DRAFT

GUIDELINES FOR PRECLINICAL EVALUATION OF SIMILAR BIOLOGICS IN INDIA

Department of Biotechnology
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Contents

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1. INTRODUCTION

In the past three decades, medicines produced using genetic engineering tools have revolutionized the treatment of many life threatening diseases. These products also referred to as biologic drugs or biologics or biopharmaceuticals or recombinant therapeutics refer broadly to substances produced by living cells used in the treatment, diagnosis or prevention of diseases**.** Whereas the market of biopharmaceuticals is continuously increasing, the patents of the first generation of these products have either expired or are likely to expire shortly. This has provided opportunities to different manufacturers to introduce follow on substitutes to original biologics, which are also known as biosimilars, similar biologics, follow on biologics, subsequent entry biologics etc. in different countries. These products promise potentially cheaper alternatives but at the same time, it needs to be ensured that the quality, safety and efficacy of such products are not compromised. Various agencies viz. World Health Organization (WHO), European Medicines Agency (EMA), Health Canada, Korean Food and Drug Administration (KFDA), Ministry of Health, Malaysia etc. have developed guidelines for dealing with these products. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has also provided useful guidance for biological therapeutics, though not specific to biosimilars. Other national regulatory bodies worldwide including US Food and Drug Administration (FDA) are in the process of developing separate set of regulations for such products.

In view of the above, the following “Guidelines for Preclinical Evaluation of Similar Biologics” have been developed to provide guidance to applicants for generating data for approval of similar biologics and regulations for evaluating the submissions.

2. RELEVANT REGULATORY FRAMEWORK IN INDIA

The Ministry of Environment & Forests (MoEF) has notified the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells, 1989 (Rules1989)" under the Environment (Protection) Act, 1986. These rules cover the area of research as well as large scale applications of GMOs and products thereof and accordingly the recombinant biological products are regulated under these rules from the research and product development stage up to its release into the environment. The relevant competent authorities involved in the approval process of recombinant pharmaceutical products include Institutional Biosafety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM) and Genetic Engineering Appraisal Committee (GEAC).

The Drugs and Cosmetics Act, 1940 and the Rules 1945 as amended from time to time also regulate the recombinant pharmaceutical products and the concerned authority is the Drug Controller General of India (DCGI).

The Department of Biotechnology (DBT) has issued a set of guidelines for preclinical and clinical evaluation of rDNA vaccines, diagnostics and other biologicals in 1999 to help in the production of relevant data for submission to RCGM/GEAC and DCGI. The DBT guidelines specifically focus on safety, purity, potency and effectiveness of the product.

To streamline the regulatory process in respect of the r-Pharma Sector under the Rules, 1989, the MoEF had constituted a Task Force on Recombinant Pharma Sector under the Chairmanship of Dr R A Mashelkar, Director General, CSIR in 2004. The report focused on bringing in more clarity with respect to the role of GEAC and DCGI in dealing with different scenarios of indigenous manufacture vs. import and also whether the end product is a living modified organism(s) (LMOs),

the Task Force recommended the rationalization of the regulatory procedures for five categories, which were adopted by Government of India in 2006:

a) Indigenous product development, manufacture and marketing of pharmaceutical products derived from LMOs but the end product is not a LMO.
b) Indigenous product development, manufacture and marketing of pharmaceutical products where the end product is a LMO.
c) Import and marketing of LMOs as Drugs/Pharmaceuticals in finished formulations where the end product is a LMO.
d) Import and marketing of LMOs as Drugs/Pharmaceuticals in bulk for making finished formulations where the end product is a LMO.
e) Import and marketing of products derived from LMOs as Drugs/Pharmaceuticals in bulk and/or finished formulations where the end product is not a LMO.

It may be noted that there is a provision in Drugs & Cosmetics rules that the submission of requirements relating to animal toxicology, reproduction studies, teratogenic studies, prenatal studies, mutagenicity and carcinogenicity may be modified or relaxed in case of new drugs approved and marketed for several years in other countries. The requirement of submitting the results of local clinical trials can be suitably relaxed as per Drugs & Cosmetics Rules (8^{th} Amendment) vide notification no. G.S.R.944(E).

In view of the above regulatory context and developments in the area of similar biologics, it is proposed to have a separate set of guidance outlining an abridged procedure for the approval of similar biologics. The “Guidelines for Preclinical Evaluation of Similar Biologics in India” are elaborated as under:

3. SCOPE

This guideline provides requirements for preclinical evaluation of those recombinant products that are claimed to be similar to already approved biopharmaceutical products, herein referred as similar biologics and therefore partly rely on the information/data from the already approved products for ensuring safety, purity, potency and effectiveness.

4. TERMINOLOGY

i. **Similar biologic:** A biological product/ drug produced by genetic engineering techniques and claimed to be "similar" in terms of quality, safety and efficacy to a reference innovator product, which has been granted a marketing authorization in India by a competent authority on the basis of a complete dossier, and with a history of safe use in India.

The products, where the reference innovator product is not authorized in India shall be considered on a case by case basis if such products have been granted marketing approval in countries with well established regulatory systems such as US FDA, EMA etc. and have been in wider use for a minimum of four years.

Such products are also referred as biosimilars, similar biotherapeutic products, subsequent entry biologics or follow on biologics in various countries.

ii. **Comparability Exercise:** Comparison of a similar biologic with a licensed Innovator product with the goal to establish similarity in quality, safety, and efficacy.

iii. **DBT:** Department of Biotechnology.

iv. **DCGI:** Drugs Controller General of India

v. **Drug:** Drug includes (as defined in Drugs and Cosmetics Act, 1940).

(i) all medicines for internal or external use of human beings or animals and all substances intended to be used for or in the diagnosis, treatment, mitigation or prevention of any disease or disorder in human beings or animals, including preparations applied on human body for the purpose of repelling insects like mosquitoes;

(ii) such substances (other than food) intended to affect the structure or any function of human body or intended to be used for the destruction of (vermin) or insects which cause disease in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette;

(iii) all substances intended for use as components of a drug including empty gelatin capsules; and

(iv) such devices intended for internal or external use in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette, after consultation with the Board.

vi. **Government / government agencies*:*** Government means Central or State Government and government agencies that are associated organizations/ bodies with Central or State Government.

vii. **GEAC:** Genetic Engineering Appraisal Committee.

viii. **Genetic engineering:** The technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material (*Rules, 1989*).

ix. **IBSC:** Institutional Biosafety Committee.

x. **Immunogenicity:** The ability of a substance to trigger an immune response or reaction (*e.g*. development of specific antibodies, T cell response, allergic or anaphylactic reaction) *in vivo*.

xi. **Impurity:** Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or excipient including buffer components. It may be either process, host cell, or product-related.

xii. **Reference innovator product:** A reference biopharmaceutical product is used as the comparator for head-to-head comparability studies with the similar biopharmaceutical product in order to show similarity in terms of quality, safety and efficacy. Only a product that was licensed by the innovators/developers on the basis of a full registration dossier can serve as reference innovator product.

xiii. **Manufacture:** "Manufacture" in relation to any drug includes any process or part of a process for producing, altering, ornamenting, finishing, packing, labelling, breaking up or otherwise treating or adopting any drug with a view to its sale or distribution but does not include the compounding or dispensing in the ordinary course of retail business; and "to manufacture" shall be construed accordingly;

xiv. **Pharmacovigilance:** The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug related problems.

xv. **RCGM:** Review Committee on Genetic Manipulation.

xvi. **Similarity**: Absence of a relevant difference in the parameter of interest.

5. KEY CONSIDERATIONS FOR APPROVAL OF SIMILAR BIOLOGICS

i. The basis of a product being treated as a similar biologic and applicability of the abridged procedure as elaborated in the present guidelines depends on its demonstrated similarity with the reference innovator product, which will provide a basis for reduction in the pre-clinical and clinical information required to support approval of such similar biologics.

ii. The development of a similar biologic involves a stepwise approach of optimizing the production process, comparability exercise for characterization of the product (physicochemical as well as biological) followed by pre-clinical and/or clinical studies. It may be noted that demonstration of similarity of a similar biologic and the consistency in production process to the reference innovator product is a pre-requisite for the reduction of requirements for pre-clinical and clinical studies required for final marketing approval.

iii. Identification of any significant differences in quality, safety and efficacy studies would mean the need for a more extensive pre-clinical and clinical evaluation and the product will not qualify as a similar biologic.

iv. In case the reference innovator product is used for more than one indication, the efficacy and safety of the similar biologic has to be justified or if necessary demonstrated separately for each of the claimed indications. Justification will depend on clinical experience, available literature data, whether or not the same mechanism of action is involved in specific indications.

6. SELECTION OF REFERENCE INNOVATOR PRODUCT

As indicated above, similar biologic needs to be studied in comparison with an approved recombinant product that is used as the comparator to establish similarity of similar biologics. This comparator is referred to as reference innovator product. The reference innovator product provides the basis for dose selection and route of administration and is utilized in the comparability studies required to

support the application for approval. As the comprehensive information on the reference innovator product is the basis for establishing the safety, quality and efficacy of a similar biologic and reduced requirement of pre-clinical and clinical data sets, the selection of reference innovator product is extremely critical. The following points should be kept in mind while selecting the reference innovator product.

- Reference innovator product must have same active substance as of the proposed similar biologic.
- The dosage, form and route of administration of the similar biologic should be same as that of reference innovator product.
- Reference innovator product should have been authorized for approval in India to confirm its quality, safety and efficacy. In cases where reference innovator product is not authorized in India, it should have been approved in countries with well established regulatory systems such as US FDA, EMA etc. and should have been in use for at least four years..
- Another similar biologic cannot be considered as reference innovator product, as the reference innovator product should be the one that has been licensed based on a full quality, safety and efficacy data.
- Same reference innovator product should be used throughout the development of a similar biologic i.e. manufacturing process, comparability exercise, pre-clinical and clinical evaluation.
- The acceptance of a reference innovator product for evaluation of a similar biologic does not imply approval for use of the reference innovator product in India.

7. DATA REQUIREMENTS FOR PRECLINICAL EVALUATION OF SIMILAR BIOLOGICS

The abridged pathway for preclinical and/or clinical testing of a similar biologic depends on establishing comparability with the reference innovator product as well

as establishing the consistency in its production and purification. Accordingly, the data requirements for approval of a similar biologic are as follows:

7.1 Manufacturing process considerations: One of the most significant challenges in developing a similar biologic is designing the manufacturing process to achieve comparability to the reference innovator product. Unlike small-molecule drugs that can typically be described by a single chemical formula and duplicated relatively easily, biopharmaceuticals are produced from living organisms and have significantly more complexity and heterogeneity. Ideally the similar biologic product should be expressed and produced in the same host cell type as the reference innovator product in order to minimize the potential for important changes to critical quality attributes of the proteins and to avoid introduction of certain types of process related impurities that could impact clinical outcomes and immunogenicity. A complete description should be provided for the manufacturing process from development and characterization of cell banks, stability of clone, cell culture/ fermentation, harvest, excipients, formulation, purification, primary packaging interactions etc and the consequences on product characteristics as indicated below:

i. **Molecular biology details**

The details regarding host cell cultures (including viral clearance), vectors, gene sequences, promoters etc. used in the production of similar biologics should be provided with appropriate drawings/figures. In case there are any amino acid substitutions made, they should be explicitly brought out in the document with protocols. The details of post-translational modifications, if any (glycosylation, oxidation, deamidation, phosphorylation etc.) should be explained in sufficient details.

ii. Fermentation process

- At least three batches of reproducible fermentation data at pilot scale (batch size adequate to give enough purified product to generate preclinical data) with detailed fermentation kinetics of single batch. Fermentation process should be carried out in controlled and monitored environment.
- Details of fermentation kinetics data from a representative batch indicating cell growth, product formation, pH, temperature, dissolved oxygen, major nutrient consumption pattern, RPM for agitation
- Concentration to be defined in terms of product/litre, yield and volumetric productivity.
- Data to validate that the specific protein yield (amount of protein per unit cell mass) remains more or less constant for all fermentation batches.
- Demonstrate that the overall productivity is reproducible and scalable.

iii. Details of downstream process for purification of the product would include:

- Steps involved in purification of protein.
- Batch size for protein purification.
- Description of each unit operation step during purification and recovery of protein along with quantitative recovery of product at each stage.
- Describe the recovery efficiency and quality of the refolded protein, if the starting material is aggregated or from inclusion bodies.
- Overall recovery of the product in each batch operation
- Consistency of recovery in 3 consecutive batches of purification from 3 independent batches of fermentation

If the protein is refolded from inclusion bodies, the applicant needs to show the details of the refolding process, specific activity at different doses, dose response curve, stability data (to confirm that the refolded protein is stable), and confirm that it is soluble and does not show aggregating behavior.

7.2 Product Characterization: The main aim of the characterization is to establish that the proposed similar biologic is similar in composition, size, structure and bioactivity etc. Characterization of active substance and formulation of proposed similar biologic should be compared with reference innovator product through physicochemical assays and biological assays.

Extensive state of the art analytical methods should be applied to detect even "slight differences" in all relevant quality attributes. Methods used should be appropriately qualified and validated with relevant information like use of standards and reference materials. If the protein is glycoprotein, the applicant needs to provide complete glycan profile, specific carbohydrate position, if any, and bioactivity at different doses.

The experiments for characterization should include samples of the applicant recombinant product, innovator recombinant product as control, known positive standard and negative control, wherever relevant. The test samples should represent randomized overall sample of produce and should be adequate to complete preclinical studies. The innovator recombinant product is used as the control to prove the similarity of applicant recombinant product with the originally approved and introduced product into the market. Known positive and negative controls are encouraged to be included for monitoring of consistency and accuracy of the methods and measurements.

To ensure the statistical analysis, each quantitative experiment should be done at least 3 times and data should be represented in terms of mean and standard deviation to know inter sample and method variability. Appropriate

statistical significance should be represented throughout the characterization data. Product specific physicochemical and biological characterization methods viz. recombinant proteins, therapeutic enzyme, monoclonal antibodies etc are given in **Annexure I-IV.** It may be noted that these annexures are suggestive but not limited to the specified method and the requirements may vary on case by case.

One of the major concerns in case of similar biologic is characterization of the contaminants, which should be undertaken as follows:

- Nucleic acid content in case of proteins/ peptides/ antibodies as products
- Protein content in case of nucleic acids as product
- Host cell proteins
- Endotoxins
- Viral validation

7.3 Pharmacological characteristics: The data about the following pharmacologic characteristics needs to be provided :

- Route of administration
- Absorption rate
- Elimination rate
- Bioequivalence range(PK/PD Parameters)
- Therapeutic Index
- Dose-response curves
- Tissue-specific localization
- Details of the formulation

7.4 Stability studies: Regarding stability data, it is necessary to test the stability of the product and formulation as most of the proteins are frequently sensitive to changes, such as those made to buffer composition, processing and holding conditions, and the use of organic solvents. Real time stability test

should be conducted to set a shelf life and storage conditions of the test product. Accelerated and stress stability studies are useful tools to establish degradation profiles, which may further contribute to a direct comparison of similar biologic and the reference innovator product.

7.5 Preclinical evaluation: Further to a thorough quality characterization, the establishment of safety of a similar biologic requires the generation of preclinical data. The preclinical studies are designed for comparative evaluation of the similar biologic with reference innovator product and aim to detect differences, if any. The requirements of preclinical studies may vary depending upon the clinical parameters such as therapeutic index, the type and number of indications applied etc. The approach to be adopted should be fully justified in the pre clinical overview, wherein each applicant must submit the following clinical information:

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- Known / proposed clinical use of the product in India, any specific indications proposed to be targeted and consideration of age, sex, pregnancy, lactating, children etc.
- Dosage schedule including quantity of each dose, number of doses/day, frequency and intervals and total duration of treatment
- Mode of administration e.g. pre filled syringe
- Details of final formulation including adjuvants, additives etc. -
- Information about diluents, if any.
- Available toxicity data in human / animals on innovator recombinant product and toxicology data of adjuvants and additives as applicable.

The applicant is also required to furnish information about the proposed test site and personnel to be involved in conducting these studies at the test site e.g. study director, principal investigator, pathologist, other investigators and quality assurance officer. The statutory approvals from Institutional Biosafety Committee (IBSC) and Institutional Animal Ethics Committee (IAEC) must be

submitted, as the case may be. The studies should ideally be conducted as per good laboratory practices(GLP) and the applicant should inform about the status of accreditation of test site ,if any.

Preclinical studies should be conducted with the final formulation of the similar biologic intended for clinical use, unless otherwise justified. In general, the dosage form, strength and route of administration of the similar biologic should be same as that of reference innovator product and in case of any differences in these parameters, justification for the differences should be provided. Further, since these studies constitute a part of overall comparability exercise, reference innovator product should be used throughout in all testing protocols to detect any significant differences between similar biologic and the reference product. The following studies are required for preclinical evaluation:

(i) ***In vitro*** **studies:** Assays like receptor binding studies or cell based assays (e.g. cell proliferation assays) should be conducted, when appropriate to establish comparability of biological activity of the similar biologic and reference innovator product. Such data are usually available from biological assays described in the quality component of the dossier and reference to these studies can also be made while submitting preclinical data in the dossier

(ii) ***In vivo*** **studies:** Regarding animal studies, at least one repeat dose toxicity is required to be conducted in a relevant species. Comparative repeat dose toxicity study is considered to provide reassurance that no 'unexpected' toxicity will occur during clinical use of the similar biologic. The use of final formulation intended for clinical use, while performing the repeat dose toxicity study also in principle helps in evaluation of any potential toxicity associated with both the active substance and product and process related impurities. The duration of the study would be

generally not less than 28 days with 14 days recovery period. However, the duration may vary depending on the dosage schedule and other parameters on a case by case basis.

Depending on the route of administration, local tolerance should be evaluated. If feasible, this evaluation may be performed as part of the above mentioned repeat dose toxicity study.

Other toxicology studies, including safety pharmacology, reproductive toxicology, mutagenicity and carcinogenicity studies are not generally required for evaluation of a similar biologic unless warranted by the results from the repeat dose toxicological studies.

Some of the key considerations while conducting repeat dose toxicity study are as follows:

(a) Choice of Animal Model: Regarding the choice of animal models, routes and doses for testing, the applicant should provide the scientific justification for the choice of animal model(s) based on the data related to scientific literature The models should be selected in such a manner where the efficacy is demonstrable.. If no relevant species is available where the efficacy has been demonstrated, the applicant may use the appropriate transgenic animal model(s). The animals should be chosen from both sexes and of appropriate ages as per product requirements. However if the relevant animal species is not available due to some justified and unavoidable circumstances, the toxicity studies need to be undertaken in two species i.e one rodent and other non rodent species, as per the requirements of Schedule Y with due permission from the regulatory agency.

(b) Route of administration: In cases when the relevant animal model is used, the route of administration would include only the intended route, whereas in other cases two routes i.e. one intended and another alternate route should be used.

(c) Dose selection and schedule of administration: The dose should be calculated based on therapeutic dose of the reference innovator product. If required, a pilot dose response study should be conducted prior to initiating the toxicity studies.Generally there would be tthree levels of doses viz low, medium and high used in the animal toxicology studies corresponding to 1X, 2X and 5X or higher test dose. Regarding the schedule of administration, the therapeutic schedules may be used as the basis.

Accordingly the study groups of animals in repeat dose toxicity testing will consist of:

i. Historical Control (Optional)
ii. Vehicle Control
iii. Vehicle Control for 5X or higher dose recovery group
iv. Formulation without protein (for vaccines), if multiple adjuvants - each to be checked independently
v. 1X Test Drug for study Duration (Lowest dose)
vi. 1X Reference Innovator product product for study Duration
vii. Medium dose Test Drug
viii. High dose Test Drug
ix. High dose Test Drug with a recovery group going beyond the end of study period for 7 to 14 days

(d) Steps to be reported: The protocols and the study reports should provide complete details of various steps in the toxicity testing as indicated below:

- Procedures prior to euthanasia eg. Blood drawing, body weight, etc.
- Events immediately after euthanasia, blood drawing, necropsy, gross – mention all organs to be weighed.
- Biochemical Parameters – Equipment and Methods used - Units of measurement and expression
- Hematology procedures and parameters – method to be used i.e. automated or manual.
- Bone marrow either examined as an aspirate /smear or on histopathology section.

(e) Histopathology observations: In case of histopathological observations, the applicants should keep in mind the following points:

- Every observation considered as deviation from described normal histology needs to be documented and the incidence of each of these in the different groups should be denoted
- Whether such a feature is significant or not can be decided on review of statistical significance or dose response or if it is within or outside the normal range of values in case of biochemical and hematological observations.
- If all organs from all animals were not examined e.g. in 5 animals only 4 livers were examined, the reason for the 1 liver not being examined should be documented.
- In case of premature death or morbidity the proposed course of action is to be included in the protocol.

7.6 Immune responses in animals: Antibody response to the product should be compared to that generated by the innovator reference product in mice. The test serum samples should be tested for reaction to host cell proteins.

For evaluating immune toxicity of the product under study, the results of local tolerance (Part of repeat dose or Stand alone test) should be analyzed with the observations regarding immunogenicity in sub-chronic study. Therefore, the immunogenicity testing should be included as part of the sub-chronic repeat dose study while developing the protocols.

The other parameters for evaluating immune toxicity include immune complexes in targeted tissues during sub-chronic repeat dose study may be kept in mind while evaluating histopathology observations, human lymphocyte proliferation assays etc.

7.7 Archiving of data

The applicant should archive all the data upto preclinical evaluation for a period of at least five years after marketing approval by competent authority in India or as per OECD, USFDA, ICH, EMA requirements. The site of archiving should be indicated in the study protocols and reports. The material that needs to be archived should also be mentioned. These may include test substance, vehicle, plasma / serum, tissues, paraffin blocks, microscope slides, documents, electronic material etc and the individual durations (e.g. test material until date of expiry). The designated authority that will be responsible for archiving and can be approached for inspection or retrieval, if required should be indicated in the study report by the applicant.

The final report of the study should reflect all the issues approved in the protocol and the following additional sections/documents:

- RCGM approval of protocol and test center
- IBSC approval of report
- IAEC approval for animal use and for the procedures
- QA statement

- Signatures of study director and all investigators who were involved in the study
- All quality analytical reports on the test material and vehicle
- Animal feed and animal health certifications
- Protocol deviations, if any
- Discussion on the results
- Individual animal data, summary data and any other data like computer analysis outputs etc
- Conclusion

8. REFERENCES:

1) Drugs & Cosmetics Rules, 1988 (8^{th} Amendment)
2) EMEA guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins London, 2007 (CHMP/BMWP/14327)
3) EMEA guideline on similar biological medicinal products containing biotechnology derived proteins as active substance: non-clinical and clinical issues. London, 2006 (CHMP/BMWP/42832)
4) DBT Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology. In: WHO Expert Committee on Biological Standardization. Forty-first report , Geneva, World Health Organization, 1991, Annex 3 (WHO Technical Report Series No. 814)
5) Guidelines for generating pre-clinical and clinical data for rDNA vaccines, diagnostics and other biologicals, 1999
6) ICH guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals (S6, 1997)
7) Rules for the manufacture, use, import, export & storage of hazardous micro organisms, genetically engineered organisms or cells, 1989

Annexure I

Physicochemical and biological characterization of nucleic acid based recombinant products

Nucleic acid - Physicochemical	Nucleic acid – biological
Sequence *(To prove if the sequence same as inventor).*	**Vector for expression of recombinant protein**
Restriction map for >1000 bp *(To check if secondary structure is same as inventor).*	• Expression pattern in actual target host cell *(To compare efficiency of expression of similar biologic with inventor in the target cell)*
Purity on HPLC *(To check if any impurities are there).*	• Expression pattern in closest animal species upon administration (along with vehicle as negative control) *(To compare efficiency of expression of similar biologic with inventor in the target cell when administered in whole animal, this will evaluate the efficiency of vector location and promoter activity in target cell).*
Gel electrophoresis (agarose/ acylamide/ urea page) *(To check quality of sample).*	• Kinetics of expression during the proposed therapeutic period of protection *(To compare half life of the similar biologic with inventor).*
Southern/ Northern blot *(Confirmation with inventor).*	• Efficacy in appropriate disease/ infection model *in vitro* and/or *in vivo (To compare therapeutic activity of the similar biologic with inventor).*
Absorption spectrum from 190 to 800 nm *(To check similarity to inventor).*	• Absence of interference of marker enzyme/antibiotic, if any *(To compare therapeutic interference and toxicity due to a marker in the similar biologic with that of inventor).*

CD spectrum from 190 to 800 nm *(To check secondary structural changes if any due to binding of impurities).*	**Vector for expression of siRNA/ snRNA etc.**
Hybridization to the target sequence. *(To confirm with original).*	• Expression pattern in actual target host cell *(To compare efficiency of expression of similar biologic with inventor in the target cell)*
Tm profile *(To check if any impurities are present).*	• Expression pattern in closest animal species upon administration (along with vehicle as negative control) *(To compare efficiency of expression of similar biologic with inventor in the target cell when administered in whole animal, this will evaluate the efficiency of vector location and promoter activity in target cell).*
Estimation of RNA and DNA using nanodrop or reagent. *(To check concentration and impurity, if any)*	• Kinetics of expression during the proposed therapeutic period of protection *(To compare half life of the similar biologic with inventor)*
	• Efficacy in appropriate disease/ infection model *in vitro* and/or *in vivo (To compare therapeutic activity of the similar biologic with inventor).*
	• Absence of interference of marker enzyme/antibiotic if any *(To compare therapeutic interference and toxicity due to a marker in the similar biologic with that of inventor).*

Annexure II

Physicochemical and biological characterization of therapeutic proteins

Therapeutic Proteins – Physicochemical	Therapeutic Protein – Biological
• Appearance, particulates, pH, osmolality, particle size (if applicable) *(To check homogeneity).*	• Biological activity in actual target host cell *(To compare activity of protein in similar biologic with inventor in the target cell).*
• MW, Sequence and amino acid composition (To check purity). •	• Biological activity in closest animal species (if available) upon administration (along with vehicle as negative control) *(To compare activity of similar biologic with inventor in the target cell when administered in whole animal, this will evaluate the efficiency of vector location and promoter activity in target cell).*
• N terminal sequence (atleast 20 amino acid) *(To check amino acid sequence and structure).*	
• Glycosylation, Phosphorylation, Acetylation, and Myristoylation, if any *(To check if active/ inactive form).*	• Kinetics of biological activity during the proposed therapeutic period of protection *(To compare half life of the similar biologic with inventor).*
• PEGylation, esterification, if applicable *(To check if modification is appropirate).* • Tryptic map (1D and 2D) *(To check if secondary structure is conserved).* • Sulfhydryl groups(s) and disulphide bridges *(To check if secondary structure is conserved).* • Size and Purity on HPLC (RP, SEC, IEX)/ MALDI *(To check if it is homogeneous and no impurities are present).*	• Efficacy in appropriate disease/ infection model *in vitro* and/or *in vivo (If available) (To compare therapeutic interference and toxicity due to a marker in the similar biologic with that of inventor)* •

• Isoform pattern, if any *(To check if secondary structure is conserved).* • Gel electrophoresis (IEF, SDS PAGE and Native PAGE, Western) *(To qualitiative check purity/ nativity).* • Absorption spectrum from 190 to 800 nm (molar absoptivity) *(To check purity).* • CD spectrum from 190 to 800 nm *(To check if secondary structure is conserved)* • Fluorescence spectrum *(To check if any impurities such as quenchers are present).* • Fourier transform IR, if applicable *(To check if any prosthetic group is present).* • NMR spectrum, if applicable *(To check if any prosthetic group is present).* • Affinity to the target receptor *(To check if required affinity to receptor is conserved).* • Helix to Coil Transition profile (To verify if the preparation is stable and impurities or isofoms are affecting the stability).	

Annexure III

Physicochemical and biological characterization of therapeutic enzymes

Therapeutic Enzyme – Physicochemical	Therapeutic Enzymes – Biological
• Appearance, particulates, pH, osmolality, particle size (if applicable) *(To check homogeneity).*	• Biological activity in actual target host cell *(To compare activity of enzyme in similar biologic with inventor in the target cell).*
• Sequence and amino acid composition *(To check purity).*	• Biological activity in closest animal species upon administration (along with vehicle as negative control) *(To compare activity of similar biologic with inventor in the target cell when administered in whole animal, this will evaluate the efficiency of vector location and promoter activity in target cell).*
• Glycosylation, phosphorylation, acetylation and myristoylation, if any *(To check if active/ inactive form).*	• Kinetics of biological activity during the proposed therapeutic period of protection *(To compare half life of the similar biologic with inventor).*
• Pegylation, estrification, if applicable *(To check if modification is appropriate).* • Tryptic peptide map (1D and 2D) *(To check if secondary structure is conserved).* • Size and purity on HPLC (RP, SEC, IEX)/ MALDI *(To check if secondary structure is conserved).* • Gel electrophoresis (IEF, SDS PAGE and Native PAGE, Western) *(To qualitatively check purity/ nativity).*	• Efficacy in appropriate disease/ infection model *in vitro* and/or *in vivo (To compare therapeutic interference and toxicity due to a marker in the similar biologic with that of inventor).*

- Enzyme activity in gel assay in the presence of chromogenic substrate *(To check activity).*
- Absorption spectrum from 190 to 800 nm *(To check purity)*
- CD spectrum from 190 to 800 nm *(To check if secondary structure is conserved).*
- Helix to Coil Transition profile (To verify if the preparation is stable and impurities or isofoms are affecting the stability).
- Fluorescence spectrum *(To check if any impurities such as quenchers are present).*

- Km with natural substrate *(To check homogeneity of biosim interaction with active site same as inventor with reference to known substrates).*
- Ki with known inhibitors (1/2) *(To check comparability of compititive biosim interaction with active site same as inventor with reference to known inhibitors)*.

Annexure IV

Physicochemical and biological characterization of antibodies

Antibodies – Physicochemical	Antibodies – Biological
• Sequence and amino acid composition *(To check purity).*	• Neutralizing activity in actual target host cell *(at least one highly prevalent Indian varient/isolate should be used) (To compare activity of similar biologic with inventor in the target cell)*
• Tryptic map (1D and 2D) *(To check if secondary structure is conserved).*	• Neutralizing activity in closest animal species (if feasible) upon administration (along with vehicle as negative control) *(at least one highly prevalent Indian variant/isolate should be used) (To compare activity of similar biologic with inventor in the target cell when administered in whole animal, this will evaluate the efficiency of vector/ antibody location and promoter activity in target cell).*
• Light and heavy chain separation *(To check antigenic recognition motif).*	• Kinetics of Neutralizing activity during the proposed therapeutic period of protection *(at least one highly prevalent Indian variant/ isolate should be used) (To compare half life of the similar biologic with inventor)*
• IgG type *(To check specificity of IgG in localization of specific tissues/ plasma).*	• Efficacy in appropriate disease/ infection model *in vitro* and/or *in vivo (If available) (To compare therapeutic interference and toxicity due to a marker in the similar biologic with that of inventor)*
• Purity on HPLC (RP, SEC, IEX)/ MALDI *(To check if preparation is free of any impurities).*	

- Gel electrophoresis (IEF, SDS PAGE and Native PAGE, Western) *(To check qualitative purity difference).*
- Absorption spectrum from 190 to 800 nm *(To check purity).*
- CD spectrum from 190 to 800 nm *(To check if secondary structure is conserved).*
- Helix to Coil Transition profiles (To verify if the preparation is stable and impurities or isofoms are affecting the stability).

- Epitopic mapping of the antibody binding to specific and non-specific epitopes with antigenic variant isolated from an Indian isolates *(To check specificity profile of similar biologic with inventor in epitope recognition, particularly in recognition of Indian variant of a host cell protein or infectious agent coded protein).*
- Anti-body dilution factors in neutralization *(To check symbiol with inventor in the neutralization strength of the antibody preparation)*

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