DRAFT
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Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology

Office of Biologics Research and Review
National Center for Drugs and Biologics

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I. Introduction

This document provides suggestions for controlling safety, purity, and potency of new drugs and biologics produced by recombinant DNA technology. The suggestions expressed herein are expected to change with time as new knowledge is acquired, and should not be regarded as being either fixed or all-inclusive. Accordingly, the points discussed below should be interpreted as being what manufacturers of such products are generally expected to consider during drug development, in applications for investigational new drug (IND) exemptions, in new drug applications (NDA), and in license applications. The general regulations applicable to other biologics (general safety, sterility, potency, etc.) are also applicable to recombinant DNA biological products. Specific concerns relevant to particular products should be discussed with the appropriate Office on a case-by-case basis.

Although considered voluntary for industry, these points represent an informed consensus relating to the general safe use of products developed using recombinant DNA technology.

New license applications or new drug applications are required for products made with recombinant DNA technology, even if the product is considered to be identical in molecular or chemical structure to a naturally occurring substance or a previously approved product produced in a conventional way.

II. General Considerations

The production of new drugs and biologics by recombinant DNA technology should generally follow the NIH Guidelines for Research Involving Recombinant DNA Molecules or other appropriate guidelines.

III. Expression Systems

Recombinant DNA technology involves systematically arranging and manipulating specific segments of nucleic acid to produce a composite molecule which, when placed into an appropriate host environment, will yield a desired product. There are three general methods for obtaining a specific coding segment: (a) reverse transcription of mRNA to complementary DNA; (b) isolation of genomic DNA or RNA; or (c) chemical synthesis as predicted from a peptide sequence.

The manufacturer should provide a description of the method used to prepare the segment coding for the desired product, including both the Cell type and origin of the source material, a detailed nucleotide sequence analysis and a restriction enzyme digestion map of the cloned segment. If a cloned polynucleotide contains more information than the coding sequence, i.e. introns or flanking sequences, then these additional sequences should be identified.

The construction of the vector used for expression of the cloned nucleotide segment into its respective product should also be thoroughly described. This description should include a detailed explanation of the source and function of the component parts of the vector e.g. origins of replication, antibiotic resistance genes, promoters, enhancers, whether or not the product is being synthesized as a fusion protein- The restriction enzyme digestion map of the entire constructed vector should be provided.

The host cell system which will generate the product is coordinated. to fit the expression vector. It is, therefore, important that a complete description of the source, relevant phenotype and genotype of the host be provided. If the host cell is of mammalian origin then data concerning its

tumorigenicity should be obtained. Various methods can be utilized to transfer an expression vector into its host, such as transfection, transduction, infection, microinjection, etc. The mechanism of transfer, copy number, and the physical state of the vector inside the host cell, integrated or extrachromosonal, should be provided. In most cases, a single host cell containing the expression vector should be cloned to give rise to the Master Cell Bank. The cloning history and methodology should be described. If new Master Cell Banks are to be generated periodically by expression vector transfer and cloning, acceptance criteria for both the new clones and the product produced by these clones should be described.

IV. Master Cell Bank

Host cells chosen to express recombinant DNA products should be maintained in seed lot aliquots under storage conditions which ensure genetic stability. The stability of both the host cell and expression vector should be investigated. In particular, the fidelity of the nucleotide sequence encoding the expression product present in each seed lot should be verified.

The purity of the cells in the seed lot should be assured by isoenzyme analysis, awotrophy, antibiotic resistance, and karyology, as appropriate.

The seed lot should be free of adventitious agents including mycoplasma, bacteria, yeast, viruses, and virus-like particles. At a minimum, mammalian host cells should be analyzed for reverse transcriptase and detection of virus-like structures by electron microscopy.

V. Production

The cells used in each production run should be characterized by analysis of relevant phenotypic or genotypic markers, and tested for adventitious agents in samples taken just prior to termination of culture.

The procedures and materials used for cell growth and induction of product expression should be described in detail.

Data on the consistency of yield of the product from full-scale culture should be maintained, and criteria for the rejection of culture-lots should be established.

Penicillin and other beta-lactam containing antibiotics should not be used in production runs.

VI. Purification

The methodology of harvesting, extraction, and purification should be described in detail, and the remdval of any toxic chemicals introduced by these procedures should be demonstrated.

The extent of purification of recombinant DNA products should be consistent with the intended use of the product. Biologicals which are to be administered repeatedly or at high concentrations should be adequately pure to prevent the development of undesired immune or toxic reactions to contaminants. Although recombinant DNA products may be demonstrated to be 99% pure by physicochemical characterization, special attention should be directed toward the removal of certain contaminants which may be present in small amounts. The purification process should specifically eliminate undesirable antigenic materials and detectable viruses, microbial contamination and nucleic acids.

The use of monoclonal antibodies for affinity purification of recombinant DNA products deserves special comment. A document entitled "Points to Consider in the Manufacture of Monoclonal Antibody Products for Human Use" already has been issued by the National Center for Drugs and Biologics. The monoclonal antibodies should be shown to be free of unwanted biologically active substances such as DNA and viruses as described in Section VII, C. Methods used for the coupling of the monoclonal antibody to the column matrix and the removal of contaminants from the affinity column should be described. The final product should be examined for the presence of antibody and column matrix.

VII. Characterization of the Product

Evidence for purity of the product and for the identity of the product with reference preparations may be derived from the results of a wide variety of tests. Such tests may include:

A. Physicochemical Characterization of Proteins

1. Compositional Analysis by Amino Acid Analyses

The complete amino acid composition of the peptide or protein should include accurate values for methionine, 1/2-cystine and tryptophan, which may require sample preparation procedures other than hydrolysis in 6N HCl. The amino acid composition presented should be the average of at least three (3) separate determinations of the same product or lot number. A positive correlation in the results of analyses of the product and reference preparations would support arguments for identity.

For small proteins or peptides with molecular weight less than 10,000 daltons, the demonstration of nearly integral ratios of amino acids would support arguments of peptide purity.

For proteins with molecular weight in excess of 10,000 daltons, the amino acid compositional analysis may not provide as useful information in support of the purity of the product as for the small proteins or peptides. However, integral values for those amino acid residues generally found in lov quantities, such as tryptophan and/or methionine, could be obtained and used to support arguments of purity.

2. Partial Sequence Analysis

Partial amino terminal (15 residues) and carboxy terminal sequence analyses can serve as important criteria for the identity of recombinant DNA produced proteins or peptides. The sequence data presented in tabular form should include the total yield for every amino acid at each cycle as well as the repetitive yield for the major sequence(s). Unexpected heterogeneity in the amino termini and carboxy termini of proteins produced by recombinant DNA technology due to proteolytic degradation has been revealed already by sequence analysis.

3. Peptide Mapping

Peptide mapping can provide a very discriminating comparison between a recombinant DNA product and an authentic sample of the natural product or a reference preparation. This is best accomplished using a pure and specific peptidase to cleave the proteins under identical conditions, followed by either two-dimensional electrophoresis or high performance liquid chromatography. In conjunction with amino acid composition and sequence analysis of each peptide,

peptide mapping can provide precise evidence for the identity of a protein. For proteins containing disulfide bonds, peptide mapping can be used to verify the proper arrangement of disulfide bonds in the final product.

4. Polyacrylamide Gel Electrophoresis (PAGE) and Isoelectric Focusing

PAGE and isoelectric focusing are valuable techniques for verifying the purity and molecular weight of proteins and peptides. The PACE gels should be run in sodium dodecylsulfate with and without exposure to reducing agents, and with appropriate molecular weight standards or reference preparations.

It is preferable to analyze duplicate samples on slab gels, one stained with Coomassie blue and the other stained with silver. The silver stain is considerably more sensitive for the detection of very small quantities of proteins and is useful in identifying nonprotein materials such as nucleic acid, carbohydrate and lipid which may be present.

For peptides of molecular weight less than ca. 8,000 daltons, most PACE methods may not be accurate for molecular weight estimates.

5. High Performance Liquid Chromatography (HPLC)

HPLC is a very useful method to determine the purity of a protein or peptide, to evaluate its molecular configuration and, under some circumstances, to confirm its identity. HPLC may be especially useful in characterizing and quantitating specific impurities in the final product. A widely used method is reverse-phase HPLC. A protein or peptide which elutes as a single symmetrical peak in two markedly different systems, including an ion-pair system, is generally quite pure.

6. Circular Dichroism and Optical Rotatory Dispersion (CD and ORD)

Comparison of the CD or the ORD spectrum of the material prepared by recombinant DNA technology with the corresponding spectrum of the native material or the reference preparation may support conformational similarity.

7. Other Characterization

Additional physicochemical characterizations may be appropriate for recombinant DNA products incorporating carbohydrates, DNA, lipids, and other nonprotein components.

B. Biological tests for Identity and Potency

Comparing the recombinant DNA product to the natural product in a suitable bioassay will provide additional evidence relating to the identity and potency of the recombinant DNA product.

Various types of bioassay may be used. ~~In vitro~~ assays are usually faster, less expensive, and more precise than animal studies, yet adequate testing of a biological product before introduction into humans may involve animal studies. Most vaccines produced by recombinant DNA techniques should be compared to the natural substance with respect to their ability to promote immune responses in animals. The extent and frequency of animal testing should be determined on a case-by-case basis.

C. Tests for contaminants

Reliable and sensitive tests will be needed to assay for trace contamination and product related impurities in individual production batches. Although physicochemical characterization can ensure a high degree of purity of a recombinant product, tests for trace contaminants will rely heavily on biological indicator systems.

1. Pyrogen Contamination

Pyrogenicity testing should be conducted by traditional methods of endotoxin detection with limulus amebocyte lysate (LAL) and direct pyrogenicity testing by injection of rabbits with the final product. Criteria comparable to those adopted for acceptance of the natural product should be used for the DNA product.

Certain biological pharmaceuticals may be pyrogenic in humans despite having passed the LAL test and the rabbit pyrogen test. This phenomenon may be due to nonendotoxin contaminants which appear to be pyrogenic only in humans. To attempt to predict whether human subjects will experience a pyrogenic response, human blood mononuclear cells can be cultured in vitro with the final product and the cell culture fluid injected into rabbits. A fever in the rabbits indicates that the product contains pyrogenic substances.

2. Viral Contamination

Tests for viral contamination should be appropriate to the cell substrate and culture conditions employed. Absence of contamination of the final product by adventitious viruses should be demonstrated.

3. Nucleic Acid Contamination

Removal of nucleic acid at each step in the purification process should be demonstrated in pilot experiments by examining the extent of elimination of added radiolabeled host cell DNA. This analysis will provide the theoretical extent of the removal of nucleic acid during purification.

A direct analysis of nucleic acid in the final product should be performed by hybridization analysis of immobilized contaminating nucleic acid utilizing appropriate probes, such as both nick-translated host cell and vector DNA. This method ought to provide sensitivity to the level of 10 picograms per

dose. Theoretical concerns regarding transforming DNA derived from the cell substr vill be minimized by the general reduction of contaminating nucleic acid.

4. Antigen contamination

Products which are administered repeatedly and/or in large doses should be assayed for trace antigenic constituents likely to contaminate the final product. Tests such as Western blots, radioimmunoassays and enzyme-linked immunosorbant assays using high affinity antibodies raised against host cell lysates, appropriate subcellular'fractions or culture medium constituents, should be used to detect contaminating antigens. Such methods can provide sensitivity in the range of 1 to 100 parts per million. Because the detection of antigens will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement but not replace silver stain analysis of SDS-PAGE gels. Patients given large or repeated doses of a product should be monitored for the production of antibodies to potentially contaminating antigens.

5. Microbial Contamination

Tests for microbial contamination should be conducted using prescribed sterility testing procedures. Absence of bacteria (aerobes and anaerobes fungi, yeast and mycoplasma in the final product should be demonstrated.

D. Toxicity tests in animals

A recombinant DNA product that is demonstrated to be identical to a natural substance for which the pharmacology and toxicology data are already develo vill generally not be required to undergo all the animal toxicity tests normally carried out in the evaluation of a neu product. Any recombinant DNA product

consisting of a natural substance with a minor modification would require somewhat more testing in appropriate animal models. A product radically altered, from the natural substance would require still more extensive animal tests, including those for carcinogenicity, teratogenicity and effects on fertility. The specific tests which might be appropriate are best addressed on a case-by-case basis with the appropriate Office.

VIII. Modified Protein Products

Using recombinant DNA procedures it may be possible to modify the structure of biological proteins to enhance their desired biological properties and/or diminish undesirable ones. Any substance which is not a natural constituent of the human body may be antigenic and also may cause unknown and possibly adverse biological effects. Whether such a product should be used in humans depends on a careful assessment of its new benefits compared to the risks identifiable during its preclinical and clinical evaluation.

IX. Clinical Trials

Clinical trials will be necessary for all products derived from recombinant DNA technology to evaluate their safety and efficacy.

NATIONAL INSTITUTES OF HEALTH
POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF
HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

WORKING GROUP ON HUMAN GENE THERAPY
NIH RECOMBINANT DNA ADVISORY COMMITTEE

OUTLINE

Applicability

Introduction

I. Description of Proposal

A. Objectives and rationale of the proposed research

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

2. Preclinical studies, including risk assessment studies

3. Clinical procedures, including patient monitoring

4. Public-health considerations

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

C. Selection of patients

D. Informed consent

E. Privacy and confidentiality

II. Special Issues

A. Provision of accurate information to the public

B. Timely communication of research methods and results *to* investigators and clinicians

III. Requested Documentation

A. Original protocol

B. IRB and IBC minutes and recommendations

C. One-page abstract of gene therapy protocol

D. One-page description of proposed experiment in non-technical language

E. Curricula vitae for professional personnel

F. Indication of other federal agencies to which the protocol *is* being submitted

G. Other pertinent material

IV. Reporting Requirements

ANTIVIRAL DRUGS FOR NON-LIFE-THREATENING DISEASES:

Draft Points to Consider for Safety Evaluation Prior to Phase I Studies

(Revised October 1985)

The Division of Anti-Infective Drug Products

Center for Drugs and Biologics

Food and Drug Administration

This document is concerned with the safety evaluation, based on preclinical studies, of antiviral drugs for non-life-threatening diseases. Because advances in technology are occurring at a rapid pace, information in this document is subject to change as new and significant findings become available. Accordingly, the discussion below should be interpreted as the general expectations of manufacturers or individual iavestigators who intend to study antiviral drugs and as points which they should 'consider during product development, in applications to the Food and Drug Administration. Existing general regulations(21 CFR, 200 series, 300 series, and 400 series) are also broadly relevant and should be consulted. These poiuts are not all-inclusive, and certain points may not be applicable to all situations. Therefore, the Division of Anti-Infective Drug Products will review the adequacy of testing any antiviral drug on a case-by-case basis. All data and information developed under these Points and submitted to or incorporated by reference in a Notice of Claimed Investigational Exemption for a New Drug (IND) are considered confidential according to 21 CFR 312.5 and 314.14.

General Considerations:

The safety evaluation and determination that some antiviral activity is present must be conducted before beginning Phase I clinical trials. Although an IND may be filed prior to completion of these preclinical studies, the clinical studies should be "on hold " until these preclinical studies are completed.

"Non-life-threatening Diseases" are defined as those diseases from which the patient is expected to recover with minimal morbidity. Thus, to use an antiviral drug for such conditions, the investigator must have data to assure that the normal life functions of the patient will not be harmed by the drug. This may, in some cases, contrast with the use of an antiviral drug for a disease which is invariably fatal and for which no approved therapy is available, since the importance of saving life may outweigh the risk of a toxic side-effect.

Investigations under an IND for phase I and II studies may be permitted if the antiviral effect has been shown in cell culture or animal models, and toxicity data corresponding to the duration of drug administration planned. For agents which do not replicate in cell culture or for which there is no suitable animal model, data from overseas clinical investigations or life-threatening infections produced by the same agent may be considered. In all cases cell cytotoxicity and animal toxicity data should be submitted.

The development of laboratory animal models for the disease to permit the evaluation of antiviral drugs should be encouraged. The use of these models will improve the safety of investigational studies. In selecting and developing animal models, differences between humans and the selected animal species in responses to the virus in question should be considered.

1.0 Safety Evaluation:

1.1 The drug should be tested in vitro to determine whether it is mutagenic, for instance whether it induces cell transformation. If positive results are obtained, transformed cells should be transplanted into syngeneic laboratory animals to determine their capability of forming tumors.

1.2 Viruses which have been exposed in vitro or in vivo to the drug, should be tested in vitro to determine whether the biological characteristics of the viruses have been altered, including whether they induce cell transformation.

1.3 Systemic absorption of a topically applied drug should be determined in animals, with attention to the surfaces which will be exposed clinically (mucosal, etc.). This may include studies of blood levels, tissue levels in various organs, and skin penetration studies.

1.4 The immunosuppressive capability of the drug should be screened. Such screening should evaluate major changes in immunologic function which might be expected to result in infection or other clinically detectable adverse result if seen later in humans. Evaluations should include effects on humoral and cell-mediated immunity and macrophage activity in appropriate laboratory animals using intact animals.

1.5 Standard toxicity tests in animals should be submitted. These are published in the "Current Views on Safety Evaluation of Drugs," FDA Papers, May 1968, and "Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use," January 1966, and include:

For systemic drugs: acute and subacute toxicity, ADME studies;

For dermatologic drugs: irritation and sensitization studies, degree of absorption.

1.6 Where extensive clinical use of a drug for other therapeutic purposes is known or where overseas clinical safety data are provided, selected requirements may be waived by FDA if there is no cause for concern as determined by the FDA reviewer.

2.0 **Preclinical** Evaluation of Antiviral Activity (to determine whether the known and **unknown** risks are warranted for the target patient population): One or more of criteria 2.1, 2.2, and 2.3 should be met for topical antiviral drugs; criterion 2.2 and/or 2.3 should be met for systemic (non-topical) antiviral drugs. In situations where the antiviral activity of a compound has been shown in overseas or published studies, justification for initiating clinical trials under an IND without meeting criteria 2.1, 2.2, or 2.3 will be decided on a case-by-case basis.

2.1 The drug should be tested in cell-free media to determine virus-inactivating activity.

2.2 The drug should be tested in cell culture to determine antiviral activity. The potential cytotoxicity of the drug should be controlled for or ruled out by parallel studies.

2.3 The drug should be tested in laboratory animals by the **route(s)** and in the **formulation(s)** intended for human use, to determine the antiviral activity. The potential cytotoxicity of the drug should be controlled for or ruled out by parallel studies.

2.4 The dose-response curve should be established for the drug in a) cell-free systems, b) tissue culture, and c) animal models. Emphasis should be placed on determining the time at which the effect of the drug is achfeved following administration. Units of potency should be developed and used.

2.5 The possibility of strain diversity of the virus studied should be evaluated. Ideally, several strains of virus should be used in some or all of the studies outlined in this document.

2.6 The ability of the drug to induce resistance in vitro should be studied.

2.7 Selected components of the formulation, other than the antiviral drug, should be tested in vitro and in vivo for antiviral activity when it is possible to isolate those components.

3.0 Later Studies:

3.1 Later in the evaluation of the IND, the sponsor is encouraged to determine the antiviral mechanism of action of the drug.