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# Insulin Detemir, Certolizumab PEGOL & Others

Technology from the group of <u>Natasa Skoko</u> at International Centre for Genetic Engineering and Biotechnology, Trieste, Italy



TechEx.in Case Manager:

Match Maker/ Biosimilars / 31 Aug 2021/DrSkoko ICGEB

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## About Certolizumab PEGOL

Certolizumab Pegol is a pegylated Fab fragment of a humanized anti-TNF IgG molecule

- Originator / reference product: The originator product, UCB's Cimzia (certolizumab pegol), was approved by the US Food and Drug Administration (FDA) in April 2008 and by the European Medicines Agency (EMA) in October 2009. The patents on Certolizumab PEGOL will expire in Europe in 2021 and in the US in 2024. (Source: GaBI Online)
- Indications: Treatment of adult patients with moderate to severe Rheumatoid Arthritis, Crohn's disease, psoriatic arthritis and ankylosing spondylitis.

## Market and Industry Overview

#### Market:

Estimated global market for Certolizumab **by 2023 is \$0.5 billion** (Source: Market Research)

### **Industry players:**

- Global: UCB, Pfenex (in the pipeline)
- India : None



## The Opportunity: Why you should be interested?

- Market interesting: a) The patents on Cimzia (Certolizumab PEGOL) will expire in the US in 2024.
  Next generation Biosimilar b) Certolizumab pegol is currently the only PEGylated anti-TNFα biologic approved for the treatment of Rheumatoid Arthritis and Crohn's disease.
- Industry not yet crowded: Sole manufacturer of Cimzia in UCB. Opportunity for other companies.

# The Technology Offering



We produce Certolizumab by fermentation in the periplasm of E.coli in a native state with approx. yield of 250 mg/L (postcapturing purification step)

SDS-PAGE analysis of Certolizumab pools:

Lane 1: Certolizumab after first chromatography capture step Lane 2: Flow through from first chromatography capture step Lane 3: Molecular weight marker

## The Technology Offering



- We developed the process for the synthesis of the branched PEG reagent for PEGylation of Certolizumab to obtain Certolizumab pegol, at gram scale with activity and purity > 95%
  - Preparation of PEGylation reagent in around 10 g scale with **yield** of over 85%, purity of over 95% and activity of over 95%.

## Selected Data: Biosimilarity- Intact mass analysis



LC-MS (RP-HPLC) of Certolizumab after first chromatography capture step **shows purity >80%**.

Intact Protein mass analysis of Certolizumab is also performed after chromatography capture step: ESI-MS determined molecular mass is 47762.64 in **agreement with the calculated average molecular mass** of 47759.98.

## Selected Data: Biosimilarity- Peptide mapping

	New interchain set0:light chain:Amino Acids					r rotein.	New interchain set0:heavy chain:Amino Acids			
Intensity coverage	e: 0.2 % (8410	050 cnts) Seque	nce coverage MS:	37.4 % Sequen	ce coveraç	Intensity covera	ge: 0.1 % (544	273 cnts) Seque	nce coverage MS:	45.9 % Seque
10	20	30	40	50		10	20	30	40	50
DIQMTQSPSS	LSASVGDRVT	ITCKASONVG	TNVAUYQQKP	GKAPKALIYS	-	EVQLVESGGG	LVQPGGSLRL	SCAASGYVFT	DYGMNUVRQA	PGKGLEWMGW
60	70	80	90	100		60	70	80	90	100
ASFLYSGVPY	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQQ	YNIYPLTFGQ		INTYIGEPIY	ADSVKGRFTF	SLDTSKSTAY	LQMNSLRAED	TAVYYCARGY
110	120	130	140	150		110	120	130	140	150
GTKVEIKRTV	AAPSVFIFPP	SDEQLKSGTA	SVVCLLNNFY	PREAKVQWKV		RSYAMDYUGQ	GTLVTVSSAS	TKGPSVFPLA	PSSKSTSGGT	AALGCLVKDY
160	170	180	190	200		160	170	180	190	200
DNALQSGNSQ 210	ESVTEQDSKD	STYSLSSTLT	LSKADYEKHK	VYACEVTHQG		210	220	230	ISLSSVVIVP	2221010111

Peptide mapping and MS/MS analysis of protein band from SDS-PAGE: MS/MS sequencing showing partial coverage in both heavy and light chains of the protein, especially in the N-termini portions.

## Selected Data: Purity and activity



RP-HPLC-UV-ELSD analysis of the PEGylation reagent for Certolizumab: the PEG reagent maleimide activated is reacted with a thiol containing tracer **demonstrating** > 97% purity and activity (peak n.2, red trace is ELSD)

# Current Status of Technology and Path Ahead

#### Stage of Development

- Protein expressed in shake flask and 10L bioreactor.
- Achieved yield of 250 mg/L (post-capturing purification step)

Development of Hypotheses and Experimental Designs

Non-clinical *in-vitro* studies: Physicochemical characterization for Biosimilarity

Non-clinical in-vitro studies: Functional characterization for Biosimilarity

Non-clinical animal studies: toxicity, PK/PD, immunogenecity

Generation of three consistent batches. Formulation development. Approvals for preclinical candidate compound from the relevant body.

Clinical studies: PK, PD, Immunigenecity

**Regulated Production, Regulatory Submission** 

Scale-up, Completion of GMP Process Validation and Consistency Lot Manufacturing and Regulatory Approvals.

Clinical Trials Phase 3 and Approval or Licensure

Seeking Industrial partners interested in:

- R& D Collaboration : To increase the production yield, optimize purification steps and develop conjugation step
- Technology co-development: To carry out further development/validation work
- Technology licensing: For commercializing Certolizumab PEGOL

### Dr Natasa Skoko's Group: Biotechnology Development Unit



#### Lead Scientist: Dr Natasa Skoko

Group Leader, Biotechnology Development Unit, ICGEB, Italy Member and reviewer, Women in Science in the Developing World Expertise: Production of biologics in bacteria, yeast and mammalian cells, bioprocessing operations such as upstream, downstream and quality control analysis following European Pharmacopoeia monographs

- Key assets and strengths of Dr Skoko's Lab:
  - Authored more than 20 publications in her areas of expertise
  - Team strength: 8
  - Well equipped labs and analytical facilities
    - Microbial and mammalian cell line facility
    - Downstream processing, chemical lab and QC lab
    - Clean rooms in Class C and D
  - Industry Project /Tech transfer
    - More that 25 years of experience in the field of biologics/biosimilars, more than 70 technology transfer agreements with companies
    - Companies from 22 countries, more than 100 scientists

trained in our lab







## For more information contact:

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