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Bridging Preclinical and Clinical Assays for Biologics Development - Challenges and Consideration for Phase I Trials

At the WRIB 14th Annual Conference, WuXi AppTec hosted a roundtable discussion around some of the challenges and considerations for Phase 1 trials and the importance of bridging pre-clinical and clinical assays for biologics. Here, we've highlighted key points and takeaways from that discussion.

The biologic drug development life cycle is complex, as is the bioanalytical program that supports the biologic drug. As shown in figure 1, the method complexity and regulatory expectations increase as the drug moves along in the development life cycle.

Some key differences in bioanalytical testing requirements between pre-clinical and clinical development of biologics are highlighted in the table 1 below.

Notably, in toxicology studies, the preclinical models are dosed with large amounts of drug. As a result, we expect higher drug concentrations in the samples. However, the course of early clinical trials focuses on establishing tolerability and dose-finding, among other goals. Therefore, dosing starts at very low levels, but escalates. Given that, the PK assay requires a Lower Limit of Quantification (LLOQ).

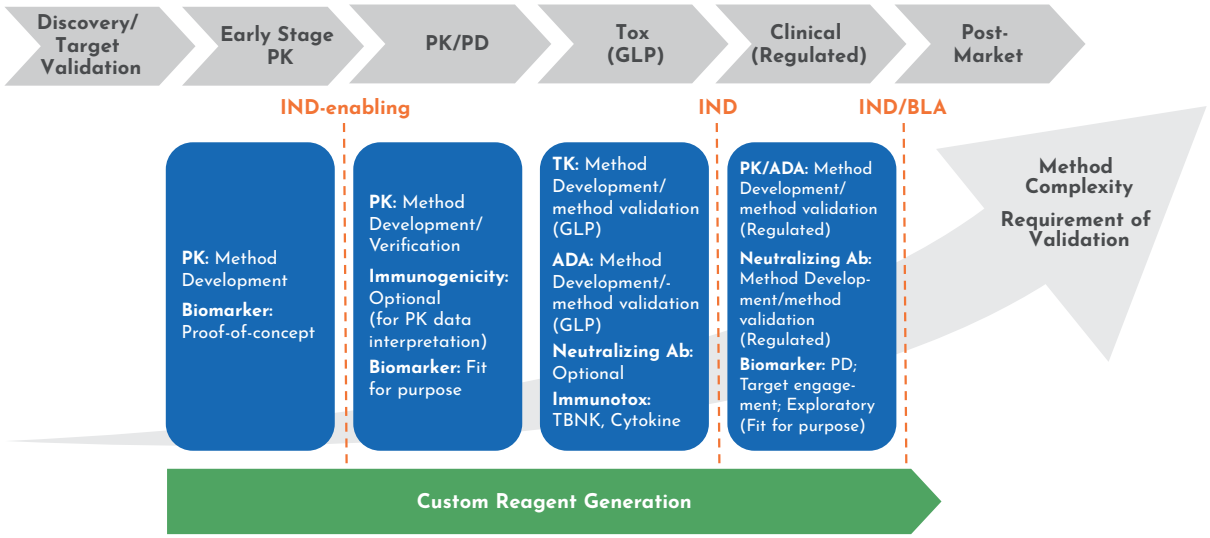

Fig. 1: Representative bioanalytical requirements for biologics development

Prior to the IND-enabling package, there is no requirement for bioanalytical method validation because the Pharmacokinetics (PK)/ Pharmacodynamics (PD) and immunogenicity assessment are running in non-GLP mode. However, when IND enabling studies commence, requirements, specifically method qualification or verification, for animal PK/PD assays increase. For toxicology studies, which are run under GLP, toxicokinetic (TK) and anti-drug antibodies (ADA) methods also require full validation.

When the program advances to the clinical stage, the PK and the ADA methods will require greater sensitivity and full validation. If there are any ADA confirmed positive samples, regulatory agencies expect the development of a neutralizing antibody (NAb) assay to determine whether an ADA-positive sample also has neutralizing activity. Depending on the context of use for certain biomarkers, (including decision-making, PD, target engagement or safety biomarkers) they may also need to be fully validated.

In pre-clinical toxicology studies, normally we measure total drug for toxicity evaluation, while in clinical studies, we are more interested in the free drug for PK assessment. In some programs, we are also interested in the ratio between the free and the bound format of the drug and the correlation with efficacy and/or safety.

In terms of immunogenicity for preclinical toxicology studies, usually the anti-whole drug antibodies need to be measured and no NAb assay is required, except for some high-risk programs. For clinical stages, if your drug has multiple functional domains, we design one whole drug screening assay, followed by confirmation and domain-binding specificity characterization. NAb assays are also required at this stage as part of immunogenicity assessment.

Key Considerations

Pre-clinical	*Clinical*	*Other Considerations*
Study design: • High dose level, high drug concentration in samples • Total drug • Immunogenicity: anti-whole drug antibody; usually NAb not required **Reagent readiness:** • Often no specific reagents available • Using target/generic anti-human Fc for PK • Prepare PC for ADA	**Study design:** • Lower dose level requiring low LLOQ of the PK assay • Free drug and/or total drug • Immunogenicity: ADA – domain specific confirmation; NAb required **Reagent readiness:** • Prepare anti-ID mAbs for PK • Multiple PC for ADA & NAb	• Drug stability • Hemolytic or lipemic • Diseased vs. healthy population • Interference

Table 1. Key considerations for pre-clinical and clinical assays

Depending on the stage of drug development, but most often at preclinical stages, specific reagents are not available. Therefore, the TK assay for toxicology studies use the target antigen or even generic anti-human Fc as a capture reagent and the generic anti-human Fc for detection in a human antibody program. The positive control (PC) for ADA assay requires a specific preparation and usually takes about six to eight weeks to generate polyclonal antibody reagents. When the program advances to the clinical stage, it is highly recommended to prepare anti-idiotype (ID) monoclonal antibodies to build your PK assay.

Meanwhile, for molecules with multiple function domains, it is expected to measure domain-specific ADAs, which may require domain-specific PCs for ADA and NAb assays.

In addition, there are many other considerations for biologic development. There may be a need to determine a longer sample stability, especially if samples are stored for a long period before analysis. Hemolytic or lipemic samples require the determination of their potential interference in clinical studies. It is also important to determine any interference between drug and ADA by the target.

When programs advance from pre-clinical to clinical, what aspects of clinical design do we need to understand in order to better plan for clinical bioanalysis?

Jon Wojciak

In my view, one of the most important aspects of the clinical design is to understand the dose levels and the dose schedule. Understanding the study endpoints is also important for determining assay sensitivity and expected C-max and C-trough levels for ADA assay development. It is also important to understand what's needed from a clinical pharmacology standpoint in order to advance the program from phase one and beyond. Dr. Wang from FDA discussed the importance of a holistic approach for communicating your bioassay strategy. It starts in the pre-clinical stage and involves open communication amongst clinical pharmacologists and clinicians.

Linglong Zou

When switching from pre-clinical to clinical for the first clinical assay, we need to look at the dose and sample collection time points. It is also important to understand the type of population used in the study, that is, healthy population versus patient population. If it is for a specific patient population, collecting the right matrix for the assay validation can be time-consuming. You will need to prepare these matrix samples ahead of time. And of course, it is also important to prepare reagents earlier for the clinical assays. It is fairly common in the pre-clinical stage to use the generic assay for a humanized or human antibody, but when you move to clinical stage, you will need to use more antigen-specific assays.

Do we have a sense of how long it takes and when should we start with reagents? When should we start planning for a clinical bioanalysis program?

Jing Shi

Generating positive controls for ADA assays requires characterization of the antibodies that you have obtained and the polyclonal antibodies takes about six to eight weeks. For the anti-ID antibodies, it can take a lot longer, between four to six months. Following a successful preparation, you need to conduct antibody pairing to pick up the best clones, which can be used to develop for your PK assay. Therefore, it is important to start planning for clinical bioanalytical assays at least six to nine months ahead of the clinical program.

Linglong Zou

I agree with the above timeline for preparing and generating the required reagents. For the anti-ID antibody preparation, in my experience, it takes around six or seven months and four to five months for polyclonal antibody preparation and reagent characterization.

From a project management perspective, what is the optimal timing for planning the clinical bioanalytical assays?

Jon Wojciak

I've worked for a couple companies where they have brought their first technology to the clinic and I've seen both sides of it. I've worked with some companies where they've had a good appreciation of the time it takes to develop and characterize these critical reagents and get these stage appropriate assays in place. And I also have experience working on programs where there was no money in the development budget for bioanalytical, and no timeline. I think it's absolutely critical to start generating these reagents early.

As Jing had presented in her slide, the custom reagents should ideally start in research, because you need to develop these stage appropriate processes for the manufacturer, for the release assays, and for various stability protocols and SOPs. The sponsor needs them in place in order to make a smooth transition from pre-clinical to clinical and evaluate whether or not these reagents are valuable and are going to work going forward. There needs to be a really strong appreciation, especially from the sponsor side early on, of what it takes to really develop these robust assays and to be able to leverage what you have learned from the pre-clinical and then apply that to the clinical.

Linglong Zou

We learn about the analyte in the pre-clinical phase to improve PK assays and immunogenicity assessment. Sometime we even need to measure the target using a PD assay. For PK assays, we either measure the total drug or free drug, each assay's design can require very different set of reagents. This is especially important for bispecific antibody programs. Whether your assay targets one-arm or two arms, different assays require a different combination of assay reagents. I mainly work in large pharma or biotech companies and we rarely generate those reagents internally, unless it's the target/ligand. If we want to generate the antibody reagents, we normally contract to an outside service provider. It is crucial to prepare a robust plan and budget accordingly.

What are the advantages of using the same bioanalytical labs to support pre-clinical and clinical assays for the same programs?

Jing Shi

From a CRO perspective, there are certain advantages in terms of continuity of knowledge about the molecule itself and the performance of the assays. Although we laid out an ideal situation where you would be generating specific reagents to prepare for the clinical assays, there may be cases where you can use the same format for the pre-clinical assay in the clinical study. The pre-clinical bioanalytical lab may already have insights into the assay's performance in human matrix using anti-target as capture and generic anti-human Fc as detection. If the same group that supports the pre-clinical method validation and sample analysis can also support the clinical development, there is no need to transfer the method. The free exchange of knowledge and techniques within the same lab allows them to retain all the minor details of the assays that are not always fully captured in the written method or SOP. From timeline and cost management perspectives, the sponsor can save a lot of time in assay transfer and related cost as well.

Linglong Zou

As a sponsor, communication is crucial. To prevent any communication gap, a single bioanalytical lab that can facilitate both pre-clinical and clinical development will make the program much easier and it can also help shorten the timeline. As Dr. Shi discussed, if the bioanalytical laboratory is already involved in the pre-clinical phase, they already have a good understanding of your program and the assay design.

Jon Wojciak

I think there are advantages to using the same laboratory for both pre-clinical and clinical development. I have never used two different laboratories which would require a method transfer. There may be some advantages from an ADC perspective of having the large molecule and small molecule lab in the same laboratory as it offers a logistical convenience.

When to perform an ADA assay? What characterization is required for the positive control and what is the optimal strategy for purification?

Jing Shi

For the ADA positive control, the preparation process involves two purification steps. A drug conjugated column is used to obtain the antibodies binding to the drug. The next step is to perform a negative depletion to get rid of the antibodies that are binding to the Fc portion of your molecule for monoclonal antibody drugs. For assay development, the affinity of the positive control antibody needs further characterization in addition to identity, purity and concentration of the antibody preparation.

Linglong Zou

We prepare the positive control antibodies for use in both pre-clinical and the clinical phases of development. Moreover, as Dr. Shi mentioned they're typically run through a purification step, firstly, affinity purification, followed by negative depletion with a frame-work sequence. If negative depletion is not used, there is a risk of retaining cross-reactive antibodies against the frame-work sequence of antibody therapeutics.

It is therefore very important to use Fab fragments to do a negative depletion and absorb these cross-reactive antibodies. Otherwise, during the clinical phase, you may find the assay sensitivity is not optimal.

For reagent characterization, at least you must know the concentration, purity and ideally, conduct affinity characterization. In our experience filling with EMA, the regulators wanted to know the isotype of the positive control antibody. This is in addition to concentration, purity and affinity.

Jon Wojciak

Affinity purification is important and it is critical to try to enrich for the anti idiotype binders. Developing really good PCs and anti-IDs, is one of the major bottlenecks and underappreciated efforts from sponsors.

Panelists

Linglong Zou, PhD

Global Head of Bioanalytical and Translational Sciences, Shanghai Henlius Biotech, Inc.

Dr. Zou is responsible for bioanalytical sciences and biomarker analysis at Shanghai Henlius Biotech Inc. Graduated with PhD from Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Dr. Zou received postdoctoral training at Baylor College of Medicine, US. He subsequently worked in several US-based biotech and pharmaceutical companies, including Tanox (Genentech) Inc. and Teva Pharmaceuticals. Throughout his career he has been involved in PK/PD bioanalysis and immunogenicity assessment of several dozen biologics in various phases, six of which were approved for marketing in U.S. and/or EU and two in China, which are HLX01 (rituximab biosimilar), the first biosimilar ever developed in China, and HLX02 (trastuzumab biosimilar). Recently, HLX02 also received EU marketing approval.

Jon Wojciak, PhD

Associate Director, Tanabe Research Laboratories U.S.A., Inc.

Dr. Wojciak has over 13 years of experience discovering and developing therapeutic antibodies and antibody-drug conjugates (ADCs). At TRL, Jon is a member of the CMC and Bioanalytical Development teams and leader of the Protein Chemistry Research group, where his roles have been to support lead molecule selection, GMP manufacturing and preclinical IND-enabling studies. He recently has been managing the bioanalytical activities for a Phase 1 FIH clinical trial of a novel ADC investigational product. Jon received his doctorate in biochemistry and molecular biology from UCLA and completed post-doctoral research at The Scripps Research Institute in La Jolla, CA.

Jing Shi, PhD

Executive Director and Global Head of Large Molecule Bioanalysis, WuXi AppTec

Dr. Shi joined WuXi AppTec in 2014 and leads the Immunochemistry Bioanalytical department, managing multisite operations. Her team provides bioanalytical method development, validation and sample testing services under GLP/GCP. Dr. Shi previously held leadership positions with AstraZeneca and Sigma in the United States. Dr. Shi has extensive experience across various stages of drug development including cell line development, process development, toxicology, biologics drug substance/drug product characterization and preclinical/clinical bioanalytical analysis. In her most recent role with Sigma, Dr. Shi was the head of the immunoassay laboratory, responsible for GMP lot release/stability testing and GLP bioanalytical testingusing immunochemistry or cell-based platforms. Dr. Shi obtained her BS in Cell Biology and Genetics from Peking University and PhD in Cell Biology from University of Virginia.

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