Clinical Immunogenicity Testing: The Regulatory Challenge - Experience with the FDA

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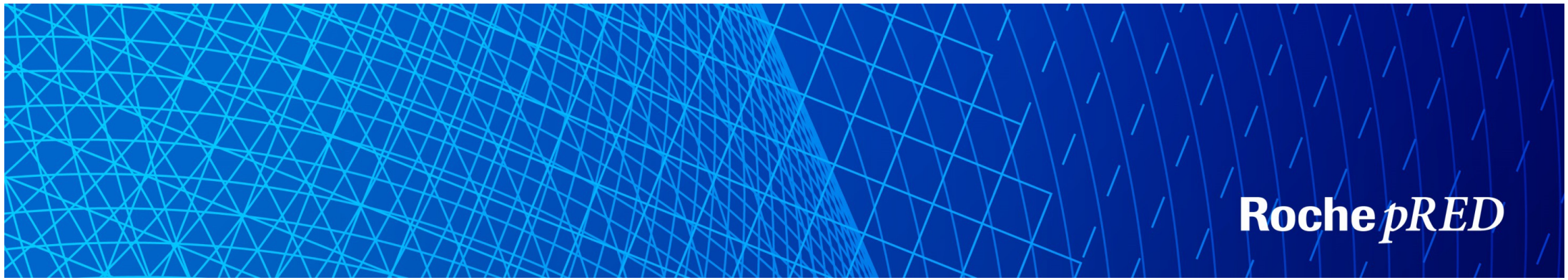


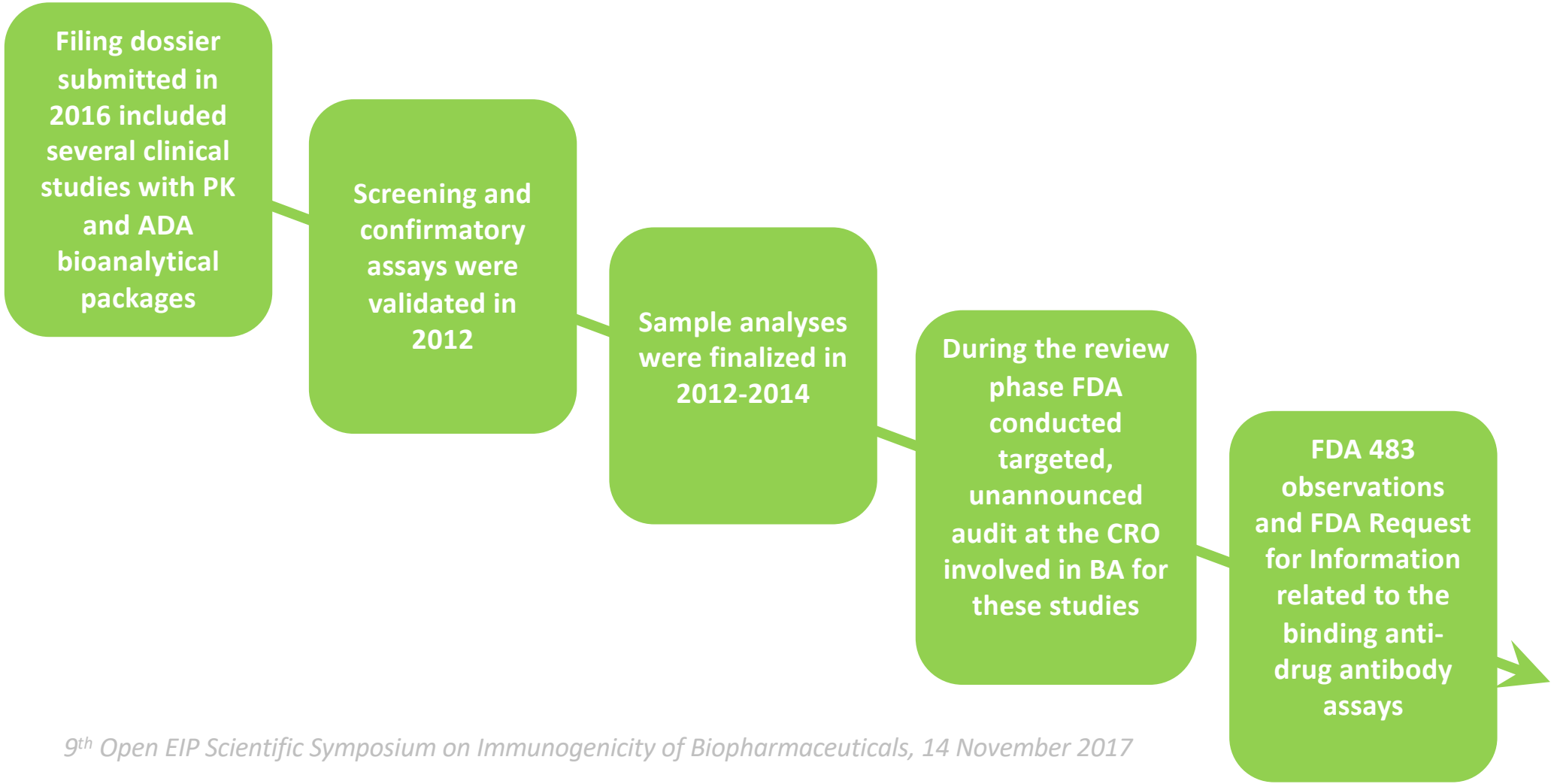
Outline

- FDA Draft Guidance 2016 on Immunogenicity Testing (Highlights)
- FDA Audit and Observations
- Response Strategy and Results
- Conclusions

Highlights from FDA Draft Guidance (2016)

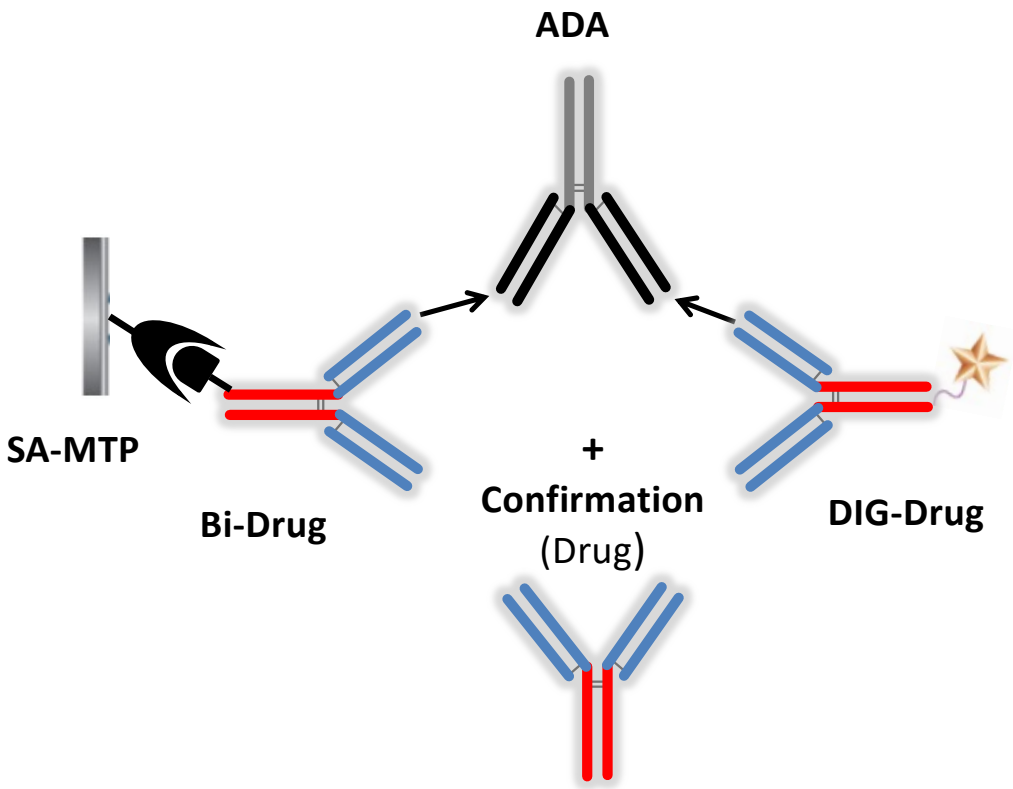
- The cut point should be statistically determined using samples from treatment-naïve subjects.
  - Footnote: Treatment-naïve subjects could be healthy individuals or a patient population not exposed to therapeutic protein product, depending on the stage of assay development or validation and on the availability of samples. Sponsors should provide justification for the appropriateness of the samples used.
- FDA recommends that screening and confirmatory ADA assays achieve a sensitivity of at least 100 nanograms per milliliter (ng/mL).
- Confirmatory assays should be fully validated in a manner similar to screening and neutralization assays
- The sponsor should examine other parameters affecting patient samples, such as hemolysis, lipemia, presence of bilirubin, and presence of concomitant medications that a patient population may be using.

FDA Audit of BA at Partner CRO



Assay Method

- Investigated drug is a classical monospecific mAb
- 1-step o/n bridging ELISA
- Detection by anti-DIG-POD and TMB



Observation #1

Description
The sensitivity of the anti-drug antibody (ADA) assay did not consistently identify low ADA-positive control samples in the confirmatory assay.

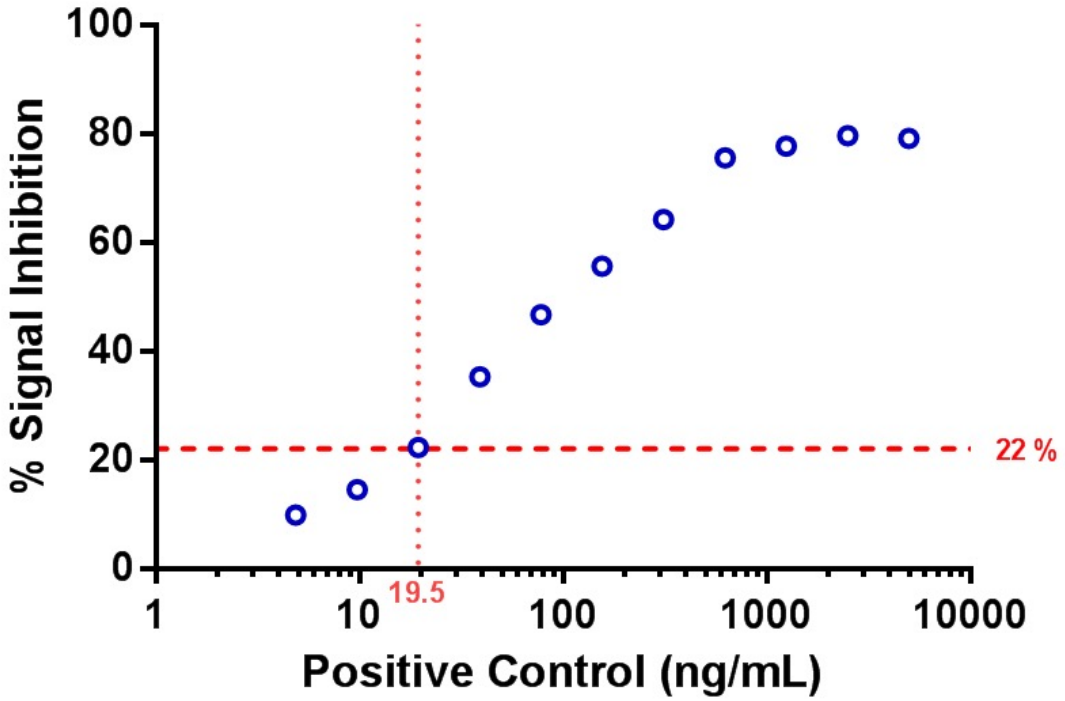
- Sensitivity of the confirmatory assay was not formally confirmed in the validation
- Validation experiments have been performed to determine the relative sensitivity of the confirmatory assay
  - A few PC levels around the screening LPC level were analyzed on several occasions
  - The lowest level that was always confirmed positive was considered the sensitivity of the confirmatory assay
  - LPC-C (confirmatory) = 10.0 ng/mL; (LPC-S (screening) = 4.06 ng/mL)

Sensitivity Determination in Confirmatory Assays

New Validations

- **Drug-spiked sensitivity curves are performed in parallel with screening sensitivity curves**
- **Statistically determined sensitivity for both assays**

LPC-C = Mean + $t_{x,df}$ x SD(Mean)



Observation #2

Description
Method validation did not completely demonstrate selectivity and precision for the ADA assay. Specifically, a. Selectivity and specificity in hemolyzed serum were not validated in the ADA screening and confirmatory assays. Specifically, 30 of 1227 samples in study X, 221 of 8777 samples in study Y, and 54 of 3015 samples in study Z had documented hemolysis.

- **Impact on ≤ 2.5% samples**
- **Effect of hemolysis was studied at NC and at least two additional levels in both screening and confirmatory assays**
- **Neither of the assays were affected by hemolysis**

SCREENING	2% hemolyzed	10% hemolyzed
NC	< CP	< CP
PC 1	> CP	> CP
PC 2	> CP	> CP
PC 3	> CP	> CP

CONFIRMATORY	2% hemolyzed	10% hemolyzed
NC	< CCP	< CCP
PC 2	> CCP	> CCP
PC 3	> CCP	> CCP

Observation #2

Description
Method validation did not completely demonstrate selectivity and precision for the ADA assay. Specifically, b. The method validation did not determine inter-assay precision of confirmatory controls. Precision of the confirmatory high positive controls (HPCs) in study runs yielded %CV values >30%.

	Absorbance HPC in absence of drug	Absorbance HPC in presence of drug	Signal Ratio	Signal inhibition (%)
Mean	2.184	0.126	0.058	94.2
SD	0.136	0.044	0.020	2.0
CV (%)	6.2	34.7	35.1	2.2
n		164		

Signal inhibition is regarded as the relevant and scientifically justified parameter

Observation #2

Description
Method validation did not completely demonstrate selectivity and precision for the ADA assay. Specifically, c. Selectivity of positive and negative confirmatory controls in serum was not determined during method validation

- **Selectivity levels using sera from 10 human individuals and pooled serum**
  - **3 times the relative sensitivity of the confirmatory assay**
  - **unspiked**
- **All samples passed in this assessment**

	0 ng/mL ADA concentration	30 ng/mL ADA concentration
NC	< CCP	> CCP
Serum 1	< CCP	> CCP
Serum 2	< CCP	> CCP
Serum 3	< CCP	> CCP
Serum 4	< CCP	> CCP
Serum 5	< CCP	> CCP
Serum 6	< CCP	> CCP
Serum 7	< CCP	> CCP
Serum 8	< CCP	> CCP
Serum 9	< CCP	> CCP
Serum 10	< CCP	> CCP

Request for Information

Description
Determination of the screening assay and confirmatory assay cut points uses healthy volunteers. We note that this assay was validated subsequent to the publication of the 2009 Draft Guidance for Industry “Assay Development for Immunogenicity Testing of Therapeutic Proteins.” The 2009 draft guidance recommended that the samples used to determine the cut point come from “patients not exposed to product”, which is clarified in the 2016 draft guidance to indicate “treatment naïve patients” as opposed to healthy volunteers. Verify the cut point with pre-dose patient sera.

Healthy volunteer cut points

- 21.5% screening positive
  - 4.3% confirmed positive
  - 17.2% false positive

Study specific cut points

- Slightly different confirmatory cut point in one clinical study
  - Identification of additional 11 transient positive samples
  - Overall picture did not change

Conclusions

- Full response to all observations and RFI within FDA deadline including additional experimental data
- FDA is reinforcing recommendations given in the 2016 draft guidance even for validations and sample analyses that had been performed before its issuance
- Confirmatory tier has to be validated as a stand alone assay
- Hemolysis effect should be tested despite lack of known cases for monoclonal biotherapeutics
- Healthy vs. disease individuals for cut point establishment in validation; strategy should carefully be defined
- Filing package was accepted indicating that responses to 483s were adequate

Acknowledgement

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... and many more

Doing now what patients need next