ANNEX-8

GUIDELINES FOR GENERATING PRE-CLINICAL AND CLINICAL DATA FOR RDNA VACCINES, DIAGNOSTICS AND OTHER BIOLOGICALS, 1999

Biotechnology is poised for economic and social progress in the developed and developing countries. The biotechnology research, development and applications are growing at a rapid rate. This would lead to availability of products and processes especially in pharmaceutical and healthcare sectors. Products and processes developed through recombinant DNA technology are already available in markets of developed and other countries. More and more such products will be available in near future. Therefore, regulatory standards for recombinant (r-DNA) products are essential. Globally flexible guidelines particularly by FDA and ICH have been developed which are under finalization. There is need to formulate appropriate guidelines for preclinical and clinical evaluations in our country. The guidelines specifically are on safety, purity, potency and effectiveness of the product.

A. SPECIFICATION AND CHARACTERIZATION INFORMATION ON r-DNA VACCINES AND BIOLOGICAL PRODUCTS

1. **Description in details of the method of r-DNA products like**
   a. host cells,
   b. gene construct,
   c. vector construction including
   d. source and diagram of the plasmid(s) used,
   e. all intermediate cloning procedures and
   f. transfection methods

2. **Description of the method of sequence verification (such as restriction enzyme mapping, PCR etc.**

3. **Description on Identity-Physical, Chemical, Immunological and Biological wherever applicable**

   a. *Description on recombinant DNA products:*

      i. Primary structure (Amino acid sequences)
      ii. Secondary structure (disulphide linkages etc.)
      iii. Post translational modification (glycosylation etc.)

   b. *Monoclonal antibodies*
      − Identity by rigorous immunochemical / physicochemical characterization.

4. **Potency (for recombinant vaccines & biologicals)**
   a. Production of specific recombinant reagent in transfected cell line,
   b. Immune responses in mice,
   c. Hypersensitivity (Guinea pig maximization test) and
   d. Permissible limits of potency

5. **General Safety Test**

   To be performed in mice and guinea pigs on each lot of r-DNA vaccines/biologicals to detect extraneous toxic contaminants potentially introduced during production. For oral vaccines general safety data as per the guidelines of US pharmacopia.

6. **Data on sterility tests as per Indian Pharmacopia guidelines.**

7. **Data on purity of recombinant product**

   a. Limits of purity,
   b. Characterization of minor impurities like RNA, protein and genomic DNA,
   c. Permissible limits of moisture, if lyophilized
   d. Pyrogenicity

8. **Description of constituent materials like preservatives etc.**

9. **Data on stability of finished formulation, as per IP (Indian Pharmacopia) guidelines.**

B.1. PRECLINICAL TESTING

1.1 General Principles:

The objectives of the preclinical studies are to define physiological, toxicological and efficacious potential of r-DNA product prior to initiation of human studies. Both in vitro and in vivo studies can contribute to evaluate the effects of r-DNA products.

Preclinical models should consider 1) selection of appropriate animal species and their physiological state and 2) the manner in which rDNA products are delivered. This should include dose regimens, route of administration and physiological, pharmacokinetics, toxicological, immunological and effectiveness parameters.

These studies are expected to be performed in compliance with Good Laboratory Practices (GLPs). However, it is recognized that some specialized test systems often needed for certain products may not be able to comply fully. Areas of noncompliance should be identified and their significance be evaluated relative to the overall safety assessment.

Conventional approaches in preclinical tests of non rDNA products may not be appropriate because of the unique and diverse structural and biological properties of rDNA products.

1.2 Biological activity/ pharmacodynamics:

Biological activity may be evaluated using in vitro assays to determine effects of the product which are related to clinical activity. The use of cell lines and/or primary cell cultures can be useful to examine the direct effects on cellular phenotype and proliferation. Due to the species specificity of many biotechnology derived pharmaceutical products, it is important to select appropriate cell lines. In vitro cell lines from mammalian cell would be required to predict specific aspects of in vivo activity. Such studies may be designed to determine for example, receptor occupancy, receptor affinity and/or pharmacological effects and to assist in the selection of appropriate animal species for further in vivo pharmacology and toxicology studies

The combined results from in vitro and in vivo studies will assist in the extrapolation of the findings to humans. In vivo studies to assess pharmacokinetics/ harmacodynamics activity including defining mechanism(s) of action would support the rationale of the proposed product in clinical studies.

For monoclonal antibodies, the immunological properties of the antibody should be described in detail including its antigenic specificity, affinity, complement binding and any unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended target. The cross reactivity of monoclonal antibodies to human tissues should be carried out by appropriate immunohistochemical procedures using a range of human tissues.

1.3 Animal species/ model selection:

The pharmacological activity together with species and/ or tissue specificity of r-DNA products often preclude standard toxicology testing designs in commonly used species (e.g. rats and dogs). Safety evaluation programs should normally include two relevant species. In certain situations, one relevant species may suffice. In these cases the rationale should be provided. All pharmacological (wherever applicable), toxicological and immunological studies may be carried out in one species of animal, while the efficacy may be studied in another species/ transgenic animal or experimental model (diseased animal).

Toxicology studies in "pharmacologically non-relevant species" are not needed and are discouraged. However, if in vitro preclinical studies have not identified a relevant animal species, due to the unique species restriction to human cells, it may still be prudent to assess some aspects of potential toxicity in a limited toxicity evaluation in a single species.

Alternative approaches, when no relevant species exist, may include the use of transgenic animals expressing the human receptor or homologous proteins. The information gained from use of a transgenic species expressing the human receptor is optimized when the interaction of the product and the humanized receptor has similar physiological consequences as is expected in humans. It should be noted that the

production process range of impurities/contaminants, pharmacokinetics and exact pharmacological mechanism(s) may differ between the recombinant product perse and the recombinant product intended for clinical use. Pharmacokinetics, toxicity needs to be generated from those.

In recent years, there has been much progress in the development of experimental animal models that are thought to be similar to the disease to be treated in humans. These animal models include spontaneous/experimental models, disease models or transgenic models of disease. These models may provide further insight not only in determining the pharmacological action of the product, pharmacokinetics and dosimetry but may also be useful in the determination of safety (e.g. evaluation of undesirable promotion of disease progression). In certain cases studies in animal models of disease may be used as an acceptable alternative in toxicology studies in normal animals. Animals models of disease may be useful in defining toxicity endpoints, selection of clinical indications and determination of appropriate formulations. It should be noted that with these models of disease there is a often a paucity of historical data for use as a reference when evaluating study results. Therefore, the collection of concurrent control and baseline data is critical to optimize study design.

1.4 Number/ gender of animals:

The number of animals used per dose has a direct bearing on the ability to detect toxicity, pharmacokinetics and effectiveness (Schedule Y, Drugs and Cosmetics [8th Amendment] Rules - 1988, Ministry of Health & Family Welfare, Govt. of India, 1991). A small sample size may lead to a failure to observe desired effects due to observed frequency. The limitations imposed by sample size, as often is the case for non-human primate studies may be in part compensated by increasing the frequency and duration of monitoring. Both genders should generally be used or justification given for specific omissions.

1.5 Administration/ dose selection:

The route and frequency of administration should be as close as possible to the proposed clinical use and should also take into account the pharmacokinetics and bioavailability of the product in the species being used and the volume which can safely and humanely be administered to the test animals. The use of routes of administration other than those used clinically may be acceptable. In case the route has been modified, the precise reasons like limited bioavailability, size/physiology of the animal species etc. may be provided.

Ideally, dose levels should be selected to provide informations on a dose-response relationship. A toxic dose and a no observed adverse effect level (NOAEL) be indicated. For some classes of products with little or no toxicity, it may not be possible to define a specific maximum dose. In these cases, a strong scientific justification of the rationale for the dose selection and projected multiples of human exposure should be provided. Where a product has a lower affinity or potency in the cells of the target species compared with human cells, testing of higher doses may be important. The multiples of the human dose necessary to determine adequate safety margins may vary with each class of r-DNA product and its clinical indication(s).

1.6 Immunogenicity:

For recombinant vaccines, preclinical assessment must include immunological potency of the vaccine. The results would be essentially required to choose a dose for clinical use. The preclinical immunological assessment be related to sero- conversion rate, geometric mean titre, cell medicated immune responses in vaccinated animals. The study should also be designed to collect information regarding the duration of antigen expression and whether long term expression will result in tolerance or auto immunity. Distribution or tropism of vaccine for specific tissue studies should define duration of vaccines/ immunogens expression and persistance of vector in somatic cell.

Antibody responses should be characterized (e.g. neutralizing or non neutralising antibodies) and their appearance should be correlated with any pharmacological and/ or toxicological changes. Specifically the effects of antibody formation on pharmacokinetic/ pharmacodynamic characteristics and/ or severity of adverse effects or the emergence of new toxic effects should be considered when interpreting the data. For viral recombinant vaccines, antibodies to single stranded or double stranded DNA may be assessed.

The detection of antibodies should not be the sole criterion for the early termination of a preclinical study or modification in the duration of the study design unless the immune response neutralizes the pharmacological and/ or toxicological effect in a large proportion of the animals. In most cases, the immune response to recombinant proteins is variable, like that observed in humans. Specific attention

should be paid to the evaluation of possible pathological changes related to immune complex formation and deposition. If the interpretation of the data from the safety study is not compromised by these issues, then no special significance should be ascribed to the antibody response.

For those DNA plasmid vaccine constructs that co-expresses cytokine genes, specific preclinical studies should be considered in relation to modification of the cellular or humoral immune responses resulting in adverse consequences like generalised immune suppression, chronic inflammation, auto immunity or other immunopathology. The significance of antibody formation in animals to the potential for antibody formation in humans is often questionable. Humans develop serum antibodies even against humanized proteins, and frequently the therapeutic response persists intheir presence. The occurrence of severe anaphylactic responses to recombinant proteins even though is rare in humans. In this regard, the results of guinea pig anphylaxis tests, which are generally positive for protein products, are not predictive for humans. Therefore, such studies are considered of little value for these types of products.

B.2. SPECIFIC CONSIDERATIONS:

2.1 Safety pharmacology:

It is important to investigate undesirable pharmacological activity in appropriate animal models and where necessary, incorporate monitoring of this activity in the toxicity studies and/or clinical studies. Safety pharmacology studies provide functional indices of toxicity. These functional indices may be investigated in separate studies or incorporated into the design of the toxicology studies. The aim of the safety pharmacology studies should be to establish the functional effects on the major physiological systems. Investigations may include use of isolated organs or other test systems not involving intact animals. The evaluation of function of specific organ systems (e.g. cardiovascular, respiratory, CNS and autonomic nervous systems and the renal system) depends on the pharmacological properties of the product.

2.2 Toxicology and pharmacokinetics (Absorption, Distribution, Metabolism, Excretion ADME):

It is difficult to establish uniform guidelines for ADME studies for r-DNA products. Single dose pharmacokinetics and tissue distribution studies for r-DNA products wherever applicable, and therapeutic monoclonal antibodies are often useful, however routine studies that attempt to assess mass balance accumulation and excretion are not useful. Differences in ADME among animal species may have significant impact on the predictiveness of animal studies or on the assessment of dose response relationship in toxicology studies. Alterations in the pharmacokinetic profile due to immune - mediated clearance mechanisms may affect the ADME profiles and the interpretation of the toxicity data. This may be kept in mind and data if necessary be generated on immune mediation effect on clearance mechanism. ADME studies should whenever possible, utilize test material that is representative of that intended for clinical use using a route of administration relevant to the anticipated clinical studies including reactogenicity be indicated.

2.3 Immunotoxicity:

One aspect of immunotoxicological evaluation includes, assessment of potential immunogenicity and hypersensitivity (see section 1.6). In addition, many biotechnology-derived pharmaceutical products are intended to stimulate or suppress the immune system. Inflammatory reactions at the injection site may be indicative of a stimulatory response. In addition, the expression of surface antigens on target cells may be altered with implications for their auto-immune potential. Immunotoxicological testing strategies should be applied to clarify any such issues. Data should be provided in relation to toxicity to potential target organs, including haemopoetic and immune system. Preclinical studies should generate data on clinical pathology, gross evaluation of histopathology of tissue, local site reactogenicity should provide clinical and histological data of the injection - site tissue obtained from biopsies or term necropsy samples. For those DNA plasmid vaccine constructs that co-expresses cytokine gene, specific preclinical studies should be conducted in relation to whether modification of cellular or humoral immune responses resulting in adverse consequences like generalised immune suppression, chronic inflammation, auto immunity or other immunopathology.

2.4 Reproductive performance and developmental toxicity:

The need for reproductive/ developmental toxicity studies is dependent upon the product, clinical indication and intend patient population. R-DNA vaccines/biologicals should be evaluated for migration to gonadal tissue and possible germ line alterations in both male and female animals. This might be studied

by PCR etc. performed on gonad derived DNA preparations from male and female animals which receive r-DNA vaccines/ biologicals.

The specific study design and dosing schedule may be modified based on issues related to species specificity and/ or antigenicity. In cases where extensive public information is available regarding potential reproductive and/ or developmental effects of a particular class of compounds (e.g. interferons) and the only relevant species is the non-human primate, mechanistic studies indicating that similar effects are likely to be caused by a new but related molecule, may obviate the need for formal reproductive/ developmental toxicity studies. In each case, the scientific basis for assessing the potential for possible effects on reproduction/development should be provided.

2.5 Genotoxicity studies:

The range and type of genotoxicity studies routinely conducted for conventional pharmaceuticals are not applicable to the active components of biotechnology derived pharmaceutical products. The administration of large quantities of peptides/ proteins may yield uninterpretable results, moreover, it is not expected that these substances would interact directly with DNA or other chromosomal material.

The concern for plasmid DNA vaccine is related to integration of vaccine in host cells resulting in insertional mutagenesis through activation of oncogenes or inactivation of suppressor genes. An integration of plasmid DNA vaccine may result in chromosomal in stability through the induction of chromosomal breaks or re-arrangements. DNA Plasmid Vaccines: The concern of plasmid DNA is related to integration of vaccine in host cell resulting in insertional mutagenesis through activation of oncogenes or inactivation of suppressor gene. An integration of plasmid DNA vaccine may result in chromosomal instability through induction of chromosome breaks or rearrangements.

DNA Plasmid Vaccines.

Preclinical studies should focus on the potential of plasmid DNA vaccine to recombine with endogenous host DNA sequences. This could be studied by various ways e.g. PCR - using primers for vaccine - to examine and to assess distribution/ distinguish integrated vs non integrated DNA plasmid in genome DNA of tissue of experimental animals or by insitu hybridigation. Local site reactogenecity should provide detailed chemical and histological evaluation of injected site tissue (biopsy/ necropsy).

With some types of products, there is a potential concern of accumulation of spontaneously mutated cells (e.g. via facilitating a selective advantage of proliferation). This could lead to concerns regarding the potential carcinogenicity of such compounds. The standard battery of genotoxicity tests is not designed to test for these circumstances. Alternative responsive in vitro or in vivo models for such conditions may have to be developed and evaluated. Studies in available and relevant systems including newly developed systems should be performed as per the ICH guidelines in those cases where there is cause for concern about the product (e.g. because of the presence of an organic linked molecule in a conjugated protein product).

2.6 Carcinogenicity studies:

A tumorigenicity study should be considered only if there is clear evidence of integration of r-DNA vaccine/ biologicals or broad tissue distribution or if biological product is intended for chronic use in life threatening situation or if r-DNA vaccine/ biological construct has extensive DNA homology to the human genome or if the vector contains sequences of known oncogenic potential.

Specific assessment of carcinogenic potential of product may be needed, depending upon duration of clinical dosing and patient population. Wherever rodents are not the elevant species for assessing toxicity and/or the product is immunogenic, conventional carcinogenicity bioassays are not appropriate in case of a concern about carcinogenic potential of the product (e.g.growth factors). Different approaches should be considered. Products that may have the potential to support or induce proliferation of transformed cells and clonal expansion leading to tumor formation should be evaluated for receptor expression in various malignant and normal human cells. The ability of the product to stimulate growth of the malignant cells expressing the receptor or to initiate malignant cell growth in normal cells expressing the receptor should be determined. When in vitro data for tumor promotion give cause for concern, further studies in relevant animal models would be required.

In those cases where the product is biologically active and non immunogenic in rodents, an assessment of carcinogenic potential in a single species should be considered. Careful consideration should be given to the selection of doses. The use of pharmacokinetic or pharmacodynamic endpoints with consideration of receptor characteristics and intended exposures in humans represents the most scientifically valid approach for defining the appropriate doses. The rationale for the selection of doses should be provided.

C. IN VITRO DIAGNOSTIC, A) RECOMBINANT REAGENTS OR B) MONOCLONAL ANTIBODIES:

1) The description in details of process leading to rDNA reagents especially
   a) host cell,
   b) gene construct,
   c) Vector construction,
   d) sources and diagram of plasmid(s) used,
   e) all intermediate cloning procedures,
   f) transfection method, &
   g) method of sequence verification like restriction enzymes mapping and PCR etc., are to be provided.
2) The description in details of the procedures in a) generating monoclonal antibodies like myeloma cell line used, the cloning procedure, ascetic fluid in animals, the purification procedures for ascetic fluid, b) isotype of monclonal, c) stability of clone, d) specificity and affinity of monoclonal antibody etc. needs to be provided.
3) The recombinant or monoclonal diagnostic reagents for in vitro use need not to undergo "Preclinical testing" as specified under item `B' of the document.
4) The data on the sensitivity, specificity, predictive positive/negative values and diagnostic accuracy of these reagents in diagnostic assay systems are to be generated and provided.
5) The data on the utility/ usefulness in terms of sensitivity and specificity at "In House" & multi-centric settings, to be provided. The data on "independent validation" of diagnostic assays utilising these reagents also to be provided.
6) The data generated by testing against indigenously/ internationally available panel of sera/ clinical materials to be provided. The reason(s) for non-compliance in generating such data may be specified.
7) In case of infections widely spread (like HIV-I/ II), reagents utilised in the diagnostic assay should have accepted level of sensitivity of 99% and the specificity of 99%. In case, the diseases/ infections having high to moderate endemicity, acceptability level of a sensitivity of about 90% with a specificity of 95% must be achieved by these reagents. In case of very low endemicity of infections or prevalence of disease being low, the minimum acceptability of sensitivity/ specificity of asay utilising these reagents well be decided by the Advisory Committee.

D. CLINICAL TRIALS:

1. Human/ Clinical Pharmacology (Phase I):

Immunogenic Potency

The potential of the r-DNA vaccine to produce immunogenic response should be monitored by titre levels of circulatory antibodies, cytokine profile and T-cell responses. The temporal kinetics of antibodies should also be determined. Its autoimmune sequelae should be monitored by circulatory autoantibodies and immune complexes.

2. Exploratory Clinical Trials (Phase II):

Preventive/ Therapeutic Efficacy

The preventive/therapeutic potential of r-DNA vaccine should be evaluated in normal subjects (of endemic and non-endemic areas)/ patients. In addition to general clinical responses, haematology and clinical chemistry, circulatory antibody titres, cytokine profile and T-cell responses should be monitored periodically. The minimum preventive/ therapeutic dose of r-DNA vaccine vis-a-vis immune responses should be determined. The duration of the immunogenic response in normal subjects and patients should be delineated and booster strategy alienated.

3. **Confirmatory Trials (Phase III):**

Newer vaccines

The multicentric trial in large number of subjects/patients as per regulatory requirement should be undertaken. The preventive or therapeutic efficacy of the r-DNA vaccine based on the efficacy data of Phase II Clinical trial should be planned. The immunological parameters should be monitored in some cases at each centre to generate data on ethnic, socio-economic and cultural variations.

Established vaccines

These vaccines are already in use. A fast clearance procedure may be adopted. The data on abnormal/ side effects, analytical subacute toxicity and sero conversions should be considered including the outline of the method of manufacturing, construction of the cell lines, clones etc. The data on post market survey should be provided. For combination vaccines also, fast track clearance procedure may be followed.