



Biosimilars

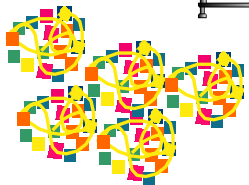
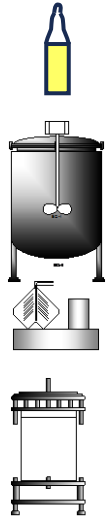
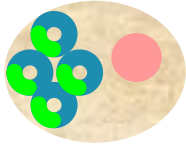
Scientific Challenges and Implications

Professor Paul Declerck
Laboratory for Therapeutic and Diagnostic Antibodies

Biological medicinal product

A well-defined **biological** product prepared by the **use of living systems**, such as organisms, tissue cultures or cells.

Recombinant Protein Production

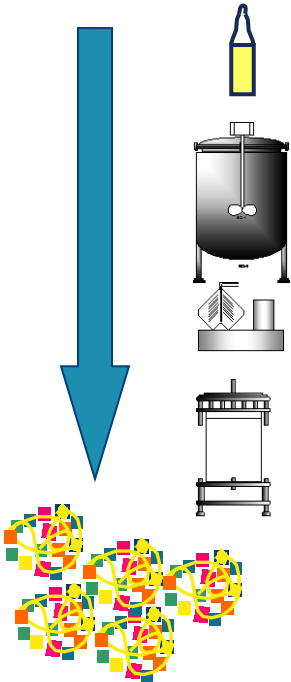
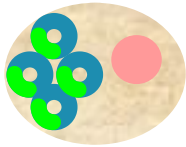


Unit Operation	Specific to Product
Cell Expansion	Cell line, growth media, method of expansion
Cell Production in Bioreactors	Cell line, growth media, bioreactor conditions
Recover through filtration or centrifugation	Operating conditions
Purification through chromatography	Binding and elution conditions
Characterization and Stability	Methods, reagents, reference standards

Bioreactor



Recombinant Protein Production



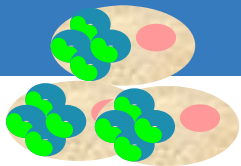
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Recombinant Protein Production

Cell Banks

10+ tests

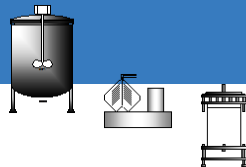
- eg,
- Karyotype
- Infectious/
oncogenic
screen
- Gene stability



Process validation

20+ tests

- eg,
- Endotoxin
spiking
- Protein
challenges
- Protein yield
- Adventitious
agents



Bulk product

20+ tests

- eg,
- Amino acid
sequence
- Peptide maps
- IEF
- HPLC
- SDS-PAGE
- RIA
- Receptor
binding
- Bioassays



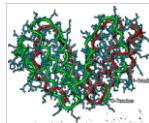
Final product batches

30+ tests

- eg,
- Peptide maps
- IEF
- HPLC
- SDS-PAGE
- Purity
- ELISA
- Potency
- Stability tests

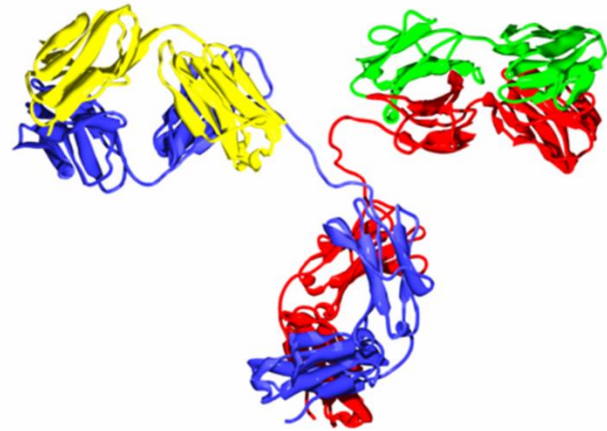


Chemical versus Biological drug



Aspirin

Interferon



Monoclonal Antibody

Chemical versus Biological drug

Small chemical entity	Large, complex biomolecule
Chemical synthesis	Cell cultures
Defined structure	Heterogeneous structures
Not or less sensitive to process changes	Extremely sensitive to process changes
Relatively stable	Variable; sensitive to conditions
Not or less immunogenic	Immunogenic

Post-translational modifications (glycosylation)

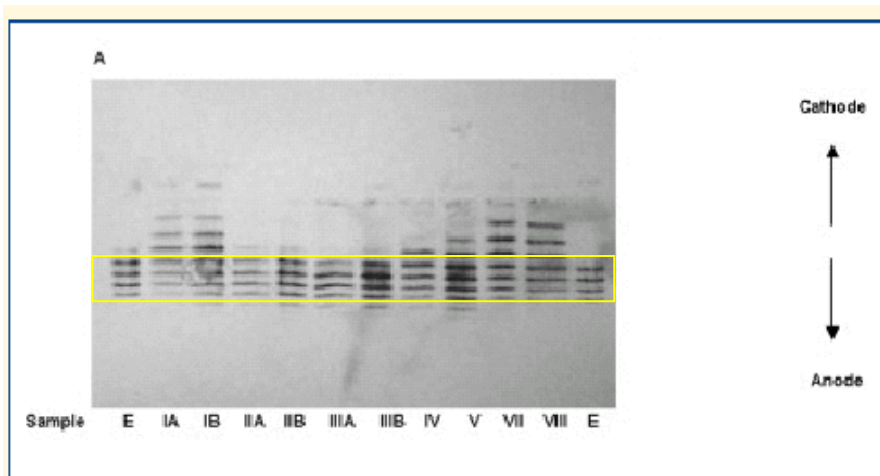


Figure 1
Isoelectric Focusing / Western Blot. Isoform distribution of each sample is shown.

Schellekens H. Nephrol Dial Transplant 2005

Molecular basis of heterogeneity

- Glycosylation
- Phosphorylation
- Sulfation
- Methylation
- N-acylation
- S-Nitrosylation
-
- cell type and culture conditions
- Deamidation (e.g. Asn to Asp)
- Racemization (L to D)
- Oxidation (Met, Tyr, His, Trp)
- Disulfide exchange
-
- External conditions (pH, additives, temperature....)

> 10⁸ variants

Chemical versus Biological drug

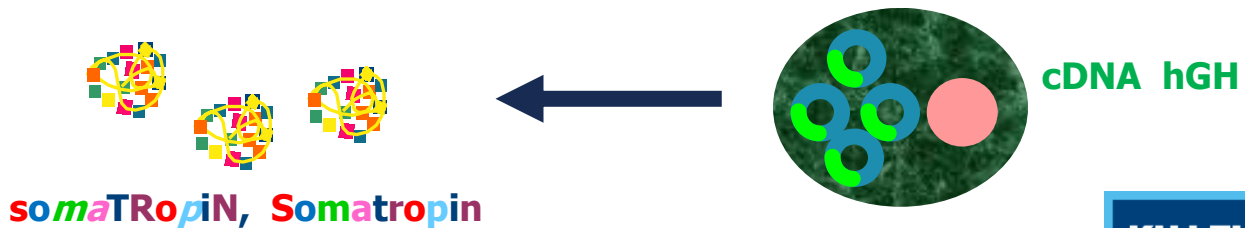
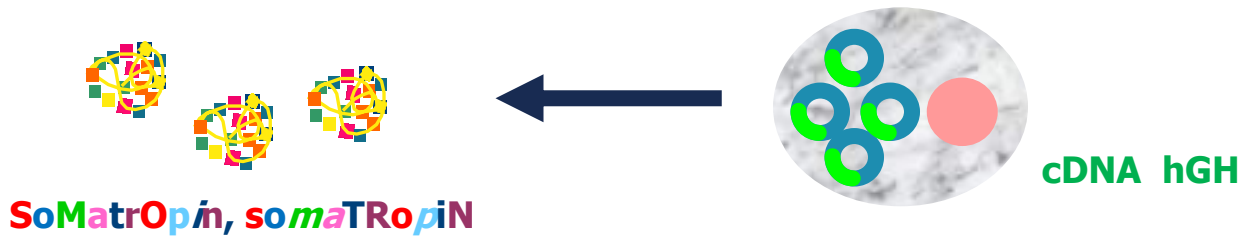
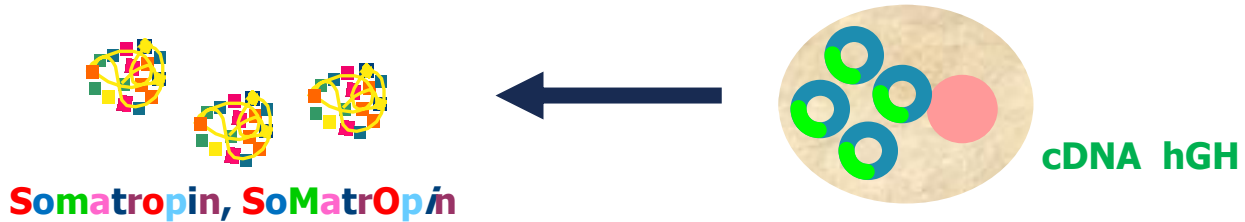
Small chemical entity	Large, complex biomolecule
Chemical synthesis	Cell cultures
Defined structure	Heterogeneous structures
Not or less sensitive to process changes	Extremely sensitive to process changes
Relatively stable	Variable; sensitive to conditions
Not or less immunogenic	Immunogenic

Biological medicinal product

- Always present
- Large number of possible variants
- Impossible to unambiguously identify
- Determined by the entire process
- Reproducibility to be guaranteed by consistency in the production process

The process determines the product

The process determines the product



A new concept

Somatropin, SoMatrOpin

somaTRopiN, Somatropin

SoMatrOpin, somaTRopiN

- Identical ?
- **Biosimilar?**
- Dissimilar ?

- Physicochemical characteristics
- Impurities
- Clinical properties

European Medicines Agency (EMA)

*‘A similar biological or ‘biosimilar’ medicine is a biological medicine that is **similar to another** biological medicine that has **already been authorised** for use.’*

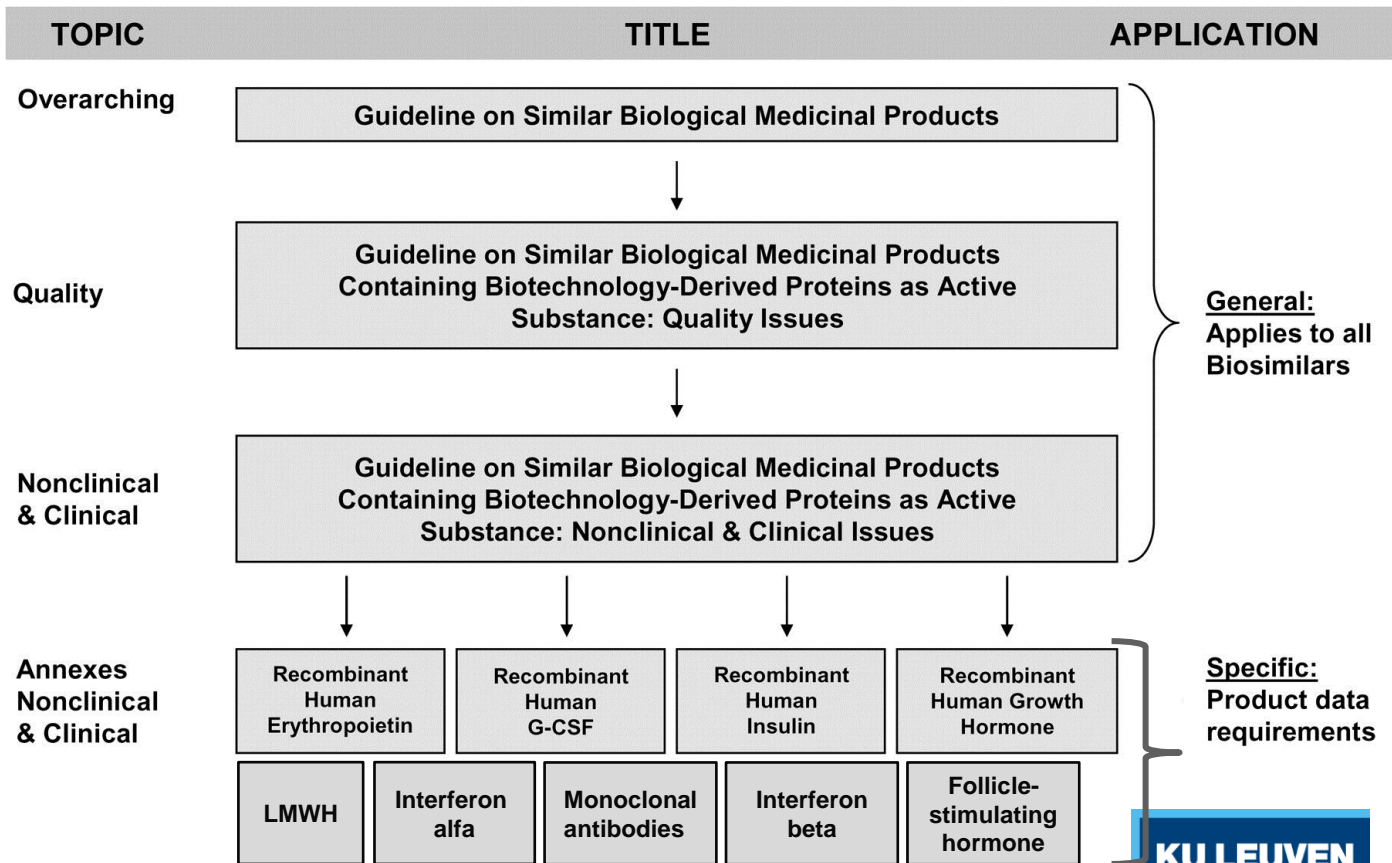
Guidelines

- Biosimilars (EMA, 2006; Australia, Canada, Japan, Korea, ...)
- Similar Biotherapeutic Products (WHO, 2010)
- Biosimilars (FDA, draft 2012; final April 2015)

- Quality, Safety and Efficacy
- Comparability exercises

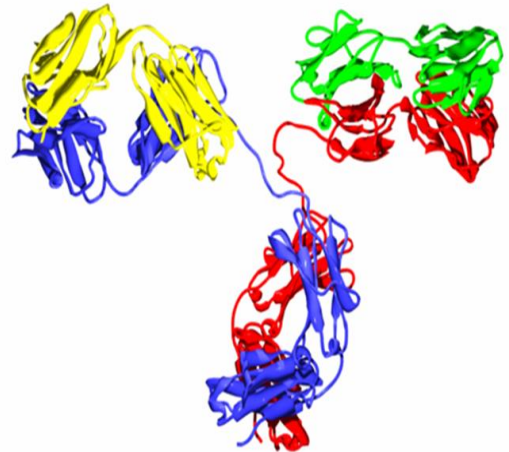
- Authorized reference product

EMA guidelines for biosimilars

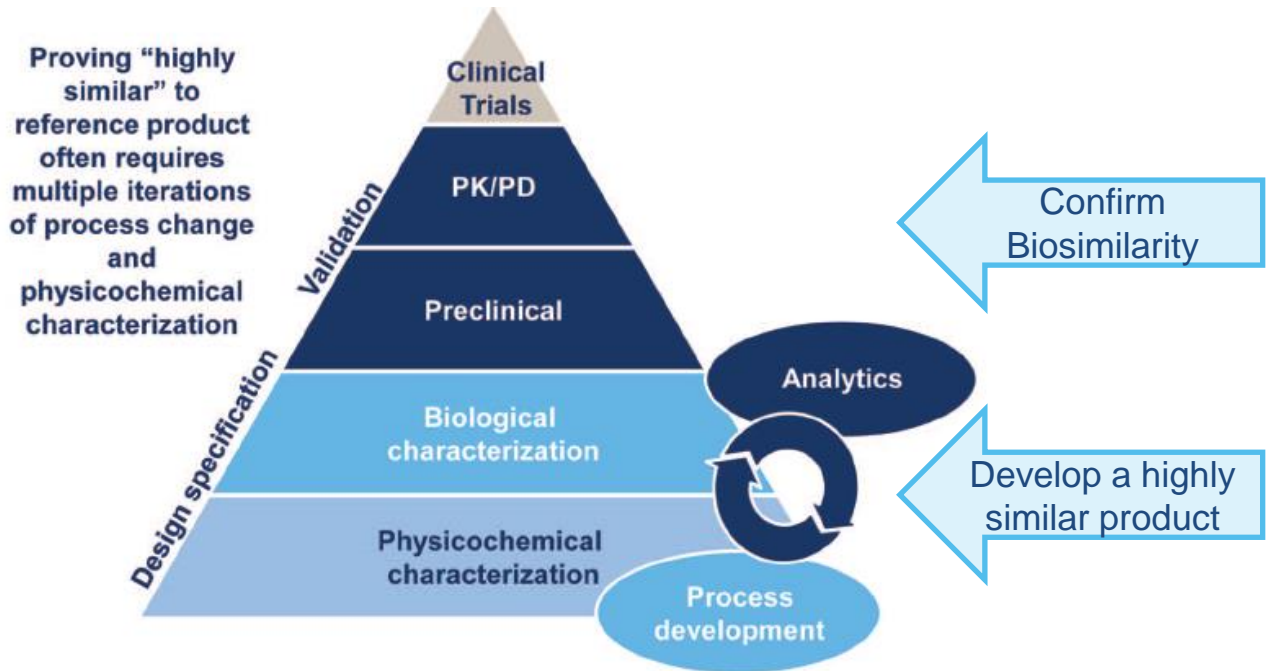


Biosimilar monoclonal antibodies

- **Binding** to target
- **Binding** to
 - FcγRI, FcγRII, FcγRIII
 - FcRn
 - C1q
- *Fab-associated functions* (*neutralization, activation, ...*)
- *Fc-associated functions* (*ADCC, CDC, complement activation, ...*)



Concept of biosimilar development



McCamish. MAbs. 2011;3(2):209-17

KU LEUVEN

Registration requirements (Original)

Quality

- Drug substance
 - Manufacture
 - Characterisation
 - Control
 - Reference standard
 - Container
 - Stability
- Drug product
 - Description
 - Development
 - Manufacture
 - Control
 - Reference standard
 - Container
 - Stability

Nonclinical

- Pharmacology
 - Primary pharm.
 - Secondary pharm.
 - Safety pharm.
 - Interactions
- Pharmacokinetics
 - ADME
 - Interactions
- Toxicology
 - Single dose
 - Repeat dose
 - Genotoxicity
 - Carcinogenicity
 - Reproduction
 - Local tolerance

Clinical

- Pharmacology
- Pharmacokinetics
 - Single dose
 - Repeat dose
 - Special populations
- Efficacy and safety
 - Dose finding
 - Schedule finding
 - Pivotal
 - Indication 1
 - Indication 2
 - Indication 3
 - Indication 4
- Post-marketing studies

Registration requirements (Biosimilar)

Quality

- Drug substance
 - Manufacture
 - Characterisation
 - Control
 - Reference standard
 - Container
 - Stability
- Drug product
 - Description
 - Development
 - Manufacture
 - Control
 - Reference standard
 - Container
 - Stability
- Comparability data
 - Analytical comparison with reference product

Nonclinical

- Pharmacology
 - Primary pharm.
 - Secondary pharm.
 - Safety pharm.
 - Interactions
- Pharmacokinetics
 - ADME
 - Interactions
- Toxicology
 - Single dose
 - Repeat dose
 - Genotoxicity
 - Carcinogenicity
 - Reproduction
 - Local tolerance

Clinical

- Pharmacology
- Pharmacokinetics
 - Single dose
 - Repeat dose
 - Special populations
- Efficacy and safety
 - Dose finding
 - Schedule finding
 - Pivotal
 - Indication 1
 - Indication 2
 - Indication 3
 - Indication 4
- Post-marketing studies
 - Safety in larger population
 - Efficacy in other indications
 - Immunogenicity

Study #1

Study #2

Registration of biosimilars (Europe)

- 2 **refused** by the EU commission:
 - *Interferon alpha-2a* (2006)
 - *Interferon beta-1a* (2009)
- 6 **withdrawn**:
 - *Insulin* (2008)
 - Insulin Rapid
 - Insulin Long
 - Insulin 30/70 Mix
 - *Insulin* (2012)
 - Solumarv
 - Isomarv medium
 - Combimarv

Registration of biosimilars (Europe)

- 21 **approved** in Europa (05/2015)
 - 2 *Human growth hormone* (2006)
 - 3 *Epoietin alfa* (2007)
 - 2 *Epoietin zeta* (2007)
 - 4 *Filgrastim* (2008)
 - 2 *Filgrastim* (2009)
 - 1 *Filgrastim* (2010)
 - 2 *Infliximab* (2013)
 - 1 *Filgrastim* (2013)
 - 1 *Follitropin alfa* (2013)
 - 1 *Follitropin alfa* (2014)
 - 1 *Insulin glargine* (2014)
 - 1 *Filgrastim* (2014)

Registration of biosimilars (Europe)

- 4 **under review** (05/2015)
 - 1 *Insulin human*
 - 1 *Etanercept*
 - 1 *Infliximab*
 - 1 *Enoxaparin*

Registration of biosimilars

- Canada
 - 2 *Infliximab* (2014)
- US
 - 1 *Filgrastim* (2015)

How similar is similar ?

Biosimilar ESA (*)

- “Differences were observed at the **glycosylation level**”
- “Phosphorylated high mannose type structures were detected at higher levels than in Reference ESA”
- “Lower values on N-glycolyl-neuramic acid and diacetylated neuramic acids as compared to Reference ESA”
- “Peptide map showed differences ... in O-linked glycan due to a higher sialylation and lower content of the **oxidized variant**”

Biosimilar hGH (*)

- “The results of this study ... demonstrate that Biosimilar rhGH produced at full scale is comparable to Reference Product”
- “The **impurity profile** of Biosimilar hGH shares some similarity with Reference hGH; however the profiles are not identical”
- “... impurities, ... , are present in the Biosimilar hGH batches and are not in any Reference hGH batches”
- “Additionally, there appears to be a higher level of **deamidated variants** in the Biosimilar hGH samples”

Biosimilar IFX (*)

- “..... all major physicochemical characteristics and biological activities of biosimilar IFX were comparable to those of the reference product”
- “...difference in the amount of **afucosylated** infliximab, translating into a lower binding affinity towards FcγR11a receptors and a lower ex vivo antibody-dependent cellular cytotoxicity (ADCC) activity....”
- “... less intact IgG , mainly due to a higher proportion of non-assembled form. unlikely to impact its biological activity”
- “a higher level of **C-terminal lysine** variability”
- “...slightly higher level of **aggregates** ...”

Similar, not identical – as predicted differences are observed

(*) Based upon European Public Assessment Report on respective biosimilars.

How similar are biosimilars ?

Primary end point: number of oocytes retrieved

	Gonal-f® n=123	Bemfola® n=249
Number of oocytes Mean (SD)	11 (6)	11 (5)

Pregnancy follow up

	Gonal-f®	Bemfola®
Pregnancy rate per patient	41 %	34 %
Take-home baby rate	41 %	32 %

From European Public Assessment Report Bemfola

Biosimilars: *extrapolation of indications*

Remicade approved indications

- Rheumatoid arthritis
- Adult Crohn's disease
- Paediatric Crohn's disease
- Ulcerative colitis
- Paediatric ulcerative colitis
- Ankylosing spondylitis
- Psoriatic arthritis
- Psoriasis



Remsima/Inflectra approved indications

- **PK study in AS (Phase I, 250 patients)**
- **Equivalence trial in RA (Phase III, 606 patients)**
- **randomised, double-blind, comparative study in active Crohn's disease planned**

- Rheumatoid arthritis
- Adult Crohn's disease
- Paediatric Crohn's disease
- Ulcerative colitis
- Paediatric ulcerative colitis
- Ankylosing spondylitis
- Psoriatic arthritis
- Psoriasis

extrapolated indications in light blue

REMSIMA European Public Assessment Report.
http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002576/WC500151486.pdf

Biosimilarity \neq Interchangeability

- **Not identical** to reference
- Claim for interchangeability **needs to be proven** (in both directions!) and holds only for the two products evaluated
- **Divergence** over time
- Two or more **biosimilars** from the same reference product **have not been compared** to each other.

Conclusions

- Complex (multi-domain) molecules
- Properties are process-dependent
- Biosimilars are similar but not identical to reference product
- Approved: pharmaceutical quality demonstrated
- Approved: limited clinical experience
- Non-interchangeable
- Follow-up measures