Early Stage Studies of Biosimilars

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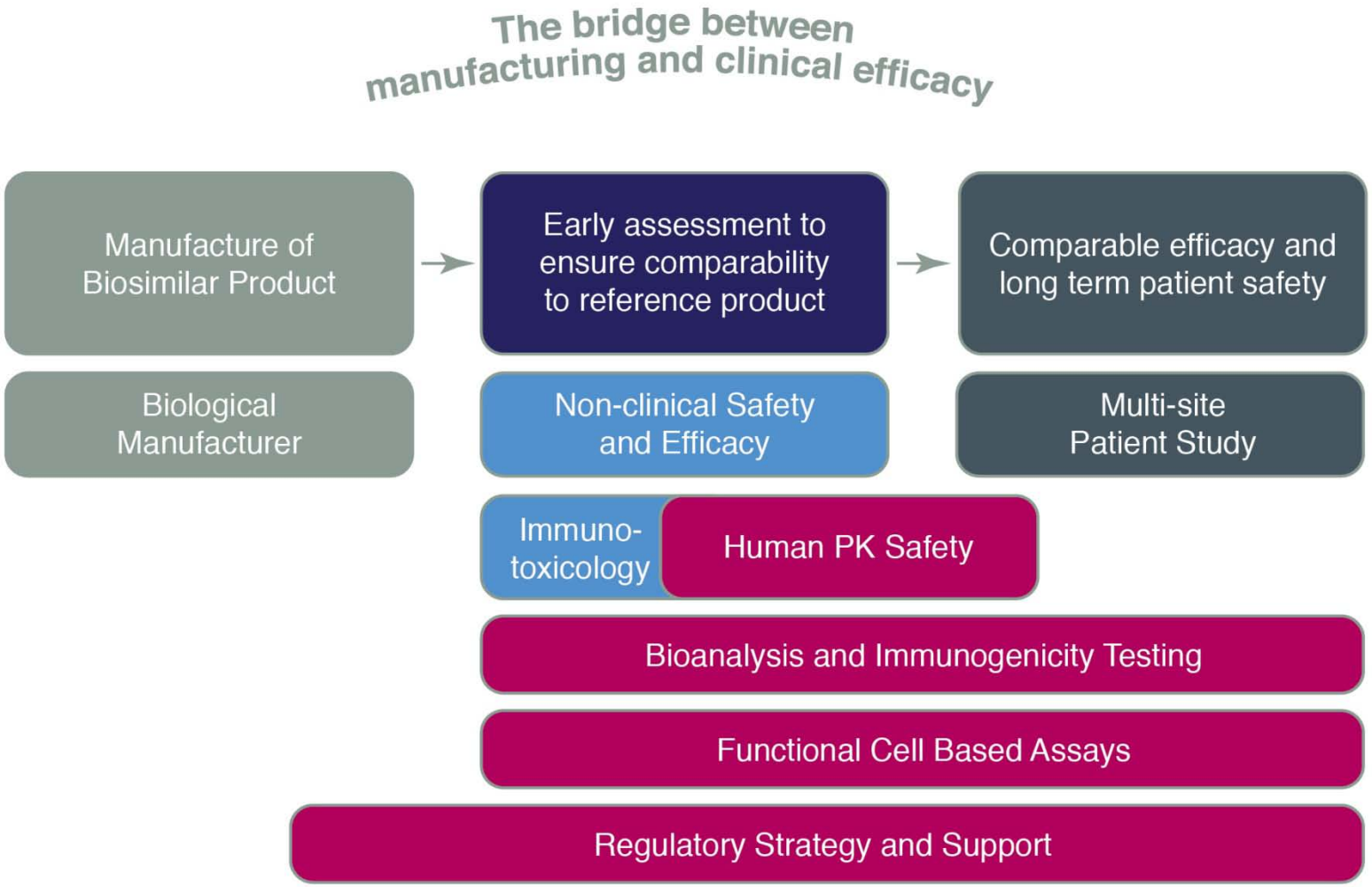
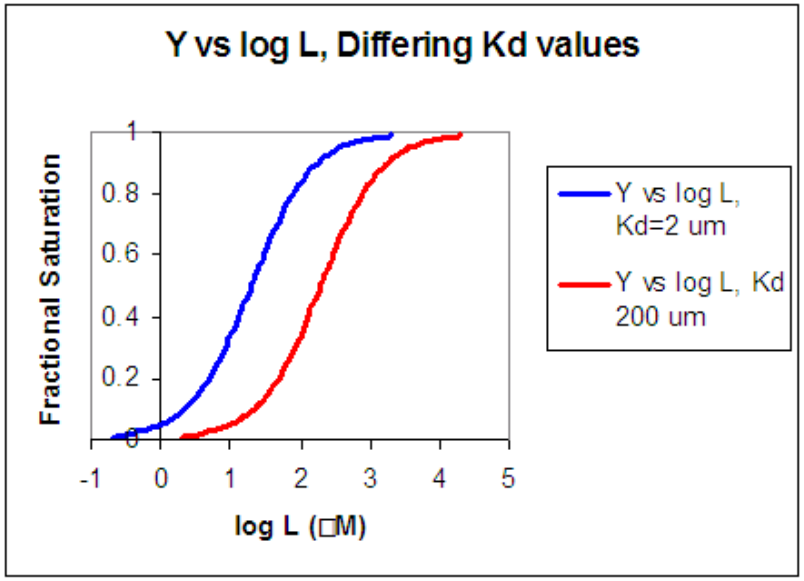
Goal – Discuss Studies that Bridge the Gap Between Newly Sourced Products and Patient Studies



Non-Clinical Studies to Meet EMA Guidelines

- EMA Concept Paper - Sep 2011
- If the reference product had an *in vitro* binding model then compare the biosimilar binding constants to the reference. **If different: Not OK**
- Compare the binding properties of the antibodies used in the bioanalytical assay to the reference and the biosimilar. **If different: OK** but may need to develop new assay antibodies for the biosimilar



- If the outcome of the *in vitro* bioassays raises concerns and if an *in vivo* model exists then you may need to conduct an *in vivo* pharmacological study:
  - A head-to-head comparison of the reference and the biosimilar in this model.
    If different: NOT OK

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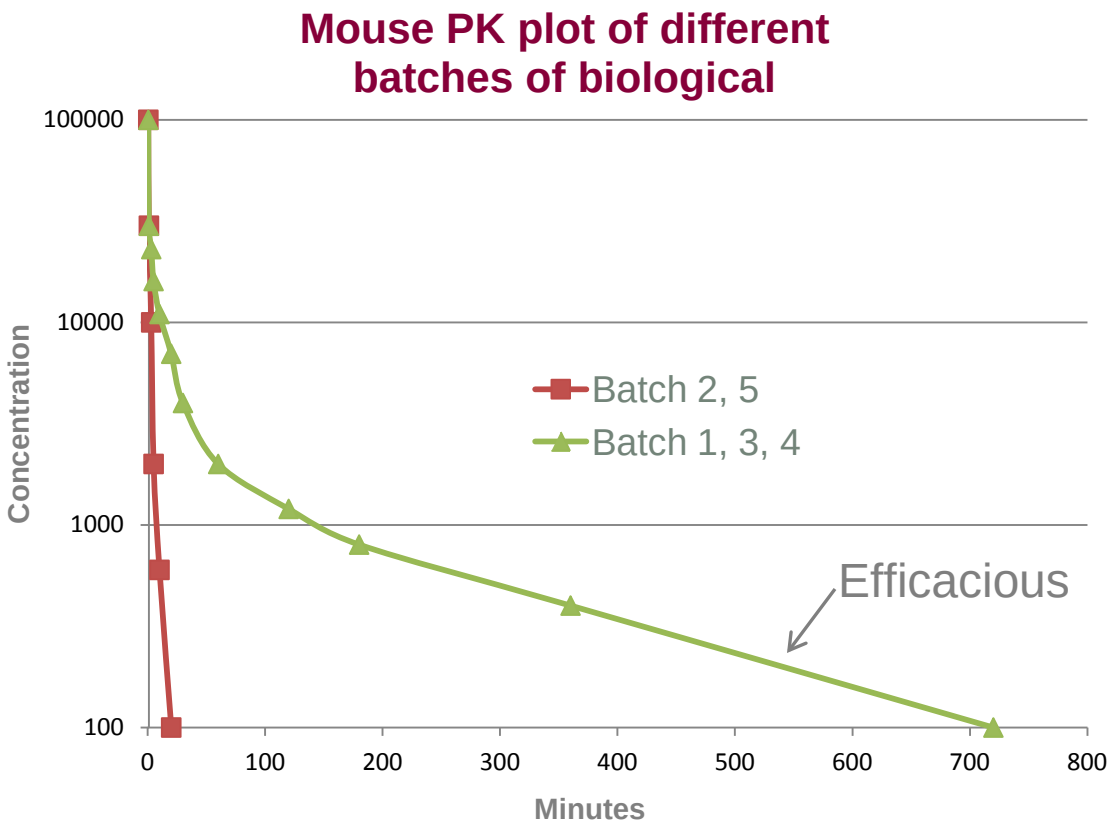
Non-Clinical Studies to Meet EMA Guidelines

- Perform a single dose PK study in mice – not required but valuable. The bioanalytical assay does not have to be validated for this study! **If different: NOT OK**



No GMP analytical differences could be found between batches!

Non-Clinical Studies to Meet EMA Guidelines

- If the outcome of the *in vitro* comparative studies raises concerns then perform the following single dose PK studies:
  - Primates – need to use primates if reference product used primates – highly likely
  - If bioactive in both rodent and non-rodent then use both
  - If NOT bioactive in rodents then it would be acceptable to perform this study only in the non-rodent (probably primates)

Non-Clinical Studies to Meet EMA Guidelines

- TRADITIONAL PATH – Establish *in vivo* comparability of toxicokinetics and immunogenicity using a relevant animal species (generally primates)
  - Dose reference product and biosimilar (head-to-head comparison)
  - Single repeat dose; 4 dosing cycles per study or as per clinical dosing regimen
    - If half-life = 2-4 days then weekly dosing cycle
    - If half-life = 2-3 weeks then monthly dosing cycle
    - One dose level, one gender and no recovery group may be acceptable (needs to be justified) – If this can not be justified then:
      - Minimum dose levels = high dose, low dose and control (may add mid-dose level)
      - 3 animals/sex/dose level for necropsy + 2 animals/sex for recovery group (if reference product didn't have a recovery group then the biosimilar does not need a recovery group)
  - Perform the standard battery of health assessment testing
  - Perform toxicokinetic measurements after first dose and last dose
  - Perform immunogenicity testing after last dose
- Other non-clinical studies such as safety pharmacology, reproductive toxicology, mutagenicity and carcinogenicity are not required

Non-clinical 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

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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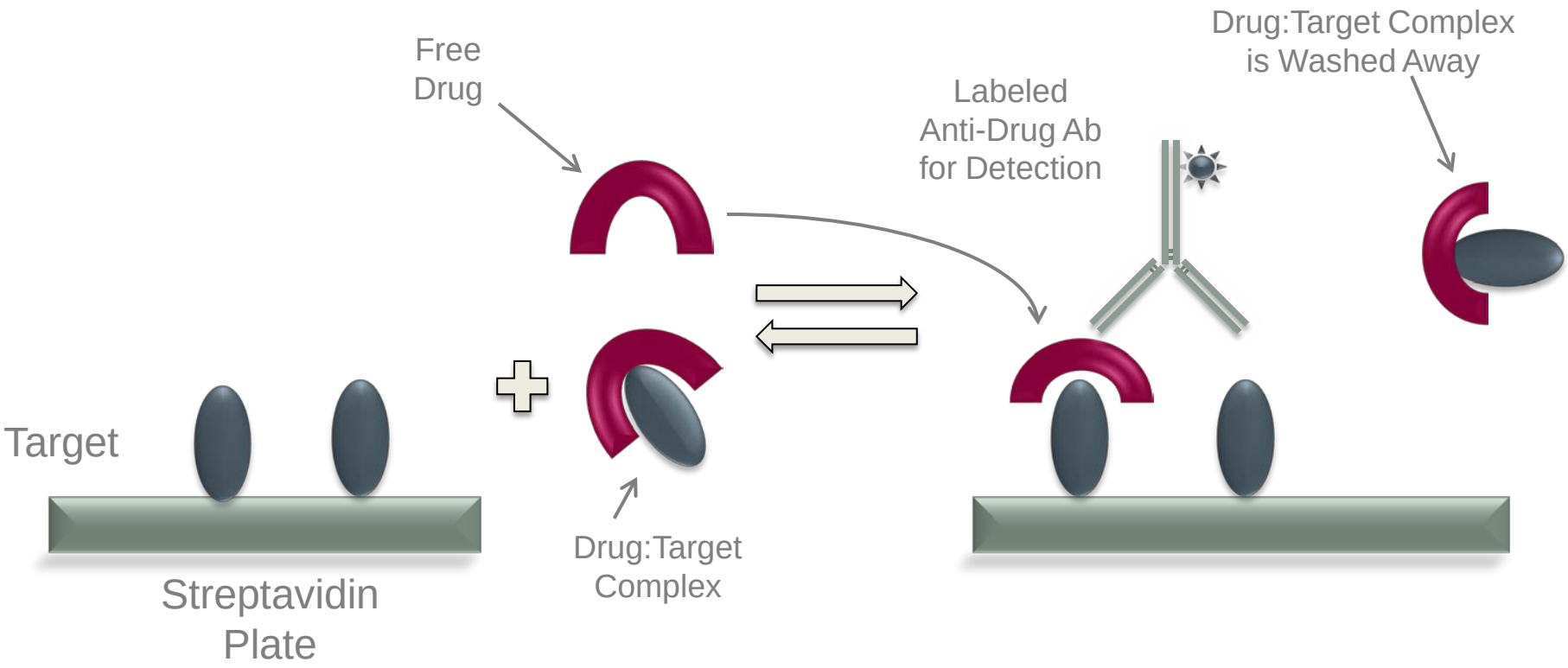
Free Drug Assay Format



Reagents required:

1. Target
2. Anti-Drug Ab for Detection

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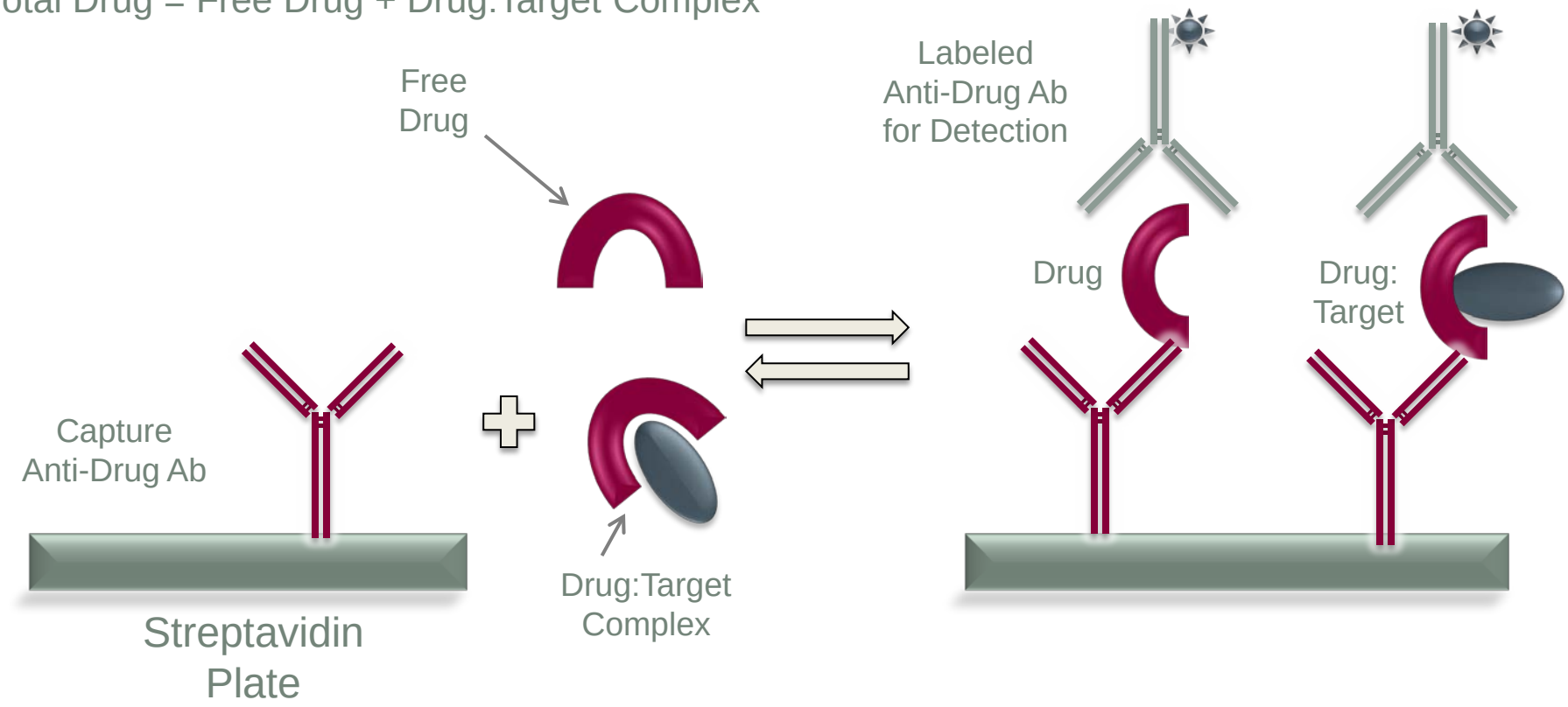
Total Drug Assay Format

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
- Grow 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 and accuracy

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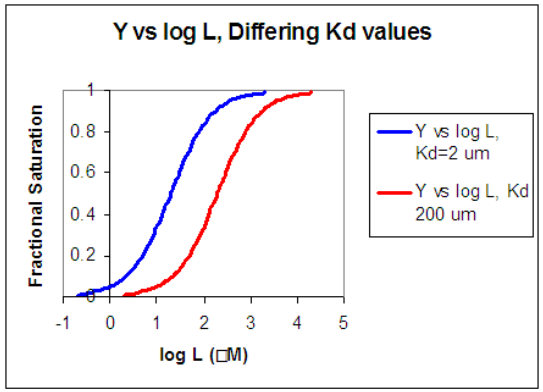
Factors to Consider During PK Assay Development

- 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 most 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

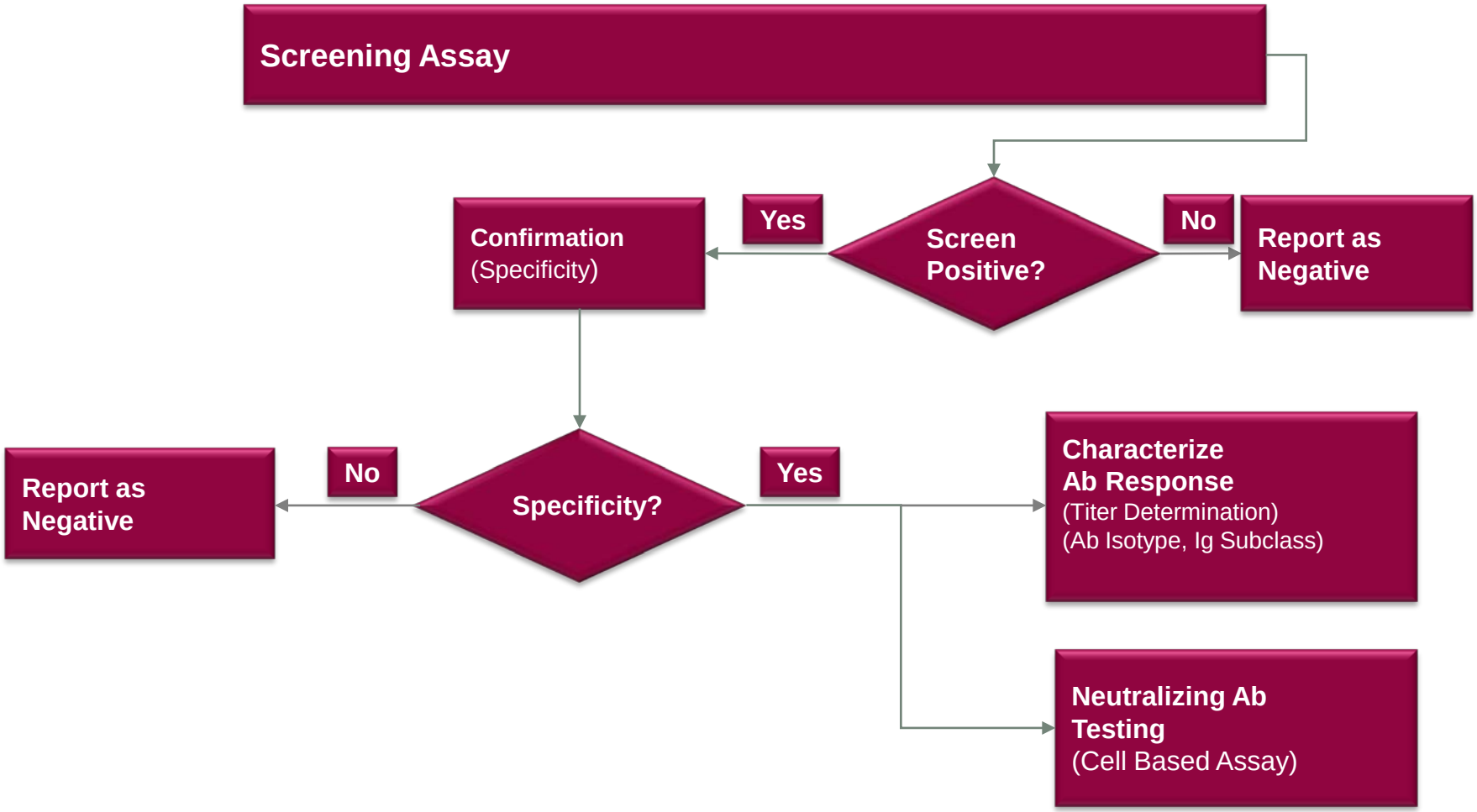
Higher risk	Lower risk
Product:	Product:
• Endogenous version exists	• No endogenous version
• Endogenous version unique	• Endogenous version redundant
• Replacement therapy	• Not a replacement therapy
• Repetitive treatment	• Single dose treatment
• Non-intravenous route of administration[a]	• Intravenous 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



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Immunogenicity Assay Development

- Information gathering
  - Intended use (pre-clinical, clinical, sample population)
  - Availability and characteristics of the analyte and assay reagents
  - Advantages and pitfalls of possible alternative assay formats
    - ELISA, ECLA, RIP, bridging assay (1-step, 2-step), direct assay

- Optimizing reagents and assay conditions
  - Direct / indirect coating
  - Labeling and titration of detection reagent / capture reagents
  - Optimizing of dilution buffers, blocking buffers, incubation conditions
  - Sample pre-treatment (e.g., acid treatment)

- Important assay parameters
  - Sensitivity (minimum required dilution, precision, background level)
  - Specificity testing (matrix effects, cross-reacting antibodies)
  - Free drug tolerance

Factors to Consider During Immunogenicity Assay Development

- Drug used for capture and detection
  - Absorptive coating or too excessive labeling may alter relevant epitopes
  - Variability between lots
- Reference standard
  - Standardized species-specific polyclonal anti-drug antibodies reference material are not generally available
  - Affinity purified polyclonal antibodies can be used for the preparation of mock positive control samples
- Sensitivity assessment
  - Highly dependent upon the antibodies used for characterization (higher affinity -> higher sensitivity)
  - No positive control can represent the diversity of antibodies found in individual subjects
- Specificity (confirmation assay)
  - Usually a competitive inhibition test => optimizing the appropriate level of drug
  - IgM antibodies may behave quite differently than IgG antibodies (more difficult to inhibit)
- Matrix interferences
  - Pre-existing anti-drug antibodies
  - Interfering substances which minimize the impact of nonspecific antibodies
  - Free drug tolerance
  - Impact of the disease state

Large Molecule Bioanalytical Capabilities
Celerion – Zurich

- Developed quantitative assays for:
  - Human insulins
  - Interleukins
  - Erythropoetins
  - Monoclonal antibodies
  - Fusion proteins
  - Hormons
  - 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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Cell Based Assays

- Advantages
  - In comparison to the *in vitro* tests such as ELISA and RIP, cell based assays provide information about how drug/antibodies may be acting on cells
  - *In vitro* tests such as ELISA and RIP demonstrate that an antigen-antibody reaction is occurring. Only a cell based assay can demonstrate that the drug is functional
  - Cell based assays can demonstrate whether an anti-drug antibody response is neutralizing or non-neutralizing
- Limitations
  - Cell lines are notoriously variable and sensitive to reagent changes – choice of appropriate cell line is crucial
  - Sensitivity/Selectivity issue – dilution of samples is required because the response of the cells is very sensitive to other signals in the matrix
  - Long development/validation time due to reagent availability

Types of Cell Based Assays

- Proliferation
- Differentiation
- Apoptosis
- Migration
- Invasion

- TF1 (*human premyeloid leukemic cell line*)
- UT7/Epo (*human erythropoietin-dependant leukemic cell line*)
- L929 (*murine aneuploid fibrosarcoma cell line*)
- A-549 (*human lung carcinoma*)
- Caco-2 (*human colon carcinoma*)
- MDCK (*Mardin Darby canine kidney*)
- ARIP (*rat pancreatic*)
- HUAEC (*Umbilical Artery Endothelial Cells*)
- HEK293MR (*human embryonal kidney*)
- HL-60 (*human promyelocytic leukemia cell line*)
- HepG2 (*human hepatocellular carcinoma*)
- EAHY (*a hybrid human cell line derived from fusion of HUVECs and A549 carcinoma cells*)

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Cell Based Assay Development

- Selection of the cell line
  - Cell number, viability, morphology, density, confluence at time of assay, passage number, cell pre-treatment, washing conditions, plates
- Selection of the assay endpoint
  - Placement of controls, sensitivity, dynamic range, specificity and positive control
- Appropriate matrix interference assays
  - Necessary to show specificity of response
  - Multiple assays/tests may be required

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Cell Based Assay: Matrix Interference Assay

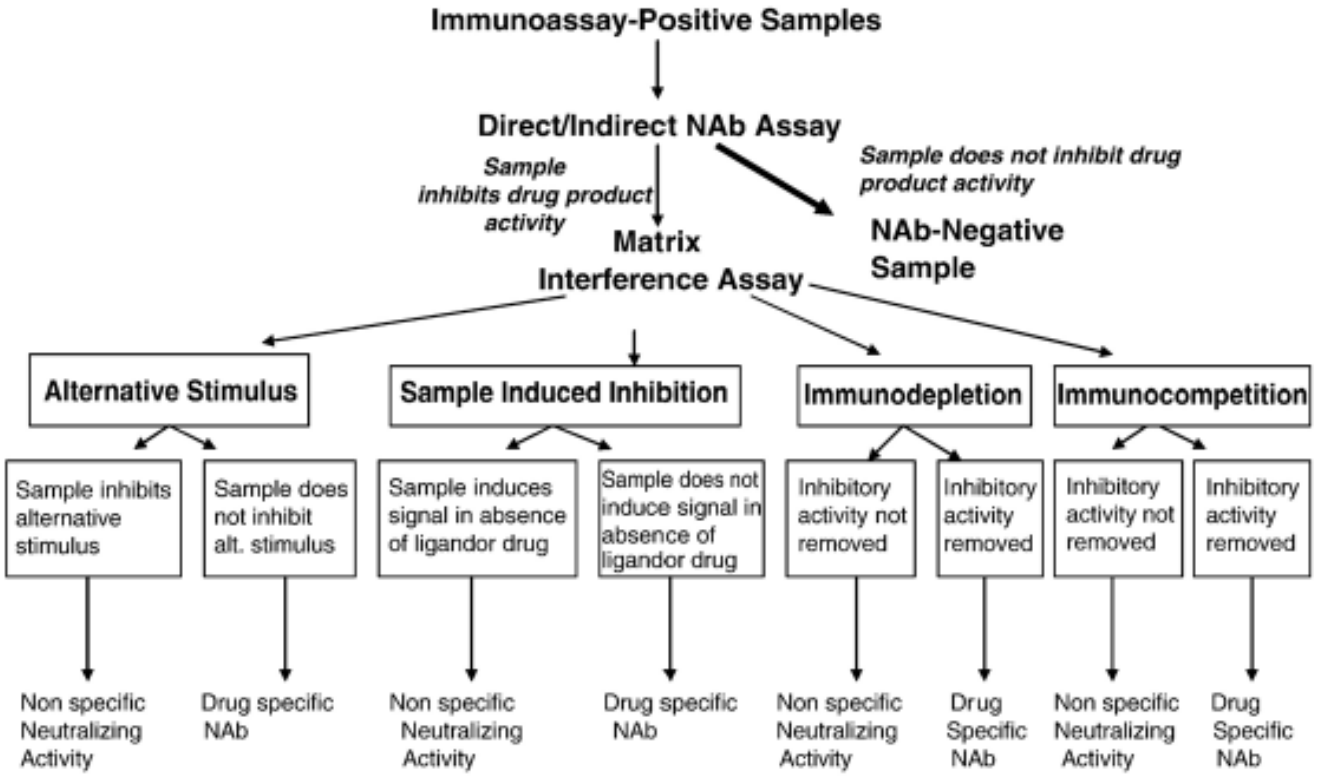

Fig. 4. Flow chart depicting use of NAb and matrix interference assays to ascertain drug product-specific neutralizing antibodies.

Source: S. Gupta et al. **Journal of Immunological Methods 321.** 2007. 1-18

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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 because of bad PK

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, Silap	Acceptance range not defined. Correction needed to meet range
Filgrastim, Hexal, Zarzio	Outside acceptance range at low doses and after multiple doses

Ref: Schellekens and Moors, Nature Biotechnology, 2011

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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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]
- The margins defining what is considered clinically comparable must be defined a priority

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

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