



Points to Consider in Drug Development of Biologics and Small Molecules

Traditionally, the treatment of human disease has been dominated by small molecule drugs. Small molecules are used to treat a variety of diseases and conditions and can be quite diverse in their mechanisms of action. Because of their small size and typical physiochemical properties, small molecules can be effective enzyme inhibitors and allosteric modifiers and can target extracellular proteins or intracellular receptors in the cytosol, nuclei, and central nervous system. Despite their perceived limitations, and with a recent resurgence, small molecules remain the major component of an ever-expanding therapeutic toolbox.

About 35 years ago, advances in biotechnology enabled the synthesis of certain biological molecules (primarily proteins) in microorganisms and other living cells using recombinant DNA technology. From these early discoveries and subsequent innovations, the realm of biologics has expanded to include a wide range of products. In addition to therapeutic proteins like peptides and antibodies, biologics also include nucleic acid-based therapies (e.g., RNAi, gene therapy, gene editing), blood components, cellular and tissue therapies (e.g., CAR T cell therapy, allogeneic transplants), and others.

Because of substantial investment and often novel approaches, the rise of biologics in recent years has been impressive. The top selling drug worldwide is Humira, a monoclonal antibody and as of early 2020, biologics comprise seven of the [top 10 best selling drugs](#). In the current post, we focus our discussion specifically on therapeutic proteins* and how they compare to small molecule drugs. In particular, we discuss similarities and differences between these two therapeutic categories and important points to consider during drug development.

Physiochemical Properties

Small molecule drugs and therapeutic proteins differ substantially in many of their class attributes. At the root of this are their disparate physiochemical properties, which affect not only the pharmacological aspects of the drug (e.g., mechanism of action, pharmacodynamics, pharmacokinetics), but also safety and efficacy, and even impact product manufacturing/quality considerations.

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Compared to biologics, small molecule drugs are relatively simple chemical compounds and can be manufactured by chemical synthesis. These compounds are typically comprised of 20 to 100 atoms and have a molecular mass of less than 1000 g/mol or 1 kilodalton [kDa]. The pharmacological activity, stability, and permeability of small molecules largely depend on chemistry as opposed to structural interactions. Because of their properties, small molecule drugs can be administered by a variety of routes, including orally. Their oral bioavailability is indeed the most obvious advantage over biologics in drug development.

Therapeutic proteins include amino acid-based molecules ranging in size from smaller peptides (1 to <10 kDa) to much larger proteins (>10 kDa) like [monoclonal antibodies](#). Even the smallest of these molecules are larger than most small molecule drugs, and they typically contain from 5,000 to 50,000 atoms per molecule. While peptides are simple amino acid chains that display little tertiary structure, larger proteins are often complex molecules that fold into unique three-dimensional structures that are integral to their biological activity. Therapeutic proteins are usually polar, heat sensitive, membrane impermeable, and subject to enzymatic degradation, although monoclonal antibodies tend to be more stable. Furthermore, unlike many small molecule drugs, therapeutic proteins are not orally active and therefore require systemic routes of administration (e.g., injection).

One example where considering the physiochemical properties of a molecule is especially important is when developing therapies to treat central nervous system (CNS) disorders. Since a large proportion of pharmacological targets are embedded in the CNS beyond the blood-brain barrier (BBB), these targets can be inaccessible to larger, polar molecules. Particularly, tight junctions within the BBB prevent the passage of molecules with masses >600 Da. This ultimately restricts up to 98% of small molecules and almost all biologics. Because of this, careful consideration must be given to product design during the discovery phase of development and to appropriate preclinical testing. These steps will help ensure that the therapy will not be ineffective in the clinic simply due to the inaccessibility of the therapeutic molecule to the intended site of action.

Pharmacodynamics

Another key difference between small molecule drugs and therapeutic proteins is the way that these molecules interact with their targets and the effect that this has on the body (i.e., pharmacodynamics). Therapeutic proteins tend to exhibit much higher specificity than small molecule drugs. This is important because specificity (or lack thereof) can impact safety.

Mechanistically, small molecules are designed to bind with targets like G protein-coupled receptors, ligand-gated ion channels, and receptor tyrosine kinases on extracellular or intracellular domains throughout the body. In addition, small molecules can disturb biological processes by mere physical interaction. However, because they lack the high specificity of many biologics, small molecules have the potential to induce off-target effects. This means that small molecule drugs can interact with tissues, cells, and cellular components in unintended and undesirable ways. Sometimes, off-target effects can be relatively innocuous, but frequently, they lead to adverse effects.

In contrast to small molecule drugs, therapeutic proteins typically bind with high specificity to their targets on cell surfaces or intracellular components. While the chemical moieties of therapeutic proteins generally do not themselves cause adverse effects, these molecules are not without risk. They can impede vital physiological functions and lead to rare but life-threatening events related to immunogenicity, cytokine release syndrome, encephalopathy, and others.

Pharmacokinetics

Compound attributes influence how the drug molecule is affected by the body. Collectively, this is known as pharmacokinetics and includes how the molecule is absorbed, distributed (include transport), metabolized, and eliminated (ADME).

Regardless of the route of administration, once a drug enters the body, its fate is determined by its interactions with various enzymes and other proteins, tissues, cells, and their components. While some drugs act locally and are not absorbed into the systemic circulation, most require absorption into the blood to induce their

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pharmacological effects. When drug molecules are absorbed into the blood, drug concentration can be assessed via routine blood sampling at relevant timepoints after administration. Following absorption, these molecules are distributed to various organs and tissues via the systemic circulation where they interact with cellular components and are subsequently broken down (metabolized) and eliminated.

In addition to blood, a less well-known but equally important circulatory system is the lymphatic system. This more “sluggish” circulatory system (~100–500 times slower than blood) is especially important for drugs with larger molecular masses (i.e., >10 kDa) and even small molecules that are targeted to the lymphatic system by association with macromolecular carriers (e.g., nanoparticles, polymers, liposomes) or that associate with endogenous macromolecules (e.g., lipoproteins and proteins) once inside the body.

Larger therapeutic proteins distribute via both the blood and lymphatic systems and move transcellularly by convective transport, receptor-mediated endocytosis, phagocytosis, and fluid-phase pinocytosis. Consequently, these molecules display distinct ADME characteristics compared to small molecule drugs. These differences include a longer half-life, a more limited volume of distribution, and a longer time to reach peak concentration for these large molecules.

Due to high-affinity receptor interaction, the elimination of therapeutic proteins is directly correlated with binding efficiency; this is also known as target-mediated drug disposition. Like endogenous substrates, therapeutic proteins are broken down into amino acids by proteases and peptidases. Most of these amino acids are then recycled by the body; the rest are secreted into bile and excreted into feces or filtered into urine (<70 kDa) and are eventually eliminated.

In contrast, nearly all small molecules are eliminated by non-targeted organs, through cytochrome P450 metabolism, or by some other metabolic process elsewhere in the body, or by the kidneys, or excreted in bile or feces.

Drug-drug Interactions

The potential for [drug-drug interactions](#) should be considered during the development of any drug, especially when defining dose modifications for specific populations (e.g., geriatric, hepatic/renal impairment). Drug-drug interactions can occur in the presence of concomitant drugs in a way that affects the ADME pathways for one or both interacting drugs. Potential outcomes can be predicted based upon class attributes or specific physiochemical properties of the individual drug.

Drug-drug interactions are less frequent for therapeutic proteins since they undergo metabolism and elimination in the same way as endogenous molecules. Nevertheless, drug-drug interactions should be assessed for novel therapeutic proteins especially when the molecule influences the expression of metabolic enzymes (e.g., cytokine-mediated changes in drug metabolizing enzymes are well documented for therapeutic proteins).

Drug Development Challenges

A common misconception is that biologics are somehow more “natural” and require less stringent testing than small molecules because they are often produced by living cells, but this could not be further from the truth. In fact, quality control measures are often even more rigorous with therapeutic proteins to avoid possible errors introduced during manufacturing. Potential errors include variation in functional groups like unintended glycosylation, methylation, or phosphorylation or a structural change that could alter the molecule’s activity in the body.

Sponsors who are developing therapeutic proteins must also consider the potential for immunogenicity. The physiochemical characteristics of these molecules (including their large size) can elicit a rapid and cascading immunological response, which can result in severe adverse effects. Because of this, patients must be carefully monitored following administration. To mitigate immunogenicity risk, therapeutic proteins may be administered alongside immunosuppressants (which come with their own safety risks) for the duration of treatment. The possibility of drug-drug interactions must also be considered whenever concomitant medications are provided.

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There are other unique challenges that sponsors may face when developing therapeutic proteins. This is especially true of more complex molecules and molecular constructs (e.g., antibody-drug conjugates, bispecific or modified proteins) or those delivered by novel routes of administration (e.g., oral or inhaled peptides). However, with rational design of both the drug and the development program, these challenges can be overcome prospectively and effectively.

Conclusions

While any drug development program can face pitfalls and uncertainties, developing biologics often presents unique challenges above and beyond those encountered for small molecule drugs. Nuventra's senior consultants and scientists have extensive experience in developing small molecule drugs, therapeutic proteins, and other biologics.

Whatever your drug development need — [contact us](#) to learn how Nuventra can help maximize your success.

Additional Resources

- [5 Common FDA Applications for Drugs & Biologics](#)
- [What are the Regulatory Difference between and NDA and BLA](#)
- [Cellular and Gene Therapies: What is My Optimal Dose?](#)
- [FDA Policies to Accelerate Development of Cell & Gene Therapies](#)
- [CAR T Cells Part I: Design, Production, and Toxicity](#)
- [CAR T Cells Part II: Hurdles to Development and Approval](#)
- [In Vivo Gene Editing Enters the Clinic](#)

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