for the bioanalytical features of the comparative immunogenicity evaluation—and individual sponsors have the opportunity to justify different approaches on the basis of scientific soundness.

12.16 ROLE OF NONCLINICAL STUDIES

The value of nonclinical *in vivo* studies for evaluation of the relative immunogenicity of related versions of any given therapeutic protein depends on the nature of the molecule and the extent of structural and functional homology to endogenous counterparts. EU and FDA guidelines (FDA, 2014b, 2015; EMA, 2015) acknowledge a potential role for nonclinical studies as part of the evaluation of the immunogenicity of biotechnology-derived products in general because such studies may have relevance for hazard identification (i.e., qualitative risks) for human subjects. For example, comparative immunogenicity studies in wild-type animals have yielded data to indicate whether related versions of a given molecule could have a markedly different quantitative profile in terms of extent of ADA formation, in a manner that can be correlated with defined differences (e.g., levels of subvisible particles) in product quality (Jeandidier et al., 2002; Fradkin et al., 2009).

However, in the case of the biosimilarity exercise involving product versions that have demonstrated highly similar structural properties, ADA data from comparative nonclinical toxicology studies was not judged to be instructive for the approval of the initial wave of biosimilars approved in the EU, even in cases where some differences were evident (Chamberlain, 2014).

For example, while filgrastim does not appear to induce ADAs in humans—due to effective immune tolerance to the exogenous protein—filgrastim is immunogenic in rats (EPAR for Zarzio) because of interspecies sequence differences in the G-CSF molecule. Epoetin alfa also induces ADAs in nonhuman species (EPAR for Binocrit and EPAR for Silapo) for the same reason. For therapeutic monoclonal antibody products, a major part of the ADA response in animals will be directed against the human IgG Fc region (van Meer et al., 2013). Therefore, differences in the immune responsiveness of animals to relevant (for humans) structural features of biosimilar and reference versions of the same molecule may be difficult to detect or interpret in the context of a stronger immune response against xenogeneic structural motifs. In addition, relatively small experimental group sizes increase the influence of interanimal variability on the assessment of relative immunogenicity.

12.17 CLINICAL IMMUNOGENICITY EVALUATION

Although immunogenicity is caused by factors that can be detected and controlled at the manufacturing and product quality testing levels, there remains uncertainty about how extrinsic (manufacturing, formulation, storage conditions, dose regimen, and patient heterogeneity) and intrinsic (B- and T-cell epitopes) factors may interact in the biological context to influence the extent of the immune response in individual subjects.

For this reason, a directly comparative, clinical immunogenicity evaluation to detect differences in the incidence and severity of immune responses induced by the biosimilar candidate and the reference product is normally expected (FDA, 2015).