My Life with Biologicals and Nanodrugs: A Twenty-Year Affair

Twenty years now
Where'd they go?
Twenty years
I don't know
Sit and I wonder sometimes
Where they've gone[1]

Twenty years ago, as Primary Examiner at the US Patent Office, I reviewed and granted many US patents on biotechnology-based drug products and nanoparticulate drug formulations, a small fraction of which were approved by drug regulatory agencies[2] and eventually commercialized. Most of these first-generation, early drug products are still on the market. Since then, I have been involved in all aspects of biotherapeutics and nanomedicines—research, patent practitioner, professor, journal editor, FDA regulatory filings, conference organizer, keynote speaker, and advisor to the drug industry. I have seen the evolution of anything and everything "biotherapeutic" and "nanomedicine." I have marveled at the cutting-edge discoveries and inventions in these emerging fields. I have also stood up to criticize inept governmental regulatory policies, spotty patent examination at patent offices, hyped-up press releases from eminent university professors taunting translation potential of their basic research and development (R&D), inadequate safety policies, and inaccurate

[1]This is an excerpt from the classic song titled *Like A Rock* by music legend Bob Seger, in which the aging songwriter laments the loss of his youth once filled with vim and vigor and wonders where time went.

[2]The primary drug agencies are the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), Health Canada (HC), and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA).

depiction of these drug products by scientists, media, government agencies, and politicians.

Twenty years later, there is a wave of "newer" therapeutics sweeping the world of medicines. Specifically, there is a rapid introduction of two somewhat distinct but overlapping categories of drugs into the pharmaceutical landscape: (1) biotherapeutics ("biologics," "biologicals," "biological products," "biopharmaceuticals," "biomolecular drugs," or "protein products")[3] and (2) nanomedicines ("nanodrugs," "nanoparticulate drug formulations," or "nanopharmaceuticals").[4] For example, biotherapeutics alone have grown from 11% of the total global drug market in 2002 to around 20% in 2017.[5] I estimate that there are over 225 approved biotherapeutics and around 75 approved nanomedicines for various clinical applications. Similarly, by my estimate, hundreds of companies globally are engaged in nanomedicine R&D; the majority of these have continued to be startups or small- to medium-sized enterprises rather than big pharma.

[3]Biologicals, including those made by biotechnology, are a special category of "drugs" or medicines. They differ from conventional small-molecule drugs derived by chemical means in that they are derived biologically from microorganisms (generally engineered) or cells (often mammalian, including human cells). In other words, these are human health products generated or produced by modern molecular biological methods, and differ from traditional biological products that are directly extracted from natural biological sources such as proteins obtained from plasma or plants. Most biologicals are large, complex molecules as compared to small-molecule pharmaceuticals. Slight variations between manufactured lots of the same biological product are normal and expected within the manufacturing process. As part of its review, the FDA assesses this and the manufacturer's strategy to control within-product variations. See: Walsh, G. (2002). Biopharmaceuticals and biotechnology medicines: an issue of nomenclature. *Eur. J. Pharm. Sci.,* **15**, 135–138: *"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."*

[4]There is no formal or internationally accepted definition for a nanodrug. The following is my definition (see: Bawa, R. (2016). What's in a name? Defining "nano" in the context of drug delivery. In: Bawa, R., Audette, G., Rubinstein, I., eds. *Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy, and Clinical Applications*, Pan Stanford Publishing, Singapore, Chapter 6, pp. 127–169): "*A nanomedicine 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.*"

[5]Data from the IMS Institute for Healthcare Informatics.

This book will focus on those biologics, biotechnology products, nanomedicines, nanodrug products, and nanomaterials that are employed for medicinal purposes for humans. Many terms used in this book are definitions that come from specific regulations or compendia, but others are being defined as they are used here. The terms "product," "drug formulation," "therapeutic product," or "medicinal product" will be used in the manner the FDA defines a "drug," encompassing both small-molecule pharmaceutical drugs, biologicals, and nanomedicines in the context of describing the final "drug product."[6] Some of the terms will be used synonymously. For example, biotherapeutics, biologicals, biological products, and biologics are equivalent terms. Similarly, nanomedicines, nanodrugs, nanopharmaceuticals, nanoparticulate drug formulations, and nanotherapeutics are the same.

Although there are major benefits touted for these "newer" therapeutics, including a reduction in unwanted side effects, their use does not guarantee the absence of side effects. For example, studies have shown that these therapeutic agents 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 pharmaceutical drugs, both biologics and nanomedicines 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 obviously can negatively affect their effectiveness and safety, and thereby limit their therapeutic application.[7] Some of the undesired immune responses

[6]Branded drugs are referred to as "pioneer," "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). As a result, a new drug application for an NCE is known as a New Drug Application (NDA), whereas a new drug application for an NBE is called 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.

[7]10–20% of the medicinal products removed from clinical practice between 1969 and 2005 were withdrawn due to immunotoxic effects. See: Wysowski, D. K., Swartz, L. (2005). Adverse drug event surveillance and drug withdrawals in the United States, 1969–2002: The importance of reporting suspected reactions. *Archiv. Intern. Med.*, **165**(12), 1363–1369.

include complement activation, tissue inflammation, leucocyte hypersensitivity and formation of antibodies associated with clinical conditions. This has highlighted the critical need to evaluate, assay, and devise strategies to overcome adverse immunogenicity of both biotherapeutics[8] and nanotherapeutics[9].

[8]Early developers of biologics assumed that as many of these drugs were based on human genes and proteins, the human immune system would not treat them as foreign and not produce antidrug antibodies (ADAs). However, this optimistic view has turned into alarm as some biologics elicit a vigorous immune response that *may sometimes* neutralize, block, or destroy them. Also, most biotherapeutics are engineered to enable dual or multiple binding sites (e.g., conjugated proteins, functionalized antibodies)—all of which could lead to them being recognized as foreign and therefore immunogenic. Specifically, ADAs may (i) neutralize the activity of the biotherapeutic, (ii) reduce half-life by enhancing clearance, (iii) result in allergic reactions, and/or (iv) cross-react with endogenous counterparts to result in "autoimmune-like" reactions. Such effects are rarely observed with conventional small-molecule drug products. For example, some studies have shown that AbbVie's HUMIRA® (adalimumab) does not work in ~20% of patients. Similarly, in 2016, Pfizer had to withdraw a promising anticholesterol biologic (bococizumab) after testing it in more than 25,000 persons. 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.

Another issue with some biologics is that they show a concentration-dependent propensity for self-association. This can induce adverse immune responses in patients that may affect drug safety and efficacy. See: Ratanji, K. D., Derrick, J. P., Dearman, R. J., Kimber, I. (2014). Immunogenicity of therapeutic proteins: influence of aggregation. *J. Immunotoxicol*., **11**(2), 99–109.

[9]Clinical application of nanomedicines and nanocarriers is also dogged by safety and nanotoxicity concerns (undesirable adverse effects), especially about their long-term use. In the case of nanomedicines, therapeutic particles 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) to the right tissue site. However, this persistence in the body may trigger immune responses. Novel "immune-toxicity" from nanomedicines may result from the unique combinations of shape, size, surface charge, porosity, reactivity, and chemical composition—all aspects to which the immune system may not have adapted to. Often, intravenously administered nanomedicines prime the immune system, leading to adverse reactions and/or loss of efficacy of the drug product. For example, it is now well established that intravenous administration of nanomedicines and nanocarriers *may* provoke "hypersensitivity reactions" (HSR) or "anaphylactoid reactions" that are referred to as complement (C) activation-related pseudoallergy (CARPA). See: Szebeni, J. (2005). Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity. *Toxicology*, **216**, 106–121 and Szebeni, J. (2018). Mechanism of nanoparticle-induced hypersensitivity in pigs: complement or not complement? *Drug Discov.*

Not all biotherapeutics, nanoformulations, and nanomaterials are created equal. Given this scientific fact, the risks for immunogenicity should be assessed on a case-by-case basis. In fact, while some biologics, particularly glycoproteins, cause the body to produce antidrug antibodies (ADAs),[8] few elicit immunogenicity in a manner that induces any clinically relevant reaction. Similarly, the diversity of nanomedicines makes it impossible to extrapolate or generalize the immunologic findings from one class of nanomedicines (e.g., nanoliposomes, solid nanoparticles, carbon nanotubes) to another. Nevertheless, the degree of risk for eliciting immune responses from biotherapeutics, nanoformulations, or nanomaterials is considered a major issue during drug R&D and administration to patients. It is now well established that any biotherapeutic, nanoformulation, or nanomaterial can *potentially* exert an immunogenic effect ("immunogenicity risks") depending on a patient's immunologic status, prior history, route/dose/frequency of delivery and unique characteristics of the administered therapeutic product. Therefore, regulatory agencies, particularly the FDA and the EMA, recommend that drug developers employ a risk-based approach to evaluate and reduce adverse immune events related to the administration of these therapeutics that could affect safety and efficacy. These must be carefully evaluated at the earliest stages of drug formulation/development as well as throughout the product lifecycle, including during phase IV. Biotherapeutic drug products containing a non-biologic 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 biotherapeutic drug products

Today, **23**(3), 487–492. These hypersensitivity reactions typically occur directly at first exposure to the nanocarriers without prior sensitization, and the symptoms usually lessen and/or disappear on later treatment. That is why these reactions are labelled as "pseudoallergic" or "nonspecific hypersensitivity." Nanomedicines causing CARPA include radio-contrast media, liposomal drugs (Doxil®, Ambisome® and DaunoXome®, Abelcet®, Visudyne®), micellar solvents (e.g., Cremophor EL, the vehicle of Taxol®), PEGylated proteins and monoclonal antibodies. Drug products other than biologics and nanomedicines such as nonsteroidal anti-inflammatory medicinal products, analgesics and morphine can also trigger CARPA. Also, see: Szebeni, J, Bawa, R. (2018). Immunological issues with medicines of nano size: The price of dimension paradox. In: Bawa, R., et al., eds. *Immune Aspects of Biopharmaceuticals and Nanomedicines,* Pan Stanford Publishing, Singapore, Chapter 2.

containing non-biologic nanomaterial components be assessed with a focus on whether the nanomaterial components possess adjuvant properties. Similarly, carriers may exhibit inherent immunologic activity that is not related to the loaded active pharmaceutical ingredient (API); this could also affect the safety and effectiveness of the drug product. Another important issue involves the approval of follow-on versions of both biologics and nanomedicines.[10] I wonder how often cost considerations drive the approval process. I suspect that there are enormous pressures on drug regulatory agencies (e.g., the Trump administration's FDA) to grant these drug products. It is no secret that in certain countries these follow-on versions are the preferred drug products and driven by government-controlled healthcare programs. However, it is critical that immune aspects of these so-called "biosimilars" and "nanosimilars" be transparently evaluated and reported during the drug approval process: *Lower drug prices should not supplant patient safety and efficacy*. The recent FDA approval of follow-on versions of Copaxone® is an example that highlights this troubling trend. I believe that accelerating the approval of follow-on versions of biologics and nanomedicines should be science-based and undertaken on a case-by-case basis.[11]

[10]Since the replication of biologics is complex and less precise as compared to small molecule drug products, the term generic has been deemed inappropriate.

[11]See: Conner, J. B., Bawa, R., Nicholas, J. M., Weinstein, V. (2016). Copaxone® in the era of biosimilars and nanosimilars. In: Bawa, R., Audette, G., Rubinstein, I., eds. *Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy, and Clinical Applications,* Pan Stanford Publishing, Singapore, Chapter 28, pp. 783–826; Bawa, R. (2018). Immunogenicity of biologics and nanodrugs: An overview. In: Bawa, R., Szebeni, J., Webster, T. J. and Audette, G. F. eds. *Immune Aspects of Biopharmaceuticals and Nanomedicines*, Pan Stanford Publishing, Singapore, Chapter 1.

Copaxone® is a non-biologic (synthetic) complex drug ("NBCD") and can be considered a first-generation nanomedicine. It is composed of an uncharacterized mixture of immunogenic polypeptides in a colloidal solution. 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

In our rapidly changing yet interconnected and globalized world, biologics and nanomedicines will continue to surprise and expand. There are numerous second- and third-generation biologics and nanomedicines at the basic research stage. Hopefully, despite enormous bottlenecks, we will find a greater number of these translated into practical patient applications. In the meantime, we need to temper our expectations yet continue to hope for paradigm-shifting advances in the bio-nano world.

Against this backdrop, the editors felt that enormous advances in the past 20 years in immunology of biologics and nanomedicines warranted an authoritative and comprehensive reference resource that can be relied upon by immunologists, biomedical researchers, clinicians, pharmaceutical companies, formulation scientists, regulatory agencies, technology transfer officers, venture capitalists, and policy makers alike. Hence, this volume aims to provide a broad survey of theoretical and experimental knowledge currently available and presents a framework that is readily applicable to develop strategies for clinical applications. Each chapter contains key words, tables and figures in color, future predictions, and an extensive list of references. The focus is on the current, most relevant information, all accomplished in a user-friendly format.

Assorted topics pertain to the immune effects of biologics and nanomedicines, both beneficial and adverse. A thorough understanding of immunology, therapeutic potential, clinical applications, adverse reactions and approaches to overcoming immunotoxicity of biologics and nanomedicines is presented.

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potentially interchangeable follow-on glatiramer acetate product: Glatiramer acetate is an immunomodulator. 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®. Due to the complexity and inexorable link between the manufacturing process and quality, any follow-on product almost certainly will differ from Copaxone®'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). Although it is not possible to fully characterize and compare these complex mixtures, differences are revealed via sophisticated analytical techniques. Despite these immunological concerns, the FDA in 2017 approved so-called follow-on versions of Copaxone®.

For instance, chapters are devoted to immune stimulatory and suppressive effects of antibodies, peptides and other biologics, as well as various nanomedicines. The state of the art in therapeutic and preventive vaccines along with their potential molecular mechanisms underlying immunogenicity is also highlighted. Adverse immune effect of certain biologics and nanomedicines, namely, complement (C) activation-related pseudoallergy (CARPA), is discussed in unprecedented detail in terms of occurrence, prediction, prevention, and mechanism. Furthermore, critical, yet often overlooked topics such as immune aspects of nano-bio interactions, current FDA regulatory guidance, immunogenicity testing of therapeutic protein products, and engineering bio/nanotherapeutics to overcome barriers to immunotherapy are also covered.

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Series Editor
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*The day my beloved *Washington Capitals* ice hockey team won the Stanley Cup for the first time in franchise history!