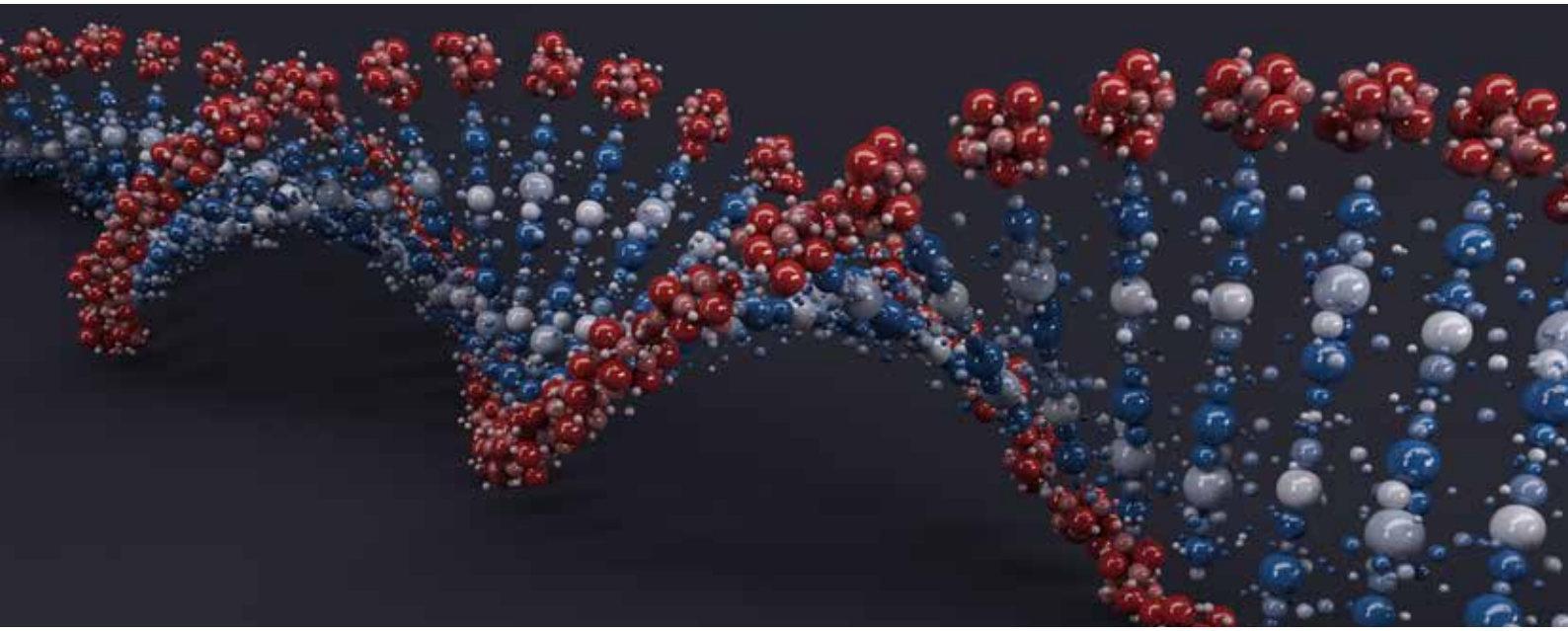


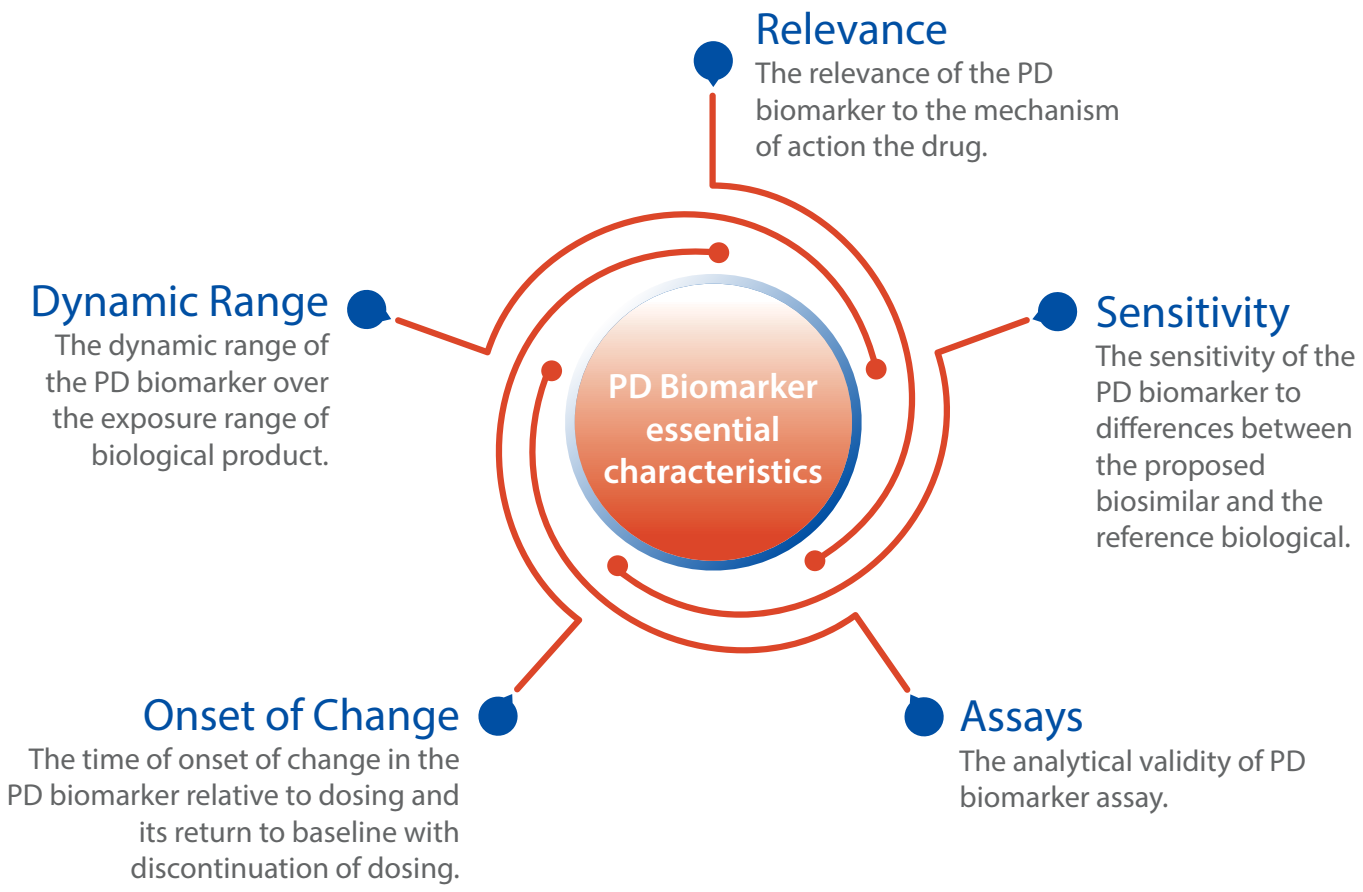
Accelerating the regulatory approval process of biosimilars by leveraging precise and reliable PK data and PD biomarker data.

The regulatory approval of biosimilars is an exacting process. Unlike generic small molecule drugs, biosimilars face a more intricate regulatory landscape. The intricate nature of biologics, coupled with the inherent variability of living organisms, demands extensive analytical and clinical data to establish similarity and demonstrate safety and efficacy. Regulatory agencies require comprehensive comparative studies to assess critical parameters such as structure, function, purity, and potency, ensuring that biosimilars are as close as possible to the reference biologic.

According to FDAs biosimilar guidance documents [1,2] the approval of biosimilars can be done based on the Pharmacokinetics (PK) and Pharmacodynamics (PD) similarity studies without an extensive comparative phase 3 clinical study in patients. Relying on pharmacokinetic (PK) and pharmacodynamic (PD) data offers the advantage of conducting shorter and more cost-effective clinical studies, often involving healthy individuals. PD biomarkers serve as indicators, revealing a drug's impact on its intended target or targets. A prime example is when the target is a receptor molecule that triggers a complex signaling process. Alterations in the levels or modifications of proteins within this signaling cascade can be recognized as pharmacodynamic responses. Consequently, these proteins hold the potential to be deemed PD biomarkers, playing a crucial role in establishing the biosimilarity of a drug. Moreover, the commitment letter for Biosimilar User Fee Amendments, also known as BsUFA III, explicitly emphasizes the pivotal role of expanding PD biomarker utilization within the regulatory science pilot program [3].

The PD markers commonly utilized for biosimilars consist of biomarkers that measure the connection between the biosimilar and its intended target. These biomarkers directly assess receptor occupancy, enzyme inhibition, or changes in specific signalling pathways linked to the biosimilar's mode of action. The choice of PD markers for biosimilars is influenced by the mechanism of action and the relevant biological pathways related to the reference biologic. Figure 1 illustrates the key attributes of the PD biomarkers.

Figure 1: Characteristics of PD Biomarkers:



The precise structure and prerequisites of PK/PD clinical trials for biosimilars can differ based on regulatory guidelines and the biologic being developed as a biosimilar. The careful selection and validation of suitable PD markers play a vital role in establishing biosimilarity and gaining approval for biosimilars. The FDA is currently assessing the possibility of replacing the conventional comparative phase III confirmatory study with PD biomarker data. Additionally, the FDA is encouraging biopharmaceutical companies to generate comparative PK and PD data for both the biosimilar and reference biologics.

Biopharmaceutical companies have the option to outsource the task of generating precise and dependable PK data and PD biomarker data to external service providers to establish the similarity of their anticipated product to the reference molecule. However, it is crucial to carefully consider the criteria for selecting a Contract Research Organization (CRO) to support the development of biosimilars. The chosen CRO should possess state-of-the-art laboratories equipped with advanced technologies for conducting biochemical assays and demonstrate expertise in biomarker analysis. An experienced CRO can expedite the designing and facilitating clinical PK-PD bioanalysis, resulting in the development of high-quality biosimilars at a significantly reduced cost.

Intox, the wholly owned subsidiary of Aragen life sciences, has established state-of-ate-art Bioanalytical Laboratory, for designing, developing and execution of biological assays allowing quick and precise screening for PK and PD response in specimens from preclinical models and early phase clinical trial studies. The scientific experts from the lab are proficient in the creation and validation of cell-based and macromolecular (DNA, RNA, protein) PD assays, crucial in supporting the evaluation of novel drugs during the early-phase of clinical trials. These assays accurately quantify biomarkers found in a variety of tissues, including tumor biopsies to blood samples. Intox follows the validated Pharmacodynamic Assay Development Pathway as designed by National Cancer Institute (Table 2).

Table 2: Pharmacodynamic Assay Development Pathway

Steps for Assay Development	1. Concept	2. Feasibility and development	3. Preparation for clinic	4. Launch
	1. Identify drug target and associated biomarkers. 2. Assess clinical application. 3.Develop assay prototype.	1. Develop standards. 2. Select optimal platform. 3. Perform analytical validation. 4. Develop assay related SOP's.	1. Conduct preclinical modelling. 2. Transfer assay between laboratories. 3. Conduct clinical validation.	1. Release to scientific community (SOP's, Training, Reagents) 2. Provide clinical trial support.
Goals during Assay Development	Develop analytically validated assays, Preclinical modelling of drug to target relationship, demonstrate drug modulates target, Internal assay transfer & clinical specimen testing, Transfer assay & train clinical laboratories			
Key Data Captured	Detect biomarker accurately & specifically, Validated assay has potential clinical utility, Biomarker reflects drug modulation of target, Preclinical PK/PD correlation, Preclinical data to inform selection of clinical dose & schedule			

Intox provides a range of potential pharmacodynamic (PD) biomarkers that can be considered for establishing biosimilarity of the molecules. Below is the list categorized by therapeutic area:

Oncology

1. Biomarkers of cell proliferation (Ki-67)
2. Biomarkers of apoptosis (cleaved caspase 3)
3. Angiogenesis biomarkers (VEGF, angiopoietin)

Immunology and Inflammatory Diseases

1. Cytokine levels (TNF-alpha, IL-1, IL-6)
2. Acute-phase reactants (CRP, erythrocyte sedimentation rate)
3. Cellular markers (CD4/CD8 ratio, T-cell subsets)
4. Inflammatory markers (C-reactive protein, interleukin levels)

Rheumatology

1. Joint swelling and tenderness assessments
2. Serum autoantibody levels (rheumatoid factor, anti-cyclic citrullinated peptide)
3. Biomarkers of bone erosion (RANKL, OPG)
4. Functional assessment (Health Assessment Questionnaire Disability Index)

Hematology

1. Hematological parameters (complete blood count, platelet count)
2. Biomarkers of clotting function (prothrombin time, activated partial thromboplastin time)
3. Biomarkers of coagulation factors (factor VIII, factor IX)
4. Biomarkers of iron metabolism (serum ferritin, transferrin saturation)

Neurology

1. Biomarkers of neuronal damage (neurofilament light chain, tau protein)
2. Neurotransmitter levels (dopamine, serotonin, glutamate)
3. Functional assessments (Timed Up and Go test, Mini-Mental State Examination)

Cardiology

1. Biomarkers of cardiac injury (troponin, creatine kinase-MB)
2. Biomarkers of myocardial stress (BNP, NT-proBNP)
3. Lipid profiles (LDL cholesterol, HDL cholesterol)
4. Blood pressure measurements
5. Echocardiography parameters (ejection fraction, fractional shortening)

It is crucial to emphasize that when choosing PD biomarkers for biosimilars, careful consideration should be given to the specific therapeutic area, disease indication, and the clinical endpoints established for the reference biologic. Regulatory authorities commonly offer guidance regarding the selection and validation of PD biomarkers to facilitate the demonstration of biosimilarity.

Typical Pharmacokinetic (PK), pharmacodynamic (PD) and Switching and Interchangeability studies that can be performed at Intox to establish the similarity between the biosimilar and the reference biologic.

Pharmacokinetic (PK) Studies:

1. **Single-dose PK:** This study assesses the PK profile of the biosimilar and the reference biologic after a single administration. It measures drug concentration levels in blood or other relevant tissues over time to determine the drug's absorption, distribution, metabolism, and elimination characteristics.
2. **Multiple-dose PK:** This study evaluates the PK profile of the biosimilar and the reference biologic after multiple administrations. It assesses drug accumulation, steady-state concentrations, and any potential drug interactions.
3. **Bioavailability and bioequivalence:** These studies compare the bioavailability of the biosimilar with the reference biologic to ensure similar systemic exposure. Bioequivalence studies establish that the biosimilar and reference product have comparable PK profiles.
4. **Immunogenicity:** PK studies also include assessments of immunogenicity, which measure the immune response to the biosimilar and the reference biologic. These studies determine the presence of anti-drug antibodies (ADAs) and their potential impact on PK parameters.

Pharmacodynamic (PD) Studies:

1. **Biological activity:** PD studies evaluate the similarity of the biosimilar's biological effect compared to the reference biologic. They assess the drug's target engagement, downstream effects on relevant pathways, and relevant biomarkers associated with the therapeutic mechanism of action.
2. **Clinical endpoints:** Depending on the therapeutic area, clinical endpoints specific to the disease indication may be measured. These endpoints could include disease-specific measures, symptom improvement, or overall patient outcomes.

Switching and interchangeability studies:

1. Switching studies assess the impact of transitioning patients from the reference biologic to the biosimilar or vice versa. They evaluate whether any differences in PK, PD, safety, or immunogenicity arise when patients switch between the two products.
2. Interchangeability studies go a step further by determining if the biosimilar can be used interchangeably with the reference biologic without compromising safety or efficacy. These studies are conducted according to regulatory guidelines specific to each jurisdiction.

Few biosimilar approvals have relied on clinical pharmacology studies, comparing PK and PD data, rather than conducting large clinical trials with efficacy endpoints. These approved biosimilars utilized established and sensitive PD biomarkers that are closely linked to the reference product's known pharmacology. These biomarkers have demonstrated a strong correlation or acted as surrogates for clinical outcomes. However, it is not mandatory for a PD biomarker in biosimilar development to have a direct association with clinical outcomes.

The use of PD biomarkers in biosimilar development aims to establish similarity rather than independently verify the safety and effectiveness of a biosimilar product. Therefore, the considerations for PD biomarkers used to support biosimilarity differ from those for new drug approvals. While having a correlation between the PD biomarker and clinical outcomes can be advantageous, it is not mandatory. PD biomarkers that reflect the biological product's mechanism of action offer heightened sensitivity in detecting meaningful differences between two products. This opens doors for biomarkers that were previously secondary or exploratory endpoints to assume crucial roles in biosimilar development programs. Additionally, if information on a suitable PD biomarker is lacking, there is an opportunity to discover new PD biomarkers using innovative methodologies.

References:

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2. US Food and Drug Administration. FDA Guidance: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product (2016).
3. Biosimilar biological product reauthorization performance goals and procedures fiscal years 2023 through 2027. (https://www.fda.gov/media/152279/download)