

Immunogenicity Concerns with Steve Becht

Interviewer: Hi, we're here with Dr. Steve Becht, a senior research scientist with our cGMP labs. Steve specializes in biologics analysis with a focus on mass spectrometry and will provide insight into immunogenicity concerns and how they drive biomolecule characterization during drug development. To get us started, could you explain the major differences between biomolecules and small molecules?

Steve: Biomolecules are polymers with a limited number of building blocks. These building blocks can, however, be arranged in any order, creating a new molecule with radically different properties from even small changes. For example, hemoglobin molecules in sickle cell anemia differ from the normal molecule by a single building block, or amino acid.

Biomolecules have more complex routes of degradation than small molecules. While many small molecules are highly resistant to acid and oxidative stresses, most biomolecules are highly susceptible to modification by these and other factors. These changes can significantly impact the activity or immunogenicity of the biomolecule *in vivo*. Hence, the characterization of biomolecules is more challenging than their small-molecule counterparts because of their sizes and complexities.

Interviewer: What information does advanced mass spectrometry offer that traditional methodology doesn't?

Steve: High resolution mass spectrometry offers a level of detail not normally available to classical methods (Read the article, "[Vaccine Characterizations Using Advanced Technology](#)"). For example, SDS-PAGE is limited to a mass accuracy of hundreds of daltons. This is not sufficient to identify most peptide chain modifications, such as oxidation, deamidation or formylation, which can have a significant effect on the properties of the biomolecules. Combining high resolution mass spectrometry with peptide mapping can provide a wealth of information about the protein and its modifications. For instance, even one dalton of mass difference resulting from deamidation can be detected. Mass spectrometry also provides the opportunity to sequence the biomolecule of interest to localize the modification ([See a PPD poster on this](#)) to a specific position, such as seen with sickle cell anemia.

Interviewer: Why is it important to have the high level of detail provided by mass spectrometry?

Steve: The FDA is now expecting more rigorous characterization of biomolecules than in the past. The small variations that can be detected by the newer, more advanced techniques can impact safety and efficacy. The immunogenicity issue associated with contaminated heparin is a recent example.

Traditional techniques often cannot adequately characterize subtle changes. Full characterization of proteins also provides insight into degradation pathways, closely related impurities and modification.

Interviewer: When should information be obtained via mass spectrometry?

Steve: Characterization of biomolecules should be well under way by the preclinical phase of drug development in support of an investigational new drug (IND) filing. Characterization requirements become more rigorous as the clinical development progresses. Most drug substance and drug product characterization must be complete by a Phase III/biological licensing application (BLA) filing. The process is time consuming and deficiencies can result in delays for drug commercialization if inadequate.

Interviewer: What role does characterization play in developing a viable formulation and manufacturing process?

Steve: Key to developing biologics is insuring a consistent product from early toxicity studies through ongoing release of commercialized product. Small changes in the biomolecule can impact immunogenicity, efficacy and stability. Such changes can result from seemingly insignificant variations in the manufacturing, storage and delivery processes. These variations are monitored and controlled throughout formulation, development and manufacturing via ongoing characterization of the biomolecule. Therefore, a more complex characterization menu is required for commercialization of biomolecular drugs.

Interviewer: Thank you very much Steve for taking the time to provide this introduction to immunogenicity concerns and how they drive biomolecule characterization during drug development.

Steve: You're welcome.

Interviewer: For further information about this topic, please visit our cGMP Web pages or fill out the [contact form](#).