Bioanalytical and Early Stage Clinical Strategies for Biosimilars

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Nonclinical and Clinical Assay Development for Biosimilars – Starting from Scratch!

- **Bioanalytical assay for PK analysis (5 – 8 months)**
  - Reagent preparation (3 – 4 months) – May have reagents from release testing
  - Method development and validation (2 – 4 months)
- **Immunogenicity testing assay (6 – 9 months)**
  - Reagent preparation (3 – 4 months) – may have reagents from release testing
  - Method development and validation (3 – 5 months)
- **Cell-based assays for functional activity (11 – 15 months)**
  - Selection of the assay procedure (1 month)
  - Breading of cell lines and feasibility study (2 – 3 months)
  - Final selection of cell line and reference antibodies (2 – 3 months)
  - Optimization and final development of assay (4 – 6 months)
  - GLP validation (2 months)
  - If reagents and cell lines are available you could save up to 6 months

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Large Molecule Bioanalytical Assay Considerations

Big Challenge: Variability of the assay should be minimized! There is a lot of variability between manufacturing batches of reference and biosimilar products and between the reference and biosimilar

Variability + variability + variability = meaningless data

When you are developing the bioanalytical assay you need to determine if the assay being developed is for free drug or total drug

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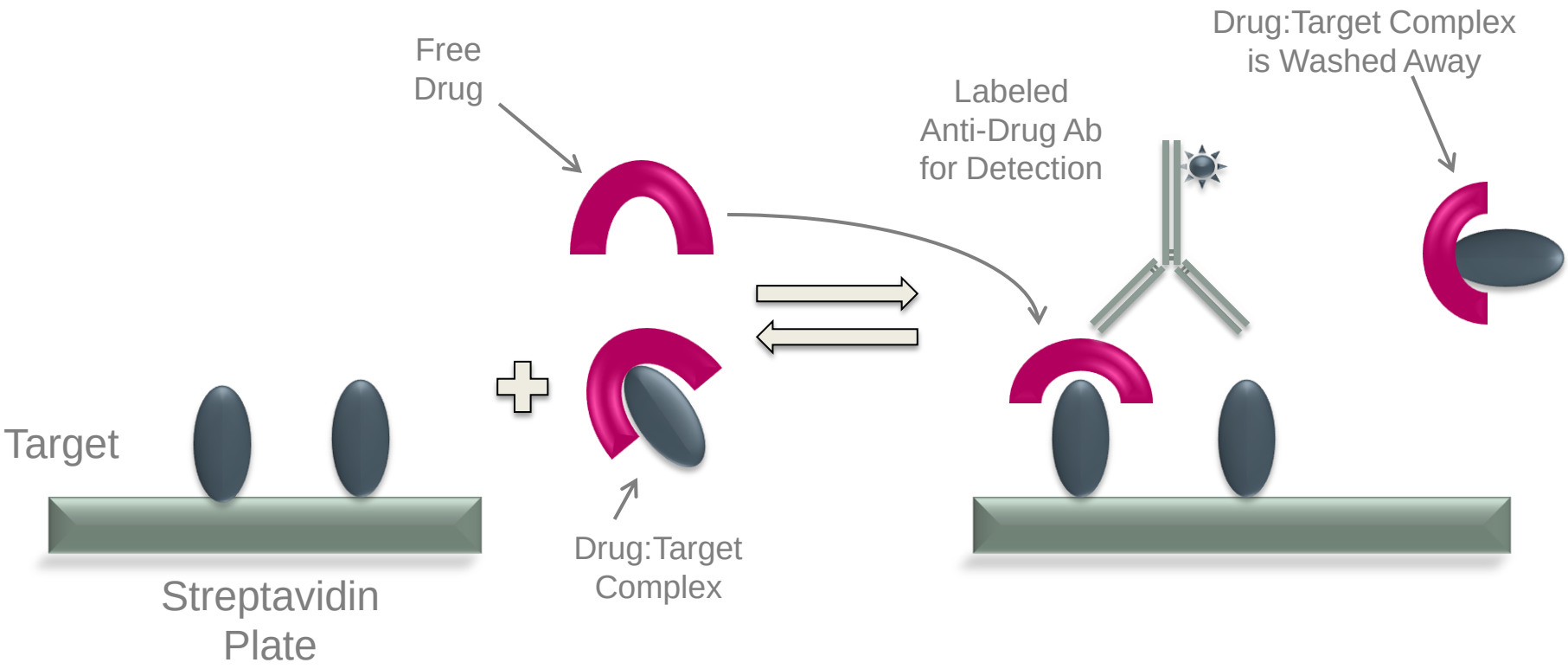
Free Drug Assay Format



Reagents required:

1. **Target**
2. **Anti-Drug Ab for Detection**

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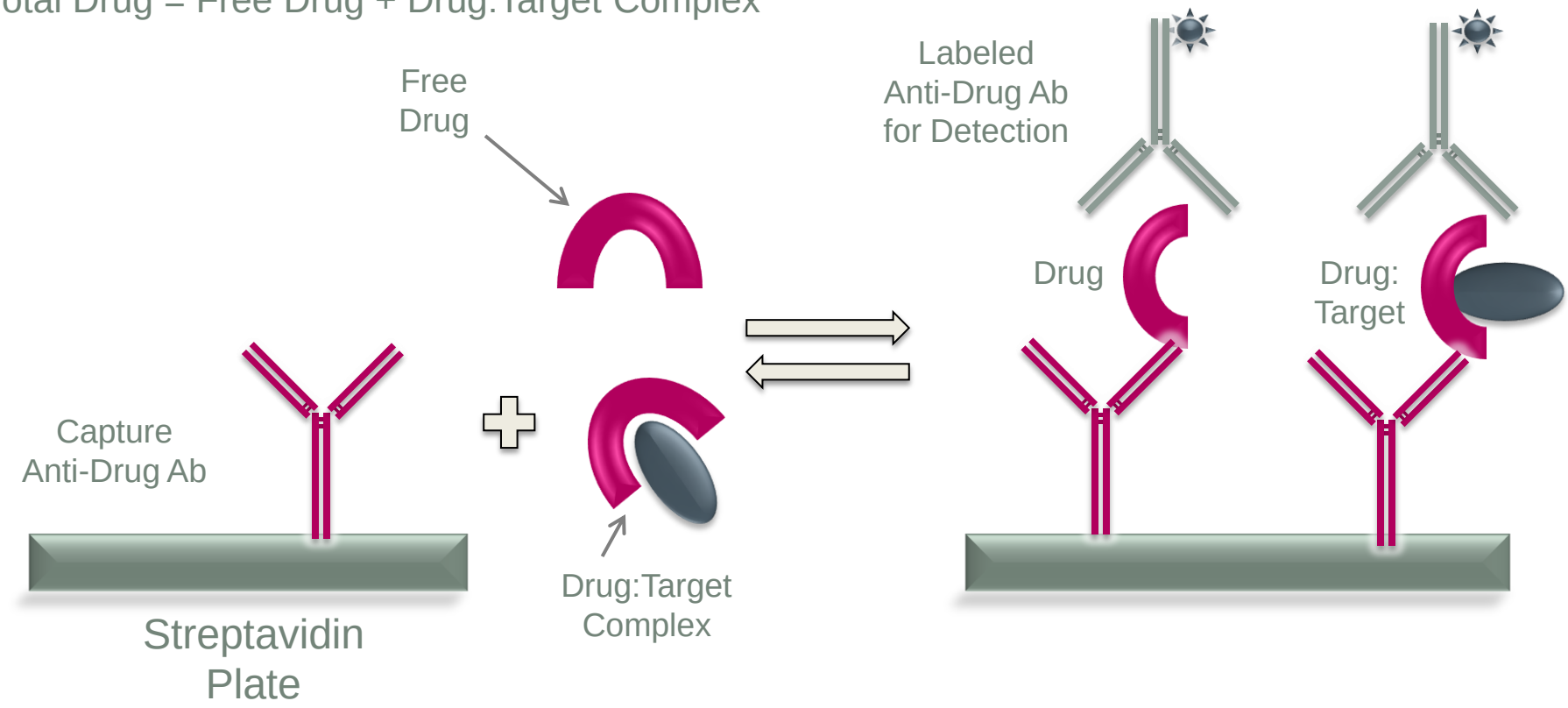
Total Drug Assay Format

Total Drug = Free Drug + Drug:Target Complex



Reagents required:

1. **Capture Anti-Drug Ab**
2. **Anti-Drug Ab for Detection**

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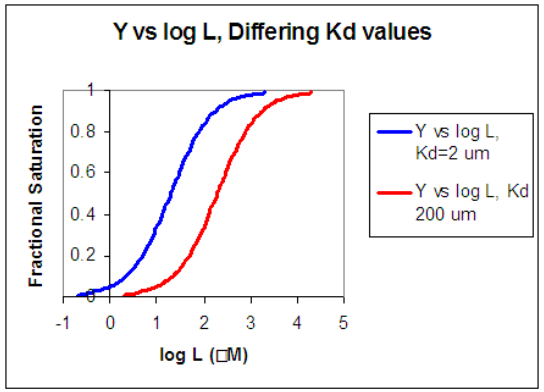
Large Molecule Bioanalytical Assay Development

- **Information gathering**
  - Intended use (pre-clinical, clinical, sample population, potential cross-reacting substances)
  - Availability and characteristics of the analyte and assay reagents
  - Advantages and pitfalls of possible alternative assay formats
    - ELISA, ECLA, RIPA; Sandwich assay (1-step, 2-step), competitive assay
- **Develop antibodies to the protein being developed**
  - If there are a lot of post-translational changes to the biosimilar product then you should probably grow antibodies to both the biosimilar and the reference (Celerion observed a 30% difference in concentration between a biosimilar and its reference due to differences in binding affinity)
- **Optimizing reagents, assay conditions**
  - Direct / Indirect coating
  - Labeling and titration of detection reagent / capture reagents
  - Optimizing of dilution buffers, blocking buffers, incubation conditions
- **Important assay parameters to be optimized**
  - Sensitivity (lower limit of quantitation)
  - Specificity (cross-reacting substances, interferences caused by drug target in matrix)
  - Selectivity (matrix interferences, spike recovery)
  - **Precision (<10%)** and Accuracy

Factors to Consider During PK Assay Development

- **Antibodies used for capture and detection**
  - These are key to an immunoassay and provide basis for specificity and sensitivity
  - Variability between lots
- **Reference standard**
  - Less well characterized than conventional drugs. Purity assessment is difficult
  - Variability between lots
- **Nonlinear calibration and calibration model assessment**
  - High-dose hook effect
  - Sigmoid curve with appropriate weighing is generally considered as appropriate for immunoassays



- **Matrix interferences**
  - Similarity to an endogenous protein
    - Impact on background response. Analyte-free matrix for calibration curve required.
  - Interfering substances (minimizing impact of nonspecific antibodies)
  - Total drug (drug bound to its target, other serum proteins or ADAs) vs. free drug in matrix
  - Impact of disease state
  - Stability of analyte in matrix

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Risk-Based Assessment of Immunogenicity (Anti-Drug Antibody) Analysis

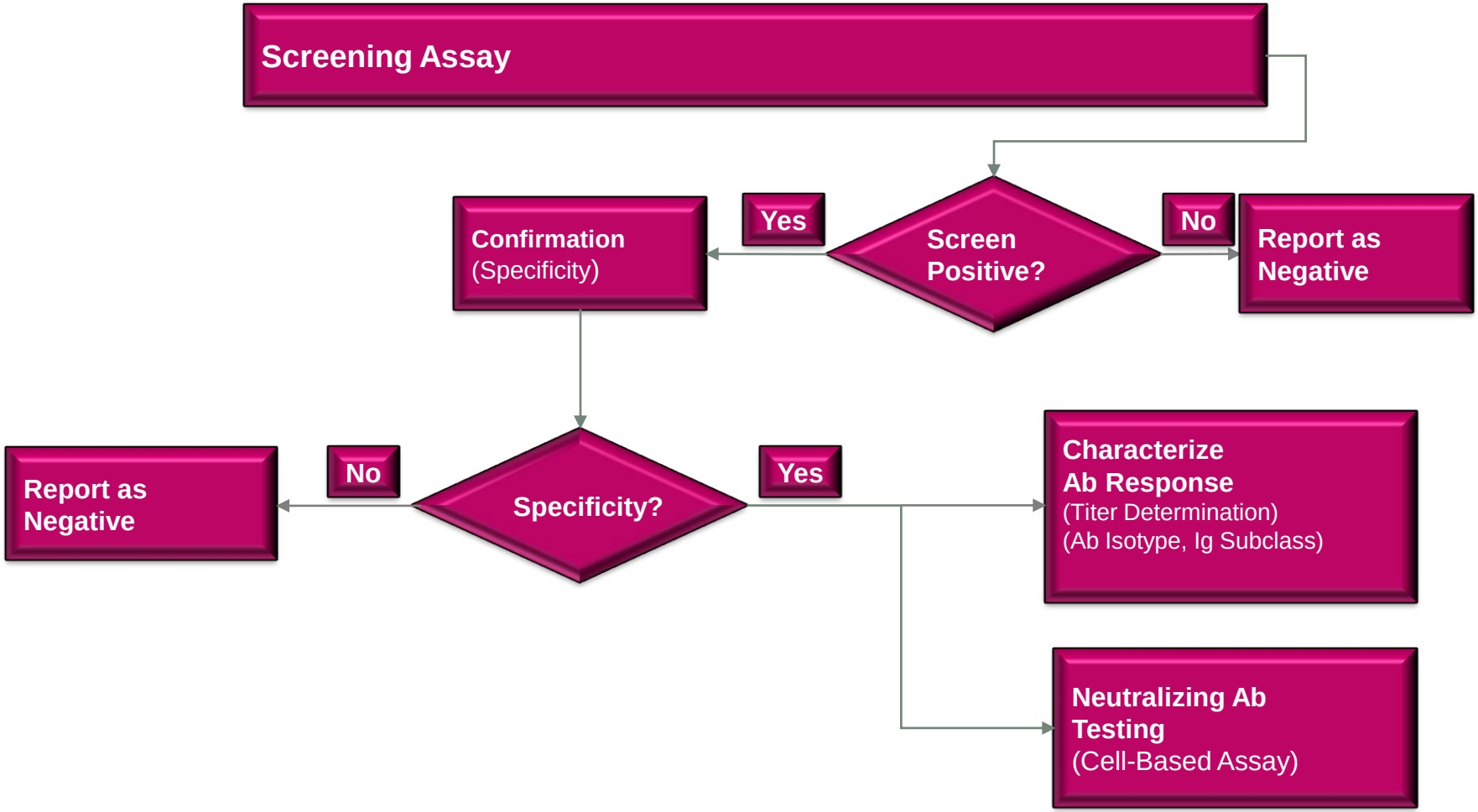
Higher risk	Lower risk
Product:	Product:
• Endogenous version exists	• No endogenous version
• Endogenous version unique	• Endogenous version redundant
• Replacement therapy	• Not a replacement therapy
• Repetitive treatment	• Single dose treatment
• Non-intravenous route of administration[a]	• Intravenous route of administration[a]
Target:	Target:
• Endogenous version exists	• No endogenous version
• Endogenous version unique	• Endogenous version redundant
• Subject/health status	• Subject/health status
• Sole therapy	• Other therapies exist
• Life threatening disease	• Not a life threatening disease
• Not immunosuppressed	• Immunosuppressed
• Autoimmune/inflammatory disease	• No autoimmunity/ inflammation

[a] The following immunogenicity of the administration route has been claimed but exceptions may exist: inhalation > subcutaneous > intraperitoneal > intramuscular > intravenous. Specific testing of individual products is encouraged.

The greater the risk, the more extensive and frequent antibody testing and characterization should be applied.

Source: Koren et al., **Journal of Immunological Methods**. 2008

Typical Work Flow



Incidence of Antibody to Therapeutic Protein with Endogenous Counterpart(s)

- **All therapeutics proteins are potentially immunogenic.**

Product Name	Occurrence of antibody response
Roferon (IFNα)	20-50%
Intron (IFNα)	0-24%
Avonex (IFNβ)	~5%
Betaferon (IFNβ)	~44%
Proleukin (IL-2)	47-74%

Source: The Regulatory Review - August 2002, Vol 5, 5

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Concerns of Therapeutic Protein with Endogenous Counterpart(s): Scenario #1

- Generation of antibody to a therapeutic protein product can affect the therapeutic efficacy of the product
  - Impact may be managed with dose adjustments or changes in frequency
- Immunological tolerance to endogenous soluble proteins not complete for proteins present at low levels:
  - T and B cells specific for low-abundance self-antigens not completely eliminated from the body
- Therapeutic protein may contribute to breaking tolerance to a normal endogenous protein and neutralize its activity.
- Anti therapeutic antibody can be devastating when it cross-reacts to an endogenous protein counterpart of the therapeutic.

Concerns of Therapeutic Protein with Endogenous Counterpart(s): Scenario #2

- Neutralizing antibody responses to therapeutic proteins are particularly dangerous when the endogenous protein counterpart of the therapeutic subserves a biologically unique function and may cause clinical deficiency syndrome.
- **For example:**
  - Neutralizing antibodies to PEG-MGDF, whose endogenous counterpart, thrombopoietin (TPO), uniquely regulates platelet production, caused severe thrombocytopenia in healthy participants.
  - Eprex, a recombinant erythropoietin alpha product, caused an increase in PRCA because the product induced neutralizing antibody that also neutralized endogenous erythropoietin (which uniquely regulates red blood cell mass).

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Concerns of Therapeutic Protein with Endogenous Counterpart(s) : Scenario #3

- Neutralization of therapeutic protein products does not produce an obvious clinical deficiency syndrome for all therapeutic protein products with endogenous counterparts, because many endogenous proteins are biologically redundant.
- **For example**:
  - Neutralizing antibody to IFN-β may diminish its effectiveness in multiple sclerosis, there is no obvious immune deficiency engendered. This is likely due to redundancy in the biological activity of type 1, α and β interferons; there are at least 22 species of IFN-α.

Source: Perini P. et. al J.Neurol. 2004;251:305-309

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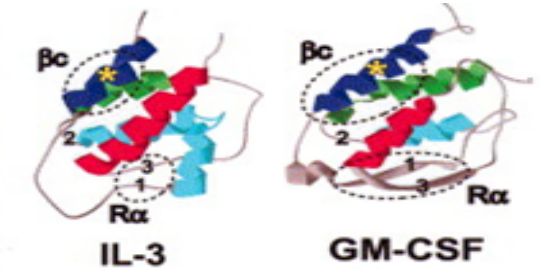
Concerns of Therapeutic Protein with Endogenous Counterpart(s): Scenario #4

- Alteration in epitopes (Fusion molecules): Fusion of a therapeutic protein with a partner molecule creates neodeterminants, which may generate immune responses. Responses to the neodeterminant may spread to conserved segments of the molecule.
- For example: The neutralization of the GM-CSF-IL-3 fusion molecule in which a specific neutralizing-antibody response to the fusion protein predominates over neutralizing responses to either the IL-3 or GM-CSF



- For fusion molecules in which both partners are self proteins, studies to define the antigenic site of antibody responses are highly recommended.
- Fusion proteins involving a foreign protein and self protein are of particular concern because of the capacity of the foreign protein to act as a "helper" determinant, efficiently eliciting T-cell help to generate a response to the self-protein partner.

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Immune System Tolerance: Low Abundance vs High Abundance Proteins

- It has been reported that the immune system is less tolerant of low-abundance proteins, such as cytokines and growth factors, compared to high-abundance proteins.
  - Natural autoantibodies to cytokines and growth factors can be found in normal healthy individuals
  - The development of antibody responses to cytokines during normal immune response
  - Immune responses can be generated to very-low-abundance endogenous factors by exogenous recombinant therapeutic products
- Example: Thrombopoietin (TPO), present at picomolar levels, immune responses were relatively easily generated, in some cases requiring only two doses, whereas for albumin, the highest abundance protein, immune responses are difficult to detect.

Challenges to Immunogenicity testing for Biosimilars

- Immune system is very sensitive to distinguish minor differences in biological products . Small differences in protein therapeutics can result in distinct immunogenicity profiles
- Current analytical testing methods may not distinguish subtle differences in innovator product and biosimilars.
- Immunogenicity testing is complex
  - Multiple assays, characterization of response, binding antibody vs. neutralizing antibody, clinical monitoring and action plan

Challenges to Immunogenicity testing for Biosimilars

- Immunogenicity may change during product life cycle
- Lack of standardization of approaches and methodologies useful to compare incidence across products of the same class
- Due to low incidence rate for some biotherapeutics, it is difficult to power clinical trials (# of patients and Rx duration) to enable statistically meaningful comparison of innovator and biosimilar.
- A validated immunogenciity method with sufficient sensitivity must be used for detection and characterization of anti-drug antibodies
- Neutralizing and non-neutralizing Abs must be identified and correlated to effects on safety and efficacy.

Neutralizing Antibody Assays

- Neutralizing antibodies (NAB) are generally of more concern than binding antibodies (BAB).
- Cell-based NAb assays are critical to understanding the ability of ADAs to neutralize biological effect of the drugs (innovator and biosimilar)
- The detection of NAb can be performed by non-cell-based competitive ligand binding assays (CLBA). However FDA prefers CBA because these assays more realistically reflect the in vivo situation.

Neutralizing Antibody Assays

- *FDA's view on Bioassays..*
  - *"Generally, bioassays have significant variability and a limited dynamic range for their activity curves. Such problems can make development and validation of neutralization assays difficult and FDA understands such difficulties. Nonetheless, we will recommend such assays because they are critical to understanding the importance of patient immune responses to therapeutic proteins."*

NAb assays – Assay format

- Assay format depends on the drug target and known signaling pathways used by the target molecule. Most appropriate signal is that associated with disease pathology or drug MOA
- Possible derivative of potency assay. Some of the examples of Nab assays are:
  - Proliferation
  - Gene expression
  - Gene reporter
  - Signal transduction

Requirements for Cell-based NAb assays

- Suitable cell line
- Cut point
- Precision
- Linearity
- Interference
- Sensitivity
- Specificity
- Robustness
- Ruggedness

Large Molecule Bioanalytical Capabilities
Celerion – Zurich

- Developed quantitative assays for:
  - Human insulins
  - Interleukins
  - Erythropoetins
  - Monoclonal antibodies
  - Fusion proteins
  - Hormones
  - Interferons
  - Pegylated interferons

- For immunogenicity screens, the lab can process up to 15,000 samples a month
- The Zurich facility has been certified according to the OECD GLP principles (Swiss Medic) and has been inspected by other regulatory agencies (FDA, AEMPS, AFSSAPS and EMA)

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EMA Regulatory Requirements for Clinical Testing of Biosimilars

- EMEA/CHMP/BMWP/42832/2005 (currently open for revision)
  - Use same reference product in clinical studies as in preclinical studies
  - Clinical studies must use drug product that was manufactured by the same process used for manufacturing the commercial material
  - Clinical PK and PD (where applicable) studies are normally performed prior to the studies that demonstrate comparable clinical efficacy and safety
  - In certain cases specific PK/PD studies may be required to establish comparability
  - Immunogenicity screening – requires one-year follow up in patients on study when product will be chronically administered
  - Sampling schedule for anti-drug antibodies should reflect onset and duration of the antibody response as known from experience with the reference product

Pharmacokinetic Studies in Biosimilar Development Programs

- Comparative PK studies are “an essential part of the comparability exercise”
  - Design should enable us to capture the PK parameters that compare both absorption/bioavailability and elimination (clearance, half-life) characteristics of the two protein products
  - Design elements (single dose, steady-state, repeated measures, crossover) need to be justified against what is known about the reference product
  - **Example** – the growth hormone somatotropin. Because this is a hormone, there is a reasonably direct connection between systemic exposure and effect, so showing PK comparability would be a good first step to demonstrate the biosimilar is not going to fail due to differences in PK

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PK Issues with Approved Biosimilars

Many approved biosimilars had PK parameters that did not meet guidelines and/or were outside the traditional BE acceptance range of 80-125%

Biosimilar	Issue
Omnitrope	No comparison to reference product
Abseamed, Binocrit and Epoetin alpha Hexal	Acceptance range not defined. AUC after IV treatment outside range
Retacrit, Silapo	Acceptance range not defined. Correction needed to meet range
Filgrastim, Hexal, Zarzio *Ref: Schellekens and Moors, Nature Biotechnology, 2011*	Outside acceptance range at low doses and after multiple doses

Therefore a PK profile that does not meet the traditional BE criteria may still support approval based upon data from clinical efficacy/safety evaluations

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Lessons Learned From Generic Drug Development

Mevacor 40 mg lots	C_{max}	T_{max}	AUC $_{(0\text{-inf})}$	$T_{1/2}$
A	3.5 ± 2.7	5.7 ± 2.4	58 ± 29	13.7
B	2.5 ± 1.2	5.0 ± 2.3	51 ± 15	14.5
C	2.8 ± 1.9	5.6 ± 5.2	62 ± 25	13.4
D	4.2 ± 2.3	5.3 ± 4.7	56 ± 28	9.2
Maximum Difference	65%	15%	25%	58%

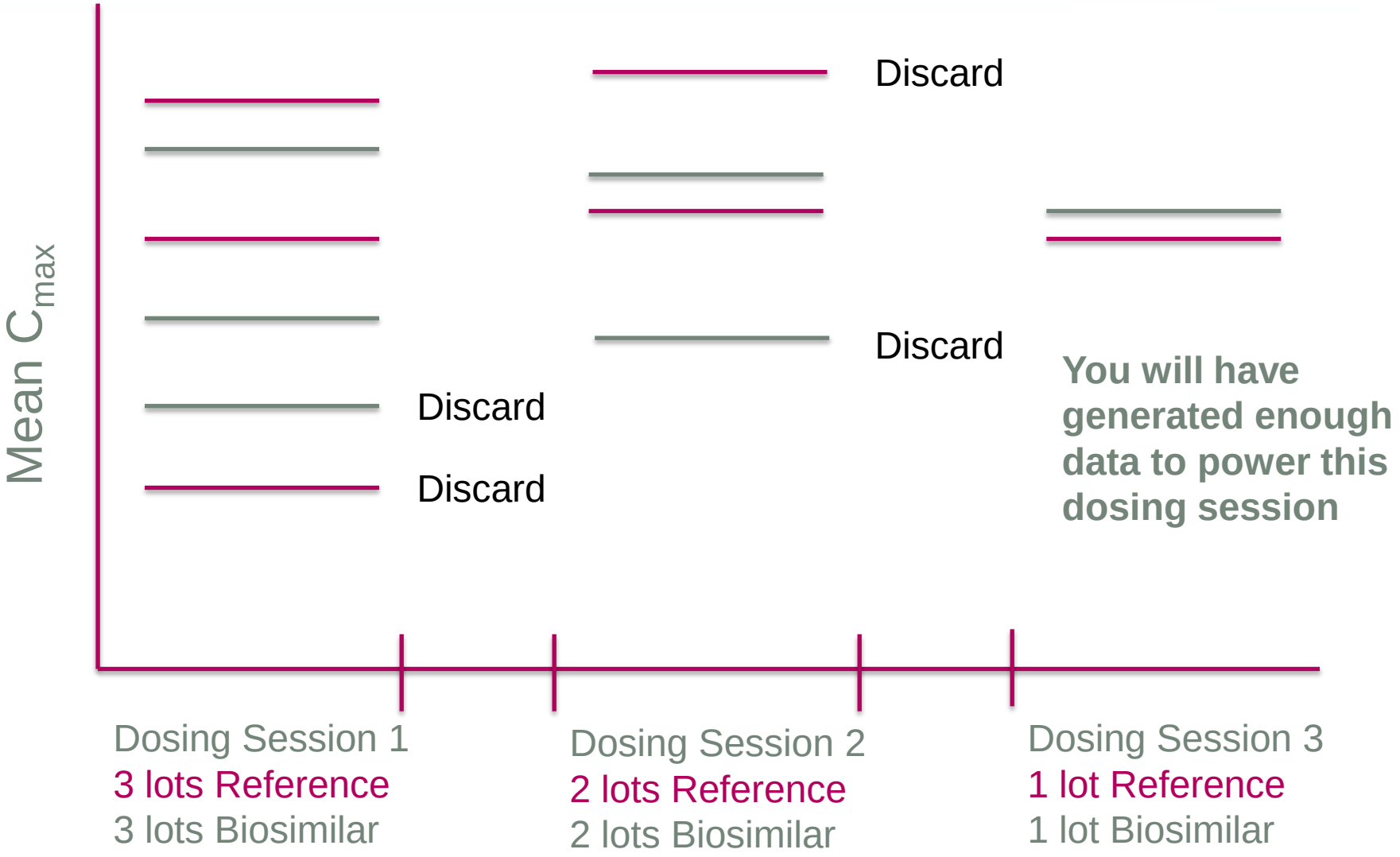
Variability between lots of reference drug

Lessons Learned From Generic Drug Development

PK Parameter	Test	Reference 1	Reference 2
C_{max}	198 ± 74.8	195 ± 135	227 ± 88.7
$AUC_{(0\text{-}inf)}$	4620 ± 3130	4490 ± 3730	4990 ± 2990

Selecting the “right” reference lot that was a close match to the generic test article improved the likelihood of success!

Adaptive Study Design for Biosimilars BE Study



Pharmacodynamic Studies in Biosimilar Development Programs

- Pharmacodynamic studies involve measuring at least one biomarker that is considered a relevant surrogate to the dynamic effect of the drug and must be conducted in a subject population most sensitive to any differences in dynamic effect
  - Usually performed in a targeted patient population at a dose in the steep part of the dose (or exposure) response curve
  - **Example 1 PK/PD Modeling** – EPO is an example where there is good PK/PD data available since hemoglobin level is a reliable and easily measurable PD marker which correlates well to the PK
  - **Example 2 PK/PD Modeling** – comparing a biosimilar for filgrastim to the reference product: "Equivalence could be demonstrated for the serum concentration profile, for the Absolute Neutrophil Count profile and, even more importantly, for the CD34+ cell count, which is a marker for the ability of the granulocyte colony-stimulating factor to mobilize stem cells."[1]

[1] Lubenau H. et.al., **BioDrugs**. 2009; 23(1) 43-51.

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