

Drugability Studies are Keys to the Successful Commercialization of Biotherapeutics

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ABSTRACT

Successful therapeutic commercialization requires the demonstration of efficacy and safety of a drug during clinical trials, as well as the commercial feasibility of drug production with consistent quality. Mitigating risk in these three areas is the key strategy for pharmaceutical development success. One of the most effective ways of risk mitigation during therapeutic development is to perform drugability assessments of the molecule. Drugability assessment studies facilitate our understanding of biotherapeutics, predict clinical outcomes, and provide rationales for molecular optimization. Better understanding of biotherapeutic drugability ensures the manufacturability, safety, and efficacy in clinical development. Therefore, drugability assessment is the key for successful biotherapeutic commercialization. Here, we reviewed current literature, and summarized the major durability studies of biotherapeutics.

Keywords: Biotherapeutics, Drug, Clinical trials.

INTRODUCTION

Successful therapeutic commercialization requires the demonstration of efficacy and safety of a drug in clinical trials, as well as the commercial feasibility for production of the drug with consistent quality. From novel drug discovery to blockbuster commercialization, risk mitigation through drugability assessment is the key strategy for pharmaceutical development success¹. The term drugability often refers to the accessibility, efficacy, and safety of a therapeutic molecule that meets clinical and

commercial needs²⁻⁵. Therefore, the drugability assessment of a molecule plays a significant role at an early stage of drug discovery. When it is performed properly, it can reduce the chance of expensive late-stage developmental failure^{3, 4, 6, 7}. While many reviews have summarized the drugability of chemical therapeutics in literature^{2, 5, 8-12}, few summarize biotherapeutic drugability assessment from current literature. Therefore, we reviewed the current literature, and summarized the durability studies of biotherapeutics, particularly those studies that involve monoclonal antibodies (Mab).



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Drugability of Biotherapeutics

When compared to traditional chemical therapeutics, biological drugs show promise in better safety, efficacy, specificity, and extended half-life. However, the molecular structural complexity of biotherapeutics underlies a variety of challenges and limitations. Examples of challenges and limitations are: long development time, inefficient penetration of cell membranes, high cost of manufacturing, unwanted immune response, and poor stability^{13, 14}. During the early stage of discovery, the drugability of a biotherapeutic molecule should be extensively evaluated to reduce the chance of late-stage developmental failure. Figure 1 shows the primary goals to be achieved and major analytical techniques that are performed to determine drugability during early stages of drug discovery. Manufacturability, safety, and efficacy are the three major aspects of drugability that need to be addressed in the early stages of drug discovery.

Manufacturability

Biotherapeutics undergo multiple chemical and physical stresses during the manufacturing process. These stresses potentially lead to modifications of the target protein drug affecting its safety and effectiveness¹⁵. Manufacturing stress induced chemical modifications include oxidation, deamidation, peptides bond hydrolysis, etc.; and manufacturing stress induced physical modifications includes denaturation, precipitation, and aggregation,

etc. Some of these induced modifications (e.g., aggregation, oxidation, glycosylation) can lead to heterogeneity and hence, significant adverse effects on the safety, efficacy, and pharmacokinetics/ pharmacodynamics (PK/PD) of the biotherapeutic drug^{15, 16}. Therefore, a quality assessment of stress induced modifications can provide necessary understanding of the physiochemical structures needed for lead candidates optimization.

Recent advances in proteomic techniques have enhanced understanding and prediction of protein modification resulting from bioprocesses^{17, 18, 19, 20}. These technologies provide diverse databases and analysis software to provide valuable information for controlling manufacturing stress induced protein modification^{17, 18, 21-23}. Sequence- or structure-based bioinformatic prediction tools have been applied in recent studies^{17, 18}. Table 1.1 presents the summary of available computational tools for structural predictions. Biopharmaceutical companies use the *In Silico* platform to assess stress-induced modification tendencies in the molecular structure of biotherapeutics. By selecting the optimal candidates via the *In Silico* platform, biotherapeutic molecules can be optimized to eliminate potential structural liabilities during manufacturing^{17, 24}. Optimized candidates are then selected for in vitro analysis including safety, yield, preformulation, and biological activity^{17, 24}.

Table 1: Prediction Database for PTMs

Database	Source
Scansite	http://scansite.mit.edu/
PREDIKIN	http://predikin.biosci.uq.edu.au/pkr/
NetPhos	http://www.cbs.dtu.dk/services/NetPhos/
NetPhos	http://www.cbs.dtu.dk/services/NetPhosK/
Big-PI-prediction	http://mendel.imp.ac.at/sat/gpi/gpi_server.html
GlycoMod	http://expasy.ch/tools/glycomod/
NetOGlyc	http://cbs.dtu.dk/services/NetOGlyc/
NetNGlyc	http://cbs.dtu.dk/services/NetNGlyc/
DictyOGlyc	http://cbs.dtu.dk/services/DictyOGlyc/
YinOYang	http://cbs.dtu.dk/services/YinOYang/
Sulfinator	http://www.expasy.org/tools/sulfinator
Oglyc	http://www.expasy.org/tools/sulfinator

Safety

Immunogenicity is one of the most common side effects for biotherapeutics. While the principal cause of the immune response is divided, immunogenicity leads to reduced drug efficacy, altered drug clearance, and shortened plasma half-life of the administered drug²⁵⁻²⁸. Therefore, immunogenicity assessment is one of the most important principal safety evaluations for biotherapeutics in development^{26, 28}.

Immunogenic response can be divided into T-cell dependent and T-cell independent responses. In T-cell independent immunogenic response, B cells bind to the therapeutic protein and respond by transiently producing IgM antibodies²⁹.

Table 2: Screening Assay Strategies for Immunogenicity

•	Physiochemical characterization
•	In silico
-	T cell epitope predictions
-	B cell epitope predictions
•	HLA/MHC binding assays
•	T cell responses
•	In vivo models

In T-cell dependent immunogenic response, the complex interplay among antigen presenting cells, T cells, secreted cytokines, and B cells occur in response to the administered proteins³⁰. Thus, anti-drug antibodies (ADA) are usually generated by T-cell dependent immunogenic responses^{25, 30}. Immunogenicity is one of the key risk attributes for clinical safety, so it is desirable to assess the potential immunogenic issues during the early discovery stages. A drug molecule should be redesigned if it is found to be highly immunogenic. One way to reduce the immunogenicity is by removing the T-cell epitopes of the biotherapeutic drug²⁴. It is feasible to predict the immunogenicity of the T-cell epitopes based on the amino acid sequence from *In Silico* because their core residues are limited to 9–10 amino acids³⁰. There are a number of computational tools for T-cell epitope prediction and assessment for potential immunogenicity evaluations³⁰⁻³². These *In Silico* predictions are usually followed by *in vitro* and *ex vivo* cell-based assays validation. Major histocompatibility complex/human leukocyte antigen (HLA/MHC) binding assays are common cell-based assays used to validate the *In Silico* predictions by measuring the affinity of predicted epitope to HLA *in vitro*^{30, 33-35}. A series of T cells will then be used to further assess the immunogenicity, including cytokine response assays, proliferation assays, naive human

Table 3: PK Characteristics Comparison of Chemical Therapeutics and Biotherapeutics

Attributes	Chemical Therapeutics	Biotherapeutics
Binding	Nonspecific	Specific
PK/PD	PK usually independent of PD; short half life	PK usually dependent on PD; long half life;
Dose	Linear PK at low doses (usually therapeutic	Nonlinear PK at low doses; linear PK
Proportionality	doses); nonlinear PK at high doses (after saturation of metabolic enzymes)	at high doses after saturation of target
Distribution	High volume of distribution	Distribution usually limited to blood and interstitial spaces
Metabolism	Metabolism by cytochrome P450 or other phase I/ phase II enzymes	Catabolism by proteolytic degradation
Excretion	Typically, biliary and renal excretion	No renal CL of intact antibody. cleared by damaged kidneys.
Immunogenicity		May be Uncommon if MW >20 kDa Maybe seen
	Not seen	

PBMC response assays *etc.*^{30, 33-35}. These *in vitro* assays also enable bioprocess related changes to be evaluated, such as antibody expression, post-translational modifications, and formulations *etc.*³⁰. *In vivo* experiments with “humanized” animal models are also performed for immunogenicity testing of therapeutic candidates to further validate the findings in cell-based assays³⁶. The data generated from the *in vitro* and *in vivo* validation assays provides an important assessment to the immunogenicity of the biotherapeutic protein to reduce clinical safety risks^{30, 32}.

Typical screening assay strategies for immunogenicity of biopharmaceuticals are

summarized in Table 2. The data derived from these immunogenicity-based assessments can be used to prioritize candidates for development when multiple candidates are available. These assessments can also be used to optimize biopharmaceutical candidates for cost-effective drug development and safety improvement via molecular redesign. Humanization is commonly used to significantly reduce immunogenicity³⁷⁻³⁹. Moreover, physicochemical modification and aggregation elimination of therapeutics can also be used to reduce immunogenicity⁴⁰. Other immunogenicity reduction strategies such as PEGylation have been explored⁴¹. However, studies show that anti-PEG antibodies have been caused by PEGylated

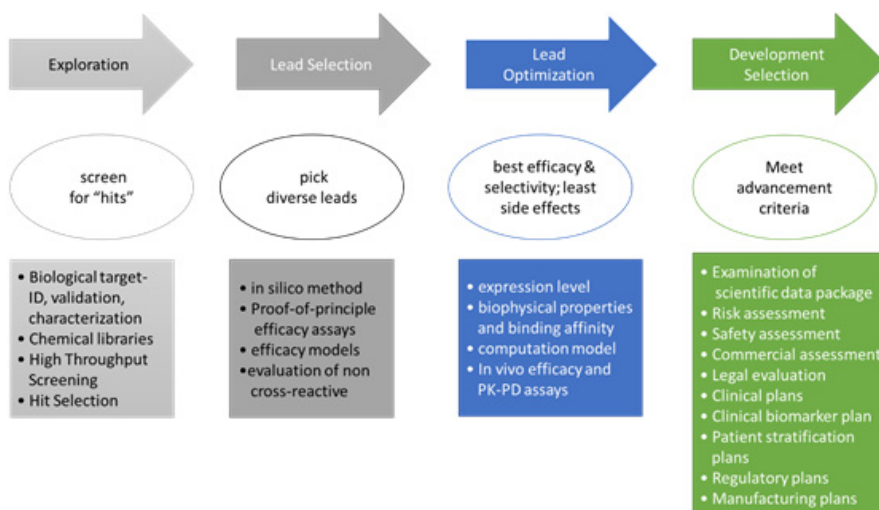


Fig. 1: Stages of biological drug discovery, primary goals, and major activities

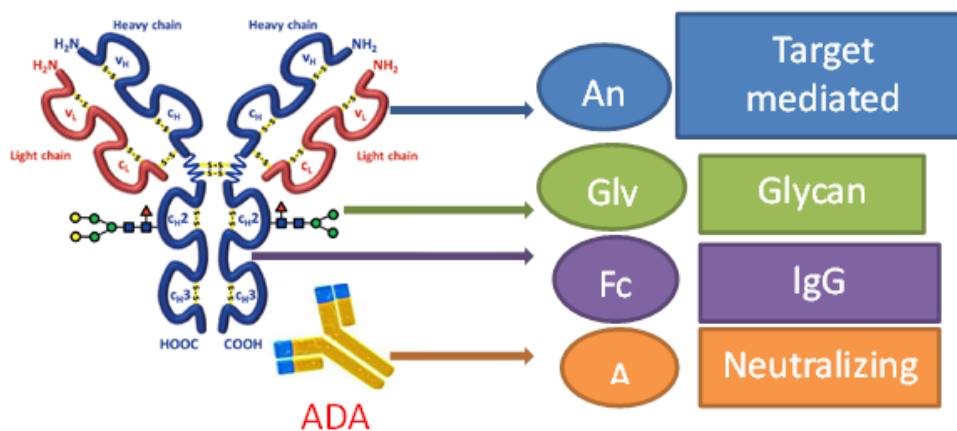


Fig. 2: Factors that impact PK properties of monoclonal antibodies

biotherapeutics⁴²⁻⁴⁶. Nevertheless, immunogenicity reducing technologies are a key to mitigating the clinical safety risks of biotherapeutic developments.

Efficacy

Efficacy is frequently linked to the pharmacokinetics (PK) of a drug because of the direct relationship between efficacy and dosage. Special considerations are applied to biotherapeutics because of their complex structures⁴⁷⁻⁴⁹. The primary PK determinant of a biotherapeutic is their FcRn-mediated recycling. However, other factors, such as glycosylation, and target mediated drug disposition (TMDD), and anti-drug antibody (ADA) response can also have tremendous influence on its PK⁴⁷. Figure 3 illustrates the general structure of IgG1 and the factors that influence PK properties. PK data is important to reference during biotherapeutic structural optimization to achieve desirable exposure, safety, and efficacy profiles^{47, 49-51}. The bioanalytical methods used for protein quantification during PK studies are mainly based on immunoassay methodologies. However, mass spectrometer based quantification techniques, including the bottom-up and top-down approach, are gaining popularity recently due to their advantages in development time, specificity, and throughput^{52, 53}. In addition, fluorescent or radioactive labeled molecules are also frequently used to test absorption and distribution of Mab related therapeutics^{54, 55}. The PK characteristics of biotherapeutics show differences from that of chemical therapeutics (Table 3). PK data of biologics such as C_{max} , Area Under Curve (AUC), half-life, volume of distribution, and clearance can help provide significant insight into their stability in blood, potential nonlinearity of PK, as well as target specific distribution assessment^{47, 48, 53}. PK data from animal studies provides the basis of extrapolation for first-in-human (FIH) dosage information in clinical studies. Linear PK, target-mediated drug disposition (TMDD), and physiology-based pharmacokinetic (PBPK) models are the most common FIH quantitative models used in order to show proper efficacy in clinical studies⁵⁶.

Linear Pharmacokinetic (PK) Models

- Protein biotherapeutics exhibit linear PK after saturation of target. PK data obtained in preclinical animal studies are typically extrapolated by simple mathematical models describing mono-exponential,

bi-exponential, or multiexponential profiles observed in plasma or serum exposure. By using allometric scaling techniques, intended dosage for human exposure predicted⁵⁷.

Target-Mediated Drug Disposition (TMDD)

Models – TMDD is applied when a significant fraction of a drug binds to its pharmacological target with high affinity such that this interaction influences the distribution and elimination of the drug⁵⁸. A well-developed TMDD model can help predict drug-related (e.g., drug elimination and distributional rate constants) and target-related (e.g., receptor expression) parameters, and estimate the *in vivo* receptor occupancy^{56, 58}.

Physiology-based pharmacokinetic (PBPK)

models - PBPK models describe physiological characteristics such as lymph flow, organ distribution, FcRn binding, and relationships with serum and tissue. In PBPK models, physiological parameters and the ADME data are integrated to represent a quantitative framework for mechanistic translation across species. This approach provides a starting point to evaluate the impact of drug-dependent properties and system-dependent properties on human PK profiles of biotherapeutics^{56, 59-61}. Although it was first developed for chemical therapeutics, PBPK models have been extensively applied to biotherapeutics⁵⁹⁻⁶¹.

Summary

Drugability of biotherapeutics is an integral concept in drug discovery. It involves the analyzation of a biological drugs' structural and physicochemical properties, biological activity, pharmacokinetics and toxicity. Drugability assessment studies facilitate our understanding of biotherapeutics, predict clinical outcomes, and provide rationales for molecular optimization. Nevertheless, many studies, such as PK drivers of efficacy and toxicity, immunogenicity response, PK extrapolation to humans, and exposure–response relationships in patients *etc*, are currently in progress to gain better understanding of biotherapeutic drugability in order to ensure the manufacturability, safety, and efficacy in clinical development. With new advances in modern biotechnology, drugability assessments are the key for successful biotherapeutic commercialization.

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