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Introductory note to the three-part series of papers by B Sharma on :

Immunogenicity of therapeutic proteins: how to assess and the role of pharmaceutical quality

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The quality, safety and efficacy of drugs are key components which need to be monitored throughout the lifecycle of the product. The research/development process is well-defined for medicinal products. In addition to pharmaceutical/biological data to be put together for documenting the quality of the medicine, non-clinical data are needed before the first administration of the drug substance in humans. The clinical development process (Figure) requires data on the proof of principle (Phase 1), dose managing (Phase 2), and efficacy in the disease population (Phase 3). Based on all the quality, non-clinical, and clinical data accumulated from early development through Phase 3 clinical data, the product can go through the regulatory process to seek marketing authorization. It is essentially during the post-marketing phase (Phase 4) that new findings, not observed during the research/development process, are reported. This situation has been illustrated with several classical pharmaceutical drugs (Table) which had to be withdrawn from the market, due to accumulation of adverse event reports during the post-marketing experience.

Therapeutic proteins inherently present a considerable degree of variability in their quality attributes (e.g. primary to quaternary structures, glycosylation, etc.) which provides these molecules with a higher degree of structural complexity compared to pharmaceutical drugs derived from chemical synthesis (Crommelin et al. 2003). Paradoxically, it is the complexity of these molecules that makes them bioactive – activity which cannot be mimicked by simpler chemical entities. The challenge for biopharmaceuticals not only relates to the intrinsic complexity and characteristics of proteins (variability and sensitivity to degradation), but also to the manufacturing process (e.g. raw materials, process-related impurities, packaging, storage and handling, etc.). All these sources of modification of the intrinsic properties of the desired molecule, which may not even be detected from one batch to another, may compromise the *in vivo* behavior of the product with either loss of efficacy and/or a change in the immunogenic properties of the molecule. One of the major regulatory concerns in the development of therapeutic proteins is the assessment of undesired host immune responses to the drug product that may abrogate efficacy and may give rise to potentially fatal adverse reactions (Committee for Medicinal Products for Human Use 2006).

The accumulated post-marketing experience of currently registered biopharmaceutical entities reveals a wide variety of host immune responses and their clinical sequel (Schellekens 2002). There can be multiple sources of immunogenicity for a therapeutic protein, some of which are due to aspects of pharmaceutical quality, which will be reviewed in the three articles to be published by B. Sharma in this journal. From these articles it can be concluded that predicting and identifying the cause of immunogenicity is almost impossible, and that one has to carefully consider any step in a given process, as well as any critical parameters in the formulation and packaging, in an attempt to diminish the risk of giving rise to immunogenic proteins.

One thing is clear, however, even if such responses occur very rarely, the clinical outcome can be very serious. Examples include the case with recombinant thrombopoietin in which patients developed a cross-reactive humoral response to endogenous thrombopoietin, resulting in irreversible thrombocytopenia (Li et al. 2001) and, more recently, the case with recombinant erythropoietin in which more than 200 cases of pure red cell aplasia were reported in chronic renal failure patients treated subcutaneously with a given formulated preparation of epoetin alfa (EPREX[®]) (Boven et al. 2005)..

Several approaches may be used to assess the immunogenic properties ("immunogenicity profile") of a product at different stages of its life cycle. Currently, these approaches are mainly based on algorithms integrating epitope response to HLA polymorphism, *in vitro* assays (e.g. epitope mapping, cell-based assay, etc.), or animal models. Although all these approaches may be very useful in identifying or ranking the relative immunogenicity of a product during development, they all have limitations and none can predict the response in humans. Only relevant clinical experience, which encompasses both clinical trials and post-marketing surveillance (pharmacovigilance), will further guarantee the safe behavior of the protein in patients (Crommelin et al. 2003)..

Assessing the "immunogenicity profile" in humans also has its challenges. Indeed, the ability to detect and measure antibodies specific for recombinant therapeutic proteins in patients is often difficult for several reasons. First, human immune sera against a particular human protein are rarely available and there are no appropriate standards or controls to normalize results. Consequently, development and validation of assays for such antibodies are usually based on extrapolation from use of monoclonal or polyclonal antibodies prepared in animals. Second, the presence of exogenous therapeutic protein or of its endogenous counterpart can interfere with the ability to detect antibodies because detection in serum is usually based on the ability of the antibodies to bind to the recombinant protein used as an assay reagent. Third, methods for expressing the quantity of a specific

antibody can vary substantially based on the assay format and reagents. Finally, assessment of the neutralizing activity of serum antibodies is usually based on their ability to modify a quantifiable response in an *in vitro* bioassay, and it is acknowledged that most cell-based bioassays are quite susceptible to variations in the assay and cell culture conditions. As a consequence, there are several ways to present immunogenicity results and comparison of results across products in the same class may lead to an inaccurate interpretation (Schellekens et al. 1997; Thorpe et al. 2005).

It is essential to harmonize methodologies used for antibody testing in human sera. The harmonization of such methods has been initiated for several years now for interferon and erythropoietin and has shown to be quite a challenging task (Schellekens et al. 1997; Thorpe et al. 2005). This is why clinical experience, through clinical trials and active pharmacovigilance programs, remains the most reliable way to assess the immunogenicity profile of recombinant therapeutic proteins in patients. Assessment is necessary all along the life cycle of the product particularly when a change is introduced to either the production process or the formulation and when biosimilar products (also called "follow-on proteins") are launched on the market.

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Table. Pharmaceutical products that were removed from the market following accumulation of post-marketing adverse events

Drug Name*	Therapy Area	Manufacturer
Accutane (isotretinoin)**	Acne	Hoffman-La Roche
FenPhen (fenfluramine or dexfenfluramine and phentermine)	Diet	Wyeth
Propulsid (cisapride)	Acid reflux	Janssen Cilag
Baycol (cerivastatin)	Anti-cholesterol	Bayer
Vioxx (rofecoxib)	Cox II inhibitor	Merck
Bextra (valdecoxib)	Cox II inhibitor	Pfizer

* US brand names are provided, although the same medicinal products were marketed under different brand names in other countries, but where also subject of the same post-marketing restriction or withdrawal

** For isotretinoin, the product was maintained on the worldwide market (princeps and generics) but with more and more stringent warning statements and restriction for use, particularly as regards contraception during treatment.

Figure. The four phases of the clinical development for innovator products

