

B & B

Proteins and Polysaccharides Advisory Panel Meeting

Tuesday February 05, 2008

Hyderabad International Convention Centre
Hyderabad, india

IPC-USP Biologics & Biotechnological Joint Working Group

IPC-USP Joint Meeting, Kasauli. Aug 8 & 9, 2007

Recombinant Protein Therapeutics & Vaccines

Topics Discussed :Biosimilars/BioGenerics/

Follow-on Biologics-Bioequivalence and QA issues

In attendance: Tina Morris (USP)-Chair, S.Anand Kumar (ABLE), M.K.Sahib (Wockhardt), Sariram Akundi (Biocon), M.Naseerulla (Shantha), Ravi Sirdeshmukh (CCMB)

USP-IPC Joint Working Group Meeting, CCMB, Hyderabad

Protein Therapeutics December 19, 2007

Topics Discussed: Protein Characterization (Ravi Sirdeshmukh)

Cell-based Bioassays for potency determination (M.K.Sahib & S.Akundi)

In attendance: S.Anandkumar (ABLE), Kumud Sampath (USPI),Murali Apparaju (USPI),Ravi Sirdeshmukh (CCMB), M.K.Sahib (Wockhardt), Anandkumar (Wockhardt), Sriram Akundi (Biocon),Rustom Mody (Intas),S.Das(Intas), B.Stephen (Reliance), S.Gariola (Serum Inst), M.Naseerulla (Shantha), K.Nagaiah (Gland Pharma), Jaspreet Singh (Biological E)

IPC-USP BIOLOGICS & BIOTECHNOLOGICAL JOINT WORKING GROUP MEETING

Thursday 08 August 2007 at Kasauli

PROGRAMME

- 0930-0940 Welcome Address: K.R.Mani, Director Central Research Institute
0940-1015 Order of Business: Kumud Sampath, Tina Morris & S.Anand Kumar
1015-1100 **Recent Developments in USP and Feed back on General Chapters
developed by IPC. Tina Morris**
1100-1130 Break
1130-1200 **Cell-based bioassays for protein therapeutics andf multi-centric validation of
assays Sriram Akundi, Biocon**
1200-1300 Discussions
1300-1400 Lunch Break
1400-1430 **Perspectives on interchangeability of biopharmaceuticals in practice- M.K.Sahib
(Wockhardt)**
1430-1500 Discussions

Biotech Drugs: The Era of Novel Therapeutic Agents

- Recombinant DNA technology : Introduction of genes from human DNA into microorganisms (Bacteria, Yeasts and animal or human cells in culture) resulting in the production of large quantities of corresponding gene products (Proteins/Peptides) which are used in the treatment of human diseases.
- Hybridoma Technology which facilitates the large-scale production of monoepitope specific antibody raised against an antigen (monoclonal antibody, mAb)
- More than 150 biotech drugs (hInsulin, hGrowth hormones, mAb s) are currently marketed with annual revenues exceeding US\$ 30 billion.
- More than 300 biotech drug products and vaccines are in clinical trials for 200+ diseases such as Cancer, Alzheimer's disease, cardiovascular disease, multiple sclerosis, AIDs, Arthritis and others
- Eleven protein therapeutics, with combined sales of \$15 billion, will have lost patent protection by the end of 2006, followed by another ten or so patent expirations by 2015

Commercially viable Gene Expression systems

- **Bacterial system: *Escherichia coli***
The earliest expression system used commercially for the production of human Insulin. Extensive knowledge on the genetics of *E. coli* available.
(minimal post-translational modifications)
- **Yeasts : *Sacchromyces cerevisiae**, *Pichia pastoris*, *Hansenula polymorpha***
Most prevalent expression hosts in Pharmaceutical production process
* Well advanced genetic knowledge available
(extensive post-translational modifications)
- **Mammalian Cells: CHO (Chinese Hamster Ovary);**
BHK (Baby Hamster Kidney);
HEK (Human Embryo Kidney);
Human Retinal Cell Line;
NSO (Mouse myeloma cell line)
Where mammalian expression is the only choice, these cell lines are acceptable to regulatory bodies (e.g., FDA-USA)
(extensive post-translational modifications)

Some Commercialized Recombinant Biologicals and their Expression systems

Insulin	E.coli	Blood coagulation Factors VII, VIII,IX	BHK cells
Interferon alpha, beta, gamma	E.coli		CHO cells
Interleukin 2	E.coli	Erythropoetin	CHO cells
Plasminogen activator	E.coli	FSH	CHO cells
Tumor Necrosis factor	E.coli	LH	CHO cells
Growth Hormone	E.coli	Gonadotropin	CHO cells
Calcitonin	E.coli	Interferon beta	CHO cells
		Tissue Plasminogen activator	CHO cells
Glucagon	S.cerevisiae	Glucocerebrosidase	CHO cells
Eutropin (hGH deriv)	S.cerevisiae	Interleukin 11-agonist	ROMI 8866
Platelet Derived Growth Factor	S.cerevisiae		(human cell line)
Hepatitis B Vaccine	S.cerevisiae		
Hepatitis B Vaccine	P.pastoris		
Insulin	P.pastoris		
Streptokinase	P.pastoris		

Companies: Novo-Nordisk; Roche, Janssen, amgen, Boeringer, Serono, Genzyme, Lilly, Novartis, Aventis, Chiron, Genentech, Wyeth, GSK

Indian Companies: Shantha, Bharat, Biocon, Wockhardt, Reddy's

Choice of the protein expression system is dictated by a combination of economics of production and the ultimate use of the product.

Challenges faced by Pharma and Regulatory Scientists

- Molecular biologists are constantly challenged by the need to improve and optimise the existing or developing new expression systems, and also develop novel approaches to face the demands of producing the complex proteins of tomorrow.
- This continuous evolution is paralleled by growing concerns about the safety of these novel pharmaceuticals, with health authorities grappling with setting appropriate standards for certification.
- Major Pharmacoeptial institutions are taking initiatives to develop evidence based guidelines derived from advances in biological and health sciences.

Parameters impacting on the potency, stability & immunogenicity of protein therapeutics

- **Post-translational & chemical modifications**
 - glycosylation, γ -carboxylation, β -hydroxyaspartic acid, acetylation, N-terminal pyroglutamate, N-terminal Met, C-terminal lysine, oxidation, S-S cleavage, deamidation, deimination,, isoaspartyl residues, glycation
- **Physical characteristic**
 - atypical conformation, aggregates, fragments
- **Adjuvant effect of process impurities/host protein**
- **Formulation (Shelf-life)**
 - liquid, freeze-dried, excipients
- **Genetics of the recipient**
 - MHC haplotype, protein polymorphisms

Pharmaceutical improvement modifications of recombinant proteins

Polyethylene glycol modifications (PEGylation)

Provide specific advantages in

- Protein Stabilization
- Protection from proteolysis
- Increased half-life in biological application
- Attenuate immune response
- Solubility enhancement
- lowered aggregation

Adds another dimension to Protein heterogeneity

Sources of Protein Heterogeneity

Product Linked:

Gene sequence related

Gene Expression system related

Factors contributing to structural heterogeneity: PTMs result in protein heterogeneity. The protein exists in multiple isoforms.

Process Linked:

Cell Culture Process Variability

Manufacture and/or storage of the drug substance and drug product

Assessment of Bioequivalence of Biosimilars

- *There are inherent variations in the protein product even if the same gene is expressed in the same host cells using similar down stream processing protocols*
- *Proteins are complex molecules. Microheterogeneity is observed even in proteins produced in the human body.*

Questions for Pharmacopeias:

- *How similar do “Biosimilars” need to be ?- Huub Schellekens Nature Biotechnology '04*
- *What Physicochemical characteristics and in vitro potency tests are acceptable for assessing comparability with innovator product*
- *What “non-clinical” safety data are essential for quality assessment ?*
Non-Clinical Safety Studies on Biosimilar Recombinant human Erythropoietin by Parnham, Schindler-Horvat & Kozlovic in Basic & clinical Pharmacology & Toxicology (2007) 100, 73-83 (PLIVA Res. Inst. Zagreb, Croatia)
- *What are the clinical trial data which establish “bioequivalence”?*
How many or how few trials are needed ? –C.Sheridan, Nature Reviews (2006) 5,445
- *What should be included in the monograph of a “Biosimilar” for debate and subsequent inclusion in the Pharmacopeias ?*
- *What should be a reference standard ?*
- *What are acceptable comparability limits ?*

Bioassays: Challenges and Opportunities

Most therapeutic proteins are pleotropic in their mode of Action

The major Challenges include:

- *Determination of Bioequivalence / Biocomparability by designing Bioassays which reflect the major physiological activities of the therapeutic protein*
- *Determination of Potency. Cell based assays to determine the ability of the therapeutic protein to exert its intended activity.*

Cell Based Bioassays and their Multicentric Validation

Sriram Akundi

Biocon Ltd

Bangalore

August 08, 2007

Perspectives on Interchangeability of Biopharmaceuticals in Practice

M.K. Sahib, PhD
Wockhardt Research Centre,
Aurangabad

Opportunities for interaction between USP and IPC

Indian Pharmacopoeial Council is preparing General Chapter/s on Biotechnology derived Pharmaceutical Products.

Objective: To derive a consensus opinion through discussions with Scientists from academia and Industry for incorporating in the Indian Pharmacopoeia general chapter on Specifications for Bioassays, cell lines , reagents, Instrumentation, assay protocols, assay validation processes and other compendial information which will be periodically updated.

Scientific Debate on the Assessment of Bioassays for Validation is key to international harmonization

USP-IPC JOINT WORKING GROUP ON PROTEIN THERAPEUTICS

Venue: CCMB-Meeting Room : Director's Meeting Room

Date: December 19, 2007

- 10:00 – 10:10 Welcome –Ms. Kumud Sampath USP
10:10 – 10:20 Address by Director of CCMB
10:20 – 10:30 Opening Remarks: Scope of Joint Working Group “The Remit of USP-IPC JWG” (S.Anand Kumar)
10:30 – 11:15 Points to Consider for setting Pharmacopeial Specifications and Standards for comparison of Biosimilars. Validation of Analytical Methods: Opening Remarks (Ravi Sirdeshmukh) Interactive session
Moderator: Dr. Ravi Sirdeshmukh
11:15 – 11:30 Break
11:30 – 12:15 Points to Consider for determining comparative efficacy of Biosimilars (Opening Remarks: S.Anand Kumar) Interactive session
Moderator: S.Anand Kumar
12:15 – 13:00 recombinant human Erythropoietin : Specifications and Efficacy (Cell-based Bioassays) Considerations

(Opening Remarks: Dr. M.K.Sahib, Wockhardt) Interactive session
Moderator: Dr. M.K.Sahib
13:00 – 14:00 Lunch Break
14:00 – 14:45 recombinant human Insulin: Specifications and Efficacy (Cell based Bioassays) Considerations
(Opening Remarks: Dr Sriram Akundi, Biocon) Interactive session
Moderator: Dr. Sriram Akundi
14:45 – 15:00 Low m.w. heparins. Specifications and assays Discussions led by Gland Pharma.
15:00 – 15:15 Break
15:15 - 15:30 Summary and Action Points (wrap-up)

Analytical Methods available for Protein Structure Determination

- Chemical methods: SDS PAGE, N-terminal amino acid analysis, Peptide mapping, amino acid sequencing (Edman degradation),
- HPSEC: High Performance Size Exclusion Chromatography for molecular weight determinations
- MS: Mass spectrometry-Maldi TOF: for Protein Chemical Structure determination, Glycosyl substituents of proteins
- X-ray Diffraction/NMR for Protein Structure determination (3D) and structures of glycosyl substituents; Low Angle Light Scattering (LALS), Raman Spectroscopy
- Circular Dichroism: Determination of degree of denaturation

Proteins are complex structures. Comparisons with Standards can be made by generating patterns and using tools of pattern recognition (e.g., Heat Maps).

Bioassays in General Chapters and Product Monographs

- Scientific basis of the assay, its relation to the therapeutic application of the pharmaceutical product
- Assay details including validation of the assay
- Complete information on the cell line, criteria for determination of purity, stability and responsiveness
- Genetically manipulated cell lines: origin of genes and mode of integration, reporter genes
- If cells are infected with a virus for cytopathic effect reduction, information on the virus

Points to Consider for Validation of Cell based Bioassays

- Good correlation between *in vivo* & *in vitro* Assays
- Robustness
- Convenient assay read-out
- Linearity
- Accuracy (Sensitivity) /Precision (Repeatability)
- Specificity
- Limit of Quantitation (LOQ)

Compatible with the guidelines of International
Conference of Harmonization

Quantitative Uses of *in vitro* Cell Culture Bioassays

- Potency determination of the manufactured drug lots
 - Bioassays are conducted in cell culture medium
- Measurement of active drug levels for Pharmacokinetic or toxicokinetic studies
 - Bioassays must function in test animal serum or plasma

QA Issues for Cell based Bioassays

- Testing of Cell Lines used in Bioassays
 - Characteristics of cell line (origin, genetics, karyotype, markers etc)
 - Purity and maintenance of cell lines
- Definition of cell response
 - methodology (reporter gene assay)
 - analytical procedures
 - Limit of Quantitation
- Choice of cell response
 - Proliferation
 - Cell death
 - Antiviral (cytopathic effect reduction)
- Reference Standard

Multi center Validation

The cell based assays require multi centre and Statistical validation for reproducibility and accuracy

USP and IPC could jointly participate in validating New bioassays for inclusion in their Pharmacopeia.



Validation of Analytical Scheme



- **Weighing Sample**
- **Solution preparation**
- **Filtration**
- **Transfer to column**
(e.g. P-10 for desalting)
- **Losses**

- **Digestion efficiency**
(Reference Standard)

**Ion exchange
Chromatography**

HPLC

- **Losses at sample transfer**
- **Dilution errors**
- **System precision**

Recovery

Repeatability
(statistical validation)