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The European Biosimilar Experience

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Biological medicinal product

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

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....)

> 10^8 variants

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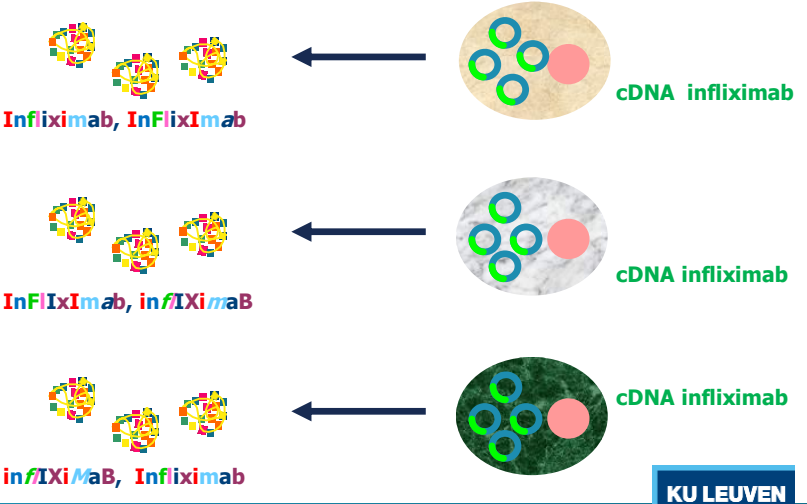
The process determines the product



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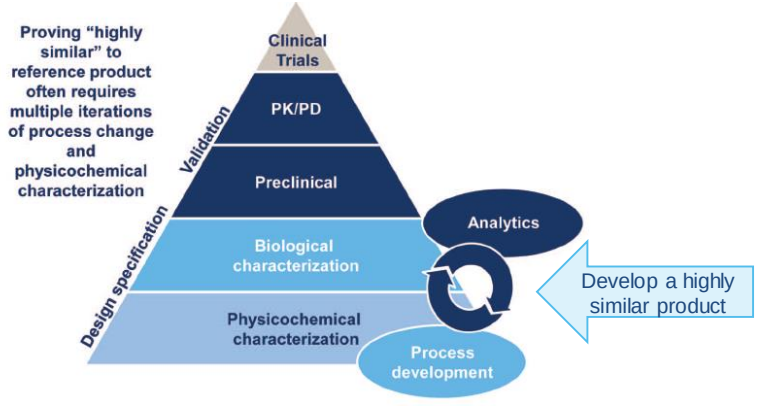
Concept of biosimilar development



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

Concept of biosimilar development

Investigate reference product quality attributes to understand target variability, create boundaries within which to be "similar"

(Different lots examined over time)

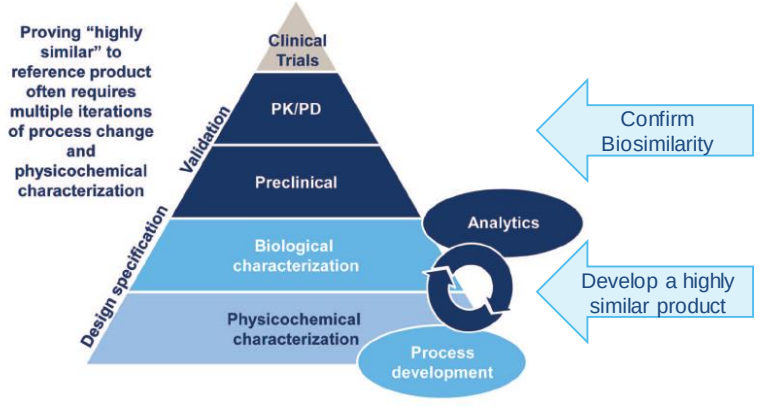
Identify genetic information that codes for desired primary amino-acid sequence and clone into host cell using vector

(Same primary amino acid sequence)(Different cell line may be used)

Iterative process used to identify clones, producing protein with target posttranslational modifications

Converge to target product profile by iterative optimization of process

Concept of biosimilar development



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

Registration requirements (Original)

Quality	Nonclinical	Clinical

• Drug substance
  • Manufacture
  • Characterisation
  • Control
  • Reference standard
  • Container
  • Stability
• Drug product
  • Description
  • Development
  • Manufacture
  • Control
  • Reference standard
  • Container
  • Stability
	• Pharmacology
	  • Primary pharm.
	  • Secondary pharm.
	  • Safety pharm.
	  • Interactions
	• Pharmacokinetics
	  • ADME
	  • Interactions
	• Toxicology
	  • Single dose
	  • Repeat dose
	  • Genotoxicity
	  • Carcinogenicity
	  • Reproduction
	  • Local tolerance
		• 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	Nonclinical	Clinical

• 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
	• Pharmacology
	  • Primary pharm.
	  • Secondary pharm.
	  • Safety pharm.
	  • Interactions
	• Pharmacokinetics
	  • ADME
	  • Interactions
	• Toxicology
	  • Single dose
	  • Repeat dose
	  • Genotoxicity
	  • Carcinogenicity
	  • Reproduction
	  • Local tolerance
		• Pharmacology
		• Pharmacokinetics (Study #1)
		  • Single dose
		  • Repeat dose
		  • Special populations
		• Efficacy and safety (Study #2)
		  • 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

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Registration of biosimilars (Europe)

- 2 refused by the EU commission:
  - *Interferon alpha-2a* (2006)
  - *Insulin human* (2015)
- 6 withdrawn:
  - *Insulin* (2008)
    - Insulin Rapid
    - Insulin Long
    - Insulin 30/70 Mix
  - *Insulin* (2012)
    - Solumarv
    - Isomarv medium
    - Combimarv

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Why refused?

Solumarv (human insulin)

- Insufficient details on manufacturing process
- Insufficiently demonstrated whether clinical study batches are representative for market batches
- Insufficiently shown that quality of proposed biosimilar is comparable to the reference product

From European Public Assesment Report Solumarv®

Why refused?

Alpheon (Interferon alfa-2a)

- Differences with reference product (e.g. impurities)
- Not enough data on stability
- Inadequate validation of process to make the finished drug product
- Lower efficacy
- More side effects
- Inadequately validated test to evaluate the potential to trigger an immunological response

From European Public Assesment Report Alpheon®

Registration of biosimilars (Europe)

- 28 approved in Europa (11/2016)
  - *2 Human growth hormone* (2006)
  - *3 Epoietin alfa* (2007)
  - *2 Epoietin zeta* (2007)
  - *9 Filgrastim* (2008 (4), 2009 (2), 2010, 2013, 2014)
  - *3 Infliximab* (2013 (2), 2016)
  - *2 Follitropin alfa* (2013, 2014)
  - *2 Insulin glargine* (2014, 2016)
  - *1 Etanercept* (2016)
  - *2 Enoxaparin* (2016)
  - *2 Teriparatide* (2016)

Registration of biosimilars (Europe)

- 16 under review (11/2016)
  - *2* ***Etanercept***
  - *2* ***Rituximab***
  - *4* ***Pegfilgrastim***
  - *3* ***Adalimumab***
  - *1* ***Insulin*** *glargine*
  - *3* ***Trastuzumab***
  - ***1 Insulin Lispro***

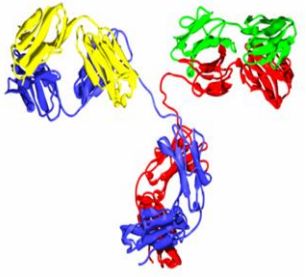
Physicochemical Comparability Tests: Analytics Set the Foundation

Test Method	Compares...
Amino acid analysis	Amino acid composition
Peptide mapping (LC-MS) in combination with MS/MS	Peptide coverage and chemical modifications
Peptide mapping (HPLC)	Tryptic peptide map by visual inspection
N-terminal sequencing	N-terminal sequences
C-terminal sequencing	C-terminal sequences
Reduced mass	Molecular weights by mass spectrometry
Disulfide bonds	Disulfide bonds location
Free thiol analysis	Amount of free sulfhydryl groups
FTIR	Secondary structures
CD	Secondary structure
DSC	Thermal stability; also determines thermal transition temperatures

Test Method	Compares…
	Purity/Impurity
SEC-HPLC	Aggregate content and monomeric purity
CE-SDS (reduced/nonreduced)	Electrophoretic mobility and purity under nonreducing and reducing conditions
	Charged Isoforms
IEF	Isoelectric point(s)
IEC-HPLC	Charge variant distribution
	Glycosylation
Sialic acid analysis	Sialic acid content
Monosaccharide analysis	Neutral and amino sugar composition
Oligosaccharide profiling	Glycosylation pattern (eg,G0F, G1F, G2F)
N-linked glycan analysis	Oligosaccharide structures, attachment sites, and distribution
	Content
UV_{280}	Protein concentration
ELISA	API content

Biochemical and functional Comparability Tests

- Binding to target
- Binding to
  - FcγRI, FcγRII, FcγRIII
  - FcRn
  - C1q



- *Fab*-associated functions (*neutralization, activation, ...*)
- *Fc*-associated functions (*ADCC, CDC, complement activation, ...*)

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How similar are biosimilars ?

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 FcγRIIIa 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 …"

Biosimilars are Similar, not identical

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

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How similar are biosimilars ?

Physicochemical / pre-clinical

CT-P13 versus Infliximab reference

- Lower degree of afucosylation
  ⇩
- Lower binding affinity to $Fc_{\gamma}RIIIa$
  ⇩
- Lower activity in the most sensitive ADCC assay

- "*However, no difference could be detected in several experiments that are more representative of pathophysiological conditions, and therefore more relevant clinically*."

From European Public Assesment Report Remsima®

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How similar are biosimilars ?

Physicochemical / pre-clinical

SB2 versus Infliximab reference

- Lower degree of C-Lys variants (⇦ CHO versus SP2/0 host cell)
- Lower % of charged variants
- Higher % of High Molecular Weight variants
- Higher binding affinity to $Fc_{\gamma}RIIIa$ (114-141% *vs.* 77-108%) but without impact on ADCC assay

From European Public Assesment Report Flixabi®

How similar are biosimilars ?

Immunological events

SB2 versus Infliximab reference

- Higher incidence of ADA formation in patients (47 % *vs.* 38 % at day 71)
- Impact of ADA on efficacy is not clear (CHMP: Divergent opinion 14/36 negative)
- Data from studies in presence of MTX → extrapolation of immunogenicity to other indications?

From European Public Assesment Report Flixabi®

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of a marketing authorisation of Flixabi as a biosimilar to Remicade and in the indications licensed to Remicade.
The reason for the divergent opinion was the following:

- Flixabi appears to be associated with a higher incidence of ADA than the originator, Remicade. It is acknowledged that it cannot be excluded that the observed difference was a chance finding or a finding associated with limitations in the immunogenicity assays that were used. However, an increased incidence was observed both in the Phase 1 and the Phase 3 studies, and it has not been substantiated that the difference was an artefact due to – for example – problems with the interpretation with the immunogenicity assays that were used.

- In the Phase 3 trial, which was conducted in patients with rheumatoid arthritis, the efficacy of Flixabi, whilst meeting the pre-specified equivalence margins, was consistently, although not universally, estimated to be lower than that of Remicade. It is not possible with reasonable certainty to exclude that the estimated reduction in efficacy of Flixabi was the result of the higher incidence of ADA. In this regard, it is noteworthy that the Phase 3 study showed that the efficacy, regardless of treatment group, was significantly lower in ADA positive patients than in ADA negative patients.

- Since the patients with rheumatoid arthritis investigated in the Phase 3 trial are treated concomitantly with immunomodulator therapy, they may exhibit less immunogenicity than patients in other infliximab-licensed indications. The consequences of any difference on ADA incidence, and consequently the impact on efficacy in these indications are unclear.

- It is considered that the uncertainties outlined above should be resolved by the Applicant before licensing.

- The proposal by the Applicant to resolve the concerns related to immunogenicity in the post-marketing setting by initiating a prospective observational cohort study in the indications of ankylosing spondylitis and Crohn's disease is considered inadequate. In addition, it is questionable to what extent a non-randomised, observational study can provide data that will effectively address the uncertainties.

In conclusion, the undersigned CHMP members consider the benefit-risk balance of Flixabi to be negative since biosimilarity to Remicade has not been established.

How similar are biosimilars ?

Physicochemical / pre-clinical

SB4 versus Etanercept reference

- Lower degree of C-Lys variants (⇦ CHO versus SP2/0 host cell ?)
- Differences in charged variants
- Higher degree of afucosylation (*considered not clinically relevant because not involved in Mode of Action*)

- Slightly less effective in a mouse model of arthritis (*not confirmed in clinical studies*)

From European Public Assesment Report Benepali®

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How similar are biosimilars ?

Immunological events

SB4 versus Etanercept reference

- Significantly lower incidence of ADA formation in patients (overall 1 % *vs.* 13 % at wk 52)
- Reanalysis excluding wk 4 and 8: 0.3 *vs.* 0.7%

- Impact of assay methodology –low drug tolerance: data affected by trough levels, the latter were different at wk 4/8, thus reanalysis after excluding ADA data at wk 4/8

- *"…. it is premature to conclude that SB4 is less immunogenic than reference …."*

From European Public Assesment Report Benepali®

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How similar are biosimilars ?

Adverse events

XM17 and Afolia versus FSH reference

	Study XM17		Study Afolia	
	Reference (n=145)	XM17 (n=152)	Reference (n=123)	Afolia (n=249)
Ovarian Hyperstimulation Syndrome (OHSS)	2.7%	4.6%	13 %	32 %

"….. The following parameters [dose, oestradiol,body weight, age] were comparable….. and cannot explain the difference that is observed in OHSS. The observed difference could therefore be a chance finding. "
(European Public Assesment Report Ovaleap®)

From European Public Assesment Report Ovaleap® and Bemfola®

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Chemical drugs

Ref

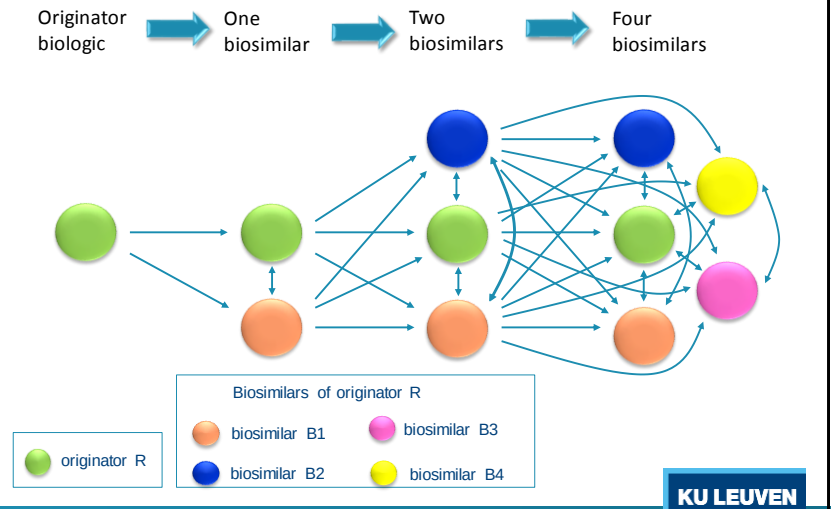
A = B

Biological drugs

Ref

A ? ? B

Patients Could Face Multiple Switches Between Biosimilars to the Same Originator



Adapted from NIBRT ©

Biological drugs

- Substitution/switching is contraindicated
- Physician control over prescribing
- Appropriate (brand)naming required
- International pharmacovigilance systems should be imposed that enable unambiguous identification of the product associated with an adverse event

Conclusions

- The concept for biosimilar development is well-defined
- The process for approval is rigorous
- Pharmaceutical quality of approved biosimilar is guaranteed
- Differences in quality attributes are always present
- Major challenges include the identification of the potential clinical relevance of differences in quality attributes and non-clinical properties

Conclusions

- Residual uncertainties (scientifically or statistically) have so far always been deemed to have no impact on safety and efficacy
- EPARs contain heterogenous information not consistent between different biosimilars for the same reference product
- To date, (multiple) switching between a reference and its respective biosimilars can not be recommended since no solid scientific data are available

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