USE OF TRANSGENIC ANIMALS IN THE MANUFACTURE OF BIOLOGICAL MEDICINAL PRODUCTS FOR HUMAN USE

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1. INTRODUCTION

Transgenic organisms contain a foreign gene which has been experimentally inserted into the normal genetic component, and currently include many plants and a number of animal species. They have been used experimentally to investigate gene function, development and disease. Transgenic animals have also been proposed as a means of testing agents for oncogenicity or virulence.

This document is concerned with the use of transgenic animals to produce biological pharmaceutical materials for use in human recipients. Transgenic animals may produce higher quantities of material in more concentrated form than existing culture methods, and therefore have considerable advantages in both the cost of producing the starting material and in its downstream processing. In some instances where very large amounts of material are required for therapy the use of transgenic animals may be one of the few viable production strategies. However in some respects the products resemble classical biologicals in that they derive from a whole animal rather than from definable culture systems. The considerations which apply are therefore a blend of those relevant to recombinant DNA (rDNA) derived materials and materials from less defined sources.

2. DEFINITIONS

Forebears: the animals from which the egg and sperm used to create the genetic founder were derived

Host: the recipient mother in whom the embryonic genetic founder was implanted

Genetic founder: the transgenic animal resulting from the introduction of the foreign DNA into the embryo or fertilised egg

Production founder: a transgenic animal used as a source for the generation of production animal herds

Production animals: the immediate offspring of the production founder

3. SCOPE OF THE NOTE FOR GUIDANCE

Many different species have been considered or developed for the production of biological medicinal products and by use of appropriate targeting sequences the transgene has been expressed in body fluids such as blood or in milk as well as in other source tissues. A wide range of host animals and source materials are therefore possible each raising specific concerns. All products must be considered on a case by case basis. However the strategy adopted should be such as to minimise potential microbiological contamination during the creation of the transgenic line including potential contamination from the host and founder

animals. Maintenance of the production animals should be such as to minimise contamination of the starting materials such as milk or blood from which the final product will be purified. The purity and microbiological safety of the final product is of major concern.

The production facilities used will probably employ agricultural animals and techniques. It is important to bear in mind that the requirements for manufacture of pharmaceutical products will be more stringent than those for agricultural production, and the production process should be designed accordingly. This document emphasises products derived from fluids of transgenic animals, particularly milk, as there is at present considerable interest in such sources, but many of the considerations will also apply to other source tissues. Other relevant notes for guidance should be taken into account including those concerned with the validation of virus removal and inactivation procedures, minimising the risk of transmission of agents causing spongiform encephalopathy via medicinal products, the production and quality control of medicinal products derived by rDNA technology and the Biotech headings for Notice to Applicants (Part II of application file).

The veterinary and environmental issues relevant to animal welfare and the consequences of release have been considered elsewhere, (see for example Directives 90/219/EEC on contained use and 90/220/EEC on deliberate release of genetically modified organisms) and the animals used in production must comply with existing regulations concerning the development of transgenic animals.

4. THE TRANSGENIC ANIMAL

4.1 Origin

Animals which have been proposed as hosts for production include among others sheep, cows, pigs, rabbits and mice, and much interest currently centres on the use of transgenes expressed in milk or colostrum. The choice of animal will be determined by a variety of factors. For example pigs breed rapidly and produce large litters, so that establishing a suitable transgenic line of animals may be technically simpler than if the same process is attempted in cows. On the other hand pigs are difficult to milk, while milk production in cows is well understood.

Each species will raise its own microbiological and virological concerns which should be addressed. Many of the potential host animals are not conventional laboratory animals, but infectious agents of agricultural significance are likely to be well known. The microbiological status of the production animal, its forebears and host animals involved in derivation of the transgenic line should be documented as far as is possible. Consideration should be given to the use of breeds of animals resistant to specific agents such as scrapie resistant breeds of sheep. The founder animals and their offspring should be shown to comply with the existing guidelines *Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products.*

4.2 The expression system

The isolation and characterisation of the gene and associated control elements should be described as should the process by which the final construct was made. The strategy used to develop the particular expression system should be described and justified. In particular the rationale for the use of regulatory sequences to ensure correct expression of the gene in the

appropriate tissue should be clearly described. The complete sequence of the final construct should be determined.

4.3 Creation of the transgenic animal

A number of methods are currently in use for the creation of transgenic animals. One favoured method involves the inoculation of the DNA into the pronucleus of a fertilised ovum, followed by implantation into pseudo pregnant females. This results in a proportion of animals carrying the transgene in the germ line which may be high in some species (e.g. mouse 5-30%) or low in others (e.g. cows and sheep, 1-5%). Depending on the time when the transgene is incorporated into the cellular DNA, mosaic animals may develop in which certain cell lineages carry the transgene while others do not. Other methods of creating transgenic animals involving retroviral infection of the embryo at an early cleavage stage in the blastocyst result in only a proportion of the cells carrying the transgene, and therefore a high proportion of mosaic animals some of which may not have the transgene incorporated in the germ line at all. Methods for predictable site specific integration of sequences into the host genome would have advantages for both controlled expression and safety.

The method used to create the transgenic animal should be described in detail, including the isolation of ova, in vitro fertilisation, insertion of the transgene, reimplantation and delivery. The use of retroviral vectors raises additional quality considerations related to preparation of the vector, its virological purity and its persistence. Consideration of guidelines related to regulatory aspects of gene therapy is advisable.

The genealogy of the production animals must be documented. A transgenic line will derive from a single genetic founder animal, and materials from different transgenic lines should not be mixed. The founder animal and the production animals should be defined as diploid or haploid with respect to the inserted sequence.

The level of expression of the incorporated gene should be assessed and the tissue distribution of expression should wherever possible be shown to be consistent with the chosen strategy of expression. Estimates of the copy number should be made and evidence as to the accuracy of the incorporated gene sequence should be presented. It is believed that while multiple copies of the transgene are usually incorporated, there is usually only a single site of integration. Thus, even where multiple copies are introduced it will be possible to identify the expressed sequence or sequences with confidence at the level of the genomic DNA.

It is of doubtful value to determine multiple sequences of the insert but evidence that the correct sequence is present should be obtained. Some sequence data for example of cDNA clones will be valuable as will restriction endonuclease maps, which will serve to demonstrate that the site of integration has not changed in offspring of the founder animal where these are used. It should be clearly stated whether the animals used for production are haploid or diploid for the transgene. The animals used in production should be characterised to ensure an acceptable level of consistency.

The virological status of the donors and host animals should be shown to be acceptable; for example calves born to mothers infected with BVDV are likely to be persistently infected, and vertical transmission of BSE has not been eliminated as a possibility. Similarly bovine immunodeficiency virus (BIV) may be transmissible through semen. These are examples only.

4.4 Stability of the gene

Transgenic animals produced by microinjection of DNA have the highest probability of incorporating the transgene into the germ line and therefore expressing it in the appropriate intended tissue. However this method often results in the insertion of multiple head to tail copies of the transgene, and rearrangements and eliminations may occur on breeding. The stability of the gene on breeding will be an issue where numbers of animals derived from a founder animal are to be used. Greater consistency of production will be achievable if a uniform production herd can be bred in a reproducible manner. The strategy used to generate a herd of animals of similar productivity should be clearly delineated. Evidence should be presented that the animals are similar, in the yield of product and genetically in terms of numbers of copies of the gene incorporated and the site of integration in the genome. Restriction length polymorphisms may be of value in providing evidence for a constant integration site.

5. PRODUCTION

5.1 Housing and animal care

There are major veterinary and ethical difficulties in raising and maintaining agricultural animals under specific pathogen free conditions although this is desirable if it can be achieved. Otherwise good husbandry and agricultural practice may contribute to virological and microbiological safety. However the general conditions suitable for satisfactory agricultural production are likely to be less stringent than those applicable to the manufacture of pharmaceutical materials, so that good husbandry and agricultural practice are unlikely to be sufficient alone to ensure adequate safety of a pharmaceutical product. The conditions under which the animals are bred and maintained should be described and precautions taken to ensure that the site is free of disease likely to affect the production animal species prior to use. Potential sources of infection may include foodstuff, animal handlers and veterinary surgeons, and the environment especially if the animals are kept outside. The health and virological status of the animals should be documented and animals subjected to regular veterinary examination. If the source material is milk the health of the udder should be subject to special examination. Administration of antibiotics and hormones for prophylactic or therapeutic reasons at any time when they may contaminate the product is not permitted. Cows should be shown to be free of bovine tuberculosis.

Many cow herds are known to be infected with bovine viral diarrhoea virus, and other infections include bovine polyoma and infectious rhinotracheitis virus which may or may not be apparent. Sheep are susceptible to many agents including orf virus and Louping Ill virus, and pigs to swine vesicular disease and porcine parvovirus. These examples do not constitute an exhaustive list. Many infectious agents of agricultural animals may establish persistent infections, and some are also able to infect humans. In general animals which are known to be infected with an agent should not be used for production.

5.2 The source material

Different litter mates have been reported to express the transgene to different levels for unknown reasons unrelated to copy number or accuracy of the incorporated sequence. During the period of lactation the expression of the gene may vary, and it may also vary between different lactations. The source material may therefore be variable, making

purification procedures potentially less consistent. The nature of the source material (for example milk or colostrum) should be clearly stated and justified. There is wide variation in the composition of milk and the purification process must be shown to be satisfactory in dealing with the range of materials expected. Acceptable limits for the level of active substance in the source material should be set. Where the source material is milk, specifications could be set in terms of product activity per unit of non fat dry solid. A single batch of source material may involve pooling separate harvests and should be clearly defined. While milk is a source material with a long history in which the safety issues are generally well understood, pharmaceutical proteins may be given parenterally, not orally, and it may not be possible to pasteurise or sterilise them in the ways which have been applied to milk.

Limits for the microbiological status of the source material should be set. Milk is likely to be contaminated with bacteria, although such contamination may be minimised by good husbandry. Contamination by certain agents, such as zoonotic mycobacteria, would make the material unacceptable. While bacteria may be removed by sterile filtration of the product, mycoplasma may not and efforts should be made to exclude them from the source material.

5.3 Purity of the active substance and validation of downstream processing

The purity of the active substance should be in accordance with criteria accepted for products of rDNA technology. Most such products are currently manufactured by in vitro culture methods involving either the fermentation of microorganisms or the large scale culture of cells from higher organisms. A transgenic animal is unlikely to be free of pathogens to the same degree as a well characterised cell bank. Validation of the purification process is therefore important in ensuring the safety of the product. Guidelines on *Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses* have been prepared. Where the source material is milk or colostrum, contamination with mycoplasma is possible, and the process should be validated for their removal, as well as limits set for their levels in the starting material.

The source material, whether blood, milk, colostrum or other tissue will contain large numbers of host derived proteins other than the desired product, some of which may be present in large amounts which must be removed. Milk is known to contain proteases, and the possible effect of these on the product should be addressed; if degradation occurs, acceptable limits should be set for the products in the final material. Care should be taken to document and if necessary eliminate host proteins homologous to the required product. Limits should be set for contaminants which may copurify with the desired material. Hypersensitivity to milk is common, and materials must therefore be of high purity.

Data on the carbohydrate components of the product should be presented. The non enzymic glycosylation or glycation of proteins in the presence of free carbohydrate such as lactose should be considered. This process is likely to be inevitable to some degree for a product derived from milk but attempts should be made to reduce it to a minimum. Glycated proteins can cause the activation of end stage macrophages to produce cytokines, and long term exposure to a glycated product is likely to be harmful.

The attractions of transgenic animals as a means of production include the ability to produce materials required on a scale which may otherwise be prohibitive because of the large amounts required in therapy. This increases the concerns associated with the immunogenicity of the proteins because of trace impurities or imperfect post translational

modifications, and close attention should be given to the purity, quality and consistency of the product.

6. CONCLUSION

Transgenic animals may have advantages over existing production methods with respect to the quantity and quality of the source material, which may reduce production costs and simplify downstream processing. Other than veterinary and environmental concerns, which are outside the scope of this document, the issues they raise are principally those of using a whole organism in production rather than a potentially more predictable cell culture or fermentation system based on a seed lot. These include microbiological and virological concerns, possible difficulties in purification and the consistency of the production and purification process.