Regulatory case studies on the applications of reference standards for therapeutic proteins

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Outline

• Recap of reference standards and regulatory expectations for therapeutic proteins

• Key considerations during method validation and system suitability testing
  – Purpose, suitability for intended use, qualification parameters, lifecycle approach, triggers for new RS, preventing drift

• Case studies and lessons learned from investigational and licensed therapeutic protein products
Recap of points to consider from FDA guidances, ICH Q2B and ICH Q6B

• “An appropriately characterized in-house primary reference material, representative of production and clinical materials” (ICH Q6B)

• “Reference materials for biological products should be representative of the manufacturing process and should be as fully characterized as practical including:
  – physicochemical characteristics, structural characteristics, biological activity, and/or immunochemical activity”
Recap of points to consider from FDA guidances, ICH Q2B and ICH Q6B

• FDA’s guidance on method validation indicates that “well characterized reference materials, with a documented purity, should be used throughout the validation study” (ICH Q2B):

  – Ref Std are critical in validating specificity for an identity test but additional Ref materials that are closely related and may be present are also recommended as negative controls (ICH Q2B)

  – Establishing accuracy of the analytical procedure by comparison to a reference material is recommended but “accuracy may be inferred once precision, linearity and specificity are established” (ICH Q2B)
    • In other words in the absence of a Std you can assign a “conventional true value” base on the above validation characteristics

• A revised draft guidance on method validation is being worked on.
Method validation and the use of reference standards

- A DS/DP Ref Std is useful in determining the validation characteristics of a method; production lots can also be used for determining many of the validation characteristics of an assay

- A single “reference standard” may not be adequate to assure method validation, especially if the method is going to be used to test in-process materials, stability samples etc. Other reference materials are sometimes necessary (i.e., impurity/purity “standards”)

- Ref Std for API are particularly critical in validating specificity for an identity test

- Establish storage, usage conditions, and handling instructions for reference standards to avoid added impurities and inaccurate analysis

- Indicate how the Ref Std is to be used and how the qualification protocol ensures that the method is suitable for its intended purpose

- Protocols should provide some reasonable limits on what is an acceptable result
System suitability and the use of reference standards

- Verifies that the analytical method is performing as expected (i.e., expected resolution, reproducibility, sensitivity, recovery, etc) and therefore, that test results on that day are meaningful

- Often achieved through the use of a Ref Std

- USP <621> defines some suitability tests and the use of Ref Std for chromatography systems

- For example for SEC, the Ref Std may be used to establish
  - Peak asymmetry
  - Height Equivalent to a Theoretical Plate
  - Retention times (resolution)
  - Recovery (e.g. total peak area)
  - Relative standard deviation
  - Consistency with historical profile of standard

From Ahrer et al., J of Chromatogr (2003)
New reference materials: 1-Tiered vs 2-Tiered approach

A = primary reference standard representative of the clinical trial material; B through F = new primary standards replaced every 3 years

- 2-Tiered: A = B, A = C, A = D, A = E, A = F
- 1-Tiered: A = B = C = D = E = F, **but** is A = F 15 years later?

- Which approach provides better assurance that over time the product continues to reflect the clinical trial material?
  - Product A is representative of the clinical trial material, if it is stored under conditions that prevent any decrease in product quality.
  - Error may be cumulative for 1-tiered approach and could allow for a drift in product characteristics over time

- Creation of new primary reference material should be carefully evaluated and need not be performed every time a change in the manufacturing process alters the product’s characteristics

- Replacing the primary Std is appropriate when the Std runs out or for certain process changes that result in change in the product quality particularly one associated with a new clinical study.
New reference materials: 
1-Tiered vs 2-Tiered approach (ICH Q6B)

“For drug applications for new molecular entities, it is unlikely that an international or national standard will be available. At the time of submission, the manufacturer should have established an appropriately characterized in-house primary reference material, prepared from lot(s) representative of production and clinical materials. In-house working reference material(s) used in the testing of production lots should be calibrated against this primary reference material. Where an international or national standard is available and appropriate, reference materials should be calibrated against it.”
New reference materials: 1-Tiered vs 2-Tiered approach

Calibration Against Last Lot

Calibration Against Primary Std
The lifecycle management of reference standards as a cGMP expectation?

- **ICH Q7A: Good manufacturing practice guidance for active pharmaceutical ingredients:**
  - VI. Documentation and records. /F. Laboratory Control Records (6.6)
    “…data on or cross-reference to the preparation and testing of reference standards, reagents and standard solutions”
  - XI. Laboratory Controls. /A. General Controls (11.1)
    “Primary reference standards should be obtained, as appropriate, for the manufacture of APIs. The source of each primary reference standard should be documented. Records should be maintained of each primary reference standard's storage and use in accordance with the supplier's recommendations. Primary reference standards obtained from an officially recognized source are normally used without testing if stored under conditions consistent with the supplier's recommendations. Appropriate testing should be performed to establish fully the identity and purity of the primary reference standard. Appropriate documentation of this testing should be maintained. Secondary reference standards should be appropriately prepared, identified, tested, approved, and stored. The suitability of each batch of secondary reference standard should be determined prior to first use by comparing against a primary reference standard. Each batch of secondary reference standard should be periodically requalified in accordance with a written protocol.”
The lifecycle management of reference standards as a cGMP expectation?

- FDA cGMP guidance for manufacturing, processing, or holding APIs (March 1998): The suitability of each lot of secondary reference standard should be determined prior to use by comparing against a primary reference standard obtained from an official source and periodically requalifying each lot in accordance with a written protocol. Primary reference standards need not be tested if stored under conditions consistent with those described in the labeling.

- FDA Biotechnology Inspection Guide: “A reference preparation for biological activity should be established and used to determine the bioactivity of the final product. Note: Where applicable, in-house biological potency standards should be cross-referenced against international…” ([http://www.fda.gov/ICECI/Inspections/Inspectionguides/UCM074181.htm](http://www.fda.gov/ICECI/Inspections/Inspectionguides/UCM074181.htm))

- 21 CFR 211.160 (b): “(b) Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity.”

- 21 CFR 211.194 (c): “Complete records shall be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions.”
Case study #1

- An IND was submitted for a protein drug product that is also a commonly used laboratory reagent. The Sponsor proposed to use a commercial, reagent-grade source of material as their reference standard and express a CQA relative to this reference standard.

- The Sponsor was advised against using the commercial sourced reference standard because it would not be representative of the Sponsor’s current manufacturing process, product, or intended clinical use.

- They were advised that it is acceptable for the development of a reference material to evolve during investigational phases as long as it is suitable for its intended use and reflects the current manufacturing process.
Case study #2

• A supplement was submitted for a new working cell bank and Ref Std where the applicant indicated that every time they created a new working cell bank, they created a new reference standard which was qualified against the current reference standard. The Ref Std was used in calibrating results of a CQA test.

• Applicant was requested to revise their Ref Std program to include primary and working (secondary) reference materials or provide a justification why these are not necessary.
Case study #3

- First Ref Std of a glycoprotein was created from the process/product representative of the pivotal clinical trials and assigned values representative of the multiple lots used in the pivotal trials. Following scale up of the phase 3 process, new Ref Stds were evaluated from the commercial scale production process.

- Specification testing included:
  - Activity/aggregation by SEC/ Identity/ Peptide Mapping/ RPHLC/ protein content/ IEF/Appearance/Endotoxin/ Sialic Acid content/ Oligosaccharide mapping/pH/SDS-PAGE with justified acceptance criteria

- Additional characterization included:
  - Free thiol/ amino terminal sequencing/CD/MW by MALD-TOF MS/ Western blot/ receptor binding/ additional activity assays/process related impurities/AUC/ peptide mapping, LC/MS/monosaccharide compositional analysis/western blot analysis

- The Sponsors approach was found acceptable because the reference material reflected the Sponsor’s current manufacturing process/clinical material and they established suitability using extended testing reflective of the intended use.
Case study #4

- A primary Ref Std was qualified by using drug substance specification testing, including the same sampling plan and acceptance criteria established in the specification.

- Sponsor demonstrated that the new standard was “comparable” to the currently approved reference material. The absolute difference in attributes was no greater than ±3 SDs.

- However, with this approach, any lot meeting specs could be a Ref Std.

- Using acceptance criteria and sampling plan established in the specifications would allow for product characteristics in the new Ref Std that are out of trend with the desired or expected attributes (tightening to mean ± 3 SDs does not help).

- The Sponsor was further advised that the absolute number does not have to be equivalent but must provide appropriate level of precision when calibrating a result. Supportive statistical analysis was also requested.
Case study #5

- During a scale-up and process change, the Sponsor qualified several new reference standards while transitioning to the new process. Some of the Ref Std were qualified by comparison to preceding Ref Std rather than the Ref Std representative of clinical trial material.

- Glycosylation pattern was a CQA for this protein. Glycan mapping peaks are reported as % of total area of Ref Std. Data showed that the amount of CQA was slightly higher in the new process. Information request was sent to Sponsor to provide clarification on this shift.
Case study #5

• A re-analysis with an orthogonal quantitative method showed the opposite trend as the previous analyses.

• Since the Ref Std changed and was not linked to a primary Ref Std, we cannot directly compare the results from old and new process. Data was also limited due to small sample size.

• Comparability between the old and new process could not be concluded from the data provided. Additional data, including calibration with a primary Ref Std, was requested to support comparability of the new process material.
Case study #6

• A new Ref Std was calibrated against a primary Ref Std for an enzyme product. The set of testing included those in the release and stability testing but with more stringent acceptance criteria for qualification of the new Ref Stds and based on intended suitability of the Ref Std.

• However, the primary reference standard was stored at the same temperature as the production lots at 2-8°C.

• The Sponsor was advised to include an appropriate storage condition for the primary Ref Stds (e.g. -70°C or below) to prevent degradation of the primary Ref Std, to the extent possible. It was acceptable to store the working standard at 2-8°C with an appropriate expiration date.

• Enzyme kinetics (Km, kcat), established to be CQAs for this product, were also advised to be incorporated into the qualification protocol for working reference standards.
Case study #7

• A Sponsor presented new information about a growth factor product suggesting that it is susceptible to oxidation and deamidation. Data from peptide mapping showed that the variants are formed during shelf-life of product. Characterization data and risk analysis suggests that variant species may impact potency, half-life, and immunogenicity.

• The Sponsor proposed to develop reference material for the two variants. The oxidized/deamidated reference material would be used to spike the test samples during validation of an LC method intended to capture the variants as identity and purity tests. The amount of variants would be expressed relative to total protein (not relative to Ref Std).

• The Sponsor was advised that their approach was acceptable. Once the method was validated using appropriate Ref Stds and shown to be suitable for detecting and quantitating variants, the Sponsor implemented the validated method for release.

• The specific reference materials were used for characterization and method validation only.
Take home messages

- Reference standard(s) can evolve over the course of development of an investigational drug.
- For licensed products, a life-cycle and cGMP approach to method validation and the application of reference standard(s) is recommended. To the extent possible, reference standards are expected to be reflective of current manufacturing process and clinical trial material.
- Appropriate primary and working reference standard(s) that are suitable for their intended purpose are expected. It is acceptable to have multiple reference materials for different attributes.
- Qualification protocols and acceptance criteria should reflect the intended use of the reference standard and aim to prevent drift, and not merely mirror release testing.
- Use of the two-tiered approach for qualifying new reference standards is recommended (see ICH Q6B).
- Store the primary reference standard appropriately to prevent degradation.
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