charles river
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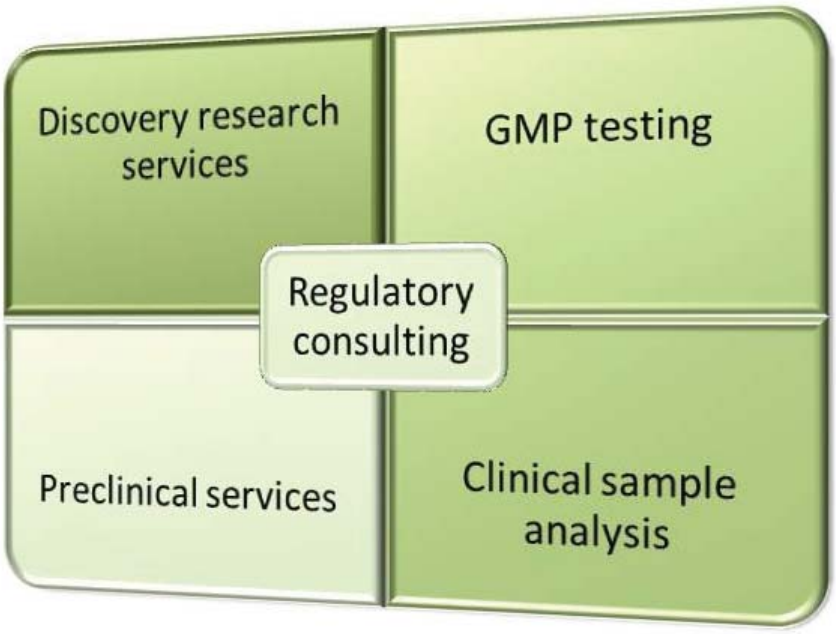
# Integrated Biosimilar Testing

## From Discovery Research Services, GMP and Preclinical Testing to Clinical Sample Analysis

Biosimilars, like their reference biologic products, are complex protein structures that are difficult to characterize. Therefore, a development program consisting of analytical and bioanalytical assays, pharmacology and/or toxicology studies in animals and clinical trials are often needed to show that biosimilars are highly similar to their reference biologic product. Production processes are highly complex, expensive and can be difficult to control, so it is essential to work with a provider that can perform the full development program while providing the control essential to product uniformity.




Biosimilars are fundamentally different from generic chemical drugs. Important differences include the size, complexity of the active substance and the nature of the manufacturing process. Because biosimilars are not exact copies of the originator products, the regulatory criteria for their approval differs from generic small molecule drugs. While standard analytical methodologies enable manufacturers of generic small molecules to show that their drugs are identical to the innovator's, which eliminates the need to re-establish safety and efficacy, the complex nature of even the most similar biological manufacturing systems makes producing identical copies of the innovative biosimilar impossible. As such, the scientific and regulatory paradigm for demonstrating that products are highly similar and that there are no clinically meaningful differences between the biosimilar product and an innovative therapeutic is necessarily much more complex than the standards set in place for generic small molecule drugs. Concerns include that undetected differences in the biosimilar product may result in reduced efficacy or different adverse reactions, especially immunological responses. Testing for the similarity between the biosimilar and innovator product is essential for the assessment of biosimilarity of follow-on biologics.

Charles River offers an integrated approach for the development of biosimilars, including regulatory consultation, discovery research services, Good Manufacturing Practice (GMP) characterization, preclinical studies and clinical sample analysis. This issue of the *Researcher* covers these services and focuses on the technical considerations necessary when developing a biosimilar.

# Integrated Service

As One Charles River, our goal is to provide the same exceptional quality of service across all of our sites and offerings. As a result, our experience and validated methods (both proprietary and client-specific) for biosimilars can be transferred between worldwide sites using cross-site validation processes. This allows for timely and comprehensive support for our clients, from discovery research services to clinical sample analysis. The basic types of testing services we offer are listed in Figure 1. Figure 2 highlights the various classes of biosimilars, including cytokines, antibodies and hormones, with which we have extensive experience. Assays that are proprietary to Charles River are listed in Figure 3.

# Discovery Research Services

The discovery research services offered by Charles River are tailored to the pharmacology and toxicology of the specific biosimilar and focus on the similarities between the innovator drug and the biosimilar. Assays that are often used include *in vitro* assays of receptor binding and function and *in vivo* animal models of efficacy. In addition, small *in vivo* pharmacokinetic (PK) and pharmacology studies are offered for the early determination of PK and efficacy parameters that can assist with the ranking of a series of biologics.

# GMP Characterization

Charles River has GMP facilities where characterization of receptor binding and function is conducted with appropriate cell lines. Potency/biological activity assays and lot release testing experience is extensive (e.g., binding assays, antibody-dependent cellular cytotoxic [ADCC] assays, complement-dependent cytotoxic [CDC] assays, antibody detection assays [ADA] and neutralizing antibody [nAb] detection assays) and is tailored to the properties of the biosimilar. Speciality assays are also routinely developed and validated via a partnership with Protagen to assess the biophysical characterization of the biosimilar compared to the innovator. Protein analysis biopharmaceutical services include identity, purity, aggregation analysis, concentration, protein sequencing, buffer composition, solubility, stability, glycosylation analysis and physiochemical properties. Note that for regulatory approval purposes, sponsors must demonstrate similarity with the reference product licensed in the given country, and it is recommended that the sponsor obtain legal advice regarding patent status.

Figure 1

| Biosimilar Testing Services |
|---|
| - Characterization of the biophysical properties and protein analysis |
| - Lot release testing |
| - Target receptor binding |
| - FcRn receptor binding for mAbs |
| - C1Q binding assays for mAbs |
| - ADCC and CDC assays |
| - *In vitro* pharmacology functional assays |
| - *In vivo* animal models of efficacy and/or toxicity |
| - Pharmacokinetic (PK), Pharmacodynamic (PD), anti-drug antibody (ADA), neutralizing antibody (nAB) integrated assessment |
| - Tissue cross-reactivity studies |
| - Speciality assays are routinely developed and validated to support specific needs to assess efficacy or toxicity |
| - Assays focus on the similarity between innovator drug and the biosimilar |

Figure 2

| Biosimilar Experience | |
|---|---|
| **Generic name** | **Indication** |
| Rituximab | Rheumatoid arthritis<br>Non-Hodgkin's Lymphoma |
| Infliximab | Crohn's Disease<br>Rheumatoid arthritis |
| Trastuzumab | Breast Cancer |
| Adalimumab | Rheumatoid arthritis |
| Erythropoietin | Anemia |
| Pegfilgastrim | Neutropenia |
| Filgastrim | Neutropenia |
| Natalizumab | Multiple Sclerosis |
| Teriparatide | Osteoporosis |
| Forsteo | Human Parathyroid Hormone |
| Adalimumab | Auto-immune disease |

# Preclinical Studies

Charles River conducts toxicology and efficacy studies in relevant animal models by various routes of administration. Our expertise covers the full range of *in vivo*, *ex vivo* and *in vitro* assessments required to determine biosimilar toxicity and efficacy, from routine hematology and clinical chemistry to specialized assays developed specifically for the biosimilars. The extent of biosimilar preclinical studies and endpoints required may vary, but should involve an assessment of similarity with the innovator drug. Charles River can design and conduct studies to compare the PK, pharmacodynamic (PD) efficacy and toxicology profiles of the biosimilar to the innovator product, including comparative immunogenicity testing.

Immunogenicity is an important issue both for biosimilar and innovator products and requires special attention, since this assessment may detect alterations in products missed by analytical methods. Immunogenicity is defined as the capacity of a substance to evoke an immune response. Every protein product has certain immunogenic potential. The immune response is typically measured by quantitation of ADA (although cell-mediated immune responses can be measured). Charles River offers the full scope of immunogenicity testing, including quantitation, titration, confirmation, isotyping and neutralizing antibody assays.

The development and validation of appropriate bioanalytical methodology for the analysis of toxicokinetic samples is routinely conducted at Charles River. Bioanalytical assays to quantitate numerous parameters (e.g., drug, anti-drug antibodies, neutralizing antibodies, biomarkers, receptor binding and functional and potency assays, such as ADCC and CDC) are developed and validated as required to support studies. Charles River toxicologists, scientists and statisticians have many years of experience designing, conducting and interpreting appropriate studies for biologics.

Tissue cross-reactivity (TCR) studies are used to assess the tissue binding profile of monoclonal antibodies (mAbs) and antibody-based therapeutic candidates in human and animal tissues from species considered for use on toxicology studies. TCR studies might also be a useful tool for comparing a biosimilar to an innovator molecule.

# Clinical Sample Analysis

Conducting discovery research, GMP and preclinical studies at Charles River – and thus obtaining valuable experience with appropriate reagents, reference products, cell lines and validated assays – eases the transition to performing assays in support of rapid clinical sample analysis. Charles River has been conducting clinical sample analysis for over 30 years in support of Phase I, II and III worldwide clinical trials. Key services for clinical sample analysis include sample kit preparation and sample management support, drug quantitation, PK analysis, immunogenicity testing, biomarkers and functional assays. Expert interpretative reports correlate these data sets, providing on-time quality data to support clinical trials.

# Regulatory Consultation

A number of regulatory authorities and organizations have issued guidelines pertaining to biosimilar drug development. These include the U.S. Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research (FDA CDER and CBER); the European Medicines Agency (EMA); the Japanese Ministry of Health, Labour and Welfare (MHLW); and the World Health Organization (WHO).

Scientists from Charles River Navigator Services collaborate with the sponsor and relevant experts within Charles River to ensure that preclinical programs are appropriate from a scientific and regulatory perspective, lay out translational plans and prepare for regulatory dialogue with the FDA. In addition, should a client need an entire program for their biosimilar drug development or only some components, client portfolio manager involvement at an early stage of discussions allows seamless integrated services.

Figure 3

**Validation – Methods**

| Compound | Assay |
|---|---|
| IFNα 2b | Proliferation Assay |
| IFNβ 1a | Proliferation Assay |
| IFNβ 1b | Proliferation Assay |
| G-CSF | Proliferation Assay |
| GM-CSF | Proliferation Assay |

**Development - Other Methods**

| Compound | Assay |
|---|---|
| PTH and modified PTH | Cell-based assay with cAMP ELISA |
| PTH and modified PTH | Homogenous time-resolved fluorescence cAMP Assay |
| EPO and modified EPO | Proliferation Assay |

**Development - Biosimilar mABs**

| Antibody | Target | Assay |
|---|---|---|
| Infliximab / Adalimumab | Inhibition of TNFα signaling | Proliferation Assay |
| Rituximab | CD20 | Binding Assay (based on flow cytometry) |
| Rituximab | CD20 | ADCC Assay |
| Rituximab | CD20 | CDC Assay |
| Bevacizumab | VEGF | Neutralization Assay |
| Trastuzumab | HER2, ErbB2 | Proliferation Assay |
| Trastuzumab | HER2, ErbB2 | ADCC Assay |
| Cetuximab | EGF-R | Proliferation Assay |
| Natalizumab | α4-integrin | Binding Assay (based on flow cytometry) |

For additional information, please visit The Source℠, a secure portal that provides registered users with direct access to the technical, scientific and educational resources available from Charles River. To register, please visit **www.criver.com/thesource**



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