remove the host cell-derived protein that co-purified with somatropin; clinical data for Ovaleap showed no impact of preexisting Neu5Gc-reactive antibodies on clinical parameters; and Alpheon was not approved due to uncertainty about biosimilarity, mainly in relation to quality aspects (Chamberlain, 2014).

12.9 REGULATORY REQUIREMENTS FOR ADA DETECTION IN THE BIOSIMILARITY EXERCISE

Regulatory guidance (EMA, 2012, 2015; USP, 2015; FDA, 2016) defining standards for bioanalytical assays to be applied to ADA assays for biotechnology-derived products in general are also applicable to the bioanalysis of nonclinical and clinical samples for the biosimilarity exercise. However, the need to design and validate assays to provide a reliable measure of the *relative* ADA response induced by *independently manufactured* versions of a therapeutic protein does create additional considerations for regulators and sponsors alike.

While sponsors are at liberty to select their preferred assay format, there is a regulatory need to ensure that the choice of assay format does not bias the detection of ADA induced by either of the product versions being compared, and has adequate sensitivity to detect ADA in the presence of clinically relevant drug concentrations.

Both the FDA and the Committee for Human Medicinal Products (CHMP) will accept a single- or a dual-assay approach in which (1) the ADA assay format is configured using only the biosimilar product as the labeled antigen ("single-assay approach"); or (2) separate assays are configured using the biosimilar product as the labeled antigen in one assay and the reference product as the labeled antigen in the second assay ("dual-assay approach"). In both cases, clinical samples should be tested in an operator-blinded manner, and the specificity of any detected positive samples should be confirmed by competitive inhibition of the signal by both the unlabeled biosimilar and unlabeled reference products.

In practice, sponsors have tended to prefer the single-assay option because this minimizes variability associated with using multiple assays and labeled antigens, and avoids redundant testing and the possibility of generating conflicting results for the same sample tested in different assays. Validation of two assays not only doubles the amount of experimental work but creates a second set of assay cut points and drug tolerance limits.

Given the abbreviated nature of the clinical program for biosimilars, sponsors should plan to have completed assay validation prior to commencing clinical studies—even though this is not a strict regulatory requirement. It is wise to seek endorsement via Scientific Advice, prior to initiation of clinical studies, for the proposed bioanalytical strategy, including technical details of assay format, schedule of clinical sampling and data analysis. This is particularly relevant for the choice of assay format to measure the neutralizing capacity of ADAs detected in the screening/confirmatory assays because for products that act as antagonists of soluble targets it is usually acceptable to apply a competitive ligand binding assay (CLBA) format in place of a cell-based bioassay—the latter being required in the case of a product whose primary mechanism of action depends on engagement of a ligand expressed on the cell surface.