Posttranslational changes in a biological molecule may affect its biological function. A change in a relevant part of the structure of the molecule is likely to lead to a change of biological activity, whereas minor variations in other parts are most likely not going to affect the functional properties of the molecule.

Qualitative or quantitative differences of product-related substances may affect the biological functions of the proposed biosimilar. It is therefore important to determine that the differences in composition of biosimilar and reference product are only minor and to establish that these minor differences do not affect safety and efficacy.

In the case of quantitative differences in protein variants that may have pharmacologic activity, an assessment of this activity is expected to be evaluated by appropriate sensitive binding affinity and functional *in vitro* assays rather than *in vivo* studies.

In IgG molecules, parts of the sugar moiety in the Fc region are known to contribute to the binding to Fc gamma receptors (FcγR) (Radaev and Sun, 2002).

A well-known example is that differences in the fucosylation of an mAb will impact the FcγRIIIa receptor binding. A higher degree of afucosylation leads to an increased binding affinity for the receptor, which may be reflected by an increased ADCC activity (Shields et al., 2002; Houde et al., 2010). However, depending on the mechanism of action, this difference may or may not be clinically meaningful. If the biosimilar is rituximab, where the mechanism of action of the drug is highly dependent on ADCC activity, this change is expected to have clinical consequences, while for mAbs where ADCC activity is not the central mechanism of action (e.g., etanacerpt), slight differences in ADCC activity may not be clinically relevant. For infliximab, the first mAb granted marketing authorization, differences in FcγRIIIa binding and ADCC in an NK cell assay were shown. Such differences were not considered relevant for the rheumatoid indications, but the relevance for inflammatory bowel disease (IBD) was discussed extensively. The company provided additional *in vitro* data to demonstrate that the observed differences were not measurable under more physiological conditions (e.g., in the presence of serum). In addition, it was demonstrated that induction of regulatory macrophages did not differ between biosimilar and reference products. These additional *in vitro* data convinced the European authorities that the observed differences in afucosylation were not relevant for all indications applied for and the IBD indication was accepted by extrapolation (Weise et al., 2014).

Qualitative differences of product-related substances may raise more concerns and will require appropriate justification. This may be the case for the presence of glycosylation structures or variants not observed in the reference medicinal product, especially nonhuman structures (nonhuman linkages, sequences, or sugars). These differences may have an effect on immunogenic potential and the potential to cause hypersensitivity. While glycosylation does not appear to play a major role, nonhuman or nonmammalian glycosylations within the product due to the expression system used can induce immune responses (Wadhwa et al., 2015).

A well-known example of qualitative differences in product-related substances is the presence of a glycan with a terminal galactose-α-1-3-galactose configuration in cetuximab because of the expression system used, causing hypersensitivity reactions in individuals with preexisting IgE antibodies against this structure. This immune