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LARGE MOLECULE PK ASSAYS: OPTIMIZING BIOANALYSIS FOR PRECLINICAL THROUGH FIH

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INTRODUCTION

During drug development, a fluid transition from preclinical research to clinical trial is key for generating high-quality, decision-critical data while adhering to budget and timeline. However, bioanalytical differences in focus and scale between preclinical and clinical investigations can make this changeover a challenge.

Preclinical studies are designed to evaluate whether a compound has the potential to move from lab to bedside. During this phase, understanding the drug's safety and toxicity is the primary goal. Typically, large dosages of the compound are administered to laboratory models to generate preliminary data. Even though multiple animal models are often tested, the preclinical phase has a relatively short duration.

In contrast, the clinical trial journey is long and has a broader focus: not only safety, but also, efficacy. In order to prevent clinical incidents and minimize clinical size effects, these studies must progress stepwise from low drug dosages and small subject pools to higher dosages and greater numbers of subjects. Ultimately, a large cohort is required to make statistically valid conclusions regarding drug safety and efficacy.

Because of these differences in scale and purpose, many preclinical assays are not suitable for transfer to clinical studies. During the clinical stages, careful planning is required to select appropriate assays and prevent unnecessary roadblocks and delays.

In this article, the key parameters (e.g., assay design, matrix interference, assay sensitivity and assay range) of preclinical and clinical assays are compared. Recommendations are then provided to facilitate the transfer of assays from preclinical to clinical phases, whether that means optimizing the preclinical assay, redeveloping the assay or changing the assay platform altogether to perform as needed for clinical development.

Assay design

Preclinical studies investigate toxicity and safety. Studies assessing drug toxicity are designed to measure all of the drug administered — the total drug, whether free or bound. Therefore, in preclinical assay design, the selected capture and detection reagents must be able to recognize drug in both free and target-bound status. To achieve this, anti-target as capture and anti-human Immunoglobulin G (IgG) as detection are popular assay settings for preclinical studies of humanized therapeutics such as monoclonal antibodies (mAb), fusion protein, antibody-drug conjugates (ADC) and bispecific antibodies.

Clinical trials, while continuing to monitor safety, are particularly focused on investigating the correlation between drug concentrations and pharmacological effects. For efficiency, a single pharmacokinetic (PK) assay is built to evaluate both efficacy and safety in the clinical trial setting. In these cases, free drug level is preferred over total drug for investigating target engagement and efficacy but can also be used for safety assessment.

Anti-idiotype (anti-ID) mAb testing may be a good choice for designing assays to measure free drug only. Depending upon its specificity, an anti-ID mAb can help detect free drug, total drug, or bound drug (Figure 1). Note that, as anti-ID generation takes 4-6 months, forethought is required to prevent delays by ensuring project timelines reflect development of such methods.

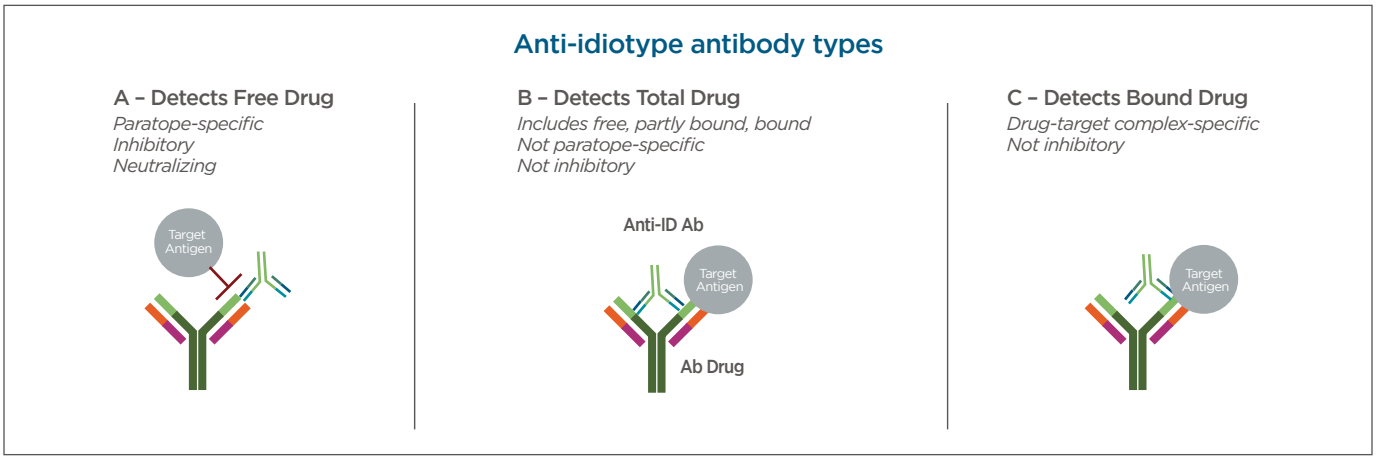

Figure 1. Anti-idiotype antibodies can be configured to target free, total, or bound drug. (A) Antigen blocking anti-ID binds specifically to the antibody drug's antigen binding site (paratope) and competes directly with the target antigen. (B) Non-blocking anti-ID binds to an idiotype on the antibody drug that is completely outside the antigen binding site, allowing both drug and target antigen to bind noncompetitively. (C) Complex-specific anti-ID only binds to an antibody drug-target antigen complex. Adapted from 'What is an Anti-Idiotypic Antibody?' [1]

Matrix interference

In preclinical studies, multiple animal models (e.g., rat, mouse, cynomolgus monkey) are typically applied for studying drug potentials. At this stage, drug developers are still uncertain about the prospects for their compounds, so fast turnaround and low cost are critical. To avoid investment in separate methods for each animal in the study, developers choose preclinical PK assays that can analyze drug concentrations in a variety of species. At the same time, these assays must be designed to tolerate interference from the various animal matrices. A convenient assay detection reagent to use for humanized drug candidates in this case is commercial anti-human IgG. Since animals do not produce human immunoglobulins, matrix interference from native antibodies will be low.

This approach often does not work for analyzing clinical samples, however. Clinical samples (e.g., serum, plasma or whole blood) contain significant amounts of natural, heterophilic immunoglobins which bind and saturate anti-human IgG. Thus, the commercial anti-human IgG will produce a measured result high in background noise. In such cases, anti-ID mAb may be required for assay detection in assay development. As they are designed to be specific for the drug being investigated, these reagents will not cross-react with naturally occurring human immunoglobulins present in patient samples.

Some drugs impact endogenous target or receptor expression. Target status such as circulating versus non-circulating target and target level before and after drug administration should be considered. In one paper[2], eight percent of expression profiles studied exhibited feedback loops that varied target expression over time. This PK effect can be linked to, for example, the development of drug tolerance. Anti-ID mAb are often also recommended as a capture reagent for eliminating biomatrix target interference in these studies.

The same holds true for studies in which patient volunteers' biological samples interact differently with the reagents than those of healthy volunteers. This phenomenon depends on disease type, patient parameters (e.g. gender, age), medications and so on. For instance, differences in expression of serum proteins can occur as part of a host response to infection, inflammation, tissue damage or cancer. Elevated levels of C-reactive protein (CRP) and serum amyloid A (SAA) can cause matrix interference,[3] as can heterophilic antibodies in autoimmune diseases such as irritable bowel syndrome or rheumatoid arthritis.[4] Drug-drug interactions (DDIs) may occur. The application of drug-specific mAb prevents interference by disease-specific matrices in clinical trials. Overall, the bioanalytical lab must ensure that the preclinical assay is suitable for analyzing clinical samples.

Assay sensitivity and range

Preclinical and clinical studies have contrasting requirements when it comes to assay sensitivity. The dose levels, dosing schedules and study endpoints are different. These factors determine the necessary bioanalytical sensitivities and assay ranges.

Since preclinical studies are focused on the assessment of drug safety and maximum tolerable dose, high drug dosages are used most samples contain highly concentrated drug levels. In these studies, assay sensitivity is less important than having an assay range that can cover high drug concentrations. This capability saves time and cost by avoiding sample pre-dilution steps.

In contrast, when the drug candidate moves to clinical trials, the situation changes. To minimize clinical adverse effects, these trials start with an ascending dose design from a very low level. A safety factor of at least a tenfold dilution of the safe preclinical dose is recommended by FDA[5] to accommodate prediction variation between animal models and humans. In this situation, a highly sensitive assay is needed to detect the drug concentration in samples from low dosage groups.

In general, the linear or parametric logistic regression between drug concentration and assay signal only spans 2-3 logs. An assay that can measure high concentrations will not be sensitive, and a sensitive assay will not be suitable for testing samples with high concentrations of drug. Therefore, an established preclinical assay's range is often not suitable for testing clinical samples.

Lifecycle considerations

The utility of any ligand binding assay depends on its reagents' binding properties. In study designs, ensuring that critical reagents will remain available for the duration of the investigation and will perform consistently throughout is paramount. While preclinical reagents may only be needed for a matter of months, clinical reagents must be available for many years to support all phases of clinical trials, including post-marketing.

Reagent management must be planned and begins with identification of critical reagents — whether in-house or commercial — that can't be changed without affecting assay results. Other considerations include ensuring consistent performance through resupply and lot changes, maintaining reagent stability, and thorough reagent-related documentation.[6,7]

Contrasts between preclinical and clinical PK assay requirements for biologics

	Preclinical assays	Clinical assays
Assay format	Receptor/target as capture; generic binding reagent as detection (e.g., anti-human IgG)	Specific drug binding reagent (e.g., anti-Fab mAb) MSD if greater sensitivity, simplicity or range is needed, compared with ELISA
Assay sensitivity	Flexible; sensitive assay is not required	Capable of measuring samples from low dosage groups
Assay range	Mid-high concentration range	Low-mid concentration range
Critical reagents	Generic reagents May be commercially available	Anti-ID Lead time: • 4-6 months for mAb • 2-3 months for pAb
	Short duration lifecycle	Long duration lifecycle
Timeline risks	IND delay	Dose escalation delay

Assay transfer checklist

For quick reference, here is a brief list of questions to help guide planning to ensure PK assays can smoothly transition from preclinical to FIH. In some cases, the original assay may be acceptable with optimization. In others, the assay may need to be redeveloped or switched to a completely different platform.

- Does the assay measure the drug at appropriate status — bound, free or total?
- Does the assay tolerate clinical matrix? (Anti-human IgG will cross-react with clinical matrix.)
- Do the assay sensitivity and range meet clinical requirements?
- Does the assay tolerate target interference?
- When disease-specific matrix is tested, does assay selectivity pass?
- Should Anti-ID be generated? How long will it take?
- Does assay robustness satisfy long term clinical trial requirements?
- Should automation be considered for high-volume sample analysis?

A smooth PK assay transition

In a clinical trial landscape with increasingly complex studies, a smooth transition from preclinical to clinical stages is crucial to obtain high-quality, actionable data within budget and timeline. However, the requirements for clinical PK assays are different, so many preclinical assays are unsuitable.

Early, holistic consideration of assay design, matrix interference, assay sensitivity, assay range and reagent lifecycle management enables drug developers to optimize trial data delivery while avoiding unnecessary delays during clinical development. Whether sponsors need to optimize an existing biologics PK assay, redevelop an assay or find an alternate assay method, Worldwide's industry-leading bioanalysis experts and powerful bioanalytical tools can help.

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