# ENFOQUE/ FOCUS

## ASSURING THE QUALITY OF MEDICINES DERIVED FROM NEW BIOTECHNOLOGIES

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Progress in the biomedical sciences and the exploitation of novel biotechnologies over the past decade or so have led, and are still leading, to the development of new biological medicines at an unprecedented rate. Developments in molecular genetics and nucleic acid chemistry enable genes encoding natural biologically active proteins to be identified, modified and transferred from one organism to another so as to obtain highly efficient synthesis of their products (1). Much genetic engineering has been carried out in *Escherichia coli* but other systems involving yeast and transformed cell lines of mammalian origin have also been developed and some are in use for production. In addition to the use of transformed mammalian cells for the production of rDNA-derived products, the fusion of B-lymphocytes from immunized animals with myeloma cells, resulting in the formation of hybrid cells known as hybridomas, can lead after cloning to the production of antibodies of highly defined specificity known as monoclonal antibodies (2). Over this period there has also been enormous progress in the ability to purify and characterize biologically active molecules. Together, these technologies allow the production of large quantities of medicinal products which were difficult to prepare from natural sources using conventional approaches, or were previously unavailable. The products include, for example, rDNA-derived insulin, growth hormone, erythropoietin, tissue plasminogen activator, human DNA-ase, hepatitis B vaccine and various cytokines, as well as monoclonal antibodies for imaging and therapy. They have extensive implications in the prevention, diagnosis or treatment of such diverse conditions as hepatitis, cancer, diabetes, cystic fibrosis and myocardial infarction.

### ASSURING QUALITY AND SAFETY

Along with these developments has come the obvious and important need to ensure the quality, efficacy and safety of such biological medicines. As with many other new technologies, a new set of safety issues for consideration by industry and regulatory bodies has been generated by these particular biotechnologies. Potencial safety concerns arise from the novel processes used in manufacture and from the complex structural and biological characteristics of the products themselves. Factors which have received particular attention include the possible presence of contaminating cellular DNA and viruses in products derived from transformed mammalian cells, and the genetic stability o the expression systems. Adequate control measures are essential both to safeguard patients against adverse effects and to ensure that they are given the full benefits of scientific innovation. Since the nature and production of these products are highly sophisticated, they require similar sophisticated laboratory techniques to ensure their proper standardization and control. Although comprehensive chraracterization of the final product is expected, considerable emphasis must also be given to "in-process" control. In this respect biotechnology derived products are considered to be similar to biologicals produced by traditional means, such as bacterial and viral vaccines, where adequate control is seen to relate to the starting materials and manufacturing process as much as to analysis of the final product. Thus, data on the quality and purity of cell cultures and on the effectiveness of purification and test methods are required for licensing. Validation of the ability of the purification process to remove unwanted material like DNA and potential viral contaminants may also be necessary.

Given the need to ensure the safety and quality of medicines produced by new technologies, guidelines on the standarization and control of these substances have been developed by several countries and organizations (3-8). These have been designed to help manufacturers develop appropriate quality control procedures and to assemble the information needed to support applications for market authorizations. The intention of the guidelines is to provide a scientifically sound basis for the manufacture and control of substances produced by the new biotechnologies and generally a flexible approach has been adopted so that requirements can be updated in the light of experience of production and use, and with the further development of new technologies.

## HARMONIZING VIEWPOINTS

Such is the speed of scientific and technlogical progress in biotechnology, it is of the utmost importance that a dialogue be maintained between the pharmaceutical industry, regulatory agencies and academia to review current requirements from time to time. Recently, the scientific criteria needed for assuring the quality and consistency of rDNA-derived medicines and in particular for ensuring genetic stability, have been the subject of much discussion and several international meetings have been held on the topic, such as those organized by the World Health Organization (WHO)/National Institute for Biological Standards and Control (NIBSC) in Potters Bar, U.K. (1992), by the International Association of Biological Standarization (IABS) in Annecy, France (1993) and by the International Conference on Harmonization in Orlando, Florida, USA (1993) (9,10,11). Of course, recombinant systems must be sufficiently stably genetically, to synthesis the desired protein product throughout the fermentation period and from batch to batch, and various approaches have been proposed for assuring genetic stability such as measuring plasmid retention or recombinant gene copy number, as well as restriction mapping and sequencing at both the DNA and polypeptide level. The current debate has centred specifically on the extent of DNA sequencing necessary for assuring product consistency. Concern had been expressed about the ability of present end product testing including peptide mapping, to detect and quantify sufficiently low levels of product with variant sequences (12). It had also been suggested that the current requirement of deriving a consensus sequence of the gene in question is of little value and that more extensive data on the leve of variant DNA sequences are required to assure product quality and consistency. Since it cannot be assumed that variant sequences introduced at the gene level are more detrimetal to the quality of the product than those resulting from translational errors and post-translational events, general opinion is that in assessing product consistency emphasis should be given to improving and validating methods for analyzing protein structures and to evaluating the contribution made by biological assays and standardization rather than to extensive DNA sequencing. The ability to characterize recombinant proteins in detail is developing rapidly and the sophisticated methods now emerging, which include electrospray and laser desorption mass spectrometry, should be used to their full extent, but not as an end in themselves. Of course, some DNA sequencing (consensus) will be required during product development, these data being necessary to confirm the identity of transfected genes and to demonstrate that constructs are not grossly unstable, rather than to detect low levels of variant sequences.

This debate (9,10,11) has been useful because it has focused attention on the problems of ensuring the consistent quality of recombinant protein and provided a platform where progress in biological assays, physico-chemical characterization and structure-function interactions could be discussed. However, the emphasis given to the issue of DNA sequence stability may have given the impression that this is the primary if not only issue in assuring product consistency. Of course, this is far from the case and in practice, the quality and consistency of rDNA-derived substances for use in medicine are best guaranteed by a range of in-process and end-product testing procedures.

## INTERANTIONAL BIOLOGICAL POTENCY STANDARDS

Since the 1920s, when insulin was first used as a life-saving treatment for diabetes, it has been recognized that for biological medicines that cannot be adequately characterized by physical and chemical means some form of biological assay is needed to assess potency. Futhermore, because of the inherent variability of such assays, over time and from laboratory to laboratory, a stable universally accepted, reference standard is essential to ensure comparability between manufacturers and to ensure that suitable but safe doses are given clinically world-wide. This concept still applies even in the case of the highly purified and highly characterized

products of rDNA-technology. This is because the relationships between the structure and function of these molecules are complex and subtle. Small changes in structure, possibly undetectable even by modern physicochemical analyses, can sometimes result in major changes in biological activity. This is well illustrated by work on the cytokines (13). Until such time when physicochemical analyses become accurate predictors of the biological activity of rDNA-derived molecules, as has now been shown for the relatively small molecular weight hormone insulin, the biological assay remains vital to the control of production of these proteins as therapeutic agents. In the case of cytokines in particular it has been important to have international standards in place early on so that products from different manufacturers are calibrated in the same units before they reach the clinic. Failure to do so can result in difficulties in assessment of dosages and inevitable risk to patient safety. A considerable number of interin reference standards and WHO International Standards for cytokines are now available (14), table 1.

**Table 1**
World Health Organization Potency Standards for Cytokines *

| PREPARATION | CODE |
|---|---|
| Interleukin 1α rDNA (International Standard) | 86/632 |
| Interleukin 1β rDNA (International Standard) | 86/680 |
| Interleukin 2 Cell line derived (International Standard) | 86/504 |
| Interleukin 2 rDNA | 86/564 |
| Interleukin 3 rDNA | 88/780 |
| Interleukin 4 rDNA | 88/656 |
| Interleukin 5 rDNA | 90/586 |
| Interleukin 6 rDNA (International Standard) | 89/518 |
| Interleukin 7 rDNA | 90/530 |
| Interleukin 8 rDNA | 89/520 |
| Interleukin 9 rDNA | 91/678 |
| Interleukin 10 rDNA | 92/516 |
| Interleukin 11 rDNA | 92/788 |
| M-CSF rDNA (International Standard) | 89/512 |
| G-CSF rDNA (International Standard) | 88/502 |
| GM-CSF rDNA (International Standard) | 88/646 |
| Leukaemia inhibitory factor rDNA | 91/602 |
| Stem cell factor/MGF rDNA | 91/682 |
| Rantes rDNA | 92/520 |
| MIP-1α rDNA | 92/518 |
| TGFβ 1 rDNA | 89/514 |
| TGFβ 1 (NAT BOVINE) | 89/516 |
| TGFβ 2 rDNA | 90/696 |
| TNFα rDNA (International Standard) | 87/650 |
| TNFβ rDNA | 87/640 |

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## THE CHALLENGE OF NEW TECHNOLOGIES-GENE THERAPY

Scientific progress over the past decade has led to the development of new therapies involving the direct transfer of genetic material into patients, process called gene therapy. The aim of these new therapies include the efficient transfer and expression of a gene in a target somatic cell population for therapeutic or diagnostic purposes. Another goal might be the transfer of a gene encoding a foreign protein into a somatic cell in which expression leads to the production of immune responses. Although in the majority of cases the intention is the addition of a new gene, the transfer of nucleic acids with the aim of recombination with an endogenous gene, thereby modifying its function is also included in the definition of gene therapy. Several approaches to the introduction of genetic material into a somatic cell are currently under consideration, including the transfer of native nucleic acid, of nucleic acid complexed with a carrier system, or the use of replication deficient viruses: the administration to the recipient of genetically modifed autologous, allogenic or xenogeneic somatic cells might also take place. Some of these systems have already been used clinically and should gene therapy be successful then products based on nucleic acid sequences linked to carriers or ligated into replication-deficient viruses are likely to be produced on a large scale and become medicinal products. In some cases, the genetically modified somatic cells themselves might be perceived to be products. For example, a therapeutic gene may be transferred to and expressed in fibroblasts, myoblasts, epithelial cells and so on, and these expanded *in vivo* to sufficient numbers for inoculation into one or more patients having the same condition.

Recenlly, a workshop on Human Somatic Gene Therapy sponsored by the European Union (EU) was held at NIBSC (September 1993) with the view to defining relevant quality and safety issues and to begin to formulate appropriate regulatory approaches (15). European Union (EU) guidelines on gene therapy products, setting out the points to consider in relation to quality, efficacy and safety in the production of vectors and genetically-modifed somatic cells, are currently being developed. The US Food and Drug Administration (FDA) has already issued a consultative document on gene therapy (16). Since gene therapy products will contain genetic and other biological materials, many of the quality, efficacy and safety considerations which apply to rDNA products and other biological manufactured by modern

biotechnological methods will apply to some stages in their manufacture. However, there will be additional considerations, the virus vectors raising particular issues regarding manufacture and safety. The viruses proposed as vectors are likely to produce pathological effects in recipients under certain circumstances and it is expected that such vectors will have been rendered defective regarding viral genes nedded for replication and packaging. Such replication-deficient viruses are propagated in special "packaging" cell lines modifed to express the viral proteins necessary for the recombinant genonmes to be replicated and packaged. The construction of packaging cell lines unable to produce replication-competent (infectious) viruses by recombination within the recombinant viral genome is seen as a crucial aspect of assuring the safety of such potential products. In the EU, gene therapy products which use viruses as vectors will also fall under existing regulations relating to the contained use of genetically modified microorganisms and to the deliberate release of such genetically modified organisms into the enviroment.

The challenge in developing a regulatory framework for gene therapy products is similar to that of a decade ago for biotechnology derived biologicals; that is to ensure public safety whilst at the same time not inhibiting the development of a new field. Historically, assuring the quality and safety of traditional biologicals, like vaccines and blood products, have been "disaster led" in that procedures seem to have been established following some problem. In the case of rDNA-derived medicines, the guidelines on their production and control have produced a climate where, to date, production and use have largely been successful and without major adverse clinical consequences. With appropriate dialogue between producers, clinicians and regulatory scientists, it should be possible also to establish a framework for ensuring the quality and safety of gene therapy products and to allow the field to develop to the benefit of the world-wide community.

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