How should antibodies against mAb therapeutics be assessed?

Meenu Wadhwa

National Institute for Biological Standards and Control
Assuring the quality of biological medicines

Immunogenicity Testing

- The immunogenicity of mAbs is complex; prediction difficult due to variability of the antibodies and factors influencing immunogenicity & its consequences

- 'Risk-based approach' based on probability and severity of clinical consequences. Recommendations cannot be generalised due to diversity of factors and mAbs & related products. Mechanism of action & structure not sufficient for attributing risk

  - Consider & identify risk factors & discuss individually their relative significance
  - This will influence decisions & provide justification of concept for design & extent of testing.

Implies mAb products should not be viewed as **'low-immunogenicity-risk'** class. Case-by-case approach warranted

Sampling strategy may depend on the risk

- **For High risk** – From early stages, frequent, sequential sampling & testing throughout the clinical programme. Analyze samples in real time.
- **For Low risk** – In late stages of development, reduced sampling possible provided no adverse events or reduced efficacy is observed. Banking of samples routinely is imperative throughout the programme. Possibility of retrospective analysis

Immunogenicity testing

- Although assay design, strategy & extent of testing are likely to vary between mAbs, certain key elements need to be addressed in designing immunogenicity assays for application during clinical testing -
- Sensitivity - Sufficiently sensitive assays to detect clinically relevant levels of antibodies
- Interference – Assay results should not be confounded by matrix interference or from residual product. Any interference needs to be evaluated and strategies to minimise/overcome this implemented
- Biological/Functional consequences – Since induced antibodies can have multiple biological effects e.g., neutralizing activity etc, assays should be designed to detect these consequences.

Every mAb needs to be evaluated for immunogenicity individually and appropriate strategies adopted for each mAb development programme

Immunogenicity testing : Some Considerations

- **Long half-life**; often administered chronically at high doses so samples are expected to contain high levels of therapeutic/immune complexes which interfere with detection of induced abs. This needs evaluation and an optimal strategy defined and built in to the assay.

  Although a suitable positive control can be used, it does not reflect the situation with clinical samples (varying isotypes, affinities etc within/between patients over time).
- **Interference from other substances** e.g., soluble target, other components, disease specific factors e.g., rheumatoid factors (RF) should be evaluated & mitigated as appropriate and built in to the assay
- **Pre-existing antibodies** – if detected, investigate reactivity and implement strategy; problematical from bioanalytical, efficacy & safety perspective
- **Antibody controls** - Positive: Human serum (ideal), Polyclonal sera from hyperimmunised animals, affinity purified, mAbs, anti-idiotypic antibodies; Negative: pre-therapy sera, irrelevant antibody, normal donor sera (individual or pooled).

Immunogenicity testing

- **Multi-tiered Approach - Valid and sensitive assays which can detect relevant antibodies**
  - For example, for heterologous e.g. rodent sequence or human chimaeric mAbs - antibodies induced against various epitopes e.g. **anti-Fab, anti-Fc.**
  - Humanised or human sequence mAbs - mainly **anti-idiotypic** & can compromise clinical responses. In some cases, antibodies induced against the **constant region of human or humanised mAbs** and impact on the immunobiological function
  - In some instances, additional assays required –

    For example, mAbs containing non-human carbohydrate structures such as gal alpha 1, 3 gal can be problematical because of the presence of pre-existing **IgE** antibodies against these structures (potential for allergic reactions); patients will need to be tested for pre-existing IgE antibodies prior to treatment – **IgE assay** needed. If mAb induces high incidence of allergic reactions on first administration, need to test
  - In certain instances, IgE antibodies may be induced by the product.
  - If mAb intended for airway/gut administration - **IgA testing**

Screening assays

- ELISAs – direct format problematical due to lack of secondary reagents that discriminate between serum abs & mAb product
  - Enzyme-labelled anti-λ to detect and mAbs (often have k light chain) to capture or labelled anti-IgG, anti-IgM as detector and mAb-Fab or F(ab')2 to capture – high background due to reactivity of serum of healthy subjects with capture reagents
- ELISAs - bridging formats popular, sensitive and robust.
- Radioimmunoprecipitation assays (RIPA) – sensitive, solution phase
  - Antigen-binding assay based on sepharose-bound protein A/G or abs to human Ig to capture ab & labelled mAb-Fab or F(ab')2 to detect
  - Two-site/Bridging assay using sepharose-bound mAb and ^{125}-labelled therapeutic
- Surface Plasmon Resonance (SPR) – detects rapidly dissociating abs
- Electrochemiluminescence (ECL) – highly sensitive & drug tolerant
- Other technologies – DELFIA, Gyrolabs, AlphaLisa etc

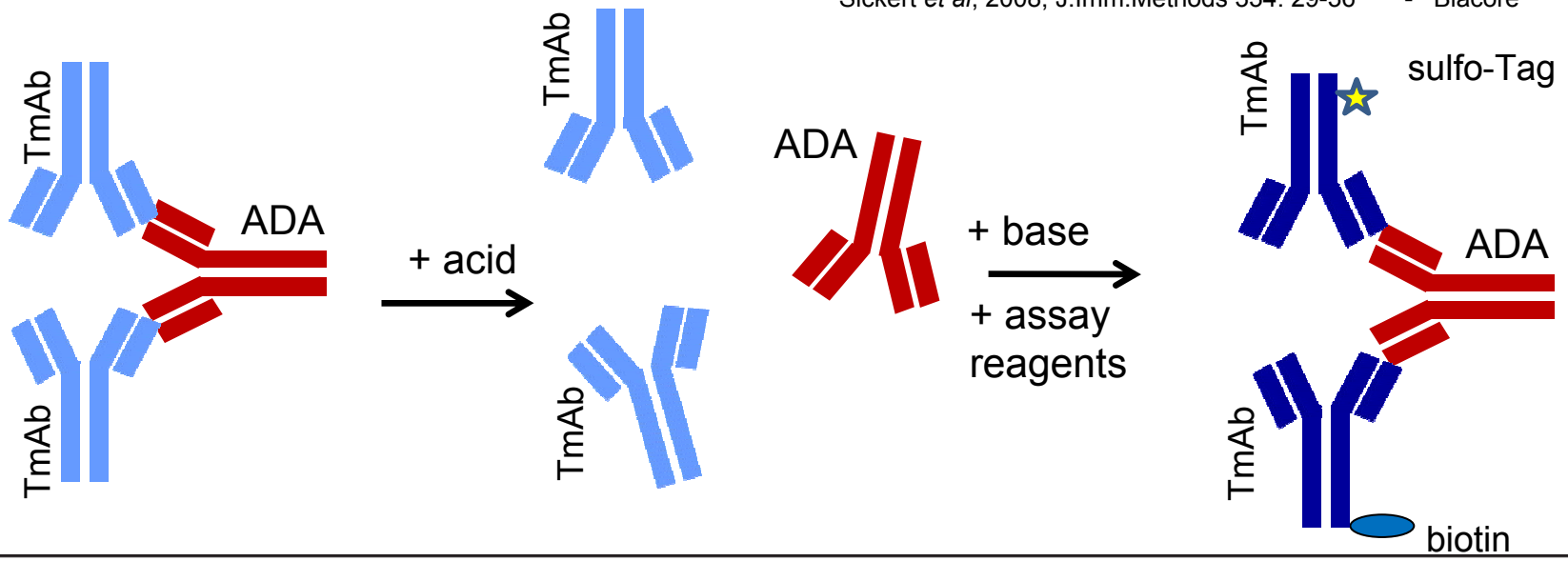
Assays – Pros & Cons

Bridging ELISA	High throughput Acceptable sensitivity No requirement for species-specific reagents	May require two antigen conjugates Antigen labeling may alter antigen & obscure epitopes Highly susceptible to interference from antigen May not detect IgG4 antibodies	Natalizumab
Electrochemiluminescence assay	High throughput. Excellent sensitivity. Large dynamic range. Minimal influence of sample matrix, tolerant of residual therapeutic	Labeling may alter antigen and obscure epitopes Requires electrochemiluminescence instrument, vendor-specific reagents	Different mAbs
Radioimmunoprecipitation assay	Moderate-to-high throughput Solution-based Good sensitivity Can be specific	Can be isotype specific. May fail to detect rapidly dissociating antibodies Requires radiolabeled antigen. Radiolabel may alter/denature antigen. Decay of radiolabel may affect antigen stability	Adalimumab
Surface plasmon resonance assay	Automated Enables detection of both low- and high-affinity antibodies. Allows isotype determination Detection reagent not required	Antigen immobilization may alter protein conformation Regeneration step may degrade antigen. Sensitivity often less than binding assay Expensive. Requires dedicated equipment, reagents are costly and vendor specific	Panitumumab

Residual mAb

Principle of acid dissociation: (AD)

Patton *et al*, 2005, J.Imm.Methods 304: 189-195 - bridging ELISA
Sickert *et al*, 2008, J.Imm.Methods 334: 29-36 - Biacore



Technology platforms	Sensitivity (ng/mL)	Drug tolerance (Drug:ADA [a])	Pros	Cons
Solid-phase ELISA	10	20:1	Generic reagents and instrumentation	Low drug tolerance
Gyros	4–20	100:1	Assay automation Assay time <2 h	Sole technology provider Fluorescent label stickiness
AlphaLISA	20	100:1	Homogeneous assay without wash steps	Sole technology provider Pipetting under restricted light conditions Hook effect
MSD ECL	10	100:1	Fewer steps than ELISA	Sole technology provider
Solution ELISA	25	200:1	Improved drug tolerance Generic reagents and instrumentation	Requires high quality streptavidin plates

[a] Drug:ADA ratio was determined at 100 ng/mL of positive control ADA and calculated using the molar values of Drug and ADA.

Mikulskis *et al*, 2011,JIM 365: 38-49;

Immunogenicity testing

- **Residual mAb**
  - Acid dissociation or a variation to dissociate & remove the therapeutic & prevent immune complex formation.

    Lofgren *et al*, 2006,JIM 308: 101-108; Bourdage et al, 2007JIM 327: 10-17; Smith *et al*, 2007, Reg.Tox.Pharm.49:230-237
  - Other options e.g., sample dilution, wash-out samples.
  - Possibly a strategy which involves a combination of above approaches
- **Other substances**
  - Multimeric soluble target, Rheumatoid factors etc need to be removed

Immunogenicity testing

- Evaluation of neutralizing capacity of antibodies is expected; deviations need to be justified
- Assays –
  - Measured using Bioassays or Competitive ligand binding assays but CLBs may be the method of ‘choice’
  - Due to the multi-faceted mechanism of action for mAbs, tests for both the blocking of binding activity and interference with an immunobiological mode of action (e.g., blocking of effector function activity) should be considered.

Immunogenicity testing

- New guideline & general guideline – need to be considered together when planning immunogenicity studies; strategy for assessment needed
- Guideline **does not recommend a particular assay**;
  - Evaluation of different platforms prior to final selection of screening assay; >1 assay platform may be needed for screening,
  - **generic assay/strategy does not fit all mAbs**; case-by-case approach
- Justification on the suitability of the chosen approach(es), taking into consideration relative merits & limitations of the methods. Other assays e.g., IgE assay or for assessment of reactivity of pre-existing antibodies

For all mAbs, a validated screening and confirmatory assay should be performed followed by a validated neutralizing assay in case of positive results in the confirmatory assay. Distinguishing between neutralizing and non-neutralizing antibodies is essential **independent of their risk level**. Correlation of antibody expression with clinical outcome is important and has to be thoroughly evaluated.