

On-Line Capillary Electrophoresis-Mass Spectrometry for Characterization of Antibody Glycosylation

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Genetech has been running CE assays for monitoring glycosylation of antibodies for several years in both the development and GMP laboratories. Assay execution involves releasing N-linked carbohydrates with PNGase-F, labeling with the fluorophore aminopyrene trisulfonate (APTS), and separating by CE with laser induced fluorescence. While CE has been found to be a robust method for analysis, characterization of the observed peaks is difficult and time and labor intensive, either involving performing spiked studies with commercially available standards or additional enzymatic hydrolysis. In contrast, in-line analysis of the elution peaks via MS would provide a straightforward means for peak characterization. However, in-line MS was not without challenges; specifically, the physical challenge of feeding the capillary outlet into the MS. The exit capillary had to be extended to enable a direct feed into the electrospray MS. In addition, the buffer system had to be modified to remove the polymer additive found in the current buffer system. Once these modifications were made, in-line MS analysis of CE peaks was performed with good results.

Biosimilars: What Does Sufficiently Similar Mean

The following are a summary of discussion points from the recent Biosimilar workshop held at the WCBP 2008 meeting in Washington, D.C.

Some Examples of FDA and EMEA Decisions Regarding Biosimilars

Generic Enoxaparin

One of the major causes cited by FDA for rejection of a generic enoxaparin was the lack of immunogenicity data accompanying the submission. This concern was raised even though the clinical data did not identify any immunogenicity issues.

The EMEA is currently drafting a guidance document for LMW heparins; clinical trial work will be a requirement per this document.

Binocrit

Binocrit is a generic form of epoetin alpha, currently approved as EPREX/ERYPO, and is manufactured by Sandoz.

Per the summary of the EMEA's approval of Binocrit, the agency found that "Binocrit has been shown to have a comparable quality, safety and efficacy profile to EPREX/ERYPO". For more details visit <http://www.emea.europa.eu/humandocs/PDFs/EPAR/binocrit/H-725-en1.pdf>.

Sandoz conducted both animal and human clinical studies to demonstrate that both safety and efficacy were comparable to EPREX/ERYPO. These included studies with Binocrit alone and studies in which patients currently being treated with EPREX/ERYPO were switched to Binocrit.

Sandoz performed a comparability study with the innovator product, including:

- Amino acid sequencing of both products
- 2^o and 3^o structural characterization using far and near UV CD studies
- Analysis of the glycosylation pattern and isoform distribution of both products

The biosimilar product was formulated using the same formulation matrix used for the innovator product

Alpheon

The active component in Alpheon is interferon-alpha-2a and was produced by BioPartners GmbH. The innovator product was Roferon-A from Roche, which was approved by EMEA in 1986. Both products were intended for the treatment of hepatitis C.

The application for Alpheon as a biosimilar was rejected due to major concerns regarding the comparability of the two products, specifically with respect to impurities. The EMEA was also concerned about the outcome of the clinical trials, where more patients experienced a return of the disease after treatment with Alpheon was halted than after treatment with Rofereon-A was halted. Patients were also found to experience greater

side effects with Alpheon treatment. Collectively, these concerns, along with drug product stability and assay and process validation concerns, led the regulatory authorities to refuse the marketing authorization for Alpheon.

More details are available at

<http://www.emea.europa.eu/humandocs/Humans/EPAR/alpheon/alpheonR.htm>.

Other discussion points

Overall, the Glycoprotein profile of the biosimilar should be within the variability of the reference material.

EMA indicated that while clinical studies will be required, the scope of the studies may be at a discount to current ICH E1A guideline, “The Extent of Population Exposure to Assess Clinical Safety for Drugs Intended for Long-Term Treatment of Non-Life Threatening Conditions”, which requires 1500 patients. As an example, the Binocrit and Alpheon applications both contained less than 500 patients, but were acceptable to the regulatory authorities.

Combination Products That include Biologics – Novel Combinations Require Novel Assessments

The following are a summary of discussion points from the recent Combination Products workshop held at the WCBP 2008 meeting in Washington, D.C.

Considerations when characterizing a combination product that includes a biologic:

- When testing the Biological part of the combination product, make sure that you separate the protein from the device without disrupting the structure/function of the Biologic (e.g. whereas treatment of the combination product with TFA will almost certainly remove the Biologic, it will also almost certainly impact the Biologics structure/function).

Considerations when submitting an application to the Office of Combination Products (OCP):

- Even though the OCP is the coordinating office, combination products require multiple branches of FDA (e.g. CDRH, CDER, CBER) who operate differently and have different regulations. Therefore, there may be inconsistencies between correspondence received from the different branches of FDA.
- Even though two branches of FDA are involved in review (CDRH and CDER/CBER), all submissions are assigned to 1 branch. Unfortunately, sometimes the lead branch doesn't consult with the other branch and so the correct people may not be present at meetings and/or discussions with the applicant
- As an example of the different requirements, CDRH requires only 1 clinical trial for devices, whereas CDER/CBER expect multiple clinical trials. The current view from FDA is that combination products involving a Biologic will also require multiple clinical trials
- An additional example of the different requirements – device applications include the IQ/OQ for the instruments used to make the device, whereas submissions to CDER/CBER include all validation documents
- A third example of the different approach to applications - CDRH on-site inspections are traditionally carried out once the entire application is complete and reviewed. In contrast, CDER/CBER typically conducts a PAI prior to final submission of the application.
- If the Biological component of the combination product has never been licensed, then the FDA will conduct a PAI assessing the manufacture of the Biologic component
- If the Biologic is already approved as a stand alone treatment, the agency is o.k. with the applicant referencing the original, approved master file in the combination product. application. However, the applicant cannot rely on the clinical results from the stand alone Biologic application in the combination application.

- Similarly, if the device is already approved, the applicant may simply reference the 510K from the device approval in the application for the combination product. Once again, separate clinical results will be required.
- The expectation is that the immunogenicity would be evaluated in each device, even if the Biologic is the same and only the device component is changing and vice-versa.
- FDA does not react favorably to changing the device component in either Phase III trials or post-approval. If the device is changed, at a minimum an animal bridging study, a human PK/PD study and full characterization of the new device-drug combination would be required.

As with all applications, the key is to have frequent and open dialog with the FDA branches involved in the review and approval of the device.