, they could pertain to both biologics and nanodrugs because of considerable overlap in their definitions (Sections 1.2 and 1.3). Copyright 2018 Raj Bawa. All rights reserved.

Although the complex field of immunogenicity assessment is still evolving, numerous hurdles persist. One major issue is the so-called “immunogenicity testing dilemma” for biologics and nanodrugs due to the recognized fact that the phylogenetic distance between laboratory animals and humans limits the predictive value for testing. For example, immune responses to biologics in conventional animal models has been rarely predictive of the response in humans. This fact is critical when evaluating human immunogenicity due to pronounced species-specific differences in antigen recognition, in immune reactivity of nonlymphoid/lymphoid cells, and in the systemic immunity at the organ level. Efforts to overcome this immunogenicity testing dilemma have not been particularly successful. For example, employing a broad spectrum of 2D *in vitro* assays in conventional culture plates based on suspension or matrix-assisted human immune cell cultures for evaluation of immunogenicity prior to

human testing is fraught with problems and still not an industry standard. Similarly, overprediction of immunogenicity risk via *in silico* methods may occur as these models depend heavily on how well computational algorithms have been created in the first place. However, some tests are slowly gaining ground and may become standard in due course. For instance, the fact that CARPA (Section 1.4.2(c)) is a major immunologic issue with intravenous nanodrug formulations, has recently prompted the FDA to list testing for complement activation *in vitro* and/or *in vivo* as one of the immunotoxicology tests [28a].

## 1.6 Entering the Era of Biosimilars

### 1.6.1 What Are Biosimilars?<sup>12</sup>

A biosimilar (Fig. 1.11) is a biological product that is “highly similar” to and has no clinically meaningful differences from an existing FDA-approved reference product. A “reference product” is the single biological product, already approved by the FDA, against which a proposed biosimilar product is compared (Fig. 1.12). A reference product is approved based on, among other things, a full complement of safety and effectiveness data. A proposed biosimilar product is compared to and evaluated against a reference product to ensure that the product is highly similar and has no clinically meaningful differences.

Biosimilars and generic drugs are versions of brand name drugs and may offer more affordable treatment options to patients. Biosimilars and generics are each approved through different abbreviated pathways that avoid duplicating costly clinical trials. But biosimilars are not generics, and there are crucial differences between biosimilars and generic drugs. For example, the active ingredients of generic drugs are the same as those of brand name drugs. In addition, the manufacturer of a generic drug must show that the generic is *bioequivalent* to the brand name drug. By contrast, biosimilar manufacturers must demonstrate that the biosimilar is *highly similar* to the reference product (Fig. 1.12), except for minor differences in clinically inactive components.

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<sup>12</sup>This US perspective on biosimilars was kindly provided by the FDA. The figures in this section have been modified by the author.



**European Medicines Agency** - A biosimilar is a biological medicine that is developed to be similar to an existing biological medicine (the ‘reference medicine’). When approved, a biosimilar’s variability and any differences between it and its reference medicine will have been shown not to affect safety or effectiveness.



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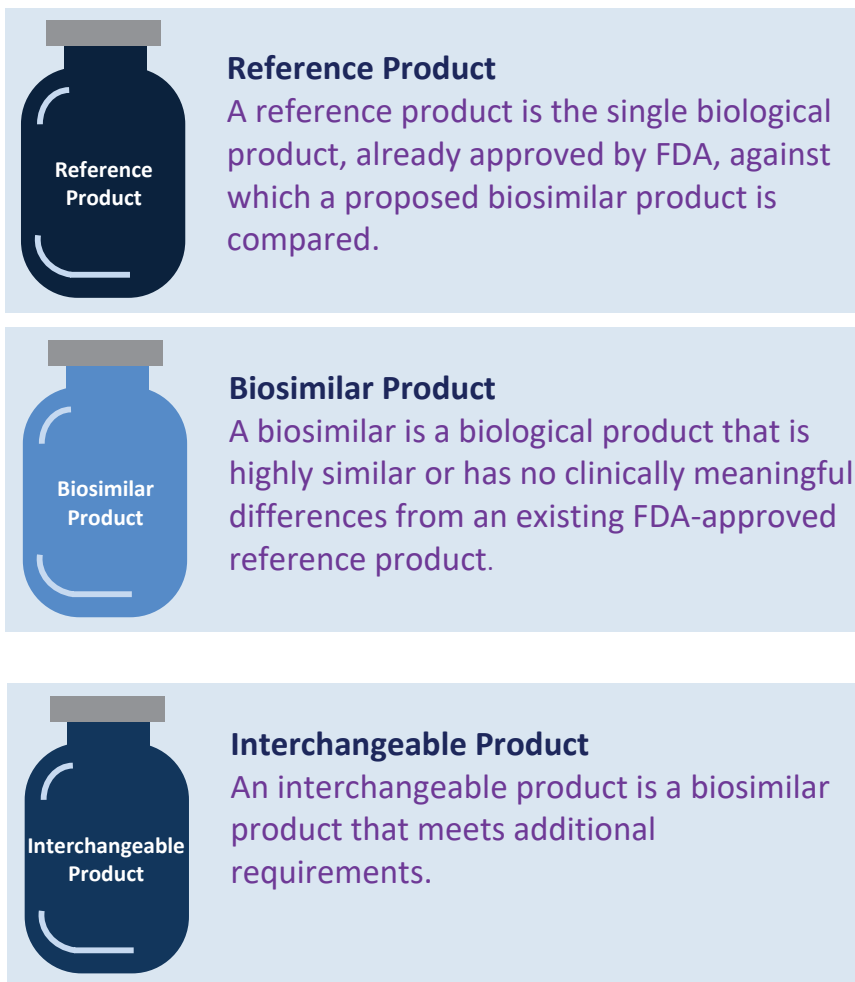
**United States Food and Drug Administration** - A biosimilar is a biological product that is highly similar to a US licensed reference biological product notwithstanding minor differences in clinically inactive components, and for which there are no clinically meaningful differences between the biological product and the reference product in terms of safety, purity and potency of the product.



**World Health Organization** - A biosimilar is a biotherapeutic product which is similar in terms of quality, safety and efficacy to an already licensed reference biotherapeutic product.



Figure 1.11 Official global definitions for biosimilars.



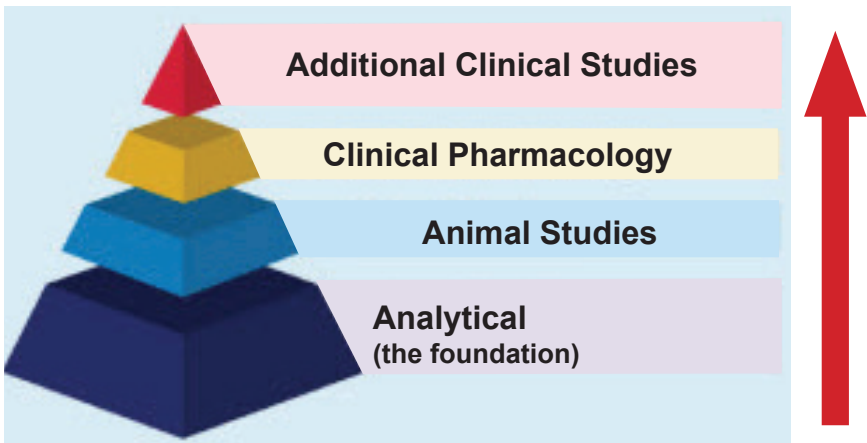
**Figure 1.12** FDA Terminology regarding biosimilars.

Biosimilar manufacturers must also prove that there are no clinically meaningful differences between the biosimilar and the reference product in terms of safety and effectiveness.

A manufacturer developing a proposed biosimilar demonstrates that its product is highly similar to the reference product by extensively analyzing (i.e., characterizing) the structure and function of both the reference product and the proposed biosimilar. State-of-the-art technology is used to compare characteristics of the

products, such as purity, chemical identity, and bioactivity. The manufacturer uses results from these comparative tests, along with other information, to demonstrate that the biosimilar is highly similar to the reference product.

Minor differences between the reference product and the proposed biosimilar product in clinically inactive components are acceptable. For example, these could include minor differences in the stabilizer or buffer compared to what is used in the reference product. As mentioned above, slight differences (i.e., acceptable within-product variations) are expected during the manufacturing process for biological products, regardless of whether the product is a biosimilar or a reference product. For both reference products and biosimilars, lot-to-lot differences (i.e., acceptable within-product differences) are carefully controlled and monitored. A manufacturer must also demonstrate that its proposed biosimilar product has no clinically meaningful differences from the reference product in terms of safety, purity, and potency (safety and effectiveness). This is generally demonstrated through human pharmacokinetic (exposure) and pharmacodynamic (response) studies, an assessment of clinical immunogenicity, and, if needed, additional clinical studies (Fig. 1.13).



**Figure 1.13** The FDA's review for licensure of a biosimilar product.

When considering licensure of a biosimilar product, the FDA reviews the totality of the data and information, including

the foundation of detailed analytical (structural and functional) characterization, animal studies if necessary, then moving on to clinical pharmacology studies and, as needed, other comparative clinical studies (Fig. 1.13).

An “interchangeable product” (Fig. 1.12) is a biosimilar product that meets additional requirements outlined by the BPCI Act (Section 1.1). As part of fulfilling these additional requirements, information is needed to show that an interchangeable product is expected to produce the same clinical result as the reference product in a patient. A manufacturer of a proposed interchangeable product will need to provide additional information to show that an interchangeable product is expected to produce the same clinical result as the reference product in any given patient. Also, for a product that is administered to a patient more than once, a manufacturer will need to provide data and information to evaluate the risk, in terms of safety (including immunogenicity) and decreased efficacy, of alternating or switching between the products. As a result, a product approved as an interchangeable product means that the FDA has concluded it may be substituted for the reference product without consulting the prescriber. For example, say a patient self-administers a biological product by injection to treat their RA. To receive the biosimilar instead of the reference product, the patient may need a prescription from a health care prescriber written specifically for that biosimilar. However, once the FDA approves a product as interchangeable, that patient may be able to take a prescription for the reference product to the pharmacy and, depending on the state, the pharmacist could substitute the interchangeable product for the reference product without consulting the prescriber. Note that pharmacy laws and practices vary from state to state.

## **1.6.2 FDA Challenges Regarding Biosimilar Approval**

According to a 2018 speech<sup>13</sup> by the FDA commissioner, about a third of new drugs approved by the FDA are now biologics while they account for about 40% of all US drug spending, and 70% of spending growth from 2010–2015. Developing a generic

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<sup>13</sup>Gottlieb, S. (2018). Capturing the benefits of competition for patients. Available at: <https://www.fda.gov/NewsEvents/Speeches/ucm599833.htm> (accessed on July 14, 2018).

version of a small-molecule drug can cost ~\$10 million. Due to the complexity of manufacturing and testing biosimilars, more significant outlays by sponsors are required: typically, \$100–\$250 million per program.

Since 2007, 31 biosimilar products have been approved by the EMA while 5 have been refused or withdrawn. On the other hand, the FDA has struggled with biosimilar approval. Since the passage of the BPCI Act, as of May 2018, the FDA has licensed only nine biosimilar products. The FDA has been justifiably criticized for the slow entrance of biosimilars into the US market. It is obvious to me that the steep cost (~\$150+ million) and lengthy development (~7–9 years) of biosimilars are untenable and need urgent addressing, possibly via appropriate regulatory adjustments. Table 1.7 lists suggested modifications to the FDA's current biosimilar guidelines.

**Table 1.7 Recommendations to the FDA for faster development and licensing of biosimilar products<sup>14</sup>**

- The FDA should remove the current default requirements of conducting bridging studies between a US-licensed product and a non-US approved comparator to establish biosimilarity.
- The FDA should present clear and open scientific views to the public, more particularly, to the prescribers that a biosimilar product has “no clinically meaningful difference” from the originator product and thus suitable for naïve patients.
- The FDA should encourage the development of *in vitro* immunogenicity testing methods to reduce exposure of test subjects on ethical grounds.
- The FDA should revise some of the specific statistical testing methodologies in establishing analytical similarity to remove certain contradictions in the guidance.
- The FDA should take a fresh look at the clinical relevance of the protocols and statistical methods used to establish PK/PD similarity, and to make these studies more clinically relevant while reducing their cost.

<sup>14</sup>Based on the Citizen Petition (CP) of Dr. S. K. Niazi of the University of Illinois College of Pharmacy to the FDA (dated May 11, 2018; docket number FDA-2018-P-1876) that focuses on reducing human testing to establish bioequivalence. It was accepted by the FDA and as of June 2018 was under the comment period. In the past, I have filed CPs on behalf of Teva pertaining to Copaxone®.

## 1.7 Immune Aspects of Biosimilars and Nanosimilars: The Copaxone® Example

Many veteran drug industry experts, including this author, believe that there are enormous pressures on drug regulatory agencies to approve follow-on versions (i.e., generic equivalents) of both biologics and nanodrugs. Frankly, judging from the rapid pace of biosimilars that were approved in the past year, the Trump administration seems to be pushing for an increase in biosimilar approvals at the FDA. Concurrently, the increase in the number of drug companies targeting generic opportunities and seeking US market exclusivity for generic versions of major branded products is on the rise. There are many factors for this, including governmental drug policy, price pressures, and statutes. However, it is critical that immune aspects of these follow-on versions of branded products be transparently evaluated in a science-based context and reported during all phases of drug R&D (from preclinical to post-marketing): *Lower drug prices, a priority for the Trump Administration,<sup>15</sup> should not supplant patient safety and drug efficacy.*

The following discussion regarding biosimilar therapeutic monoclonal antibodies (TMABs) highlights the fact that such follow-on biologic approval by a regulatory agency must be carefully evaluated on a case-by-case basis for clinical data based on the “totality-of-evidence” [32]:

*“By contrast with generic small-molecule drugs, clinical performance of a biologic pharmaceutical is a function of its structural complexity and higher-order structure (HOS). Biomanufacturing controls of such complex products cannot fully ensure chemical similarity between an innovator product and putative biosimilar because minor differences in chemical modifications and HOS can significantly alter a product’s safety and efficacy. Therefore, to substantiate claims of clinical functionality, a demonstration of bioequivalence is inadequate for biosimilar pharmaceuticals. This is different from regulatory approval for generic drugs, in which bioequivalence demonstration is adequate. The overall challenge in approving biosimilar pharmaceuticals is to enable scientific inference of similarity*

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<sup>15</sup>Sterling, J. (2018). President reveals plan to cut drug prices. *Genet. Eng. Biotechnol. News*, **38**(12), 1, 30.

*in safety and efficacy for a new biologically derived product compared with an innovator without repeating burdensome clinical studies.... So although they are helpful, biological and/or functional assays may not fill a gap in analytical assay sensitivity to detect minor conformational differences between biosimilar TMABs and innovator products. It is important to note that no analytical test or combination for HOS has yet been sufficiently validated for analytical testing as a substitute for clinical studies in the development of a biosimilar TMABs drug substance."*

In this context, the recent FDA approval of multiple generic versions of Copaxone<sup>®</sup> is an example that merits discussion as it highlights this problematic issue [33]. Copaxone<sup>®</sup> is a nonbiologic complex drug (NBCD) [34] but can also be considered a nanodrug (Section 1.3). However, it also shares features with biologics and given the loose definition of biologics (Section 1.2), it can be classified as a biologic as well. In this chapter, it will be considered a NBCD, a nanodrug, and a biologic. Owing to the complexity of NBCDs and nanodrugs, showing equivalence is more challenging for their follow-on versions. Therefore, the interchangeability or substitutability of nanosimilars and their listed reference product(s) cannot be taken for granted. In the past, nanosimilars have been approved via generic pathways but differences in clinical efficacy and safety have been reported in the scientific literature following approval [35].

#### ***What Is a Nonbiologic Complex Drug (NBCD)?***

"A medicinal product, not being a biological medicine, where the active substance is not a homomolecular structure, but consists of different (closely) related and often nanoparticulate structures that cannot be isolated and fully quantitated, characterized, and/or described by physicochemical analytical means. It is also unknown which structural elements might affect the therapeutic performance. The composition, quality, and *in vivo* performance of NBCDs are highly dependent on the manufacturing processes of both the active ingredient and the formulation. Examples of NBCDs include liposomes, iron-carbohydrate (iron-sugar) drugs, and glatiramoids."

Source: [35]

Copaxone® is composed of an uncharacterized mixture of immunogenic polypeptides in a colloidal solution. The active ingredient in Copaxone®—glatiramer acetate—is a heterogeneous synthetic mixture of polypeptides comprising four amino acids found in myelin basic protein (L-glutamic acid, L-alanine, L-lysine, and L-tyrosine) in a defined molar ratio. Glatiramer acetate has immunomodulatory effects on innate and acquired immunity and is indicated for the treatment of patients with relapsing forms of multiple sclerosis (MS). Copaxone® is not a single molecular entity but a heterogenous mixture of potentially millions of distinct, synthetic polypeptides of varying lengths, some containing up to 200 amino acids with structural complexity comparable to that of proteins, or even more complex than proteins. It is presently impossible to isolate and identify its pure components even via the most technologically sophisticated multidimensional separation techniques. The complexity of glatiramer acetate is amplified by several aspects: (1) The active moieties in glatiramer acetate are unknown; (2) the mechanisms of action are not completely elucidated; (3) pharmacokinetic testing is not indicative of glatiramer acetate bioavailability; (4) pharmacodynamic testing is not indicative of therapeutic activity and there are no biomarkers available as surrogate measures of efficacy; and (5) small changes in the glatiramer acetate mixture can change its immunogenicity profile. There is one aspect of Copaxone® that raises special safety and effectiveness concerns that merit heightened vigilance with respect to the approval of any potentially interchangeable follow-on glatiramer acetate product: *Glatiramer acetate is an immunomodulator* [33]. In other words, Copaxone® is intended to achieve its therapeutic effects by interacting with and modulating a patient's immune system over an extended period. For this reason, Copaxone®'s package insert warns that chronic use has the potential to alter healthy immune function as well as induce pathogenic immune mechanisms, although no such effects have been observed with Copaxone®.

For small-molecule drugs, regulatory approval of generic versions is based on factors like molecular identity of the active ingredient, identity in strength, purity and quality, and bioequivalence. In other words, a demonstration of bioequivalence can result in regulatory approval of a small-molecule generic



without having to conduct the full set of clinical trials that prove clinical safety and efficacy. However, this strategy cannot be followed for biologics like Copaxone<sup>®</sup>. Even if a biosimilar were to have the exact same primary amino acid sequence as the innovator, the innovator's manufacturing process is usually proprietary and not in the public domain. *Hence, biosimilars are, by definition, manufactured using different processes than the innovator (Section 1.6). Obviously, these differences in manufacturing process, no matter how subtle, can generate unique heterogeneities within a potential biosimilar product as compared to the branded product. This can have different pharmacologic effects or adverse immune effects on the patient. Therefore, biosimilars necessitate careful consideration for safety and efficacy.* With this backdrop, it is clear that due to the complexity and inextricable link between the manufacturing process and quality, any Copaxone<sup>®</sup> biosimilar almost certainly will differ from Copaxone<sup>®</sup>'s structure and composition of active ingredients because it will be made using a different manufacturing process than that developed by the branded product developer (Teva Pharmaceutical Industries Ltd., Israel) [33]. Although it is not possible to fully characterize and compare these complex mixtures, differences are revealed via sophisticated analytical techniques. In the past few years, purported generic glatiramer acetate follow-on versions have been approved in India, Argentina, and Mexico. More recently, the FDA has also approved substitutable generic glatiramer acetate formulations.<sup>16</sup> A variety of physicochemical tests performed by Teva have been done on these generic products and they have been proven to be similar to Copaxone<sup>®</sup> in some basic features [33]. However, they are different in the bulk composition of constituents when analyzed via methods for analysis of complex closely related molecules [33]. In this regard, a widely used analytical tool for characterization of complex mixtures of biologics in the context of biosimilars is ion mobility mass spectrometry (IMMS). The ion mobility method applies multidimensional separation techniques based on size, shape, charge, and mass of the molecules in the sample mixture and can

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<sup>16</sup>Teva Pharmaceuticals USA, Inc. v. Sandoz, Inc., 574 U.S.\_\_(2015), is a landmark Supreme Court patent law case pertaining to Teva's Copaxone<sup>®</sup> patent. Available at: [https://www.supremecourt.gov/opinions/14pdf/13-854\\_o7jp.pdf](https://www.supremecourt.gov/opinions/14pdf/13-854_o7jp.pdf) (accessed on June 21, 2018).

separate isomeric peptides that chromatographic techniques cannot. The analysis produces a three-dimensional “heat map” to highlight intensity differences of peptides at various mass/charge ratios and drift times. The difference between the intensities of heat maps for the generics tested by Teva as compared to Copaxone<sup>®</sup> (result of subtraction of generic heat map from that of Copaxone<sup>®</sup>) show highlighted areas indicating different polypeptide populations compared to those of Copaxone<sup>®</sup> lots tested. Clearly, these results indicate a profound difference in size, shape, and charge of the constituent polypeptides in Copaxone<sup>®</sup> as compared to the purported generic products tested by Teva [33].

What does this mean in the context of immune aspects of Copaxone<sup>®</sup>? Because Copaxone<sup>®</sup> is an immunomodulator, a follow-on product characterized by different constituent population could have significant and unpredictable differences from Copaxone<sup>®</sup> in its immunological mechanisms, raising major safety and efficacy concerns. The potential risks associated with such follow-on products include increased immunogenicity, immunotoxicity, induction of additional autoimmune disorders, lack of efficacy, and exacerbation of the MS disease processes. Moreover, because of the nature of both RRMS and Copaxone<sup>®</sup>, these risks may not develop for months or years and, once apparent, may be irreversible. Since the active amino acid sequences in the glatiramer acetate mixture responsible for its efficacy are unknown, it is impossible to predict whether already-approved and future follow-on products will have the same efficacy as Copaxone<sup>®</sup>. They could have a weaker anti-inflammatory effect and/or enhance a pro-inflammatory environment, further exacerbating MS pathogenic processes. A reduced anti-inflammatory effect may provide less effective control of MS relapses, which would be difficult to detect in the post-marketing environment because MS relapses and progression of disability are not completely abolished by any MS therapy. On the other hand, creation or amplification of a pro-inflammatory environment would likely increase relapse rate and progression of disability or worse (e.g., have a profound encephalitogenic effect).

Finally, the potential for the development of cross-reactive neutralizing antibodies must be assessed before any regulatory authority approves any follow-on glatiramer acetate product

intended to be used interchangeably with Copaxone®. Switching between two complex polypeptide products with subtle differences in structure and/or composition may increase the chance of cross-reactivity, a phenomenon that has been observed with interferon beta products. Upon switching from Copaxone® to a follow-on product or using them interchangeably, antibodies formed against Copaxone® may neutralize the activity of the proposed generic product and *vice versa*. If this were the case, patients would be left without any effective treatment. Again, there is no evidence that progression of neurologic disability associated with untreated MS can ever be reversed.

*It is thus critical to ensure that any proposed follow-on product has a long-term immunogenicity profile that is comparable to Copaxone®'s before approval. This can only be done based upon data from appropriate clinical testing.*<sup>17</sup> Surprisingly, despite these immunological concerns, the FDA recently approved so-called generic versions of Copaxone®.

## 1.8 Concluding Remarks and Future Directions

Immune regulation is mediated by a highly complex network of cells and signaling pathways, massive and dynamically interacting gene networks, host–pathogen interactions, and nutrition–microbiota–host interplay. Therefore, dysregulation of immune pathways (i.e., when immunoregulatory mechanisms deviate or fail) is central to many diseases. In fact, immune-mediated diseases are often multifactorial, exhibit enormous patient-to-patient variability, and are often hard to treat via traditional therapies. There continues to be a lack of understanding of the physicochemical determinants underlying immune mechanisms as they relate to biologics and nanodrugs. Despite enormous advances in medicine in the last hundred years, there exist major

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<sup>17</sup>Tyler, R. S. (2013). The goals of FDA regulation and the challenges of meeting them. *Health Matrix*, 22(2), 423–431: “[W]ith respect to drugs, there is no substitute for a well-controlled clinical trial to establish a drug’s safety and effectiveness and conducting such a trial is beyond the competence of individual consumers. Consumers, unprotected by regulations requiring such trials, are unable to judge the safety and effectiveness of a drug...Nevertheless, the regulatory framework is unsettled and there are now, as there have been in the past, demands in Congress and elsewhere to change the laws under which FDA operates.”

gaps in our current understanding of immunological responses and immune mechanisms. We are on a steep learning curve with respect to fully comprehending the extremely complex mechanisms, side reactions, and interactions of various immune cells.

Unwanted immunogenicity of biologics and nanodrugs is a major safety and efficacy concern during drug development and clinical use. Hence, assessment of immunogenicity remains a key element during drug R&D. Unfortunately, extensive testing during drug development does not guarantee that the approved product will be free of immune issues, including immunogenicity, that could adversely affect drug effectiveness and patient safety. There are basic underlying reasons responsible for this unpredictability. For example, the medical and/or scientific concepts related to immunogenicity are incompletely understood. The pressure to develop effective and safe drugs for disease states with unmet medical needs adds another wrinkle to the mix.

Pharmacovigilance is, therefore, critical for biologics and nanodrugs. Due to unpredictability of their immunogenicity profile, managing it is essential not only during the drug R&D phases but all the way to postmarketing surveillance (PMS). In this context, a multidisciplinary approach is called for to better understand and minimize immune issues associated with biologics and nanodrugs. The assessment of unwanted immunogenicity can be improved by using immuno-prediction tools, optimizing immunoassays, and monitoring patients receiving these drug products. In fact, routine immunogenicity and drug level assessment in patients receiving biologics and nanodrugs should become a healthcare standard to better understand their underlying immune mechanisms. Basically, we need to identify various modulating factors that could reduce drug immunogenicity below clinically significant levels. An early indicator of a potentially highly immunogenic drug, before it enters clinical phase testing, will avoid an unnecessary safety risk to patients and save time and resources. Although the etiology of immunogenicity is still not fully understood for these drug products, advances in approaches to mitigate immunogenicity that are currently underway involve rigorous immunogenicity characterization, advances in animal models, and

*in silico*, *in vitro*, and *in vivo* prediction tools. Future biomedical research must expand and standardize analytical methods.

Another broader problem impacting immunogenicity assessment is that there are many defects in the current drug research environment. The “evidence” from clinical studies of drug effects, including immune potential, and why such evidence might fail in the prediction of the clinical utility of drugs is an issue of much concern to me. Although the standards used by the regulatory agencies have evolved and expanded over the past two decades, serious concerns persist with the current approach [36]:

*“Problems in clinical studies are an indication of missed opportunities to successfully define the real-world effectiveness and safety of drugs. Driven largely by commercial interests, many clinical studies generate more noise than meaningful evidence to guide clinical decision making. Greater involvement of nonconflicted bodies is needed in the design and conduct of clinical studies, along with more head-to-head comparisons, representative patient populations, hard clinical outcomes, and appropriate analytical approaches. Documenting, registering, and publishing study protocols at the outset and sharing participant-level data at study completion would help ensure transparency and enhance public trust in the clinical research enterprise. Such an approach is needed to generate evidence that is better suited to the tasks of predicting the clinical utility of drugs and providing the information needed by patients and clinicians. Future efforts should focus on engaging the industry, researchers, regulators, clinicians, patients, and other decision makers in discussions to develop transformative ideas with the aim of tackling the numerous defects in the current research environment. Emerging ideas should be piloted and subjected to scientific scrutiny before they are widely implemented and touted as solutions.”*

Many concerned experts highlight another key issue that affects the entire pharma enterprise. It is referred to as the “institutional corruption of pharmaceuticals” and is due to an interplay of key players with often-serious conflicts of interest: physicians, Congress, and the drug industry. Naturally, this jeopardizes the safety and effectiveness of all drug products, not

only biologics and nanodrugs. One apparent consequence for patients are serious ADRs [37]:

*“Institutional corruption is a normative concept of growing importance that embodies the systemic dependencies and informal practices that distort an institution’s societal mission. An extensive range of studies and lawsuits already documents strategies by which pharmaceutical companies hide, ignore, or misrepresent evidence about new drugs; distort the medical literature; and misrepresent products to prescribing physicians... First, through large-scale lobbying and political contributions, the pharmaceutical industry has influenced Congress to pass legislation that has compromised the mission of the Food and Drug Administration (FDA). Second, largely as a result of industry pressure, Congress has underfunded FDA enforcement capacities since 1906, and turning to industry-paid “user fees” since 1992 has biased funding to limit the FDA’s ability to protect the public from serious adverse reactions to drugs that have few offsetting advantages. Finally, industry has commercialized the role of physicians and undermined their position as independent, trusted advisers to patients.”*

Advances in immune aspects of biologics and nanodrugs over the past decade have created tremendous opportunity to accelerate the discovery and development of these novel therapeutic agents to treat devastating human diseases. However, despite enormous advances, wide gaps persist. *So, what to expect in the next decade in this vast field regarding efforts to blunt adverse immune reactions and design safer biologics and nanodrugs? What tools, techniques, and analytical methods will be leveraged? Will these advances to come leave us poised on a threshold of innovation?*

I expect that in the next decade there will be an intense competition for targets, introduction of second- and third-generation biologics and nanodrugs, more follow-on versions on validated targets, expiration of blockbuster patents, spotty patent examination at patent offices, nomenclature confusion, poor regulatory guidelines from regulatory agencies, third-party payor pressures, sky-rocketing prices of biologics, and governmental pricing pressures—all impacting and reshaping the drug industry landscape. I also expect that due to limited current experience

with the evaluation of biologics and first-generation nanodrugs, manufacturers, regulatory agencies, clinicians, patients, and patent offices will face challenges not only regarding second- and third-generations of these two drug classes but also on the biosimilars, nanosimilars, and NBCD similars front.

Immunogenic effects are likely to be especially challenging to evaluate for highly complex biologics, combination products such as theranostics, and later generation nanoformulations. So, for the time being (this decade), immune reactions to biologics and nanodrugs will be common and regulatory agencies will continue to approve drugs based on an analysis of the risk-benefit ratio that changes significantly depending on the treatment modality. However, as more drug products are developed, information will accumulate on the structure and function of biologics and nanodrugs. As a result, the description and understanding of these drug products and their functionality will be revised, as applicable, and supported with characterization data. Moreover, as the intricacies of the human immune system are further elucidated, we will learn more about the interactions of these therapeutics with immune cells. In the meantime, all medicinal products, including biologics and nanodrugs, will continue to be evaluated by regulatory agencies on a case-by-case basis.

Academic immunology research is generally lagging industry and other medical research fields in incorporating modeling approaches. Due to the high failure rates, long time line (10–17 years), high attrition rate, and enormous R&D costs (estimated at \$2.6 billion total)<sup>18</sup> involved in the approval of a new drug, pharma has increasingly turned to computational and mathematical modeling at all levels—modeling drug-receptor interactions, PK and pharmacodynamic (PD) modeling, *in silico* clinical trials. Given this trend, I predict that we will glean greater information regarding the immune aspects of biologics and nanodrugs as we expand our arsenal of both *in vitro*, *in silico*, and *in vivo* analytical methods as well as instrumentation to evaluate potential immunotoxic effects. Computer-driven computational methods followed by *in vitro* and/or *in vivo*

<sup>18</sup>DiMasi, J. A., Grabowski, H. G., Hansen, R. W. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. *J. Health Econ.*, **47**, 20–33.

testing of any potentially immunogenic epitopes will help in minimizing immune responses. In future, due to the great cost and time needed for comprehensive animal studies, researchers will increasingly develop various *ex vivo* mimics of *in vivo* biological environments to study the interactions of these drug products with the immune system. Artificial intelligence (AI) is expected to change the drug discovery process as machine learning and other technologies are likely to make the hunt for new drugs quicker, cheaper and more effective [38]. Specifically, AI will be employed in this arena to analyze large data sets from clinical trials, health records, gene profiles, and preclinical studies. Technically, a sufficiently large medicinal chemistry database of transformations could provide novel approaches to improving drug discovery [39].

#### ***Drug Discovery Technologies: Current and Future Trends***

“[D]rug discovery remains perhaps the most challenging applied science largely due to the complexity of human biology, the vastness of chemical space, the discontinuous impact of functional group changes on molecular properties, and the inability to optimize a single variable (potency, selectivity, permeability, metabolic stability, solubility) without having simultaneous and sometimes detrimental effects on other critical parameters. For these reasons, a successful drug discovery campaign often emerges after investigating dozens of pharmacological targets, with each one requiring thousands of chemical hits to be triaged and hundreds of close-in synthetic analogues to be evaluated. A recent 2016 publication based on 10<sup>6</sup> new drugs from 10 pharmaceuticals firms estimated that the overall investment in discovery and clinical development approaches \$2.6 billion for each successful launch...Technologies that enable more effective selection of productive biomolecular targets provide novel ways to engage targets, or appropriately guide design to the most effective regions of chemical space will lead to transformative improvements in drug discovery efficiency.”

*Source:* Noe, M. C., Peakman, M-C. (2017). Drug discovery technologies: Current and future trends. In: Chackalamannil, S, Rotella, D, Ward, S, eds. *Comprehensive Medicinal Chemistry III*, vol. 2, pp. 1–32, Elsevier.



Single-cell genomics involving cell capture and accurate analysis of DNA, RNA, and protein of single cells will certainly transform our understanding of the immune system. Single-cell genomic analysis of blood samples or biopsies will be routine in the next decade, and the entire immune composition of patients will be analyzed and compared with all known healthy and diseased states [40].

I am not a fan of the various accelerated approaches currently underway and on the rise at global regulatory agencies, primarily at the FDA, EMA, and PMDA. For serious or life-threatening disease, the FDA can approve drugs through its accelerated approval review track based on surrogate end-points (rather than hard clinical end-points) that are “reasonably likely to predict clinical benefit.” This pathway was designed in the early 1990s to speed drug development. Various accelerated approaches include breakthrough therapy designation, accelerated approval, and conditional marketing authorization—collectively referred to as “facilitated regulated pathways” (FRPs). A greater uncertainty is introduced into the regulatory approval process via FRPs. This could translate into unwanted immunogenicity.

A comprehensive map of molecular drug targets is currently lacking. Gaps and opportunities need to be identified to shed light on the so called “druggable genome”—the subset of genes (~3,000 of the ~20,000 total protein-coding genes in the human genome) encoding proteins that could bind drug-like molecules.<sup>19</sup> In fact, out of ~3,000 of these druggable genes, less than 700 are currently targeted by FDA-approved drugs [41]. This is because big pharma focuses on relatively well-characterized proteins as targets for drug development to mitigate risk. However,

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<sup>19</sup>The phrase “drug-like molecule” implies that certain properties of a chemical compound (drug candidate) confer on it a greater propensity to become a successful drug product. The standard method to evaluate “druglikeness” or to determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans is to check compliance of “Lipinski’s Rule of Five” that covers certain features or properties of the compound: the numbers of hydrophilic groups, molecular weight, and hydrophobicity. See: Lipinski, C. A., Lombardo, F., Dominy, B. W., Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.*, 23(1-3), 3-25.

it is hard to fault it for following this path: There is a lack of consolidated information on the druggable genome and also a scarcity of high-quality technologies to characterize the function of protein family members. Hence, there is a critical unmet need to expand our basic knowledge of the druggable genome and to increase our catché of potential drug targets by studying druggable gene families [42]. This will aid in determining the relevance of drug targets to human health and disease as well as in the identification of off-target effects of existing drugs and drug candidates. An important long-term outcome of this would be the development of new drugs for immune targets. Also, rapid and precise gene editing technologies, including CRISPR-Cas9,<sup>20</sup> can be applied to build systems of greater physiological relevance and disease significance.

The impact of noncrystalline single-particle cryo-electromicroscopy (cryo-EM) on structural biology cannot be understated in the context of immunogenicity. Although, cryo-EM has been used to determine the structure of biological macromolecules and assemblies, its potential for application in drug discovery has been limited by two issues: the minimum size of the structures that can be used to study and the resolution of the images [43]. However, recent technological advances, including the development of direct electron detectors and improved computational image analysis techniques, are leading to high-resolution structures of large macromolecular assemblies [43]. These improvements should further enable structural determination for “intractable” targets that are still not accessible to X-ray crystallographic analysis. Therefore, negative staining techniques and cryo-EM, which have both been employed previously for both linear and conformational epitope mapping analysis, should further enable epitope mapping for designing novel biologics and nanodrugs as well as for determining epitopes at the amino acid level that are critical to immune aspects of these therapeutics. This could also aid in anti-ADA vaccine design in future.

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<sup>20</sup>What are genome editing and CRISPR-Cas9? U.S. National Library of Medicine. Available at: <https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting> (accessed on July 7, 2018).

In the coming years, the study of immune complex (IC) biology specific to biologics will shed more light on the role and relationship of ADA to clinical outcome measures. The formation and contribution of ICs (Section 1.4.1(a)) is central to most of the downstream sequelae that are seen following development of ADA [17]. IC formation and corresponding risks could persist if the same treatment continues unabated even with symptomatic remediation of adverse effects. One central question for now is: *Why do some individuals develop clinically significant ADA titers while others do not?* Attention is also warranted to address the discrepancies currently seen when measuring ADAs with different assays. This can lead to biased clinical interpretation and treatment modalities. Hence, accurate immunogenicity measurement, as reflected by the presence and magnitude (titre) of ADAs, is essential towards assessing, predicting, and mitigating unwanted immunogenicity in a clinical setting. Ultimately, this can lead to safer and more effective drug products.

Compared to conventional small-molecule drugs, further understanding will be essential about the interactions of biologics, nanodrugs and their carriers with biological tissues. Even if these drug products are declared nontoxic according to standard regulatory assays, more robust testing of their interaction with the immune system needs to be performed. Specifically, the impact of intrinsic (e.g., disease, age, sex) and extrinsic factors (e.g., co-administered drugs, presence of impurities, dosing frequency, disease state of the patient) exposure and response, the role of enzymes and transporters in their disposition and their immunogenic potential will be essential to advancing the safe use of these drug products (Fig. 1.5). *In future, drug companies will need to increasingly prove to regulators that neither their manufacturing processes nor later use of the final drug product generates CARPA, immunogenicity, ADAs, or ICs in a manner that causes adverse reactions impacting safety or efficacy.* Regulatory agencies must hold biologics and nanodrugs to strict safety and efficacy standards now so that corresponding follow-on versions later (biosimilars, nanosimilars, NBCD similars [34, 35, 44–46]) are also safe and efficacious. The FDA and the EMA, in particular, should formulate regulatory pathways that are science-based

and follow the “totality-of-the-evidence approach” for highly complex drugs like biologics and nanodrugs.

The ever-expanding landscape of innovative technology, techniques, and assays makes it critical for immunologists, protein chemists, drug formulators, nanotechnologists, medicinal chemists, analytical chemists, structural biologists, screening biologists, and computational scientists to expand and integrate their efforts into cross-disciplinary collaborations and to become more familiar with a multitude of areas outside their expertise. Only then can we provoke transformative change in this complex field and address issues regarding immune aspects of biologics and nanodrugs. Ultimately, developing biologics and nanodrugs that have minimal, or no adverse immune aspects, will only be addressed in a comprehensive manner with firm commitment and cooperation between all stakeholders—the public, researchers, pharmaceutical and biotechnology companies, government policymakers, patients, and regulatory agencies. After all, our common mission of building a bridge from “bench-to-bedside” is quite simple: *enhancing translation of biologics, nanodrugs as well as their follow-on versions.*

## **Disclosures and Conflict of Interest**

The author declares that he has no conflict of interest. No writing assistance was used in the production of this chapter and the author has received no payment for its preparation. The author is a scientific advisor to Teva Pharmaceutical Industries Ltd. (Israel). The author is not aware of any affiliations, memberships or funding that might be perceived as affecting the objectivity of this chapter.

## **Corresponding Author**

Dr. Raj Bawa  
Bawa Biotech LLC  
21005 Starflower Way  
Ashburn, Virginia, USA  
bawa@bawabiotech.com

## About the Author



**Raj Bawa, MS, PhD**, is president of Bawa Biotech LLC, a biotech/pharma consultancy and patent law firm based in Ashburn, Virginia, that he founded in 2002. He is an inventor, entrepreneur, professor, and registered patent agent licensed to practice before the U.S. Patent & Trademark Office. Trained as a biochemist and microbiologist, he has extensive expertise in pharmaceutical sciences, biotechnology, nanomedicine, drug delivery, microbial biodefense, FDA regulatory issues, and patent law. Since 1999, he has held various positions at Rensselaer Polytechnic Institute in Troy, NY, where he is an adjunct professor and where he received his doctoral degree in three years (biophysics/biochemistry). Currently, he serves as a scientific advisor to Teva Pharma, Israel, is a visiting research scholar at the Pharmaceutical Research Institute of Albany College of Pharmacy in Albany, NY, and is vice president of Guanine, Inc., based in Rensselaer, NY. He has served as a principal investigator of SBIRs and reviewer for both the NIH and NSF. Currently, he is principal investigator of a CDC SBIR Phase 1 grant to develop an assay for carbapenemase-resistant bacteria. In the 1990s, Dr. Bawa held various positions at the US Patent & Trademark Office, including primary examiner from 1996–2002. He is a life member of Sigma Xi, co-chair of the nanotech and personalized medicine committees of the American Bar Association, and founding director of the American Society for Nanomedicine. He has authored over 100 publications, co-edited four texts, and serves on the editorial boards of 14 peer-reviewed journals, including serving as an associate editor of *Nanomedicine* (Elsevier). Some of Dr. Bawa's awards include the Innovations Prize from the Institution of Mechanical Engineers, London, UK (2008); Appreciation Award from the Undersecretary of Commerce, Washington, DC (2001); the Key Award from Rensselaer's Office of Alumni Relations (2005); and Lifetime Achievement Award from the American Society for Nanomedicine (2014).

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