
# The EBF Perspective on the FDA draft guidance on Assay Development for Immunogenicity Testing of Therapeutic Proteins

Presented by:
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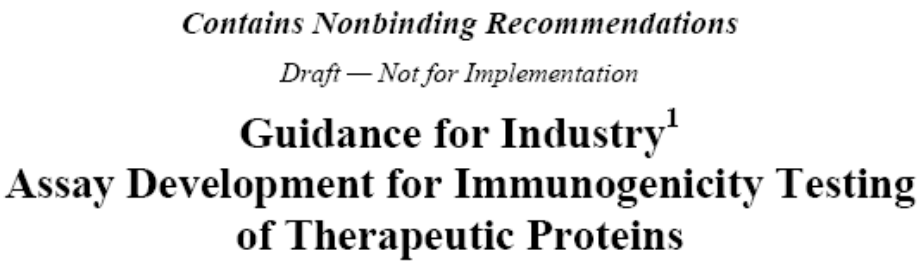
at the EBF open meeting in Barcelona 2010

# Introduction

- In December 2009 the US Agency (FDA) presented a draft guidance for immunogenicity assessment of bioproducts:



*Contains Nonbinding Recommendations*

*Draft — Not for Implementation*

**Guidance for Industry[1]**
**Assay Development for Immunogenicity Testing of Therapeutic Proteins**

- FDA requested comments to this draft guidance
- EBF comments were submitted to the FDA (collected from a total of 13 EBF-IGM member companies)

Bayer Schering Pharma AG, Boehringer-Ingelheim, Crucell, Ferring, Johnson & Johnson, H. Lundbeck A/S, Merck Serono, Micromet, Hoffmann-La Roche, Sanofi-Aventis, Merck Sharpe and Dohme, Solvay Pharmaceuticals, UCB

# Introduction

- Outline of the draft guidance:
  - I: Introduction
    - (rationale and purpose)
  - II: discusion
    - (general, immunogenicity testing, principles of immunogenicity testing)
  - III: Approach to assay development
    - (overview of design elements, screening assay, neutralization assay)
  - IV: Clinical aspects of assay validation
    - (critical considerations and caveats, determining the minimal dilution, assay cut point)
  - V: Assay validation
    - (validation of screening assay, validation of the neutralization assay, validation of immunodepletion/competitive confirmatory assay)

# Introduction

- Outline of the draft guidance, continued :
  - VI: Implementation of assay testing
    - (obtaining patient samples, concurrent positive and negative quality controls, cut point normalization, reporting patient results, pre-existing antibodies, specific considerations)
  - VII: Other aspects of immunogenicity testing
    - (isotypes, epitope specificity)
- During this presentation the highlights of the comments of the EBF-IGM on the guidelines are discussed

# Section II: Discussion

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| General | "Clinicians rely on the information re immunogenicity rates observed during clinical trials. This makes the development of valid, sensitive immune assays a key aspect of product development." | Explain what can affect these rates (dose, regimen, formulation, healthy volunteers versus patient, immunosuppressed patient, indication etc.) |
| | Banking and possible re-assessment of patient samples | Might contravene with ICH and GCP guidelines on patient consent |
| Immunogenicity testing during product development | Head-to-head comparison of products in patient trials using standardized assays | Standardization of assays is challenging<br>Difficult to obtain competitors material for clinical trial use |
| | Usage of a standardized assay that has equivalent sensitivity and specificity for both products | Standardized assay with equivalent sensitivity and specificity for both products is impossible |

# Section II: Discussion

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Principles of immunogenicity testing | Assays should have a “sufficient sensitivity” to detect clinically relevant levels of antibody | What is clinical relevant? |
| | “Immunogenicity tests should be designed to detect functional or physiological consequences” | Immunogenicity tests cannot detect functional consequences. The read out will always be the pathologic or clinical alteration. |

# Section III: Approach to Assay Development

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Overview of design elements (Multi-tiered Approach) | Multi-tiered Approach | Presented as a general Requirement. Not the case for low-risk compounds or compounds with extremely low immunogenicity rate. |
| | "Neutralizing antibodies (NAbs) generally of more concern than binding antibodies (BAbs)" | For preclinical safety assessment, knowledge of BAbs is essential (hypersensitivity Type I, III and autoimmune reactions) |
| Overview of design elements (Aspects of Assay Development) | Initial screening should be very sensitive | Since the definition of sensitivity is always based on the available possible control the statement "very sensitive" should be clarified |

# Section III: Approach to Assay Development

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Screening assay (Selection of Assay and Reagents) | Development of positive and negative controls | - Purification of positive control can result in enriching for strong binding Ab<br>- Producing a positive control which mimics antibodies found in subjects during an entire immune response is difficult |
| | "The applicant should use antiproduct antibodies to assess assay validation parameters such as sensitivity, specificity, and reproducibility." | Drug tolerance should be added as one of the key validation parameters |
| Neutralization Assay (Selection of Format) | Selection of Format | We suggest to perform PD measurements on the clinical samples if possible. Together with the PK profiles it generates direct information on the impact of immunogenicity on the product efficacy |

# Section IV: Clinical Aspects of Assay Validation

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Determining the Minimal Dilution (importance) | Validation of Minimal Residual Dilution (MRD) | MRD determination (also done as recovery experiments) should be part of development and not necessarily of validation |
| Assay Cut Point (Determination) | Sample size of 50-100 is statistically more reliable to effectively define the cut point | What is the statistical basis to use 50 – 100 individual samples for cut point determination? |
| | "When establishing the cut point, the applicant should also consider the removal of statistically determined outlier values" | More guidance on the removal of outliers in Cut Point determination is expected (statistical criteria) |
| | "Using immunodepletion approaches, the applicant should identify those samples with pre-existing antibodies and remove them from the analysis" | Handling of pre-existing antibodies need to be better specified (e.g. use of immunodepletion approaches) |

# Section V: Assay Validation

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Validation of Screening Assay (Sensitivity) | "..the applicant should evaluate antibody avidity before and after purification as part of reagent characterization" | What is the rationale to evaluate avidity of surrogate ADA used for validation? |
| Validation of Screening Assay (Precision) | "Samples should include negative controls and positive samples with values in the low, medium and high levels in the dynamic range of the assay" | Immunogenicity assay is rather a qualitative assay therefore QC samples should inlcude NQC, LQC and HQC – MQC can be omitted |
| Validation of Neutralizing Assay (Specificity) | "The applicant should also confirm the absence of alternative stimuli in patient serum" | Determining if unexpected alternative stimuli exists is impossible – delete this requirement from the guideline |
| Validation of Immunodepletion/ Competitive Confirmatory Assay | Identifying the degree of inhibition or depletion that will be used to ascribe positivity to a sample | Refer to the publication of Shankar *et al*. (2008) for the specifics of confirmatory cut point determination (CCP). Drug naive samples should be used for CCP determination. |

# Section VI. Implementation of assay testing

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Obtaining patient samples | "Optimally samples taken 7-14 days after exposure can help elucidate an early IgM predominant response" | Assessment of immunogenicity 7-14 days post exposure is only feasible for drugs with short half-life or if acid dissociation can be implemented |
| Reporting Patient Results | "Reporting in terms of titers is more appropiate and is well understood by the medical community. We believe attemps to convert such data into mass units by using standard curves or other data conversion methods are generally confusing and inaccurate" | The prerequisite of having to demonstrate parallelism in order to make use of the quasi-quantitative assay approach is not supported by EBF. It is not believed to be achievable given the high biological variability of an immune response (Difference in response between subjects: titers can not be compared) |

# Section VII. Other aspects of immunogenicity testing

| Sub-section | FDA guidance | EBF-IGM comment |
|---|---|---|
| Isotypes | "While the initial screening assay should be able to detect all isotypes" | With current technologies it is not possible to detect all isotypes with the same sensitivity using only one screening assay |
| | "Consequently, determining if antibody responses occurring upon prolonged exposure to therapeutic proteins are associated with this isotype may be useful" | EBF suggests to include that "determining whether antibody responses are associated with a specific antibody isotype (e.g. IgE and IgG4) may be useful, but not required"<br>Isotyping should be case-by-case and risk based |

# Final remarks

General comments of the EBF-IGM on the entire document:

- Overall EBF-IGM appreciate this very comprehensive guideline that provides clarity to the industry.
- PK and PD read-outs do often provide more information on *in vivo* effects of the binding antibody responses than any in vitro assay may give
- The guideline favors the titer based approach for screening opposed to quasi-quantitative mass units. EBF-IGM recognizes that both approaches have their limitations
- The need to specify concentrations with regard to assay sensitivity is not understood as the immunogenicity assays are not quantitative but qualitative assays. The proposed change is delete any "concentration" values from the guideline

# Final remarks

General comments of the EBF-IGM on the entire document:

- The intension to provide a ‘generic’ guide to assay development for biological therapeutics may cause difficulties to interpret certain aspects of the guideline
- EBF-IGM would appreciate clarification on the present guidance whether it refers only to clinical studies or also to preclinical studies and a strategy that may be applied
- It would be supportive to get recommendation on the content of immunogenicity risk assessment (categorization into high- and low-risk compounds).This should trigger the extent of immunogenicity testing in a tiered approach in drug development process

# Final remarks

General comments of the EBF-IGM on the entire document:

- To avoid diverging documents EBF-IGM strongly supports harmonization of the regulatory effort between FDA and EMA
- The complete comments of the EBF-IGM on the draft guidance were submitted to the FDA and can be viewed on the FDA website