Assessing Similarity of Biosimilars to Innovator Biopharmaceuticals:
A Reviewer's Perspective

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Introduction

- In South Africa potential manufacturers of biosimilars lack a roadmap for comparing their products to the originals.

- How can the bio/generics industry demonstrate pharmaceutical and therapeutic equivalence of biosimilars?

LMW drugs *vs* Biopharmaceutical

- LMW drugs can be reversed engineered and a synthesis devised within days.
- Protein drugs are complex and created by living systems. Even slight differences in manufacturing processes can result in a product that behaves very differently from the originator.
- Since innovators are not obliged to disclose their manufacturing processes, potential developers of biosimilars must invent their own methods and hope the end result is a protein product that behaves similarly to the original biopharmaceutical.

Objectives of Biosimilar Manufacturers

- Prove that their products possess sufficient structure and function similarity to the original protein.
- Convince regulators that extensive pre-clinical and clinical trials are not necessary to prove safety and efficacy.

Question

- *Is the available technology sufficiently advanced to satisfy these objectives?*

Terminology

- *Biosimilar:* A recombinant protein product which is intended to be a similar version or a duplicate of an already approved or licensed recombinant protein product (K. Webber, 2005).

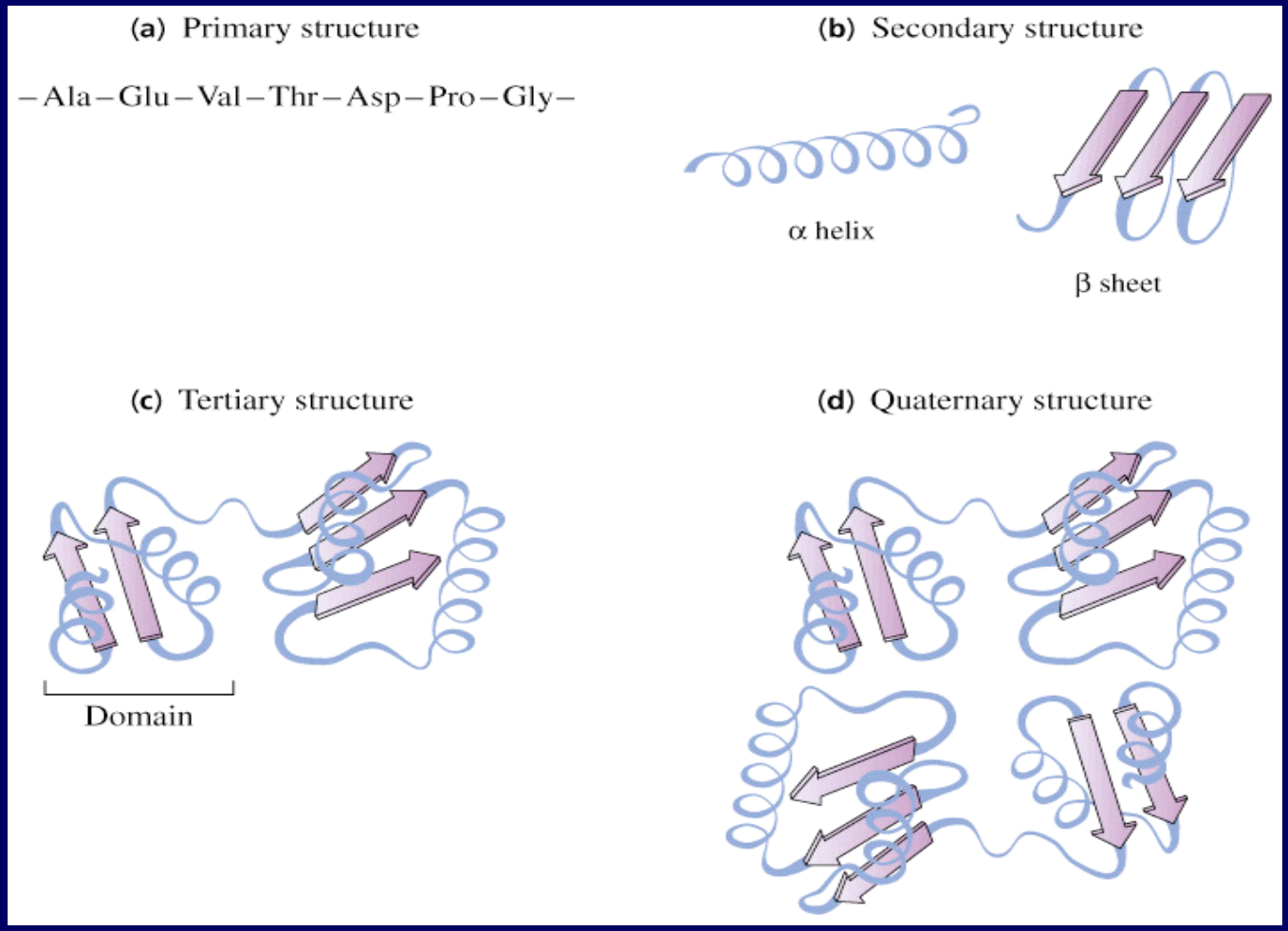
- *Comparability:* Used in context of biopharmaceutical produced from a single, validated production process (i.e., how different batches from the same manufacturer *compare* with one another i.t.o. physicochemical properties).

- *Similarity:* Essentially has identical meaning, except it refers to products from different manufacturers.

Technology Requirements

- Protein structure cannot be solved by a single or a few methods.
- Multiple orthogonal analytical techniques must be employed, both for characterizing new proteins and for comparing them to innovator products.
- Comparisons between biosimilars and originator molecules is complicated by the diversity of protein structure, including higher order folding and associations as well as post-translational modifications.

Complexity of protein structure



Taken from Horton HR, et al. Principles of Biochemistry. 3rd ed. 2002.

Analytical Techniques to Examine Molecular Heterogeneity: Primary Structure

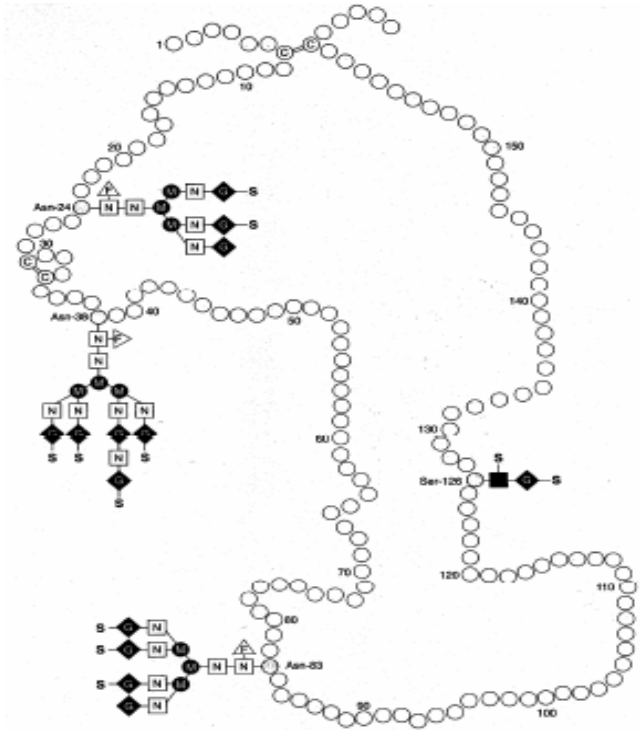
- Primary structure:
  - Amino acid sequence plus post-translational modifications → function of protein.
  - Post-translational modifications:
    - Glycosylation (addition of sugars)
    - Acetylation (acetyl groups)
    - Methylation (methyl groups)
    - Phosphorylation (phosphate groups)
  - Post-translational modification operates combinatorially to create several variants of the same protein (heterogenous protein).
  - Heterogeneity can affect safety and efficacy.

Techniques to Characterise Post-translational modifications

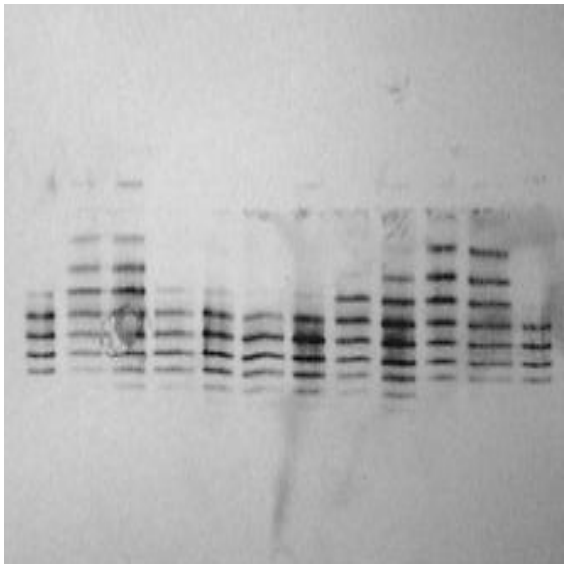
- Mass Spectrometry:
  - In conjunction with chromatography which resolve different protein variants by first purifying them and then determining their mass.
  - Possible to define the entire primary structure of all acetylated, phosphorylated and methylated isoforms (variants).
  - However, MS techniques for resolving heterogeneity due to glycosylation has been elusive.
  - A comprehensive strategy for sequencing glycan adducts to proteins is essential since more than half of all proteins are glycosylated.

Protein Structures

Erythropoietin MW 34 000 D



Isoform Profile of Different EPO Products



Schellekens H., Eur J Hosp Pharm 2004; 3:43-7

Secondary & Tertiary Structures

- The higher order structures of protein pharmaceuticals are affected by every condition and operation experienced during manufacture and subsequent processes of isolation, purification and formulation.
- The impact of changes in the higher order structures on therapeutic efficacy and safety are difficult to measure or predict.
- Multiple techniques are necessary to determine secondary and tertiary structures of biopharmaceuticals and to prove congruence of structure between two proteins

Techniques for Higher Order Structure Elucidation

- Optical methods:
  - UV Absorption
  - IR spectroscopy
  - Fluorescence spectroscopy
  - Circular dichroism

These methods are extremely sensitive to minor structural changes

Data produced by each technique are combined to construct an empirical phase diagram (vector) which is visually represented so that two proteins can be compared side-by-side without referring to the raw data.

Quaternary Structure: Aggregates

- Protein aggregation is highly undesirable primarily because aggregates tend to induce sometimes serious immune reactions.
- Clinically the immune response to aggregates can be enhanced by non-protein substances (adjuvants).
- Several precise methods exist for measuring aggregation, including light scattering, ultracentrifugation, field-flow fractionation and atomic force microscopy.
- Electron spray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry techniques are particularly attractive because they can handle very large masses of aggregates and measures how these structures fall apart to their component proteins

Aggregation = Aggravation

- Protein aggregates, which can be soluble or insoluble, provoke immune responses that profoundly affect therapeutic protein quality.
- The extent to which these aggregates resemble microbes (size, etc) determine how robustly they will active an antibody response.
- For innovator products the aggregate profile is related to patient outcome through clinical trials so developers know the implications of a particular aggregate profile.
- Comparability analyses between a biosimilar and original product will require some sort of relative quantitation of aggregates as well as qualitative analyses in the biosimilar and original product.

Effect of manufacturing Process on the Product

- With advances in manufacturing process technologies, downstream processing and analytical techniques, is it possible to produce the same product by two different methods?

Process = Product?

- Can dissimilar processes produce the same product?
  - In theory – Yes
- The hurdles will be product specific and related to the complexity of the product:
  - Molecular complexity
  - Heterogeneity (isoforms)
  - Critical quality attributes (potency, impurity profile)

Process = Product?

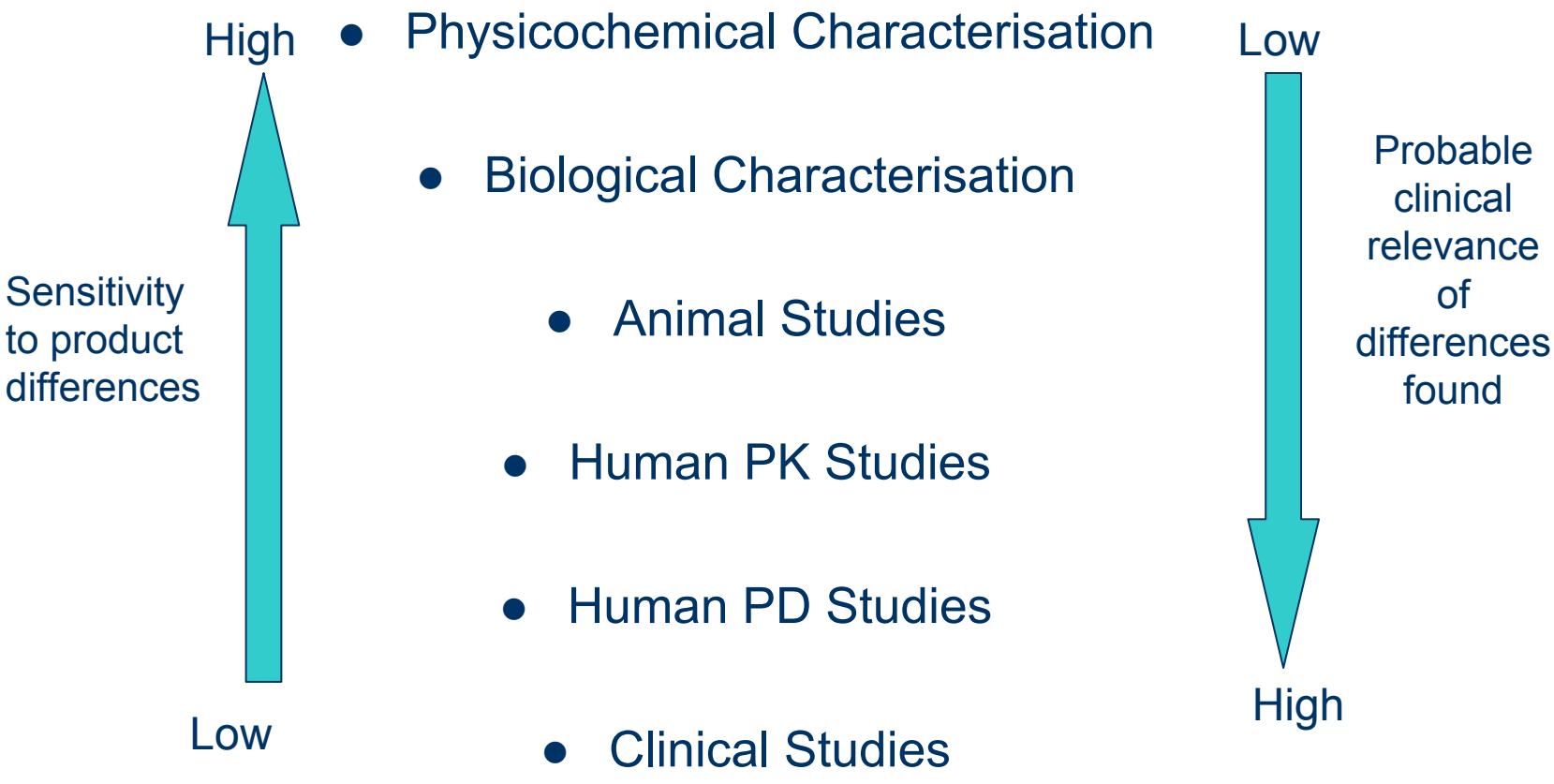
- Yes…
- Subtle process modifications DO change the product composition
- Several expression systems – bacterial, yeast, plants, insect cells, transgenic animals – each generating proteins with distinctive degradation profiles, post-translational modifications and structural features, any of which can affect protein similarity and, ultimately, safety and efficacy.

Role of Process & Analysis

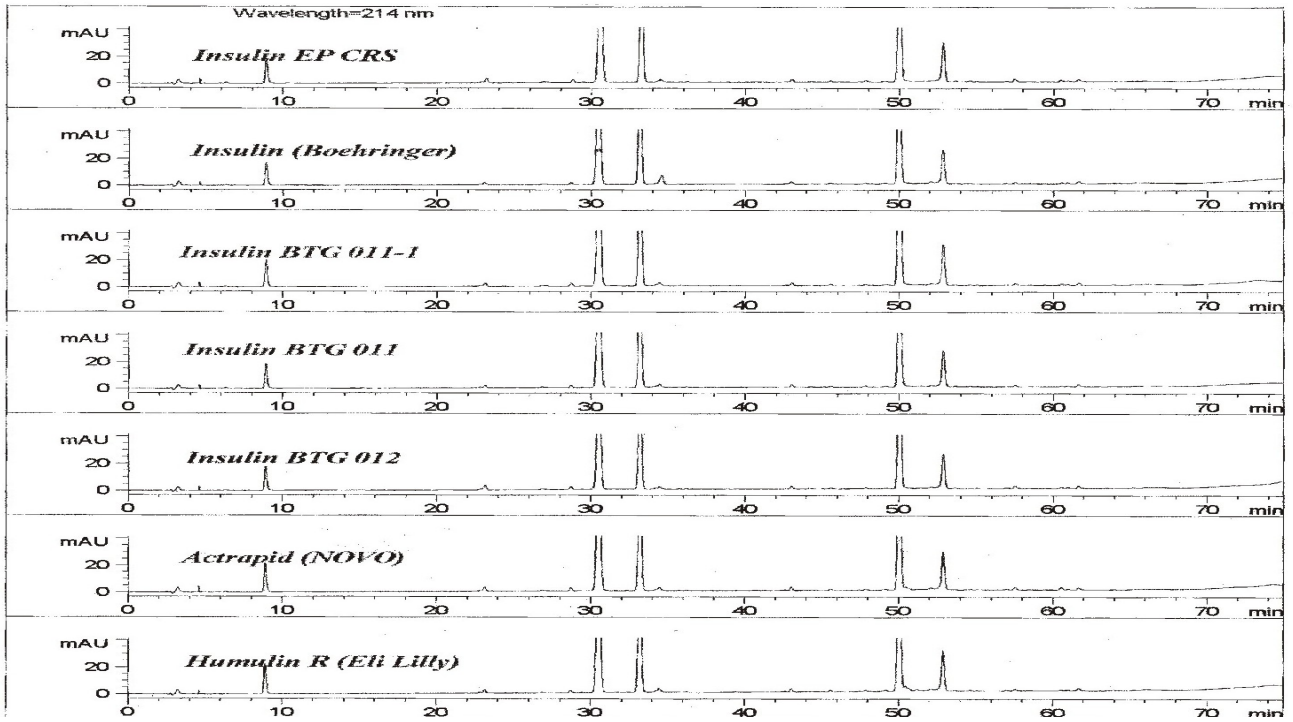
- Process often evolves in multiple steps during clinical trials, pre- and post-approval.
  - Different but related processes, all yielding comparable products
  - These process changes justified by comparability testing, guided mainly by physico-chemical and *in vitro* biological assays.

Are minor changes clinically relevant?

Comparartive Tools



Comparison of Insulin Preparations by Different Manufacturers (peptide mapping)



Jacob Hartman, Ph.D., Director, Development
Bio-Technology General (Israel) Ltd.

Role of Manufacturing Process

- The process along with the numerous analytical and biological assays used to control it, is one, but not the only, acceptable way to produce a comparable product.
- What ultimately defines a product is the composition and properties of the finished material “in the vial” together with its packaging components.

Analytical Testing: Safety & Efficacy

- Is it possible to predict safety and efficacy of a biosimilar from analytical testing?
  - For example, can monographs such as the USP adequately define safety, identity, potency and quality of biological compounds as to allow an approval of a biosimilar based on analytical comparisons?

Predicting vs Assuring

- Analytical testing cannot predict safety or efficacy *de novo.*
- Comparative analytical testing against a reference product known to be safe and efficacious can provide assurance of safety and efficacy.
- The extent of this assurance depends on complexity of the two products and the closeness of the match.

Pivotal Question

- When would it be appropriate to streamline or eliminate certain animal or human studies during development of a biosimilar product?

Is Streamlining Possible?

- Human and/or non-human data should always be necessary
  - Non-human
    - Pharmacological studies
    - Toxicological studies & biodistribution
  - Human
    - Biomarker/surrogate
    - Immunogenicity
    - Disease modification
- The extent of testing depends on patient risk and product complexity

Therapeutic Equivalence

Products	Characteristics	Possible Criteria
Insulin, hGH	Microheterogeneity and process variability does not affect activity. Accepted pre-clinical models. Accepted surrogate for activity	Animal and human PK/PD studies. (Post-marketing immunogenicity testing)
INF-α, β; GM-CSF	Microheterogeneity affects activity. Immunogenicity uncertain. No accepted pre-clinical or surrogates for activity.	Animal and human PK/PD studies. Clinical bridging study and/or post-marketing immunogenicity study.
EPO	Adverse immunogenicity demonstrated.	Animal and human PK/PD studies. Pre-approval immunogenicity study. Clinical bridging study and/or post-marketing immunogenicity study

Conclusion

- Whatever road we may decide to take – whether it be the development of our own criteria for the registration of biosimilars or the adoption of criteria of other regulatory authorities – it is essential that we be guided by science.
    - *Science is nothing but trained and organised common sense… - Thomas Huxley*