F·star THERAPEUTICS

Characterisation

Practical Aspects of Immunogenicity

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CONFIDENTIAL

What does ADA Characterisation mean?

- Characterisation is the further assessment of the immune response to a therapeutic after identifying that a response has been generated.
- *"Samples identified as positive in the confirmatory assay should be further characterized in other assays, such as titration and neutralization assays."*
  - Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection FDA Jan 2019
- *"Further characterization **if required** should include e.g., antibody class and subclass (isotype), affinity and specificity and assays used for these should be qualified for their intended purpose."*
  - Guideline on Immunogenicity assessment of therapeutic proteins- EMEA/CHMP/BMWP/42832/2005 Rev1
- The requirement for, and extent of, characterisation depends on where you are in the development process and the particular immunogenicity risks of your molecule.

When to perform Characterisation assessments

- The decision to perform, and the timing of, these assessments is based on your Immunogenicity risk profile for your drug.
- The minimum characterisation required is:
  - Assessment of Antibody amount (e.g. Titre)
  - Neutralising antibody (unless justified (e.g. single dose low risk therapies)
- However other risk factors that may require additional characterisation of immune responses include:
  - Homology to endogenous protein
  - Multi-domain products
  - Dose route
  - Patient Population and Clinical risk
- These assessments will be required at submission but should be included earlier if there is a risk to patients, or a business risk.

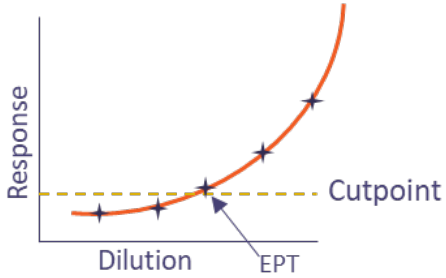
Relative amounts of Antibody in samples

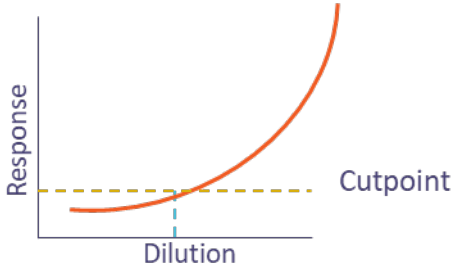
- Screening response in an assay is affected by a combination of antibody concentration, antibody affinity/avidity and potentially Ig Isotype.
- These can change between samples from the same patients, let alone between patients.
- Despite this a normalised ratio of screening response to the NC response (S/N) can be a useful estimate of antibody concentration [1]
  - Especially useful early on in development
- Sample Titre is more widely used and described in regulatory guidelines
  - Approach comes from Vaccine development and is well understood outside bioanalysis
  - Sample Titres only correlate weakly with impact of ADA
  - Titration is imprecise and only an estimate of antibody concentration
  - Titres can also be interpolated from dilution curves

[1] Manning *et al,* **Bioanalysis** (2017) 9(23), 1849–1858

ADA Titration

- End-Point Titre
  - Traditional method of calculating Titres
  - Samples serially diluted until response drops below the screening cutpoint (other cutpoints can be used)
  - Dilution factor of last sample dilution above cutpoint reported
  - Precision reported as how many dilutions from the modal titre
- Interpolated Titre
  - Sample dilution series plotted as curve
  - Titre interpolated using cut-point response
  - Cutpoint choice key for this method
  - May require more dilutions for good curve fit
  - Can generate false impression of Precision





Epitope Mapping/Domain Specificity

- Epitope mapping is not normally performed for ADA
  - Heterogenous response
  - Resource intensive
- Domain Specificity can be more useful
  - Multi domain products
    - Bi specific Abs
    - PEGylated proteins
    - ADC's etc.
- Timing of Domain Specificity will vary
  - Risk to patient safety primary driver for early assessment
- Normally only performed on samples confirmed positive using whole molecule
- Not performed pre-clinically
  - Response will be different in clinic

Cross reactivity to Endogenous Proteins

- Where a drug product has a similar structure to part, or all of an endogenous protein there is a risk that an ADA response will generate auto-antibodies to that endogenous protein
- The level of characterisation of this cross reactivity will be related to the clinical risk of these autoantibodies
- The type of assay used will depend on this risk, but as a minimum:
  - The presence of antibodies to endogenous proteins can be investigated using Inhibition testing in the Drug ADA assay
  - Where there is a high clinical risk a separate protein specific assay may be required
- When a risk is identified this may require real time testing of ADA for patient safety

Isotype Characterisation

- Isotype characterisation is not normally performed as a routine test
  - Most standard ADA responses are overwhelmingly IgG responses
- There are some risk factors that may require the assessment of subtypes.
  - Products where anaphylaxis is high risk (or similar products have anaphylaxis risk)
    - Measure IgE response
  - Treatments where a specific isotype/ subtype response is associated with immune responses
    - Factor VIII treatment or EPO.
    - Assessment of specific subtypes should be driven by clinical concern.
  - Treatments administered by mucosal routes (e.g. nasal)
    - IgA is a major isotype in mucosal immune responses
- Individual assays can be difficult to design, but Multiplex formats can be useful where multiple isotypes can be present.

Neutralising Antibodies

- ADA responses can interfere with Drug efficacy in two ways
  - Neutralising ADA prevent the Drug MoA by inhibiting activity
  - Non- neutralising ADA can affect Drug PK (usually increasing clearance- but not always)
- Nab assays should demonstrate impact of ADA response on drug potency
  - For drugs such as MAbs Demonstration of Binding inhibition may be sufficient
    - Especially in early development where detailed knowledge about Nabs is not required
  - For other drugs the GMP potency assay is often re-purposed and adapted
    - Impact of serum and residual drug in ADA sample must be controlled
- Timing of Nabs analysis is dependent on many factors
  - Not required in early clinical development
    - Exception may be oncology due to lack of efficacy markers.
  - Frequency and intensity of Immunogenic reactions
  - Business requirements

Summary

- Characterisation is dependent on factors such as
  - Clinical risk
  - Drug structure and homology to endogenous proteins
  - Business risk
- Requirement for Characterisation can change through development programme
  - Response to immunogenicity profile seen in earlier studies
  - Supporting evidence
    - Improved PD markers
- Golden Rule:
  - There are no rules- each immunogenicity profile is drug programme specific and should be guided by best science.