TNF gene construct, for products used in rheumatoid arthritis (Keffer et al., 1991). The outcome of a comparability exercise comparing the pharmacological activity of a biosimilar and a reference product in such models depends on the robustness of the model. Variable growth of xenotransplants and semiquantitative scoring of pathological features would, however, decrease the usefulness of these models to detect differences in biological activity (van Aerts et al., 2014).

It is always important to understand the limitations of animal studies when interpreting the results. In the case of animal studies for biosimilar mAbs development, these limitations include, next to target specificity, small sample size and intraspecies variations. If the proposed biosimilar has already shown a high degree of *in vitro* similarity, it is recognized that animal studies in standard species or in animal models of disease rarely provide additional information needed for a decision. This limitation has led to the strategy proposed in the guideline on the nonclinical and clinical issues of the development of biosimilar mAbs: the major decisions on similarity should be based on similarity of quality attributes and comparable performance in *in vitro* studies. This is considered the foundation of biosimilar mAbs development.

Where in previous guideline recommendations on biosimilars a nonclinical package was expected to consist of comparative studies, including a pharmacodynamic study (bioassay) and a repeated dose toxicology study, a new paradigm emerged for biosimilar mAbs in which the use of animals was obviated in most cases by a thorough stepwise approach of testing. Moreover, the conduct of large comparative toxicological studies in NHPs is unacceptable from both a scientific and an ethical point of view.

The EU biosimilar mAbs guideline (EMA, 2012) indicates that relevant assays on the binding of the complementarity determining region (CDR) to its primary target and the binding to representative isoforms of the relevant three Fc gamma receptors (FcγRI, FcγRII, and FcγRIII), FcRn and complement (C1q) need to be performed. Since both the Fab as the Fc portion of the molecule may elicit several effector functions, both need to be evaluated, even though some may not be considered essential for the therapeutic mode of action. Notably, Fab-associated functions like neutralization of a soluble ligand, receptor activation or blockade, and Fc-associated functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and complement activation need to be evaluated. As these assays are considered paramount in the nonclinical comparability exercise, the qualification of these assays is of utmost importance. Variability due to assay format and reagents utilized needs to be minimized.

6.6 A PARADIGM SHIFT IN EUROPE

The discussions on the relevance of *in vivo* animal testing for biosimilar mAbs were soon broadened to other biologicals, since target specificity is not a property that is specific for mAbs only. Target binding, such as to a receptor, ligand, or substrate, and the resulting functional effects can be evaluated *in vitro* for most biological medicinal products. If there is a high degree of confidence that the *in vitro* functional assays are reflective of the mode of action of the biological, these assays could be of