New tools to enhance the antibody drug pipeline

Therapeutic antibodies are the fastest growing class of drugs, with continuous increases in the number of innovator and biosimilar drugs in development. Drug development and clinical trials necessitate the ability to specifically, sensitively and reproducibly measure the concentration and bioavailability of these drugs in pharmacokinetic assays. We discuss the rise of therapeutic antibodies in drug discovery in favour of traditional small molecules and issues around the development of these biologics, including their tracking with anti-idiotypic antibodies. We will examine new tools that are entering the market to offer solutions that can speed development timelines and address problems of reagent reproducibility.

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Biological therapeutics continue to demonstrate viability and effectiveness, along with excellent safety profiles within the clinic. Combined with an increased financial return on investment, the incentives for the pharmaceutical industry to develop these technologies as new therapies are clear. As research yields an increased number of viable disease biomarkers, developing new drugs to these targets is the next step in bringing these discoveries to application. Additionally, patents on many successful biologics currently available in the clinic are beginning to expire, including trastuzumab for breast cancer treatment[1], adalimumab for rheumatoid arthritis treatment[2] and bevacizumab for the treatment of many cancers such as colon and lung cancer[3], raising the prospect of a lucrative biosimilar market.

The rise of biological therapeutics has created a concurrent increased need for tools for their specific analysis within patient samples. It is essential to be able to monitor the level and distribution of potential therapeutics in animal models in pre-clinical studies, and in patients throughout clinical trials. However, high levels of endogenous antibodies are present within the serum, sometimes in up to a million-fold excess[4], potentially masking the biotherapeutic molecule. Consequently, highly-specific and sensitive reagents are key for enabling the specific detection and accurate quantification of the therapeutic antibody within patient serum samples.

Anti-idiotypic tools and essential characteristics

Anti-idiotypic reagents (anti-ID), including antigens, antibodies and anti-ID antibody mimetics, are the specific tools that enable monitoring of therapeutic antibody levels and their distribution within pharmacokinetic (PK) assays. An idiotope is a specific set of unique antigenic determinants, typically found in the variable portion of an antibody, which defines that particular antibody in contrast to all other antibodies. An anti-ID antibody can specifically bind to the idiotope of the target antibody, such as a therapeutic antibody, to act as a capture or detection reagent in PK assays.

Bioanalytical scientists can generate a variety of data through use of anti-ID antibodies, such as quantification of bound drug, free antibody, or total drug levels. Developing such highly-specific anti-ID reagents can be challenging, and development of anti-ID antibodies is often time-consuming, with poor success rates[5-8]. Additionally, the

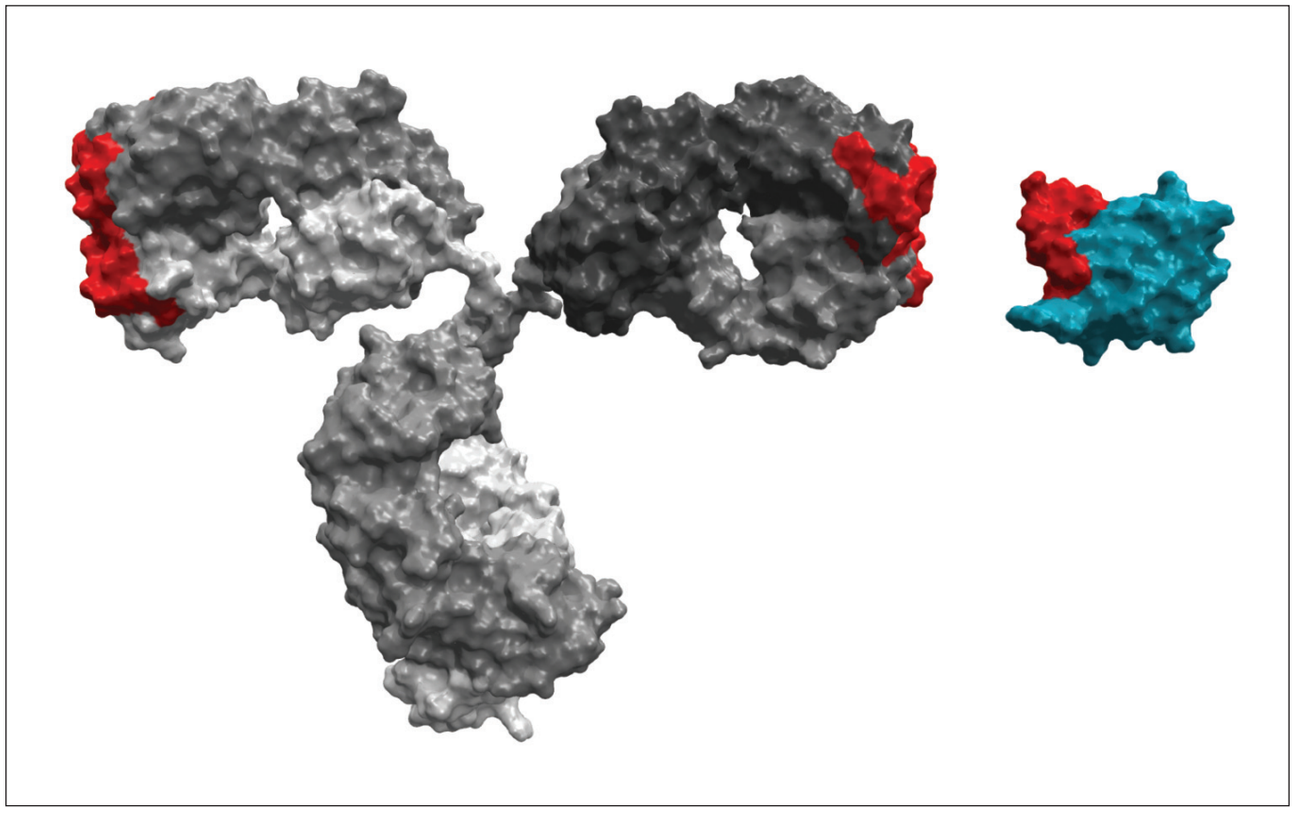

Figure 1
A variety of anti-idiotypic reagents have been developed to specifically function within pharmacokinetic assays. Ranging from standard monoclonal antibodies to antibody alternative protein scaffolds, they offer a range of different benefits

sensitivity achieved from screens of naïve recombinant antibodies is often insufficient, requiring an additional affinity maturation step which adds to reagent costs and extends development timelines[9,10]. Rapid development times are a key consideration for anti-ID reagents, as the drug development process relies on the availability of suitable bioanalytical reagents to develop and validate PK assays.

With any anti-ID reagent, ensuring consistent performance and supply involves one of two options: obtaining small batches of the reagent and carrying out multiple batch-to-batch standardisation processes over the course of drug development; or obtaining larger reagent batches, requiring fewer batch-to-batch standardisation processes, but with consideration of the shelf-life and stability of the reagent[5]. Having a reliable source of critical reagents that can be produced consistently at scale is therefore a benefit.

In addition to shelf-life stability, selected PK assay reagents must also show functional stability across a range of critical ligand binding assay conditions. Many biological molecules are sensitive to variations in assay conditions, such as pH and temperature, causing variability in performance and potentially resulting in erroneous assay results. It is essential that any selected PK assay reagent shows consistent high target affinity, specificity and sensitivity across the desired assay range in the presence of biological components from patient samples when used within the specific assay containers, without showing matrix effects[5-8].

Regulatory background for critical reagents

From a regulatory perspective, it is clear that anti-ID reagents must fulfil high quality standards for use in clinical settings[5-7]. In addition to consistent batch-to-batch control, high specificity, sensitivity, and reproducibility in assay performance are key attributes as they may be used for several years over the lifetime of the drug evaluation process. It is therefore crucial that a reagent continues to perform to the same standards throughout the drug development lifecycle[8,11,12].

There has been recognition from the industry that all ligand-binding assays are dependent upon the critical reagents used within the assays, with the essential assay features of specificity, selectivity and sensitivity all being determined by the affinity reagent used[5]. Extensive established methods exist for the analytical validation of critical

reagents for use in PK assays, with the FDA, OECD and Clinical and Laboratory Standards Institute guidelines all emphasising the need to characterise reagents appropriately and providing guidance on key characteristics[13-15]. Consequently, a standard battery of bioanalytical assays has been developed to meet regulatory authority criteria for the approval of potential new therapeutics. While subtle differences exist between the given regulatory requirements for PK assay reagents from the various controlling regulatory authorities, all available guidance documents require demonstration of selectivity, accuracy, precision, sensitivity, stability, a lack of matrix effect and reproducibility for any specific reagent that may be incorporated into such assays[16].

Significant collaboration and communication across industry and the various health authorities has existed for more than 25 years, resulting in the generation of evidence-based regulations and recommendations governing the use of ligand-binding assays[13-15]. Until recently these recommendations have remained largely region specific, but the formation of the Global Bioanalysis Consortium in 2010 brought together scientists from various associations and different countries with the aim 'to harmonise and merge existing or emerging bioanalytical guidance to create one, unified consensus document that can be presented to the regulatory bodies/health authorities in various countries'[17].

The fundamental nature of critical reagent control to the quality and long-term performance of ligand-binding assays makes the consensus and regulation of these reagents throughout the drug lifecycle essential to safety. However, despite the global convergence on regulatory authority guidelines for the approval of critical assay reagents, there has previously been an apparent lack of guidance on their lifecycle management and stability. To address this, a panel of industry experts formed the Large Molecule L4 Harmonisation Team of the Global Bioanalysis Consortium to lay out clear recommendations and best practices for the lifecycle management of critical reagents in bioanalysis assays, for both internally-developed and commercially-available reagents[8]. These recommendations detail the management of lot changes for any critical reagent, stability management and the documentation of critical reagents within ligand-binding assays. The guidelines highlight the very real challenges presented by the lack of reproducibility in PK assay reagents and the need for new reagents that can overcome these pitfalls in lot-to-lot variability.

Available anti-idiotypic reagents

An array of anti-ID reagents has been developed to specifically identify and measure the concentration of potential biological therapeutics within PK assays (**Figure 1**). Each of these is associated with different advantages and disadvantages, with newer reagents having been developed to overcome many of the drawbacks of traditional reagents[18].

Antigens

The capture of a potential therapeutic agent from a patient sample within a PK assay was traditionally achieved using the original protein or biological antigen to specifically bind the antibody therapeutic. This method of capture can offer specificity for the target antibody so as to allow subsequent quantification as part of a PK assay, though matrix effects are often observed with human serum samples, complicating the interpretation of any results.

By design, capturing the antibody therapeutic via its antigen only allows for the capture of free antibody molecules, not antibody-target complex, preventing full interpretation of the therapeutic antibody's metabolism. Another significant drawback of the use of antigen as anti-ID reagents within PK assays can be the availability of sufficient quantities of the antigen at acceptable cost. Even with the use of recombinant antigens, supply issues may remain, due to the inherent variability of different antigen proteins[19].

Monoclonal antibodies

Monoclonal antibodies can offer the benefits of high target affinity and specificity for use as PK assay reagents. Furthermore, they can be developed to recognise both bound and free forms of any therapeutic antibody, thus allowing the full repertoire of therapeutic biologic to be analysed within any patient sample, providing additional bioanalytical information.

A number of commercial laboratories, including Genscript, Creative Biolabs and R&D Systems, have shown success with this strategy, producing monoclonal antibodies for use as PK assay reagents. Each of these companies offers custom anti-ID antibody production from a range of species, in addition to a catalogue of standard anti-ID antibodies targeting the most common antibody therapeutics, for use in the growing field of biosimilar therapeutics development. Furthermore, R&D Systems offers additional characterisation services to ensure any selected antibodies function as desired within a specific assay system, and the ability to convert any polyclonal or monoclonal hybridoma into recombinant antibodies.

Table 1: Key features comparison for non-antibody protein scaffolds

	TEMPLATE	PRODUCTION	TM	SIZE OF VARIABLE REGION (AMINO ACIDS)	SIZE OF SCAFFOLD
Affibodies (Affilogic)	Z domain of protein A	E. coli or peptide synthesis	42-71°C	13	5-6 kDa
DARPins	Natural Ankyrin repeat proteins	E. coli, up to 200mg/mL	66-89°C	18 (split across three conjugated DARPin units)	18-20 kDa
Nanobodies (Ablynx)	Single-domain HC-only antibody fragments	E. coli, up to 150mg/mL (post-translational modifications and disulphide bridges)	60-80°C	9-12	15 kDa
Affimer proteins	Human protein Stefin A and plant Cystatin	E. coli, up to 200 mg/mL	42-100°C	18	12-14 kDa

One of the major disadvantages of anti-ID antibodies is the reliance upon animals for their initial generation[20]. The selection of an appropriate anti-ID antibody relies on the probability of finding a good candidate produced as part of the animal's natural immune response. Such candidates represent only a small proportion of the antibody repertoire generated, requiring the screening of a large number of clones, with a variable rate of success. Once a promising candidate has been selected, further work is often necessary to increase the target affinity of the molecule. Consequently, long development times and associated costs can become problematic.

Beyond selection and optimisation, these large, complex molecules also present issues when it comes to production, as only costly eukaryotic cell systems can be used. Additionally, the product must be carefully monitored for changes in glycosylation and drift in expression throughout production, to ensure consistency. Furthermore, as monoclonal antibodies are highly sensitive to changes in pH and temperature, their storage and use within complex assay matrices may affect downstream performance.

Recombinant antibodies and antibody fragments

There has been a conscious shift across the industry toward recombinant antibodies, in order to overcome issues of batch-to-batch reproducibility associated with standard monoclonal antibodies[20, 21]. Benefits include the ability to manufacture using *in vitro* systems, offering increased reproducibility between batches of reagents, and the ease of returning to the starting material in the face of genetic drift of the expressing cell line.

Recombinant reagents may also be isolated from phage libraries, assuring maintenance of the structure during screening and allowing anti-ID selection to be driven by project aims and requirements, increasing the potential of identifying specific, high-affinity binders to a target antigen. Being able to specifically drive the selection of binders in this manner also ensures that recombinant antibodies can be selected to both free and bound formats of a potential therapeutic antibody, for use in a wide range of bioanalytical PK assays.

Despite the reproducibility advantages of recombinant antibodies over monoclonal antibodies, the same issues around manufacturability of these complex structures remain. One strategy to prevent these problems has been the adoption of antibody fragments in the place of full antibodies. As these structures are smaller and less complex, it is possible to produce them in prokaryotic culture systems, which are simpler and less expensive.

Prominent recombinant antibody suppliers include Bio-Rad and Creative Biolabs. Both companies are able to offer rapid initial selection of binders in up to eight weeks, due to the advantages of library screening for recombinant anti-ID binders. However, as Bio-Rad notes within its literature[22], further affinity maturation of the selected binder may be required to increase affinity and specificity for the target post-purchase, whether recombinant antibodies or antibody fragments,

which can extend development timelines and increase costs.

Antibody alternatives

Engineered affinity reagents are growing in popularity for use in research and development applications, designed to overcome the apparent limitations of antibodies. Comprising both nucleic acid and protein molecules, which adopt a defined conformation that matches a binding surface on the target, it is possible to achieve high affinity specific interactions with great specificity[18]. While many examples of affinity reagents have entered the marketplace for research and therapeutic purposes (**Table 1**), a scaffold particularly well-suited for commercial anti-ID applications is the Affimer® protein.

Affimer binders are screened and selected from phage libraries, allowing targeting towards certain antigens and the isolation of high affinity binders that specifically identify either free or bound therapeutic antibodies. As this recombinant scaffold contains no post-translational modifications or disulphide bonds, production within inexpensive prokaryotic systems is simple, and batch-to-batch reproducibility is assured, reducing regulatory issues with the incorporation of this reagent into PK assays, for use throughout a project lifecycle[23].

A significant benefit of Affimer proteins over other available anti-ID reagents appears to be the lack of requirement for affinity maturation of selected binders. A number of molecules have been developed that show excellent specificity to individual therapeutic antibodies, with complete selection and characterisation of the binders possible within just three months. These benefits would clearly offer reduced development and production costs, while maintaining the high-quality characteristics of the resulting reagent to ensure accurate and reliable bioanalytical assay results. DDW

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