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Cancer Research Campaign Operation Manual for Control Recommendations for Products Derived from Recombinant DNA Technology Prepared for Investigational Administration to Patients with Cancer in Phase I Trials

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1. INTRODUCTION

1.1 *Aim*

THE AIM of this document is to establish guidelines for the quality and safety of products produced in research departments by recombinant DNA technology, and to establish principles on which phase I trials of these agents will be conducted. It will also establish a basis of information which should be collected into a single document and submitted in support of phase I clinical trials to funding bodies, ethical committees and other interested parties.

Guidelines for phase I clinical trials of cytotoxic drugs, hormones and antibodies produced from hybridomas have been agreed by the Cancer Research Campaign Clinical Trials Committee. These have been used as a basis for the proposed

procedures for clinical trials of products derived from recombinant DNA technology with appropriate modifications and additions.

1.2 *The present situation*

Biological products of peptides or protein nature such as cytokines, growth factors, enzymes and antibodies have been shown to have specific biological effects in experimental systems which may be beneficial to patients with cancer. Initially these agents made available in the clinic were derived from a variety of sources and often the preparations were crude and contained more than one active ingredient. The biological agents currently provided for clinical use are often produced using recombinant DNA technology in prokaryotic or eukaryotic cells. It is important to investigate the efficacy, safety and quality of these new agents so that any advantages may be offered to patients with cancer without undue delay.

1.3 *The problem*

New biological agents produced using recombinant DNA technology require careful preclinical evaluation to provide the clinician with information concerning the likely biological effects on normal and cancerous tissues and enable appropriate planning of phase I/II clinical studies. These preparations must be produced in a regulated manner in order to provide a formulated product with appropriate purity and biological activity. The preparation must then be submitted to appropriate preclinical examination using appropriate experimental systems.

It is recognised that the requirements of the licensing authority in regard to physicians undertaking a limited trial of a drug in their own patients on their own responsibility for the products used are less demanding than those required by a drug company seeking a clinical trial certificate [1]. The cost of toxicity would be prohibitive and destructive to the venture if the safety requirements are set too high.

1.4 *The special position of drugs for therapy of cancer*

Human cancers are unique and although similar cancers can be induced in many animal species, only generalisations about human cancer and its course are obtained. For this reason predictions of likely antitumour properties are often inaccurate and the problem may be compounded by species specificity in terms of the agent's biological properties in normal tissues. The recognition that tumour growth is regulated by a close interplay between the neoplastic cells and normal tissues provides a challenge in this context.

1.5 *The inadequacy of animal models*

The lack of reliably predictive animal models of human cancer implies that an agent's therapeutic potential can only be assessed following careful studies in man. There is, however, a strong argument in the case of anticancer drugs to adopt the minimum toxicology requirements which are consistent with safety so that promising candidates may proceed rapidly to clinical testing. The only valid information obtainable from animals is an *indication* of a useful biological effect or of tumour targeting, an *indication* of antitumour activity, an *indication* of target organ specificity and an *indication* of an appropriate dose level with which to start a human phase I trial.

1.6 *Toxicological requirements*

The present document summarises recommendations on the preclinical data required before a phase I study of a biological agent is carried out taking into account the feasibility of obtaining this information in the laboratory.

2. PRODUCT DEVELOPMENT

2.1 *Name of product*

The product should be named in such a way that its origin and production history can be unambiguously identified.

2.2 *Expression systems*

The host cell and expression vector used in production must be fully documented. This should include details of the origin and identification of the gene which is being cloned and the construction, genetics and structure of the expression vector. The method by which the vector is introduced into the host cell and the state of the vector within the host should be described. The association of the vector and host cell may be permanent, allowing continuous expression of the product; or self-limiting, for example where the vector is an acceptable cytopathogenic virus.

Where the product is expressed in eukaryotic cells, especially continuous cell lines, it is advisable to use lines which have already been used in the commercial manufacture of licensed products as the basis for production. Cells must be obtained from a reputable source, such as a national type collection recognised cell depository. Cells from other sources should be characterised in detail.

2.3 *Sequence of the cloned gene*

Full details of the nucleotide sequence of the gene insert and of flanking control regions of the expression vector should be recorded. Relevant expressed sequences should be clearly identified.

2.4 *Expression*

The strategy by which the expression of the relevant gene is promoted and controlled during production should be documented. The choice of inducers and other agents used to control expression should be considered carefully for potential toxicological implications.

3. SEED LOT

3.1 *Description*

A seed lot is a homogeneous suspension of cells already transformed by the expression vector containing the desired gene and aliquoted into individual containers for storage (e.g. in a liquid nitrogen refrigerator). The aliquots should be treated identically during storage, and once removed from storage, the containers must *not* be returned to the seed stock. In some cases it may be necessary to establish separate seed banks for the expression vector and the host cells.

3.2 *Control of seed lot*

It is essential that production is based on a well defined seed lot system. The history and genealogy of the cell line should be

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available. Extreme care should be taken to avoid contamination of the seed lot with other cells. During the establishment of the seed no other cell line should be handled simultaneously in the same production area or by the same persons. Attention should be paid to the stability of the host–vector expression system in the seed stock under conditions of storage and recovery. It should be shown that the correct gene insert is retained at seed lot stage and after production scale fermentation. For example, restriction digestion of plasmid DNA and electrophoresis to demonstrate correct insert size, or restriction mapping of the cloned insert should be carried out. Any known instability of the insert or variability in the expression should be documented since this may determine the future production process.

Ideally the seed lot should be shown to be free from contamination by other cell strains. Where higher eukaryotic cells are used for production, distinguishing markers of the cell may be of use in establishing the identity of the seed. For example, specific isoenzyme and immunological features or karyology may be provided. Likewise, where microbial cultures are used, specific features which form a basis for identification of the seed should be described.

For higher eukaryotic cells, such as mammalian, avian and insect, evidence that the seed lot is free from potentially oncogenic, where appropriate, or pathogenic adventitious agents (viral, bacterial, fungal or mycoplasmal) must be provided. It is strongly recommended that a well characterised cell line, already used for the commercial manufacture of other licensed products, is used as the basis for production, as much of the necessary data concerning tumourgenicity and viral contamination will have been obtained. Evidence should be available that pathogenic viruses have not been introduced during the manipulations leading to the establishment of seed stock. Consideration should be given to the purification process and its ability to remove or inactivate viruses which may inevitably be present in the seed as an endogenous agent or part of the expression vector.

4. PRODUCTION

4.1 *General principles*

Production and purification should take place in dedicated areas using dedicated equipment. Care should be taken in designing the purification process and production area so as to prevent contamination of purified products with unwanted materials, including impure intermediates from earlier stages of the process. A detailed record of use should be kept for all production areas and equipment, for example, incubators, fermenter vessels, concentrators and purification columns. This enables identification of possible reasons for processing failures or unexpected contamination.

Standard operating procedures (SOP) should be drawn up for the individual stages of the production process. These should describe how the recommendations will be put into practice in the individual laboratory. A full record of each production run should be kept.

4.2 *Cross contamination*

Ideally, not more than one cell line should be cultivated in a production area. If more than one cell line is cultivated in the same facility, records must be kept of the cell lines handled and evidence presented for the absence of cross-contamination between them.

4.3 *Production at finite passage (single harvest)*

Details of the fermentation or culture used to manufacture the product should be documented. Wherever possible, cultivation methods should be suitable for subsequent scale up.

4.4 *Continuous culture production (multiple harvest)*

This approach should only be undertaken when special consideration has been given to the control of production based on continuous culture. These considerations should include stability of the expression system and the possible introduction of microbial contaminants. The harvests pooled for processing as a batch should be clearly identified and related to the monitoring schedule applied to the culture.

4.5 *Method of purification*

Methods of harvesting, extraction and purification should be described in detail. The purification process should be designed to remove or inactivate infectious agents and to reduce non-product contaminants. The possibility of contamination by agents used in the purification procedure, such as monoclonal antibodies used in affinity chromatography should be borne in mind.

Wherever possible methods should be based on those used in the manufacture of pharmaceutical materials.

4.6 *Reproducibility of the purification procedure*

The reproducibility of the purification process should be adequately demonstrated regarding its ability to remove contaminants and to give a product of acceptably consistent composition.

5. FINAL PROCESSED PRODUCT

5.1 *Characterisation of the purified active substance*

Characterisation of the active substance by chemical and biological methods using a range of analytical techniques is required. The appropriate techniques will depend on the nature of the product, but may exploit size, charge, isoelectric point and biological properties. Modifications such as glycosylation may differ from those found in a natural counterpart and may influence the biological, immunological and pharmacological properties of the product.

5.2 *Purity*

Data should be provided on contaminants whose presence is anticipated in the final processed product. The level of contamination considered as acceptable, and criteria for acceptance or rejection of a product batch should be defined. The techniques used to demonstrate purity should encompass as wide a range of methods as possible, including physico-chemical and immunological techniques. Emphasis should be placed on tests for unwanted materials of host origin, as well as on materials which may have been added during the production or purification processes. Specific activities of highly purified material (units of activity/weight of product) are of particular value.

5.3 *Potency*

The potency of each batch of the product should be established (e.g. units of biological activity per ml) using a reference preparation calibrated in units of biological activity. Potency measurements involving biological tests should be consistent with the results of physico-chemical methods of assay.

6. FINISHED PRODUCT

6.1 *Specification and reference*

Evidence of identity, purity, potency and stability of the product should be provided. Details of preservatives and recommendations for reconstitution, if lyophilised, should be included. Where possible, samples representative of each batch should be retained for at least 2 years after completion of the clinical phase I study. The future need for reference materials should be borne in mind. The studies described in Section 5 will contribute to the definition of a reference material should a phase I trial seem promising.

6.2 *Biological characterisation*

Where possible biological characterisation using experimental models *in vivo* should mimic the intended clinical approach. However, many biological agents are species-specific and immunogenic in non-host species and, therefore, relevance of many animal tests may be limited. The product should be calibrated using physico-chemical tests and biological assays should be used to confirm that the product is biologically potent within stated limits.

6.3 *Toxicity studies*

Toxicity should be tested in an appropriate species and if possible this should be a species in which the product is shown to be biologically active. Animal studies supporting the dose, route of administration, frequency and duration of dosing should be provided. All animals should be sacrificed at least 14 days after dosing. Macroscopic investigation of all relevant tissues and a total red cell count, white cell and platelet count together with differential assessment of white cell counts are to be obtained. Where toxic effects are observed, histopathology should be carried out on any tissues which are macroscopically abnormal.

Selection of safe starting dose in humans would depend on the concentration of biological agent which produces the desired effect *in vitro*. Toxicity in animals should be performed using at least 10X the proposed starting dose in man, on a weight for weight basis.

If mutagenic agents are used for production or any other known toxic agents used in manufacture, appropriate data concerning the mutagenicity or toxicity should be obtained. When toxicity is observed information regarding reversibility of the effect is valuable.

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