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Immunogenicity Testing of Large Molecules & the Challenges of Developing ADA Assays

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Rise of the Large Molecules
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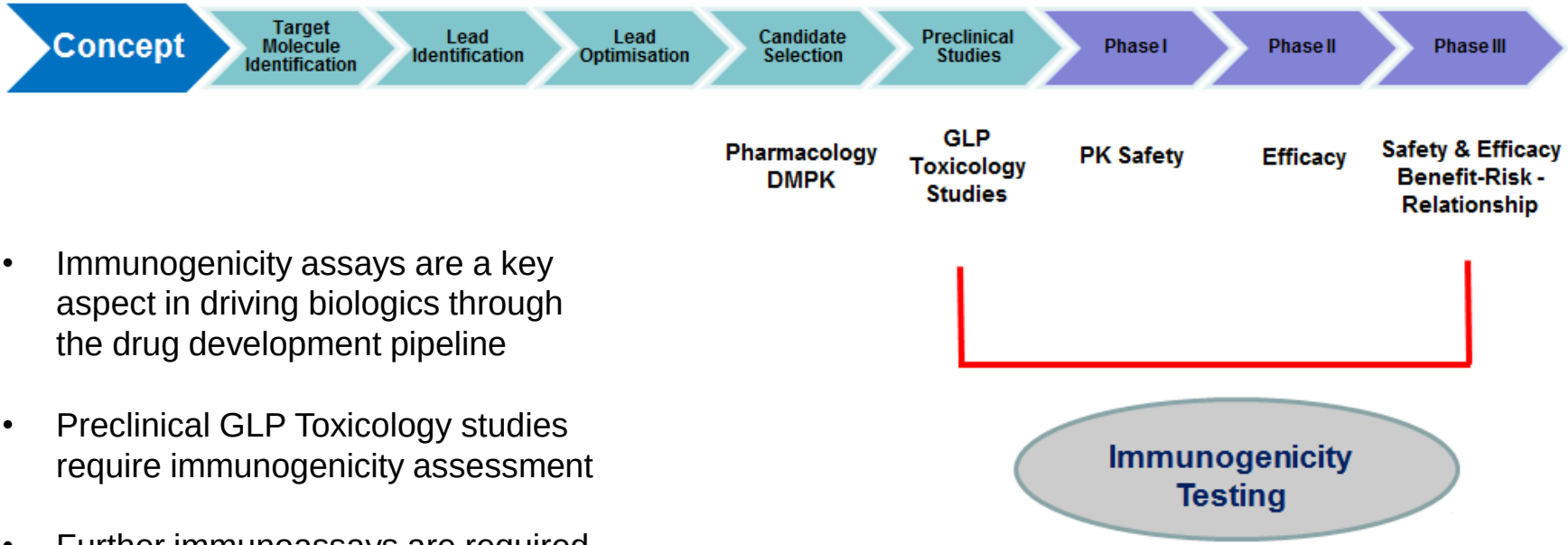
Content

Immunogenicity

- When the patient produces antibodies to the drug – Anti-Drug Antibodies (ADA)
- Assessing the immunogenic potential of drugs is critical to ensuring safety of the patient - can be related to adverse effects
- Large molecules by nature are more prone to eliciting an immune response
- ADA can have varying impacts, in particular to PK/PD
- Multi-factorial – Product? Patient?
- Regulatory requirement

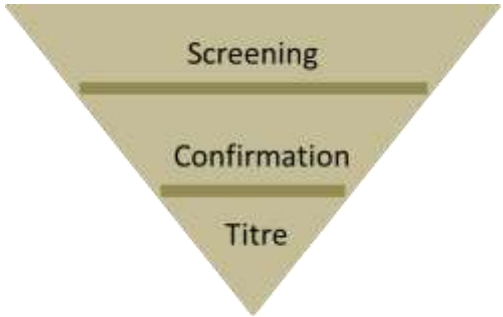
Immunogenicity testing is a mandatory requirement at various stages in the drug development process



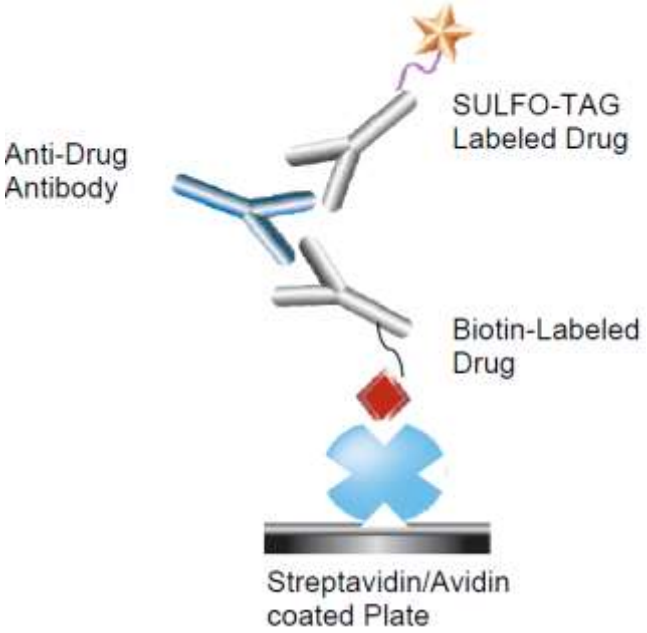
- Immunogenicity assays are a key aspect in driving biologics through the drug development pipeline
- Preclinical GLP Toxicology studies require immunogenicity assessment
- Further immunoassays are required for clinical development

ADA assays are fundamentally different to PK assays

PK	ADA
Single assay	Multi tiered
Quantitative - utilises a standard curve	Not quantitative - uses a cut point
Defined reference standard - the drug	Uses a surrogate positive control
Potential interference from matrix substances	Potential Interference from matrix substances **and circulating drug**
Capture/detection reagents often commercially sourced	Drug molecule is labelled for use as capture and detection reagents



MSD Bridging Assay is commonly used in Immunogenicity Assessment

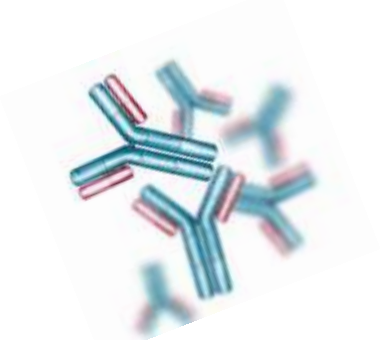


- Potential for better sensitivity
- Homogenous solution phase incubation simplifies assay
- Potential for shorter assay times
- Not species-specific
- Generally better drug tolerance than ELISA

Meso Scale Discovery®, Bridging Immunogenicity Assays

Selection of a Positive Control (PC) is the first critical step in developing and ADA assay

- Ideally the PC should reflect the anticipated immune response although it is impossible to obtain a PC that is truly reflective of in-vivo ADA responses



- Options include:
  - Hyper immunisation of animal
  - Serum from patient population
  - IgG antibodies generated against the drug through hybridoma

- Need to test the selected PC for sensitivity, specificity and reproducibility

Screening and confirmatory Cut Points provide thresholds for determining and confirming positive/negative status of samples

- Screening cut point should be determined from negative control samples (i.e. from subjects not exposed to product)

Development

Validation

  - ➢ Statistically determined with appropriate false positive rates (pre-clinical 1%, clinical 5%)

- Confirmatory cut point is determined by measuring inhibition of signal when samples are incubated in presence of drug
- May need to also establish SCP and CCP in patient population

Summary

- Immunogenicity assessment is vital to understanding the immunogenic potential of drugs and hence the impact on patient safety
- A tiered approach is used to initially screen samples for positive ADA response, followed by confirmation and characterisation of the response
- The assay is only as good as its positive control
- Drug tolerance assessment helps us understand the limit of our assay and whether we need to improve it
- Labelling of reagents is a critical step in assay development and needs to be tailored to the properties of the molecule

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