This is performed in a comparative clinical study in which the relationship of the ADA response to relevant clinical parameters is evaluated over a suitable treatment period.

In justified cases, that is, where no impact on clinical properties is detected when tested under suitably sensitive conditions, it could be acceptable for the biosimilar to have higher or lower amounts of particular product-related variants. For example, the relative level of C-terminal clipped-human IgG is not expected to influence the immunogenicity of therapeutic monoclonal antibodies. In general, the low levels of nonhuman glycans, including *N*-glycolyl neuraminic acid (Neu5Gc) added by some mammalian cell substrates, has not been associated with enhanced immunogenicity of therapeutic proteins—even though a relatively high proportion of human subjects possess preexisting antibodies reactive with Neu5Gc (Amon et al., 2014).

In the case of cetuximab, however, a relatively high level of Gal-α-1,3-Gal linked *N*-glycans, as well as of Neu5Gc, is associated with each of the Fab arms of the molecule (Qian et al., 2007). The quantity and spatial disposition of the Gal-α-1,3-Gal linked *N*-glycans in cetuximab have been attributed as the causal factor of severe IgE-mediated hypersensitivity reactions in subjects who have been previously sensitized by environmental or dietary exposure (Chung et al., 2008). As a consequence of the immunogenicity-related risk identified for the reference product, it might be justifiable for a biosimilar cetuximab candidate to be manufactured in a different cell line from that used for the reference product (e.g., CHO instead of Sp2/0), on the basis that equivalent efficacy could be achieved with an improved safety profile (i.e., lower incidence of severe immune-mediated adverse events) and that adequate similarity for other quality parameters was demonstrated.

The recognition that product aggregates were a plausible causal factor for increased incidence of amPRCA in renal anemia patients treated with subcutaneous administration of an originator version of epoetin alfa (Rossert et al., 2004) was instrumental in increasing rigor of analysis and control of levels of oligomers, aggregates, and subvisible particles. There is accumulating evidence (Joubert et al., 2012; Rombach-Riegraf et al., 2014) to support a role for aggregates in increasing the mass balance of antigen uptake and processing by antigen-presenting cells, as well as for directly stimulating B-lymphocytes to bypass B-cell tolerance. It appears that even relatively low levels of subvisible particles are able to provide co-stimulatory signals to enhance antigen-specific T-cell responsiveness, and that such additional stimulation may contribute to enhancement of immunogenicity, depending on the intrinsic immunogenic potential of the molecule (Ahmadi et al., 2015).

Choice of primary container can represent an influential variable because different groups have demonstrated an association between residual tungsten particles in glass prefilled syringes and an increased tendency for protein aggregate formation (Bee et al., 2009; Jiang et al., 2009; Liu et al., 2010). Accordingly, risk mitigation for biosimilar products should include careful selection of the primary container and demonstration of comparative (vs. reference product) stability of the drug product–primary container formulation to be commercialized.

Residual host cell-derived protein levels cannot be directly compared for the biosimilar versus reference product because quantitation depends on the availability of process-specific assay reagents. Nevertheless, the product quality dossier for the biosimilar product will need to demonstrate effective clearance of host cell-derived