SLS Cell Cure Technologies
Predictive Diagnostics & Cell Therapies

Best Practices in Product Characterization

4th International Summit on cGMP, GCP & Quality Control
October 26-28, HICC, Hyderabad

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Predictive Diagnostics & Cell Therapies



SLS Cell Cure Technologies Pvt. Ltd

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Introduction to SLS

Business Streams



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Phase – I

Molecular Diagnostics

- Infectious Disease screening
- Predictive Diagnostics
- Pharmaco-genomics.

Phase – II

Cell Therapies

- Autologous Fibroblast therapy.
- BM derived MSC's
- Cord Blood Derived MSC's
- Peripheral Blood Derived MSC's
- Allogenic Therapies

Molecular Diagnostics

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A

Infectious disease screening

- PCR based screening For viral, bacterial, fungal pathogens
- qPCR for Viral loads
- Food pathogens

B

Predictive diagnostics

- Platform based technologies
- Microarray based
- Genetic screening
- Life style diseases
- Cancer panel
- STD panel
- Familial Hyper
- cholestremia

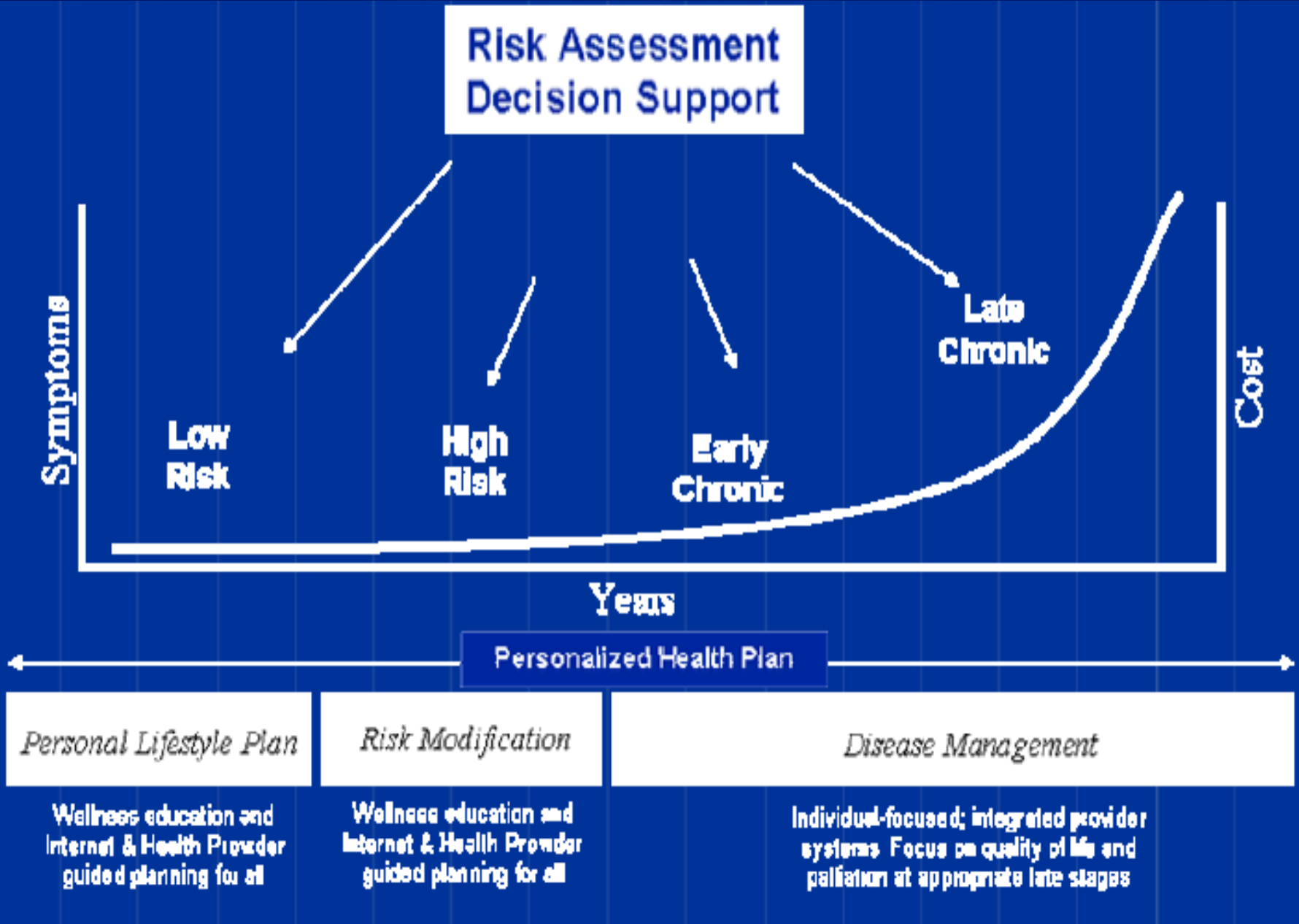
C

Pharmaco-genomics

- Patients are different
- Medicines are not differentiated
- Health risk analysis:
- Monitoring of therapy
- Identification of drug resistence
- Target validation molecule becomesTherapeutic molecule

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Prospective Health



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Biosimilars

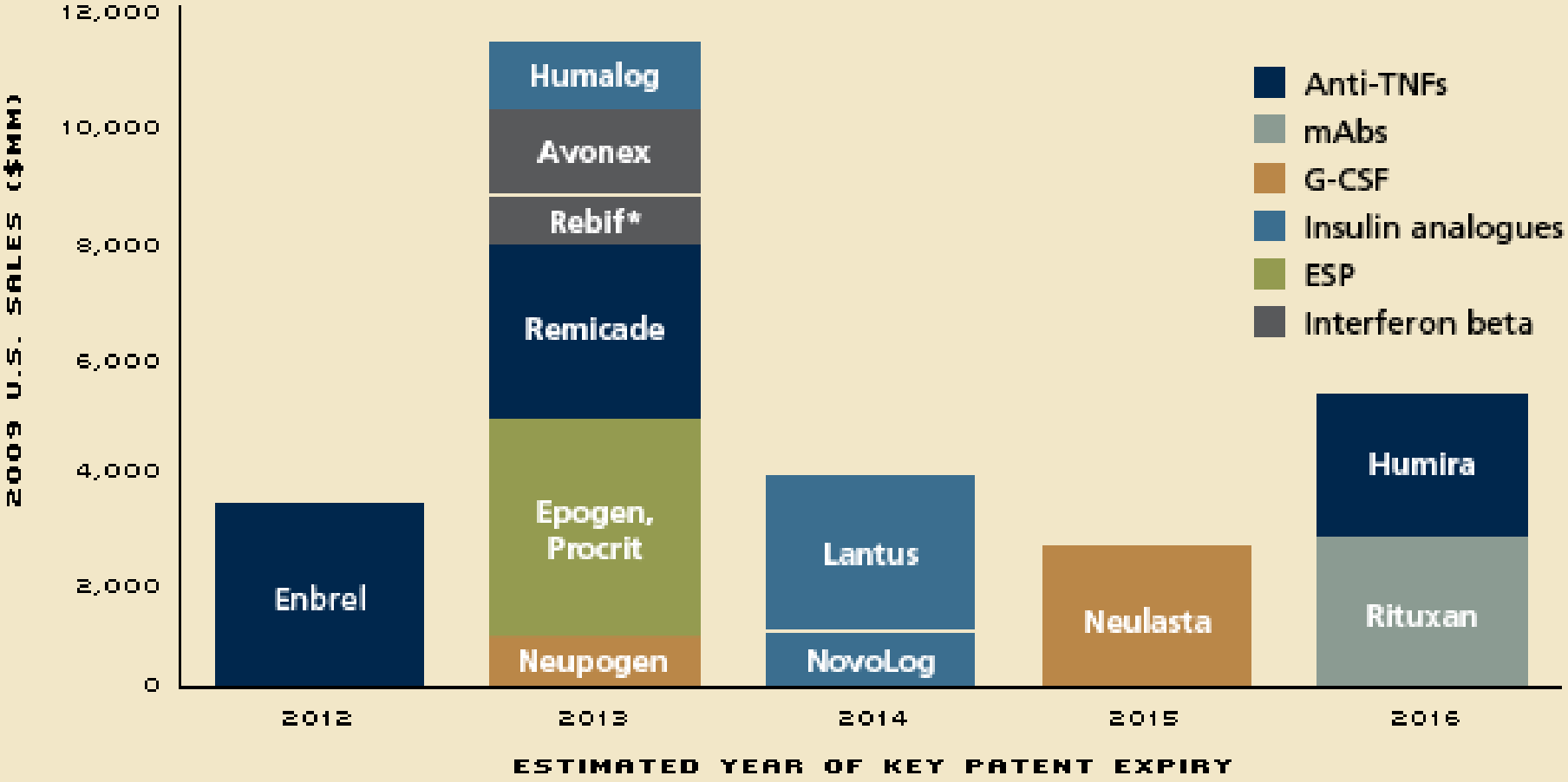
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Patent Cliff

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KEY BRANDED BIOLOGIC REVENUES AT RISK WITH BIOSIMILAR ENTRY

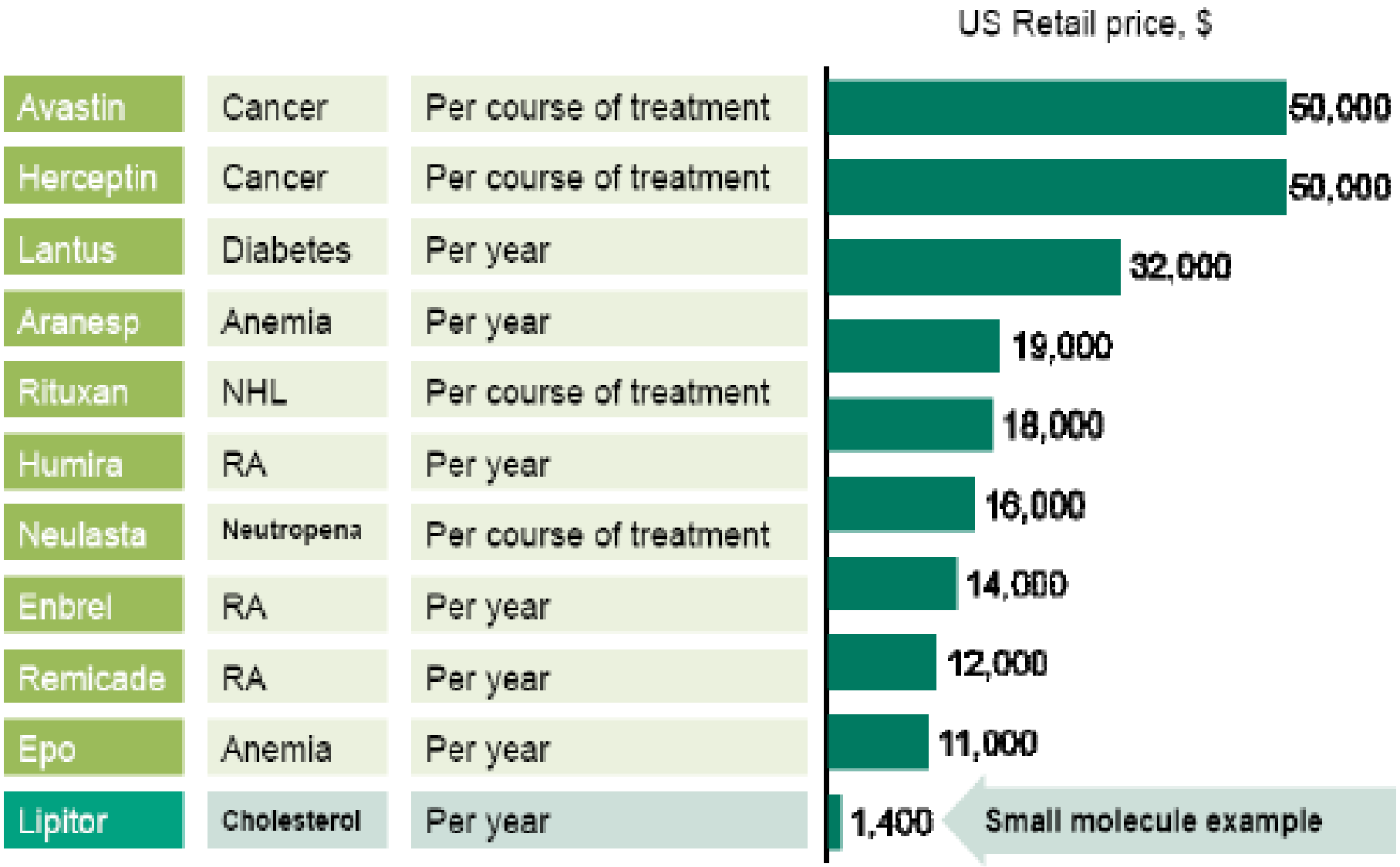
AROUND $25bn Market



* 12-year data exclusivity period ends in 2014

Source : *Analysis Group Health Care Consulting Bulletin (Fall/Winter 2010)*

Traditional Biologics are Expensive



F. Pieters, Teva Pharmaceuticals

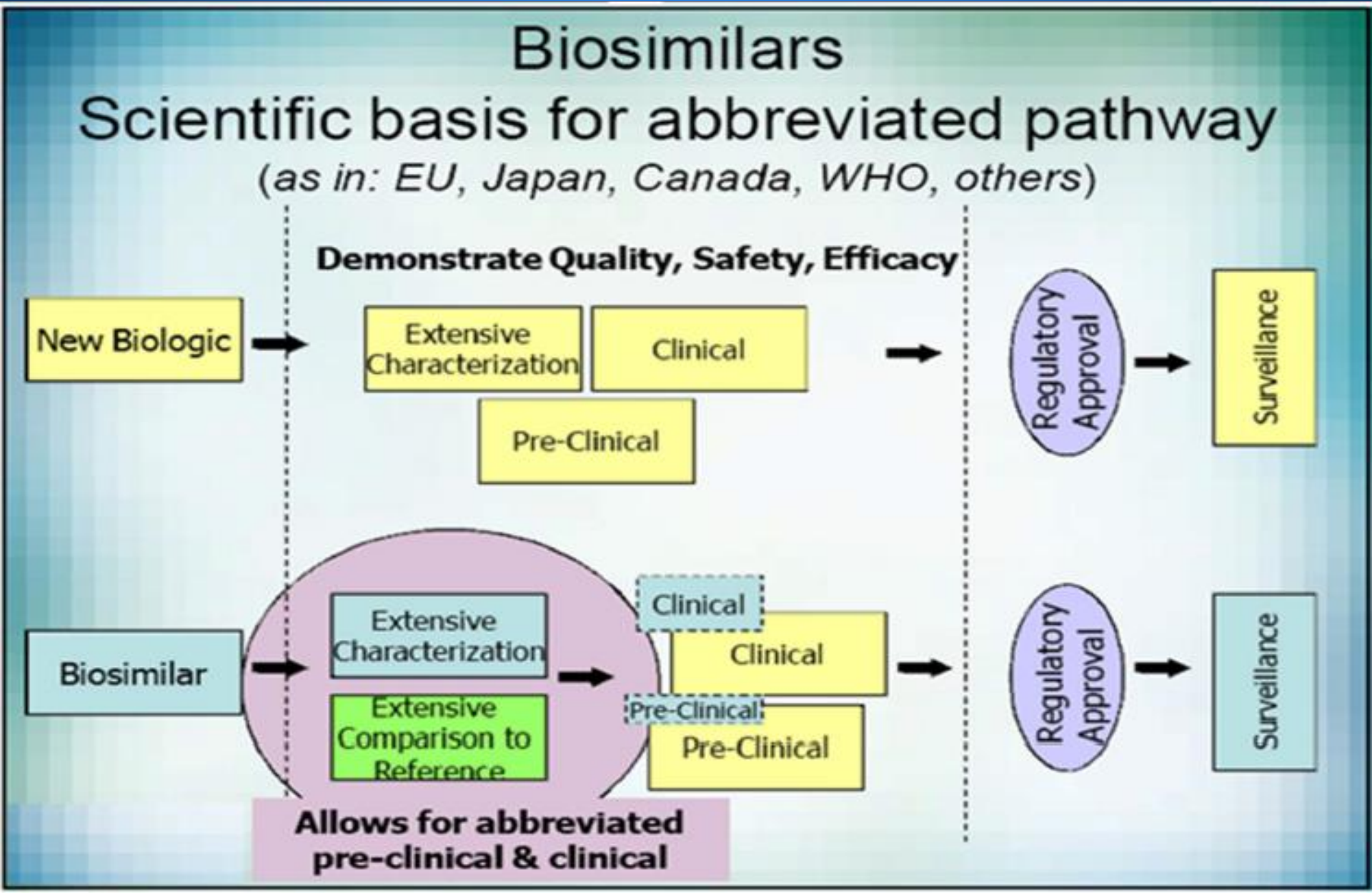
Development Of Biosimilar Regulatory Framework Worldwide

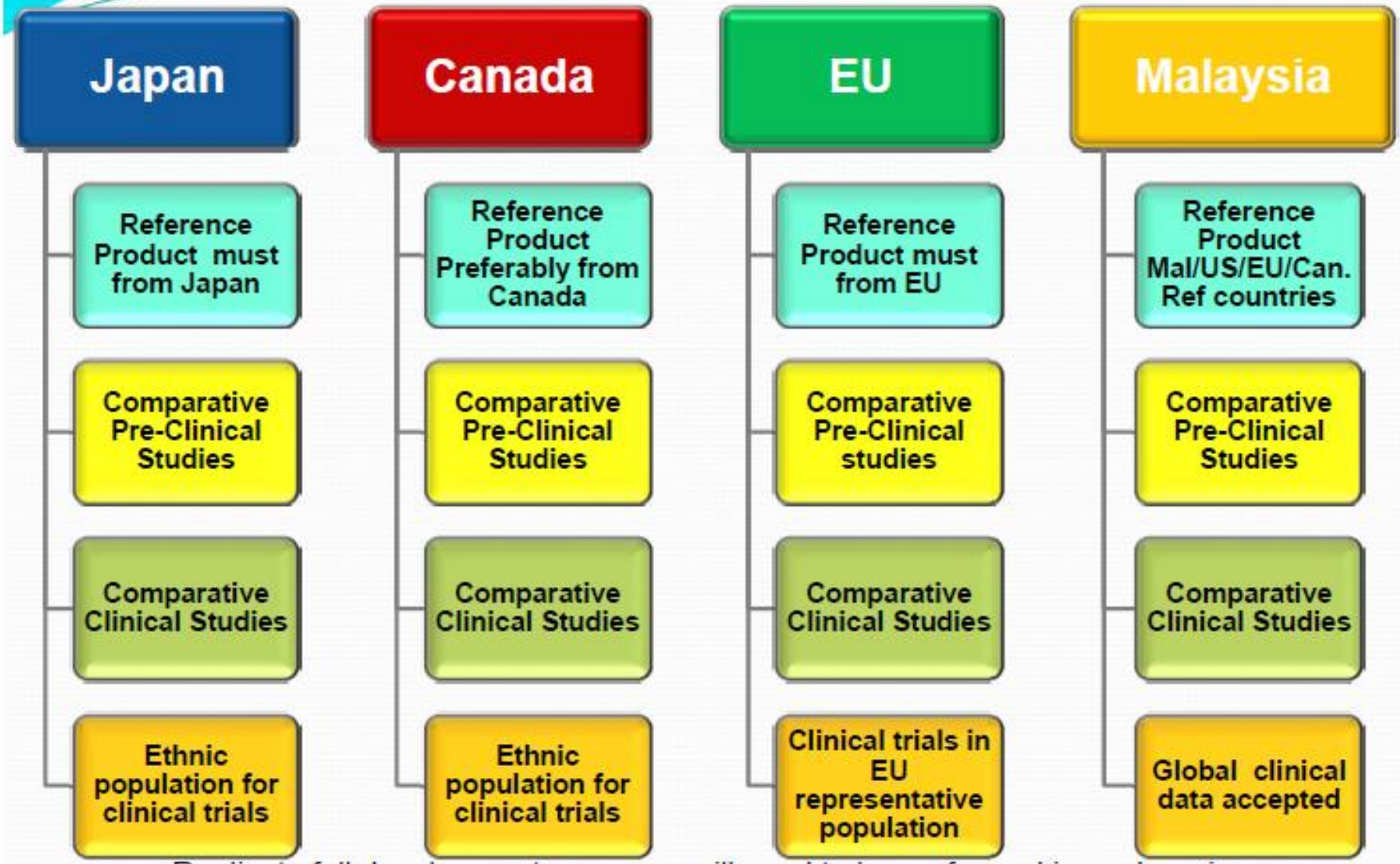


Biosimilars

Scientific basis for abbreviated pathway

(as in: EU, Japan, Canada, WHO, others)




Japan
Reference Product must from Japan
Comparative Pre-Clinical Studies
Comparative Clinical Studies
Ethnic population for clinical trials
Canada
Reference Product Preferably from Canada
Comparative Pre-Clinical Studies
Comparative Clinical Studies
Ethnic population for clinical trials
EU
Reference Product must from EU
Comparative Pre-Clinical studies
Comparative Clinical Studies
Clinical trials in EU representative population
Malaysia
Reference Product Mal/US/EU/Can. Ref countries
Comparative Pre-Clinical Studies
Comparative Clinical Studies
Global clinical data accepted

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Biosimilarity

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Clarifying Terminology – Comparability is often used in different ways and for different purposes

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Innovator Comparability

Innovator comparability testing measures quality attributes of a single product after a manufacturing process change

Also referred to as:

- Innovator product manufacturing change
- Manufacturing change comparability
- Manufacturing comparability

Biosimilar Comparability

Biosimilar testing involves the analytical, pre-clinical, and clinical comparison between two different, but related products

Also referred to as:

- Biosimilarity Exercise
- Comparability Exercise
- Biosimilarity Comparison
- Biosimilar Reference Product Comparison

Innovator Comparability ≠ Biosimilar Comparability

Manufacturing Comparability ≠ Biosimilarity Exercise

Post manufacturing change Assessment vs Biosimilar Development

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Key Considerations

- ‘Similar’ does not equal ‘same’
- Small alterations can make BIG differences
- USFDA & EMA Clearly distinguish the requirements for manufacturing comparability and Biosimilarity.
- Knowledge produces consistency and confidence

Two Different Processes Create Two Non-identical Biologic Products

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START

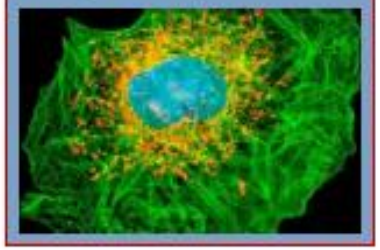
Both may use the same gene sequence



Different vectors to insert the gene



Different host cells to grow the protein



Different fermentation/ culture conditions

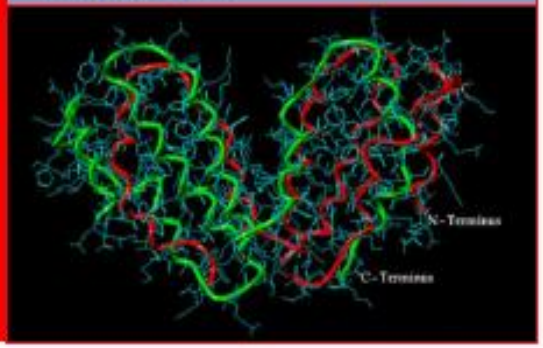


Different downstream processing



END

Different biophysical characteristics in final product



Biologics Manufacturing Control at Every Step

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For Comparability, the innovator has a rich testing data base, from every in process step of every batch, the biosimilar has access to only final product

Cell Culture



Cell Bank



Bioreactor



Harvest

Purification



Chromatography
1, 2, 3

Final Dosage Form



Virus Filter



Concentration



Bottling

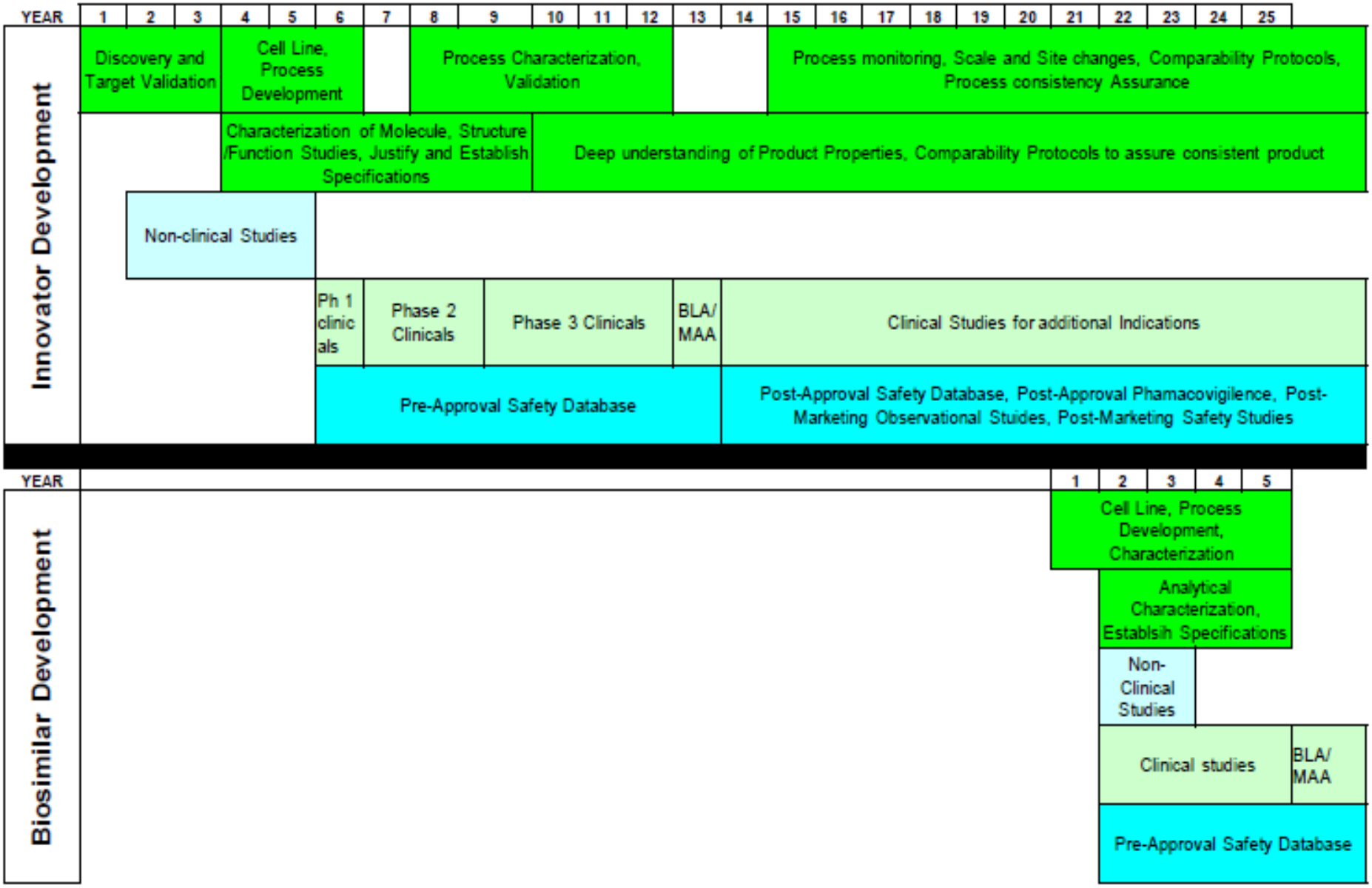


In Process Testing Data From Every Process Step

Final Tests

Accumulated experience and knowledge generates sustainable quality and predictability

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Case Study: EMA Biosimilar Applications Rejections and Withdrawals

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Biosimilar vs. Innovator	Year	Differences	Consequence
Alpheon *(interferon alpha 2a)* vs. Roferon-A®	2006	• Differences identified between the two medicines (such as impurities) • Non-validated finished product evaluation process • Lack of stability data • Rates of return of disease after treatment discontinuation, and more side effects[1]	• CHMP recommended that Alpheon be refused marketing authorization • No new trials being conducted for Alpheon
Human Rapid Marvel, Human Long Marvel and Human 30/70 Insulins vs. Humulin® S, I and M3 Insulins, respectively	Feb 2008	• Clinical differences in rates of lowering blood sugar levels[2] - "Trend in favor of Humulin" • Inadequate submission of active or finished product process • Non-validated manufacturing process	• Marvel withdrew its applications for marketing authorizations
Solumarv, Isomarv and Combimarv vs. Humulin® S	Nov 2012	• New bioequivalence data needed to be in line with new requirements in the EMA biosimilar insulin guideline (currently being revised) • Questions raised on clinical study size and patient population as well as the sensitivity of the clamp study	• Marvel withdrew its applications for marketing authorization • Intends to repeat and submit new bioequivalence on each PK/PD data clamp study

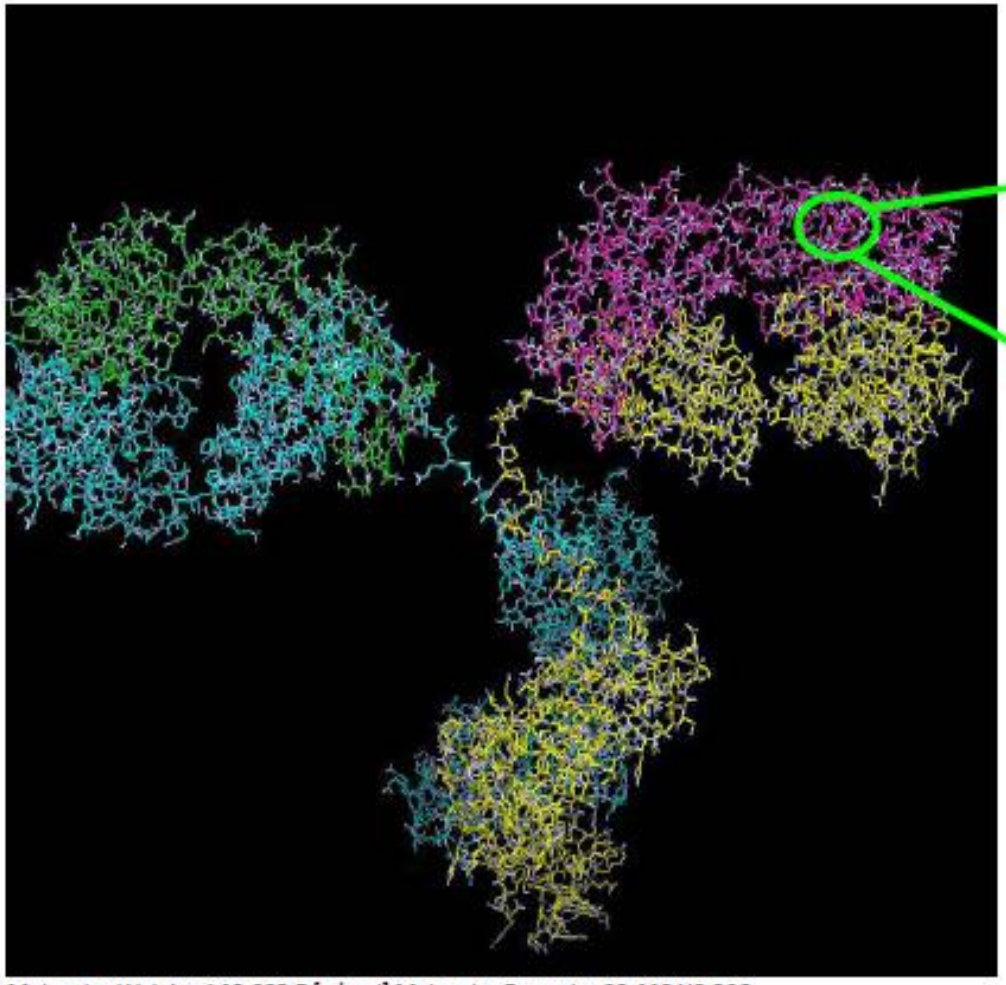
Humulin and Referon-A are trademarks of Eli Lilly, and Roche respectively

(1) Questions and Answers on Recommendation for Refusal of Marketing Application for Alpheon. 2006 Doc. Ref. EMEA/190896/2006.
(2) Questions and Answers on Recommendation for Refusal of Marketing Application for insulin Human Marvel 2008 I Doc. Ref. EMEA/4193/2008.
(3) Press Release. Marvel LifeSciences Ltd withdraws its marketing authorization application for Solumarv, Isomarv and Combimarv (human insulin). 2012 Doc. Ref. EMA/747975/2012.

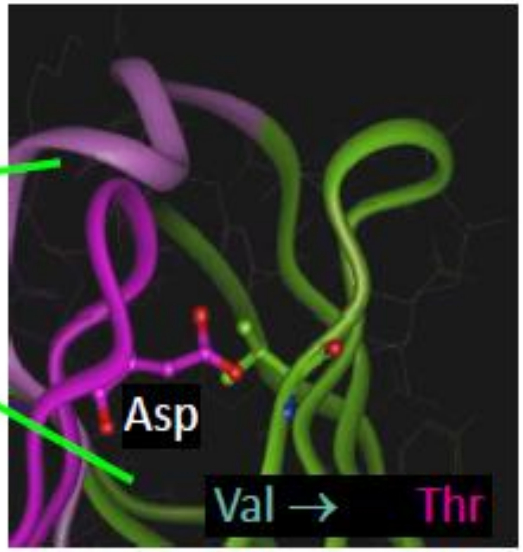
Which Changes Matter? Which don't?

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We don't know unless identified and clinically tested!



Molecular Weight: 148,683.5 [g/mol] Molecular Formula: C6,440 H9,928 N1,704 O2,011 S56



Charlie Hutchens – Abbott Labs

A single additional H-bond increases thermodynamic stability and could change the aggregation.

Does this impact safety/efficacy?

Case Studies:
"Not So comparable" Manufacturing Changes

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Innovator process changes resulting in significant clinical impact

Product	Change	Impact
Myozyme/Lumizyme[1] (glucosidase alpha)	160 to 2,000 liter scale produced glycosylation differences	New clinical trial, biologics regulatory submission, and name change from myozyme to lumizyme
Eprex (epoetin alpha)[2-4]	Replaced HSA with sorbitol-80 stabilizer using un-coated stoppers in PFS	Increased incidence of neutralizing antibodies and PRCA

PRCA: Pure Red Cell Aplasia. HSA : human Serum Albumin, PFS: Pre Filled Syringe

1.http://www.in-pharmatechnologist.com/ingredients/Myozyme-becomes-Lumizyme-after-biologic-scale-up
2.Kuhlmann M. et. Al 2010:90 British Journal of Diabetes. Lessons learned from biosimilar epoetins and insulins
3. Schellekens, H. Nature Biotechnology 2006;24(6):613-14(4) Bennet C et al. N.Engl.J. Med:2004 Sep 30, 351(14):1403-8

Regulatory Perspective of Manufacturing "Comparability"

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- Manufacturers make changes when:
  - -Maintaining state of the art manufacturing process
  - -Increasing scale
  - -Improving product stability
  - -Complying with changes to regulatory requirements

- Relevant Quality attributes are evaluated
  - -Manufacturers evaluate potential impact of process modifications on clinical safety and efficacy of the drug.

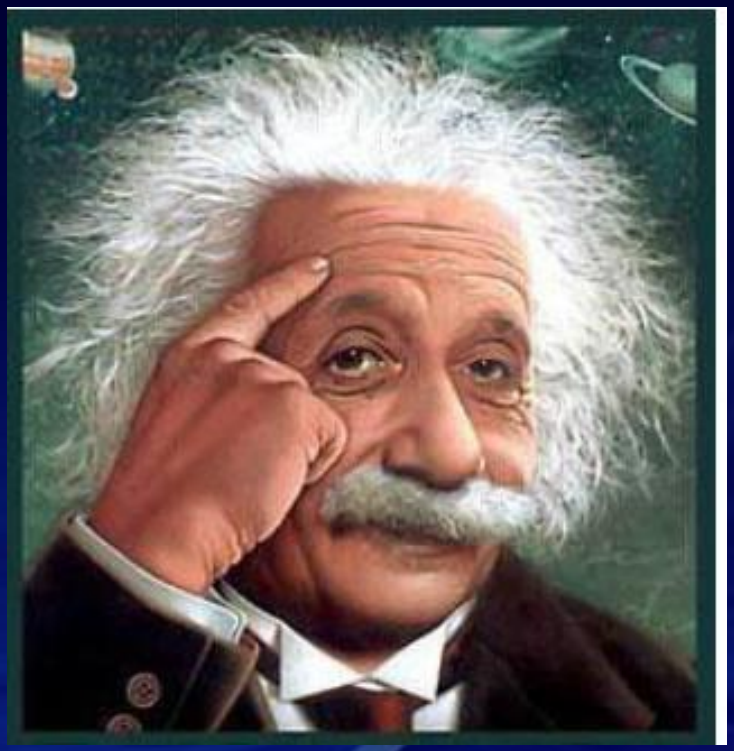
- Such an evaluation should indicate whether or not confirmatory non-clinical or clinical studies are appropriate
  - -This is known as comparability exercise

- How does this differ from the development of a Biosimilar?

Ref: ICH Q5E Comparability of Biotechnological /Biological Products subject to changes to their Manufacturing process

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Forward Path

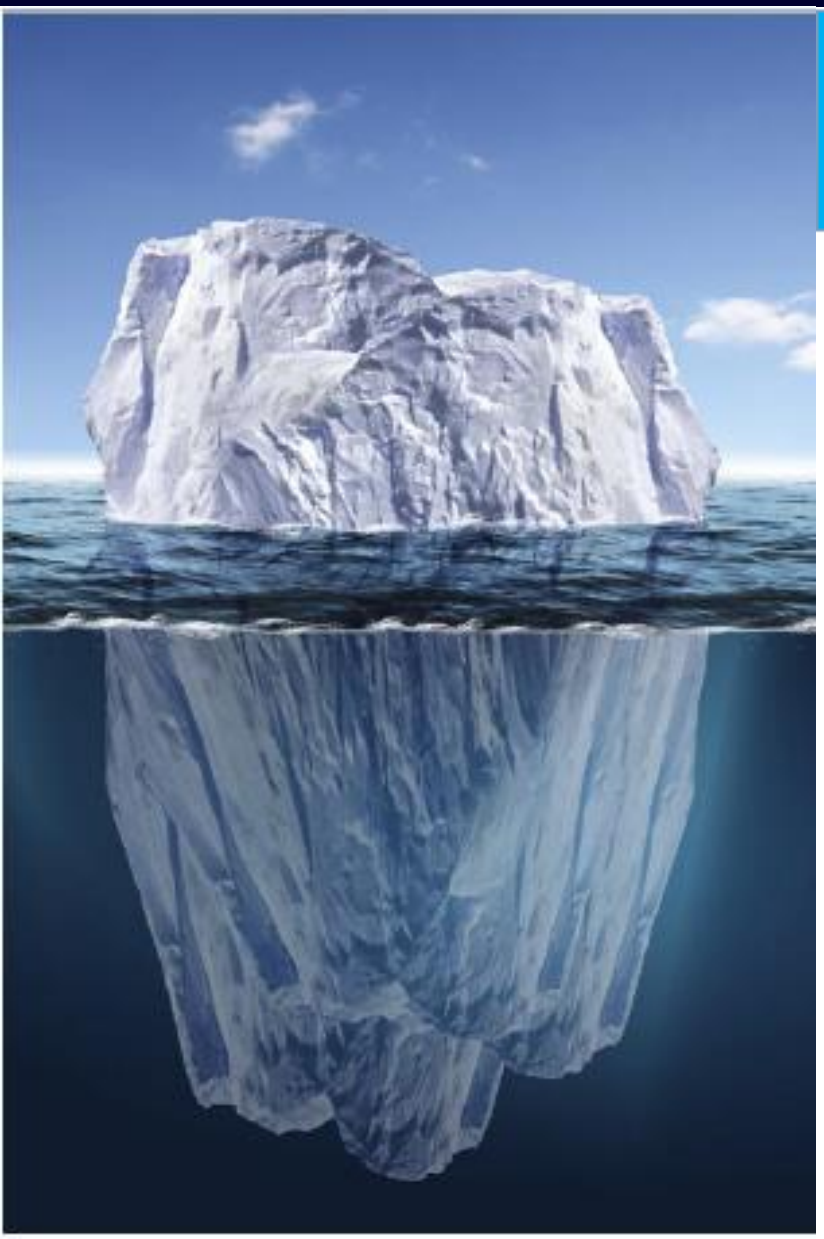


"The only source of knowledge is experience."

- Albert Einstein

How well do we understand our Biologic?

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GOAL: Consistent manufacturing yielding consistent product therefore producing consistent SAFETY + EFFICACY

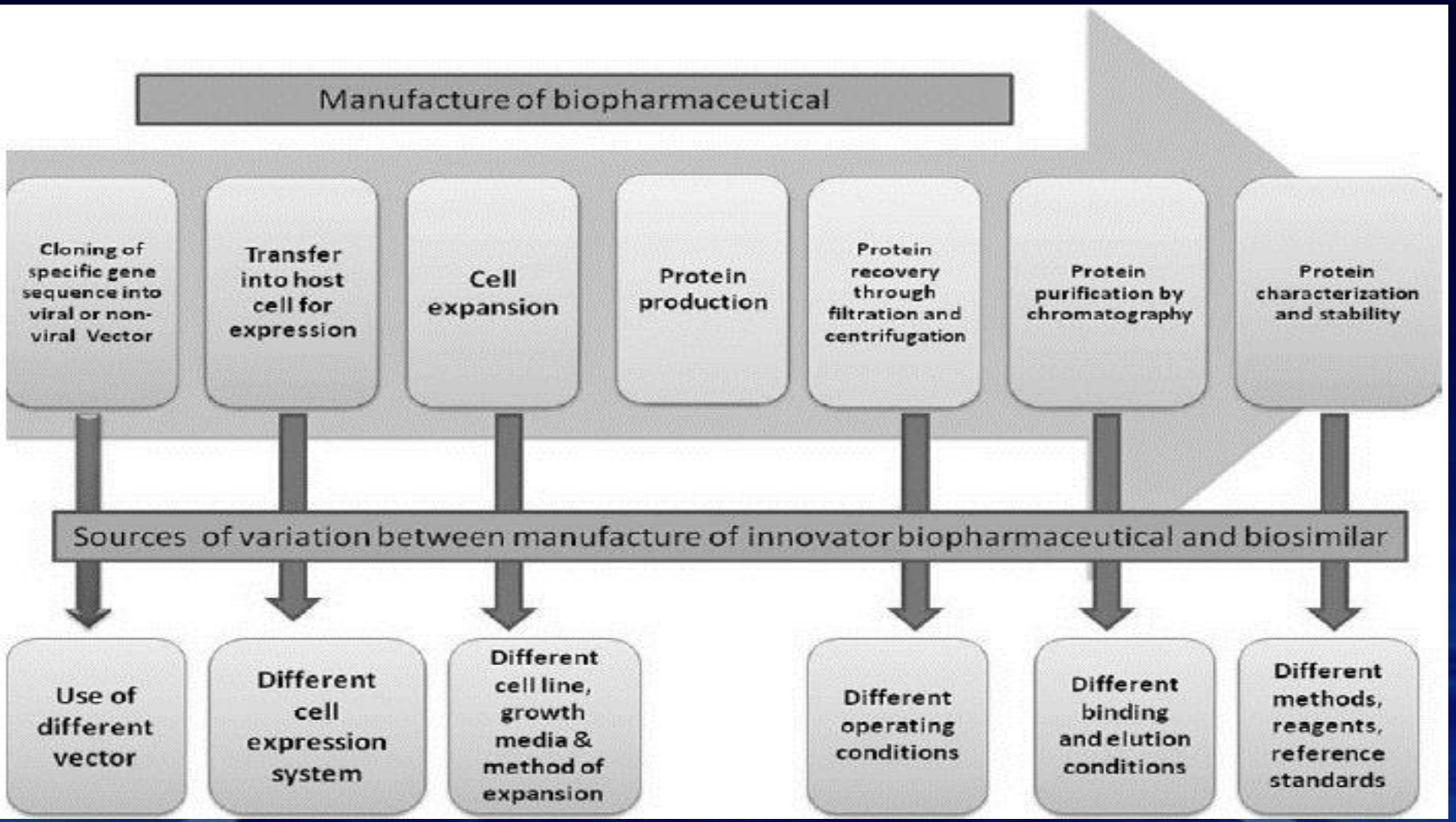
Release Tests
- Certificate of Analysis

Characterization Tests
- Process characterization
- Extended product characterization and comparability

Process Control
- Process and product impurities
- Raw materials
- Process monitoring and in-process testing
- Controls, setpoints, ranges, hold times
- Process validation

Where Does This Difference Come From ?

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Sources of variation between manufacture of innovator biopharmaceutical and Biosimilars

Source: Misra M , **Biosimilars: current perspectives and future implications**, Indian J Pharmacol.,2012 Jan;44(1):12-4.

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Differences of Biosimilar/ Follow-on Products

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Known, detectable differences

- Genetic construct and cell line
- Cell culture/fermentation conditions
- Purification process and in-process controls
- Characterization test and specifications
- Micro heterogeneity for glycoforms
- Impurities and variants

Unknown, hard-to-detect differences

- Biological activities
- Structural/conformation
- Immunogenicity
- Efficacy/safety

Goals of Quality, Non-clinical, and Clinical Studies

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Quality
- To demonstrate comparability of the product to a reference product- the most critical step.

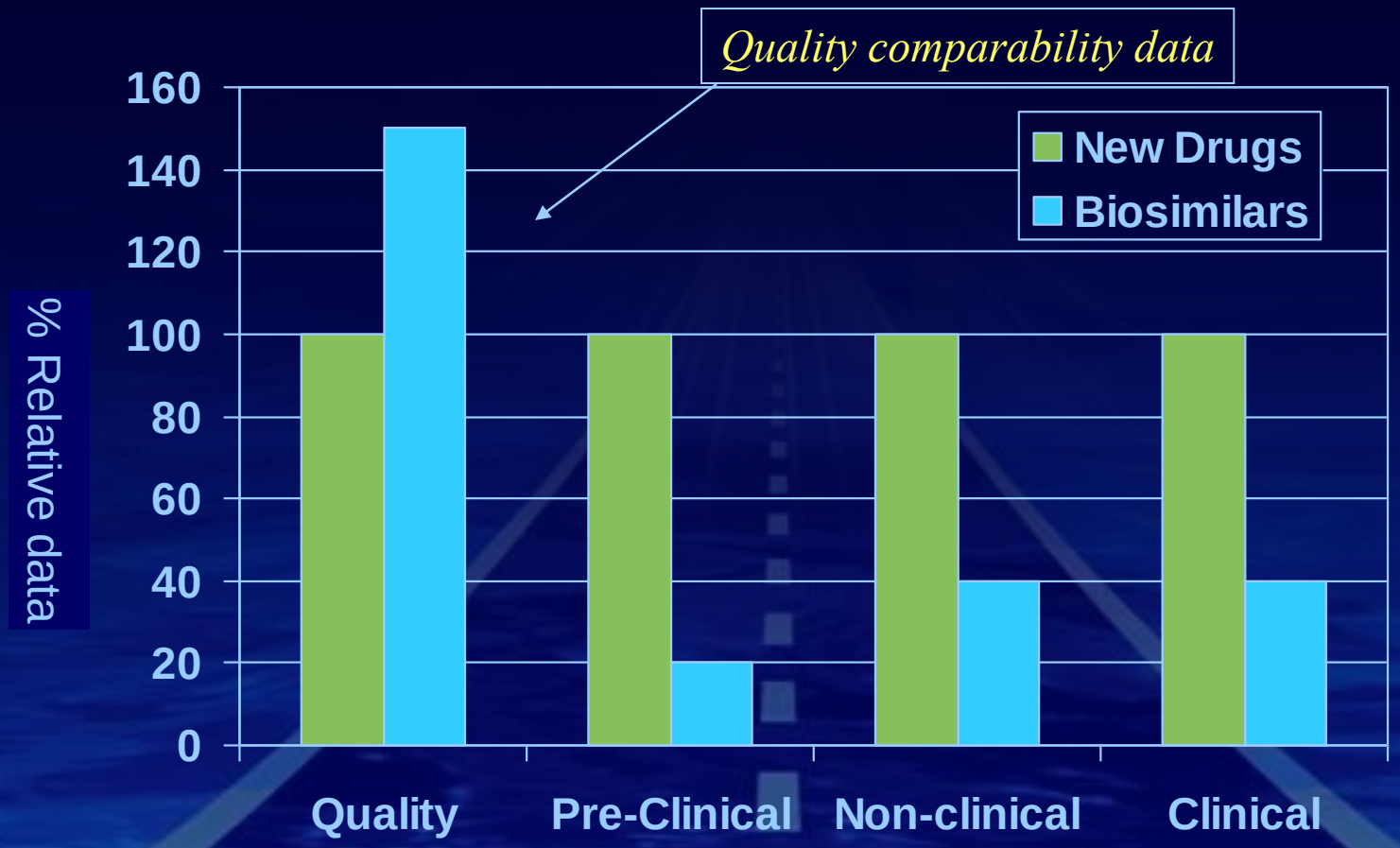
Pre-clinical toxicology
- To confirm therapeutic index and safety profile.
- To qualify impurities by short-term animal studies .
- Full animal toxicity studies are not necessary.

Non-clinical PK/PD studies
- To confirm dosing regimen by PK profiles.
- To confirm the mechanism of actions by biomarkers (PD).

Clinical safety
- To compare immunogenicity and/or hypersensitivity with the reference products

Efficacy
- To conduct confirmatory clinical trials (smaller scale).
- Use of complementary bio-markers , or surrogate endpoints.

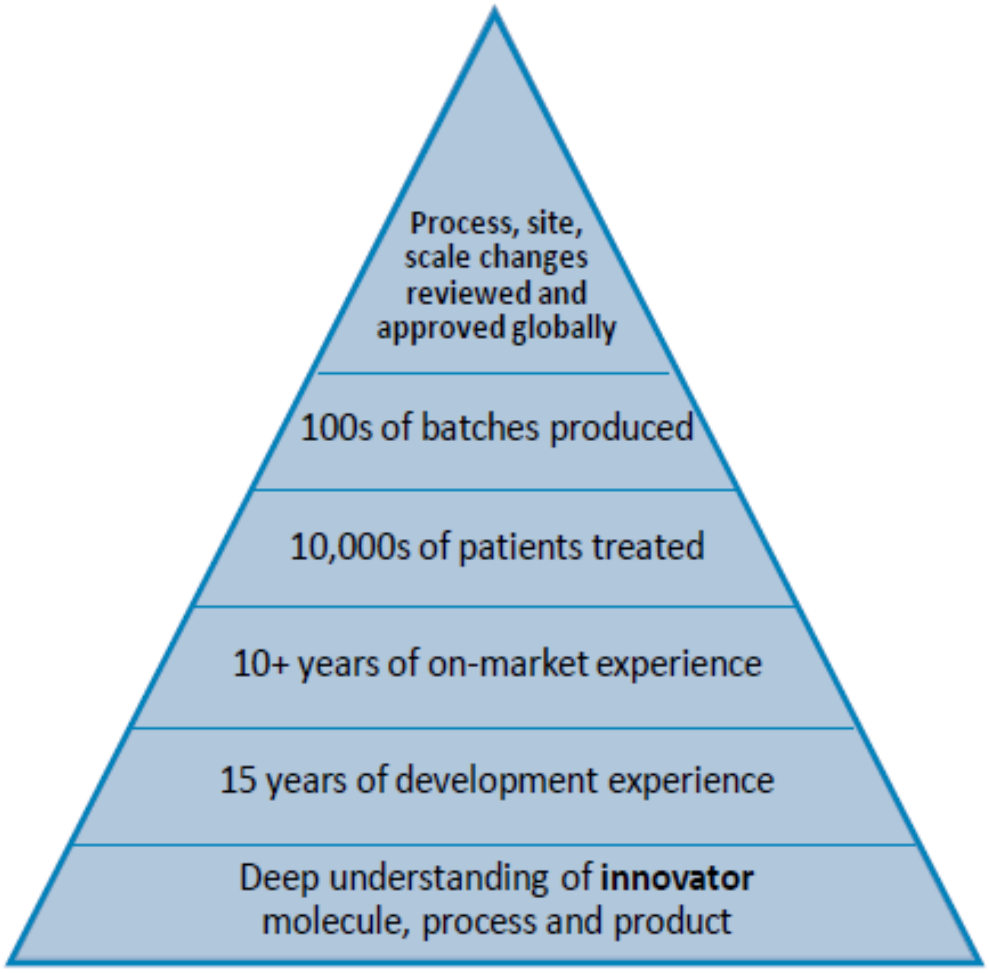
Comparability Concept for Biosimilars/Follow-ons



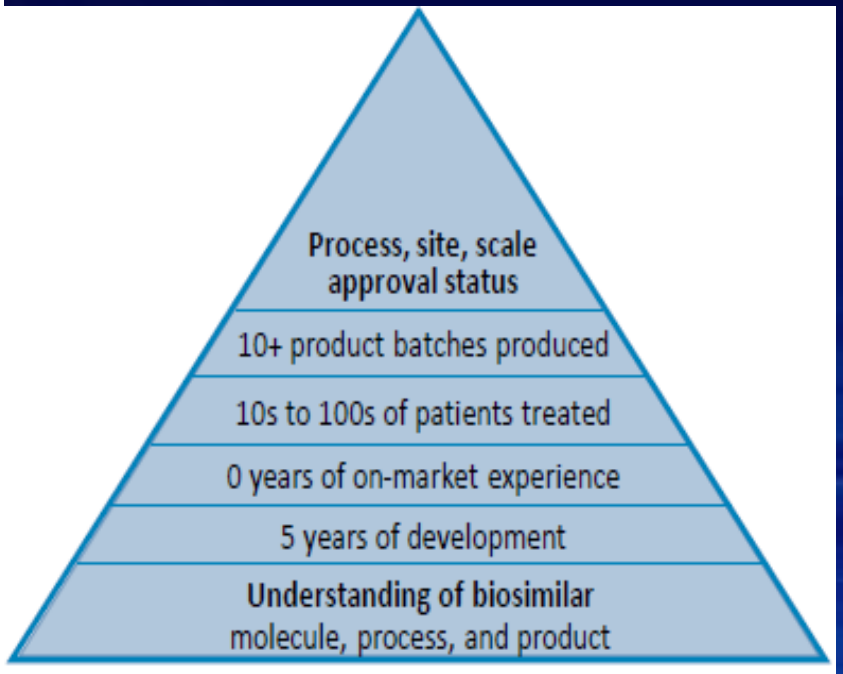
Experience brings Confidence

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Innovator Biologic
Justification for Changes

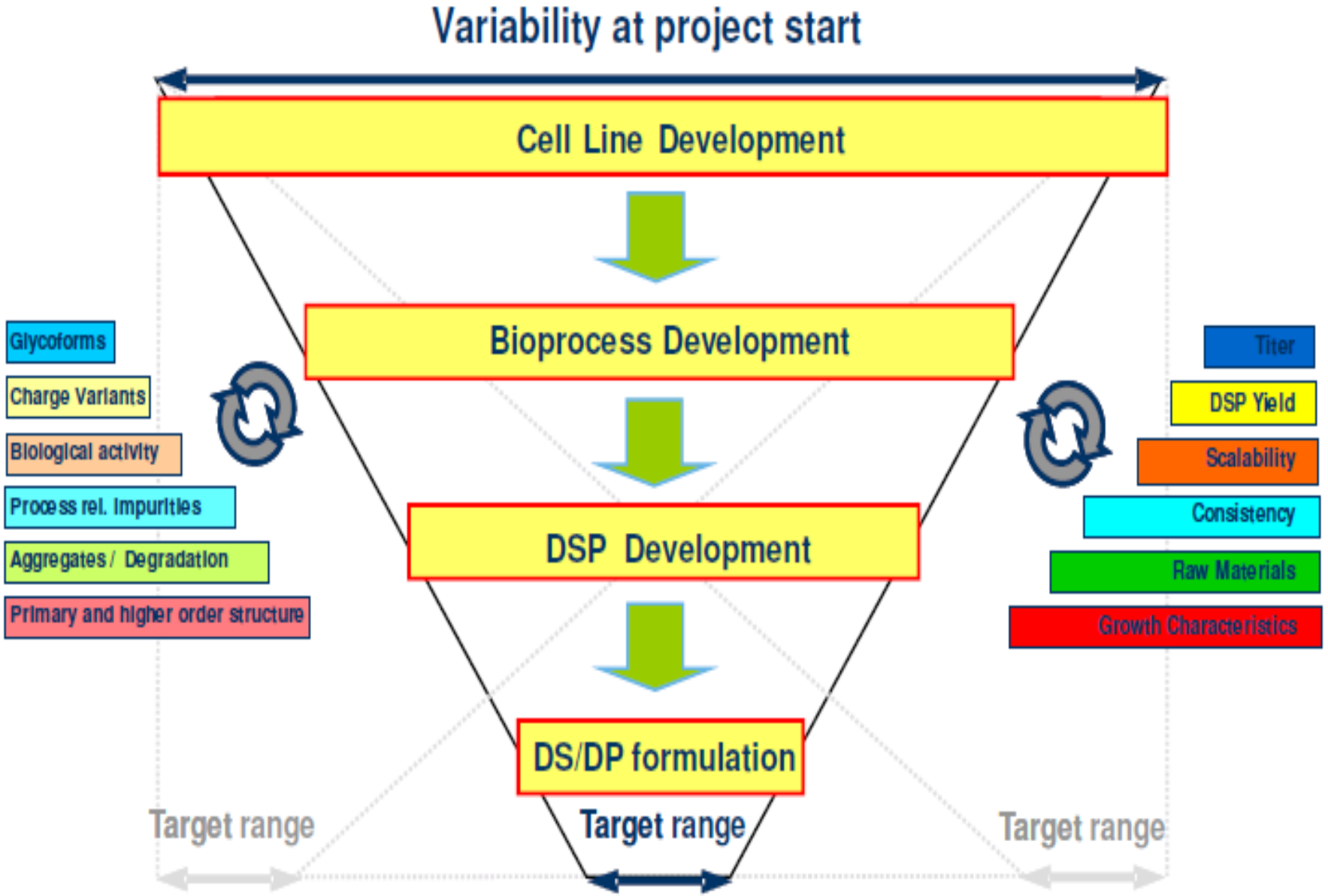


Biosimilar Biologic
Basis for Approval

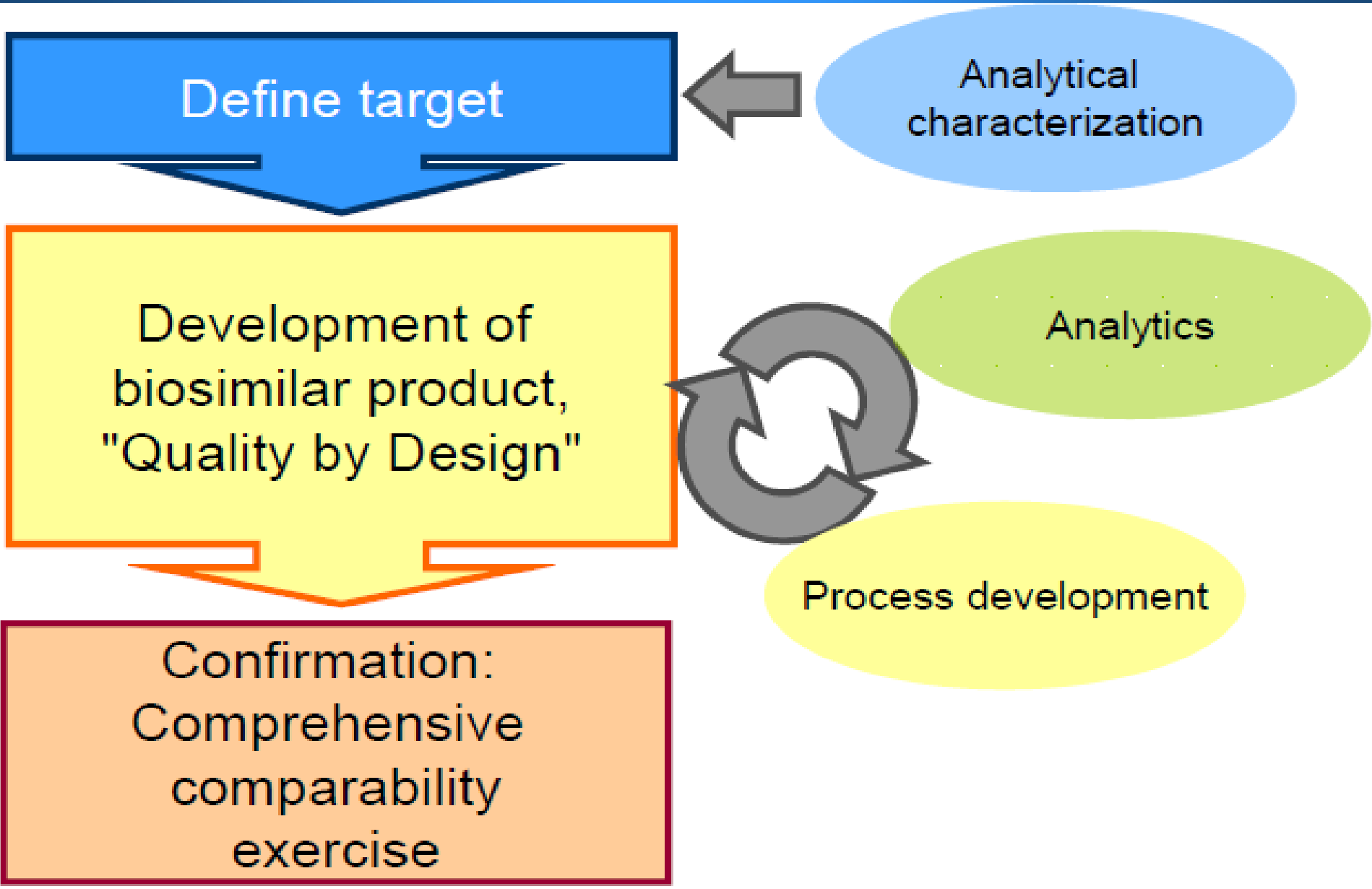


The numbers and years shown for Innovators and Biosimilars are estimates, based upon time of biosimilar approval, and may differ in some cases.

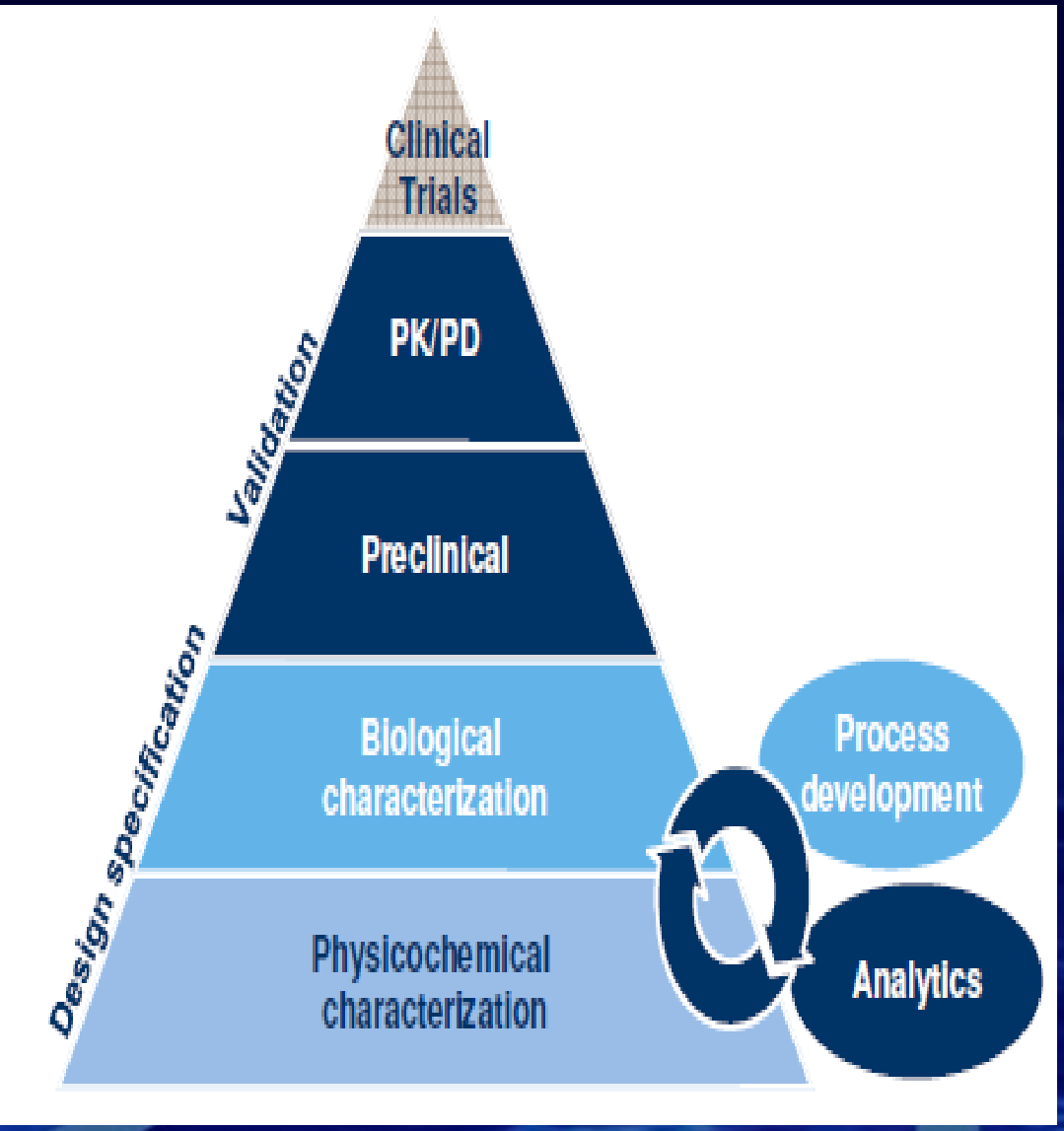
Managing Variability : QbD Biosimilar Development



Development of a Biosimilar Product



Proving Biosimilarity with comparability with reference product at all stages



- Appropriate Clinical Trials to show safety and Efficacy.
- Design Manufacturing Processes to ensure Comparability.
- Science based Process Development to deliver Target Quality.
- Characterization to prove that the product is safe and efficacious

Physiochemical and Biological Methods **Frequently** used with Well-Characterized Protein Products

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METHOD	ATTRIBUTE	TYPICAL USE
pH (if liquid)	General quality	C, R, S
Karl Fisher (if lyophilized)	Moisture, integrity	C, R, S
Appearance	General quality	C, R, S
UV Absorbance	Concentration	C, R, S
SDS-PAGE (R/NR)	Identity, purity, integrity	C, R, S
SEC-HPLC	Identity, purity, integrity	C, R, S
RP-HPLC, IEX-HPLC, HIC-HPLC	Identity, purity, integrity	C, R, S
Peptide Mapping	Identity, integrity	C, R, S
Mass Spectrometry	Identity, integrity	C, R, S
Isoelectric Focusing	Identity, integrity	C, R, S
Capillary Electrophoresis	Identity, integrity	C, R, S
Immunoassay/ELISA	Identity, integrity	C, R, S
Ligand Binding Assay	Identity, potency, integrity	C, R, S
In Vitro Bioassay	Identity, potency, integrity	C, R, S
N terminal Sequencing	Identity	C, R
Amino Acid Analysis	Identity, concentration	C, R
Product Residuals	Purity	C, R
Process Residuals	Purity	C, R
Monosaccharides	Identity	C, R*
Oligosaccharide	Identity	C, R*
Sialic Acid	Identity	C, R*
Circular Dichroism, FTIR	Conformation	C
AUC	Impurities (Aggregates)	C

[only in specific instances for selected glycoproteins]*

Typical Performance Capabilities of Bio-molecular Methods

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- Spectrophotometry (Direct Measurement)
  - ≤ 3% CV intra; ≤ 5% CV inter
- Colorimetric Assay (Reaction-based, non ELISA)
  - ≤ 8% CV intra; ≤ 10% CV inter (cuvette)
  - ≤ 8% CV intra; ≤ 10% CV inter (plate w/meniscus +Q10)
  - ≤ 10% CV intra; ≤ 15% CV inter (plate w/o these)
- ELISA
  - ≤ 10% CV intra; ≤ 15% CV inter (plate w/meniscus +Q10)
  - ≤ 15% CV intra; ≤ 20% CV inter (plate w/o these)

Typical Performance Capabilities of Bio-molecular Methods

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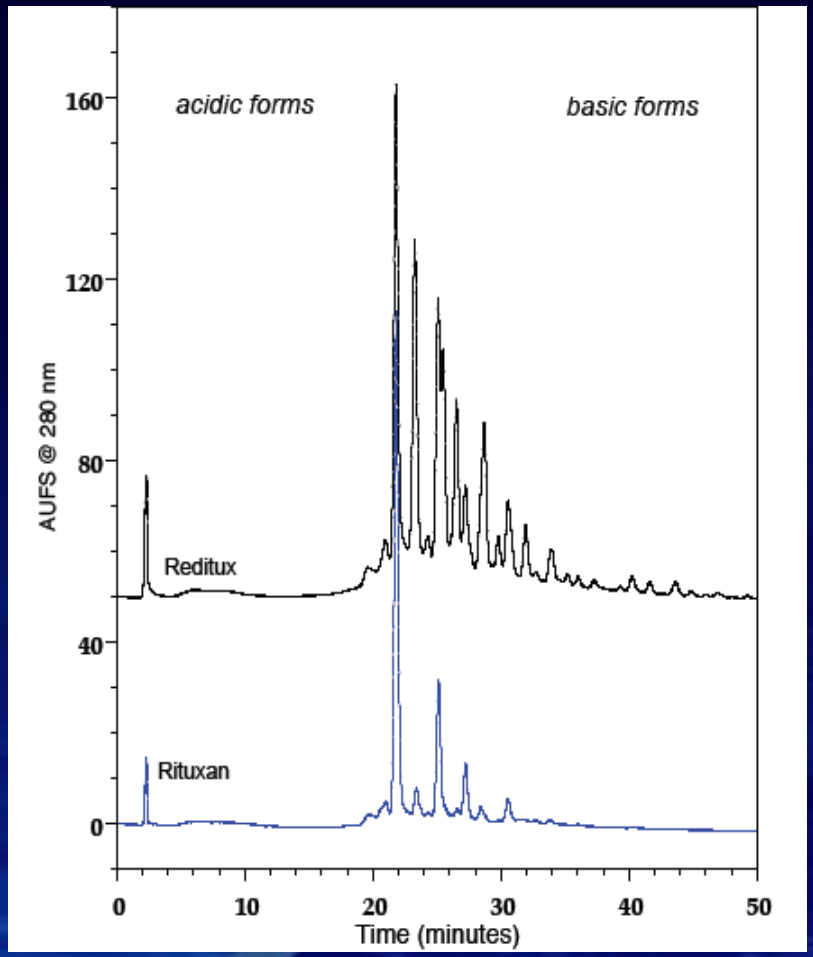
- Electrophoresis with densitometry
  - ≤ 10% CV intra; ≤ 15% CV inter (Area, w/ defined scanning)
  - ≤ 15% CV intra; ≤ 20% CV inter (Area, w/ undefined scanning)
  - ≤ 20% CV intra; ≤ 30% CV inter (MW, software calculation)
- RP-HPLC
  - ≤ 3% CV intra; ≤ 5% CV inter (RRT)
  - ≤ 8% CV intra; ≤ 10% CV inter (peak area)
- SEC, IEX, HIC HPLC
  - ≤ 5% CV intra; ≤ 8% CV inter (RRT)
  - ≤ 10% CV intra; ≤ 15% CV inter (peak area)
- cIEF
  - ≤ 5% CV intra; ≤ 10% CV inter (peak area ratios)
- cSDS
  - ≤ 10% CV intra; ≤ 20% CV inter (peak area ratios)

Typical Performance Capabilities of Bio-molecular Methods

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- Carbohydrate Analysis
  - Monosaccharide composition (peak areas)
    - ≤ 15% CV intra; ≤ 20% CV inter
  - Sialic acids (peak areas)
    - ≤ 10% CV intra; ≤ 15% CV inter
  - N-linked oligosaccharides (peak ratios)
    - ≤ 15% CV intra; ≤ 20% CV inter
- Bioassays
  - Cell based (defined SOP steps + Q10)
    - ≤ 15% CV intra; ≤ 20% CV inter
  - Cell based (less defined SOP w/o Q10)
    - ≤ 20% CV intra; ≤ 40% CV inter
- BIACore Binding Assays
  - ≤ 10% CV intra; ≤ 15% CV inter (defined SOP +Q10)
  - ≤ 15% CV intra; ≤ 20% CV inter (less defined SOP w/o Q10)

Challenges for MAbs Quality Comparability



Reditux approved in India in 2007

Analysis by Genentech:

- Identical amino acid sequence and molecular weight
- Glycoforms not comparable
- Charge distribution not comparable
- Aggregate content not comparable
- Effector function not comparable
- Higher host cell protein content
- Clinical data with Reditux in NHL comprised 17 patients only (immunogenicity?)

Reed Harris, Genentech, Presentations at FABIAN 2008"Biopharma, Biosimilar, Biogenerics? Bioanalysis", Groningen, the Netherland, 2008 and "Biogenerics 2008".

Major Challenges by Companies from Asia

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- Regulatory requirements and patent issues.
- Cell line and process for manufacturing of products meeting comparability criteria.
- Capacity of manufacturing and compliance with internationally recognized GMP standards.
- Comparability issues after changes in site and scale.
- Product-specific issues on comparability testing.
- Ability to secure reference products for comparability testing including pre-clinical and clinical studies.
- Design of non-clinical and clinical studies that meet regulatory requirements.
- Development costs and competitions from 2nd generation products, bio-betters and Bio-novel.

Challenges

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•**Price :** Key challenges to the originator companies to anticipate the way in which bio-similars will act on price. (discounting for bio-similars is only about 20-30%).

•**Duration of Therapy:** Nature of drug use –chronic use (mab's) vs small molecules.

•**Established Experience:** General acceptance of high similarity between original brands and bio-similars well established in Europe – eg:EPO's and GCSF's.

•**Familiarities & Trust:** Clinicians already familiar with the concept of cost benefits of initiating treatment s with generic versions of non –biologics. What about Biologics?

•**Patient Role:** growing role of patients in treatment decisions.

•**Technology :** Technical hurdles for manufacturers of Biosimilars.

•**Volume effects:** Potential for significant volume effect on biologics consumption as observed in case of G-CSF – UK & Sweden.

•**Competitive Landscape:** ??

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Thank you

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Forward Path

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