The Role of Biostatistics in Immunogenicity Testing

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Immunogenicity of Biopharmaceuticals

ADA detection methods – common issues

- Qualitative; there is no reference standard.
- Require a “cut-point”.
- *ADA is not “an analyte”; it is a spectrum of analyte/reactivity*
  - Species specific
  - Epitope specific
  - polyclonal (probably), varying avidities
  - In humans, ADA could be expressed as IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgE; Other species produce other isotypes
- Assay development depends upon availability of analyte (a positive control) Assay performance is optimized for THE positive control analyte on hand; we are lucky when more than one positive control is available. Defines assay sensitivity and drug tolerance.
- So how good is a test method based on a single analyte, in detecting “a spectrum of analyte” in the subjects?

The Challenges We Face…

- *Statistics for Qualitative assays? Are you crazy or just being mean?* ☺
- Anti-drug antibodies can impact safety. Assay results are relevant to clinical outcomes. *So, we need to be conservative…*
- Tiered ADA testing scheme for practical reasons: Screen-negative samples are not tested anymore. *So, we need to be conservative…*
- ADA assays don't just produce +/- results. They produce a continuum of signal, of which non-specific binding ("background") must be differentiated from specific binding
- Some drug naïve patients, and also some healthy volunteers, have high reactivity in the ADA assay. Is that non-specific, pre-existing ADA, or a specifically binding interferent (false-positive for ADA)?
- Pre-existing antibodies versus treatment emergent antibodies

For all these reasons, we must make our best effort to reduce subjectivity & increase objectivity

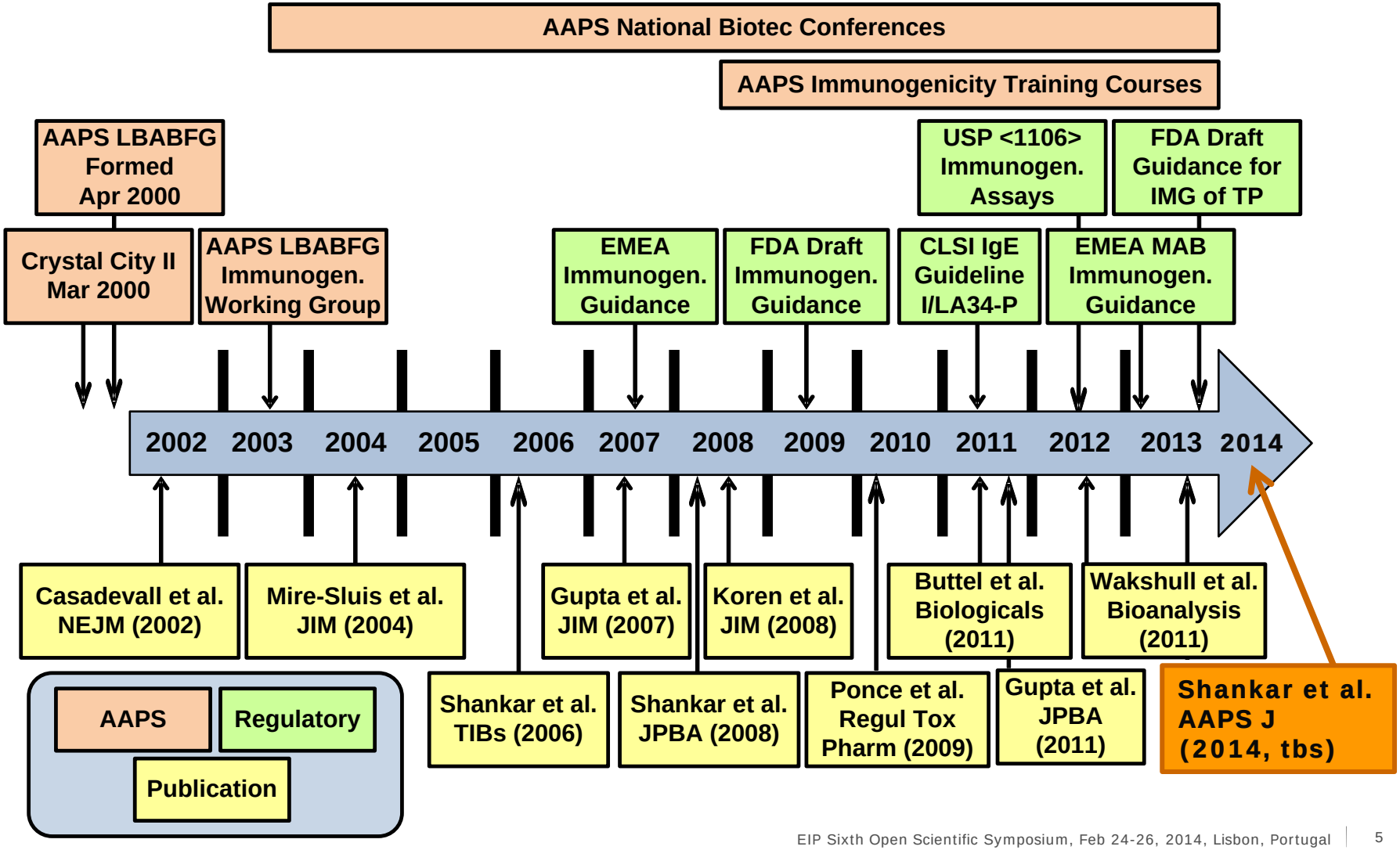
How did we do it before any of the consensus publications and regulatory guidance documents?

1. **Variable and subjective approaches to screening cut point**
   a. No false-positive rate built-in
   b. LLOQ or LLOD approach
   c. Based on low positive control value
   d. Drug and ADA naïve sera, but using 2SD or 3 SD
   e. Without eliminating outliers
   f. Without eliminating true-reactive samples (pre-existing antibodies)
   g. Etc.

2. **Specificity confirmation cut point***
   a. No competitive inhibition approach used. Dilution or titration, or orthogonal assay, used to confirm
   b. Competitive inhibition using positive control
   c. Etc.

The 2000's – Biotech's *Immunogenicity Decade*

Adapted from Dr. Ronald Bowsher's presentation



ADA Immunoassay Development

2004

ELSEVIER

Journal of Immunological Methods 289 (2004) 1–16

JIM Journal of Immunological Methods

www.elsevier.com/locate/jim

Standardization

Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products

Anthony R. Mire-Sluis[a,*], Yu Chen Barrett[b], Viswanath Devanarayan[c], Eugen Koren[d], Hank Liu[e], Mauricio Maia[f], Thomas Parish[g], George Scott[h], Gopi Shankar[i], Elizabeth Shores[j], Steven J. Swanson[d], Gary Taniguchi[k,†], Daniel Wierda[l], Linda A. Zuckerman[m]

KEY MESSAGES:

- ADA immunoassays qualitative (screening) or quasi-quantitative (titration)
- Assay quality controls: positive and negative controls should be used
  - When possible, polyclonal antibody positive controls are preferred
  - Depending on the assay type, species–specific assays should preferably have species specific controls
- Use a risk-based approach to assure that low positive samples can be identified, and false-negative samples are limited. The screening assay cut point should be computed to allow (theoretically) the selection of of 5% false-positive samples
- Immunoassay sensitivity: clinical – 250 to 500 n/mL; non-clinical – 500 to 1000 ng/ml

ADA Immunoassay Validation

2008

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Review

Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products

Gopi Shankar[a], Viswanath Devanarayan[b], Lakshmi Amaravadi[c], Yu Chen Barrett[d], Ronald Bowsher[e], Deborah Finco-Kent[f], Michele Fiscella[g], Boris Gorovits[h], Susan Kirschner[i,1], Michael Moxness[j], Thomas Parish[k], Valerie Quarmby[l], Holly Smith[m], Wendell Smith[n], Linda A. Zuckerman[o], Eugen Koren[p,*]

KEY MESSAGES:

- *Objective decision criteria are critical; subjective approaches should be eliminated/minimized*
- *Alternate objective approaches may also be applied*
- *Application of statistics is important (balanced experimental design, outlier exclusion, etc)*
- *Assay means and variability across runs drives choice of screening assay cut point: fixed, floating or dynamic*
- *Specificity cut point should be based on analytical and biological variation (like screening cut point)*
- Positive controls used to validate methods may not represent the analyte (ADA)
- CAUTION: over-reliance or dependence on quantitative data (sensitivity, drug tolerance) generated using positive control “standard” reagents

Assay performance characteristics for validation

Where is a statistical approach critical?

1. Screening cut point*
2. Specificity confirmation cut point*
3. Titer cut point
4. Sensitivity
5. Interference
    - Drug tolerance
    - Target tolerance
6. System suitability control (“QC”) criteria*
7. Precision*
8. Robustness
9. Stability

Final Remarks

- To ensure objective criteria, use of statistics is important

- The analyses need not be complicated; can do without an expert statistician, where possible

- But let's see what our statistician friends have to say today…