assays to assess any structural difference. These methods are relevant to known MOAs, biological functions, safety, and immunogenicity profiles, and are further derived from the knowledge of the conserved attributes for the same class of molecules; e.g., IgG1 exhibits effector functions.

It is noteworthy that over the past two decades, the science of protein analytics has significantly changed, and the regulatory agencies expect the biosimilar developer to use the most advanced and novel methods. The electronic revolution presents us with methodologies that are millions of times more sensitive, such as mass spectrometry and nuclear magnetic resonance. This can be challenging for some developers since the cost of establishing this analytic apparatus can be onerous, not just in the equipment but also in the qualified personnel to perform and conduct these analyses. Whereas some developers may find it less capital intensive to outsource their testing, and there are several very good choices available, an in-house analytical testing program cannot be obviated given the speed and the frequency of testing required in the development programs.

The analytical similarity exercise begins with securing both the lots of reference product as well as the reference standards where available; the U.S. Pharmacopoeial Convention has recently added several monographs of recombinant protein products and reference standards have become available. However, the difference between the two should be clearly understood. Similarity should be assessed against criteria established based on the reference product and not against the standard. Monograph standards may or may not have any relationship with the reference originator product and may or may not capture all attributes of clinical relevance. The similarity is assessed against the reference product, while specification normally centers on the standard. This can create a situation that when the standard is close to the mean of reference product range, the specification also centers on reference product range, but when the standard is close to the edge of reference product range, specification no longer centers on reference product range. This should be clearly demonstrated in regulatory submissions as a justification for establishing specification. The standards used to measure biosimilar activity should represent the reference product, with attention to strength and biological and functional properties.

The sample age at the time of testing should be factored in when comparing stability-induced attributes. One way to satisfy this requirement is to collect, where possible, reference product lots of different ages and develop a range of attributes over the course of the expiry of the product; this will allow overlapping the biosimilar product analytical results over an appropriate course of the plot. This may apply to some testing more than others. For example, purity and product-related impurities are prime testings. This includes potency, aggregates, and impurities.

Similarity assessments are performed on drug product lots manufactured from unique drug substance lots using the to-be-commercial process that will support marketing applications. The last requirement may