TechEx.in is a Regional Tech Transfer Office supported by:

Insulin Detemir, Certolizumab PEGOL & Others

Technology from the group of **Natasa Skoko**
at **International Centre for Genetic Engineering and Biotechnology, Trieste, Italy**

TechEx.in Case Manager:
Pradnya Aradhye, pradnya@venturecenter.co.in

Outline

- About the **International Centre for Genetic Engineering and Biotechnology**, Trieste, Italy
- About **Biotechnology Development Unit**
- Technology 1: Insulin Detemir
- Technology 2: Certolizumab PEGOL
- Other Capabilities and Offerings

ICGEB International Centre for Genetic Engineering and Biotechnology

80+ Signatory Countries, 60+ Member States, 3 Components:
Trieste (Italy) - New Delhi (India) - CapeTown (South Africa)

TIMELINE

1995 Biotechnology Development Unit (BDU) is launched

1997 IFN alfa2a/b technology

1998 First Technology Transfer (in-house training)

2000 EPO alfa technology

2002 GCSF technology

2005 PEG-IFN alfa 2a technology

2007 Pilot scale IFN alfa 2a

2009 Pilot scale GCSF

2012 PEG-GCSF technology

2013 PEG-EPO technology

2014 Insulin technology

2015 EPO beta technology

2016 Growth hormone technology

2018 BDU receives grant from the FVG for the construction of a new facility for development of biosimilars

2019 Long-lasting insulins technology

2020 First video-based Technology Transfer training

2021 Opening of a new BDU facility and GMP-certification for QC lab

Match Maker

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

Biotechnology Development Unit-upstream and downstream

Parallel bioreactors (2x2L)

Bioreactor for bacteria/yeast (30L)

Parallel single use bioreactors (4x250ml)

Bioreactor for mammalian cells (2.5L)

Clonpix2+imager

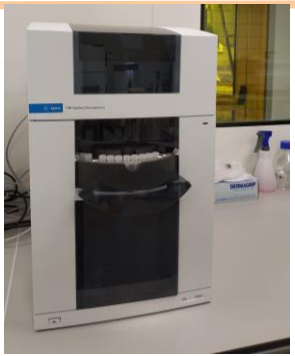
3 AKTA pure (25 and 2x150)

Biotechnology Development Unit-Quality Control lab

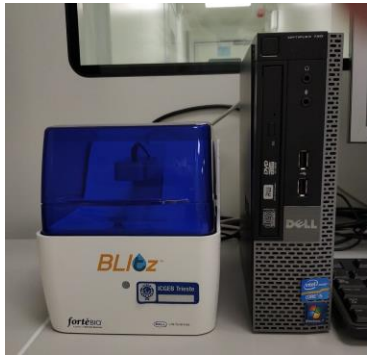
UPLC

HPLC

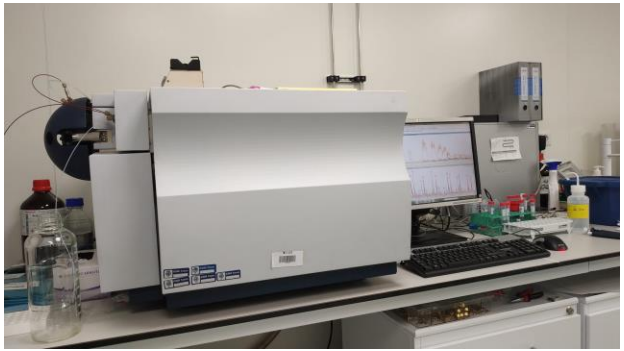
Capillary Electrophoresis



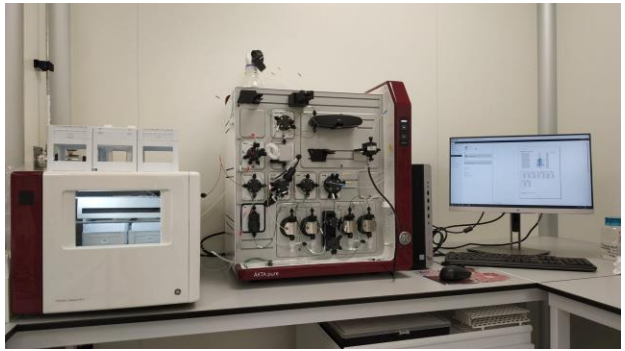
Blitz system (Bio-Layer Interferometry)



Mass spectrometer



AKTA pure 25 ml/min

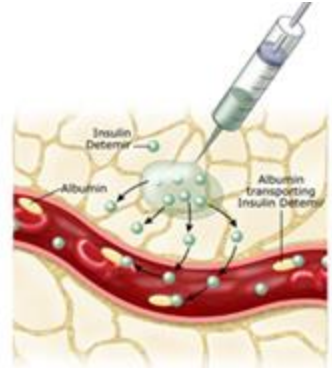


Technology 1: Insulin Detemir

About Insulin Detemir

Insulin detemir is a **long-acting** (up to 24-hour duration of action) recombinant **human insulin analog.**

- **Originator / reference product:** Long lasting insulin analogues Detemir (Novo Nordisk's Levemir®, the **patent expired in 2019 in US.** (Source: Novo Nordisk Annual Report)
- **Indications:** Treatment of **Type 1 and 2 Diabetes Mellitus**

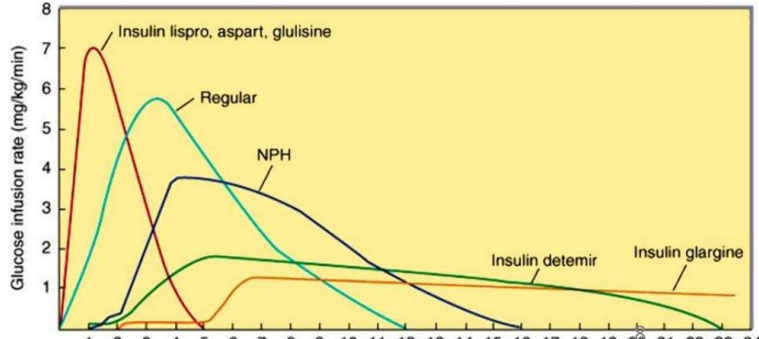


Detemir

[LysB29-tetradecanoyl, des(B30)]human insulin

once-daily administration

Detemir differs from human insulin in that the amino acid threonine in position B30 has been omitted and a C14 fatty acid chain has been attached to the side chain of amino acid B29Lys.



Market and Industry Overview

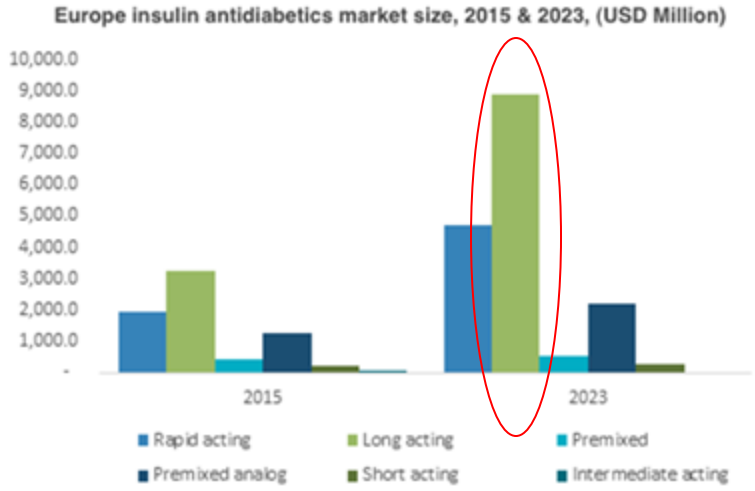
Market:

The global basal insulin market (Detemir, Glargine and Degludec) is expected to register **a CAGR of 8.2% during the forecast period of 2019–2024**, the market is estimated to reach **$11.4 billion by 2019**. (Source: Research and Markets)

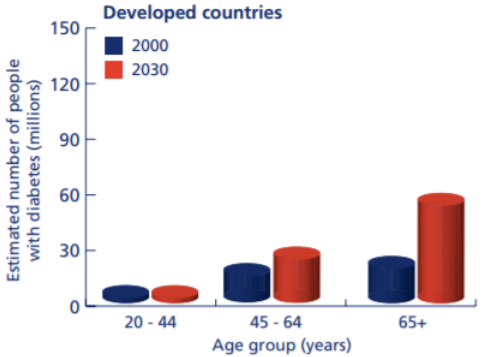
Industry players:

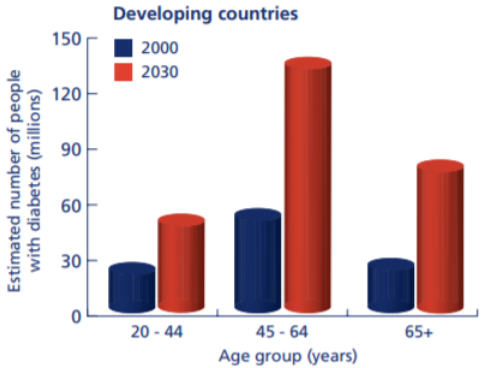
- Global: Novo Nordisk, MNKD, Bristol-Meyer Squibbs, Emisphere

- India : Biocon



The Opportunity: Why you should be interested?





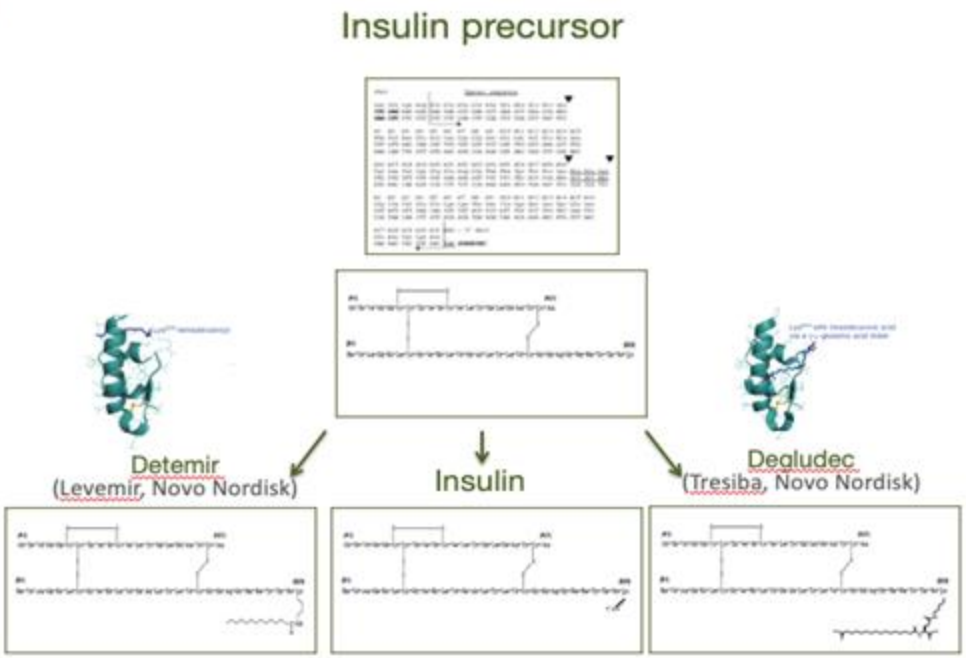
Estimated number of adults with diabetes.

- **Market interesting: A rising global burden:** According to World Health Organization (WHO), the number of people with diabetes will more than double over the next 25 years, to reach a **total of 366 million by 2030**. Most of this increase will occur as a result of a **150% rise in developing countries.** (Source: WHO)
- **Cost still high:** Levemir (Insulin Detemir) treatment (taken once a day) costs around **$500/month** and annual cost of treatment is around **$6,000 (very high, if it is to be taken for a lifetime)**. Insulin Detemir is to be taken for a lifetime.
- **Industry not yet crowded: Few players** manufacturing long acting insulins

The Technology Offering

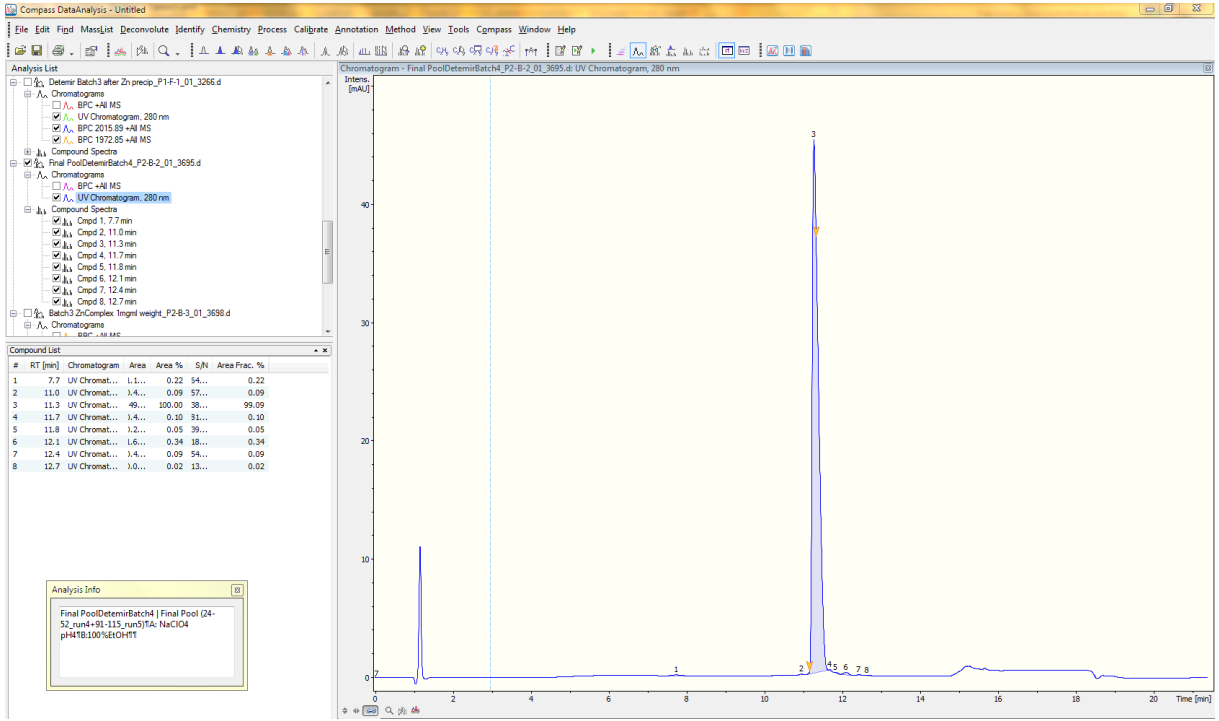
Insulin precursor

- Insulin precursor is produced in the yeast ***Pichia pastoris*** .
- From the insulin precursor we developed three technologies: **short lasting insulin and two long-lasting insulins (detemir and degludec).**
- **Yield** of Insulin precursor: **3-4 g/L**



Selected Data: Biosimilarity- Physicochemical characterization

RP-HPLC analysis: The purity of purified Detemir pool is **> 98%**

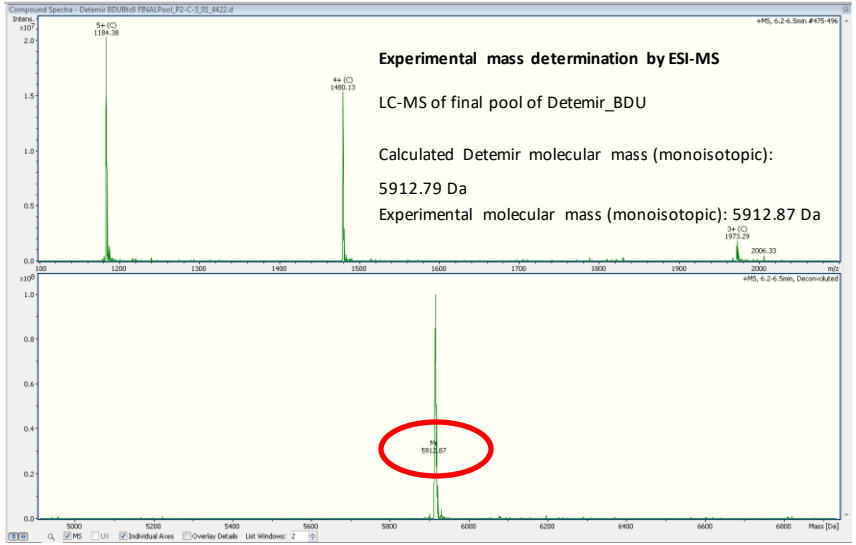


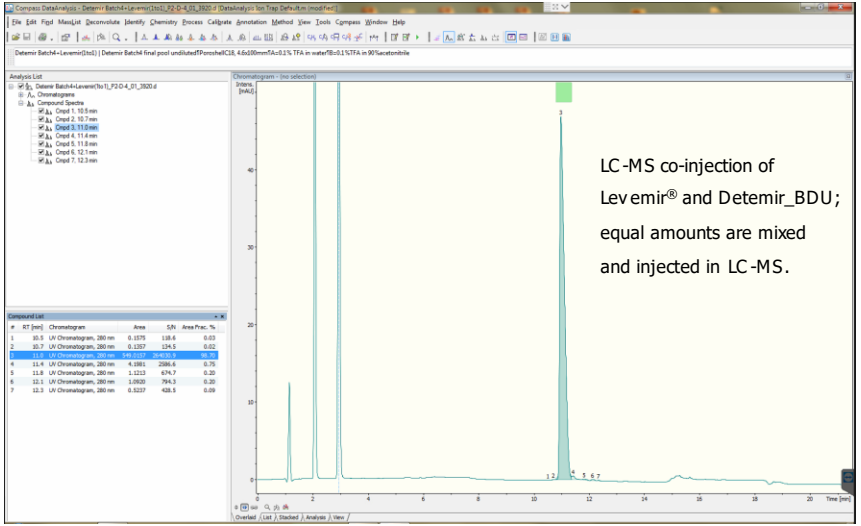
Selected Data: Biosimilarity - Intact mass analysis

Biosimilarity - Intact mass analysis and co-injection with originator

LC-MS with full protein MS: Electrospray Mass Spectrometry **confirms the correct molecular mass of Detemir**.

LC-MS co-injection of Levemir® and Insulin Detemir_BDU confirms **that Detemir BDU has the same Retention Time as commercial Levemir®**

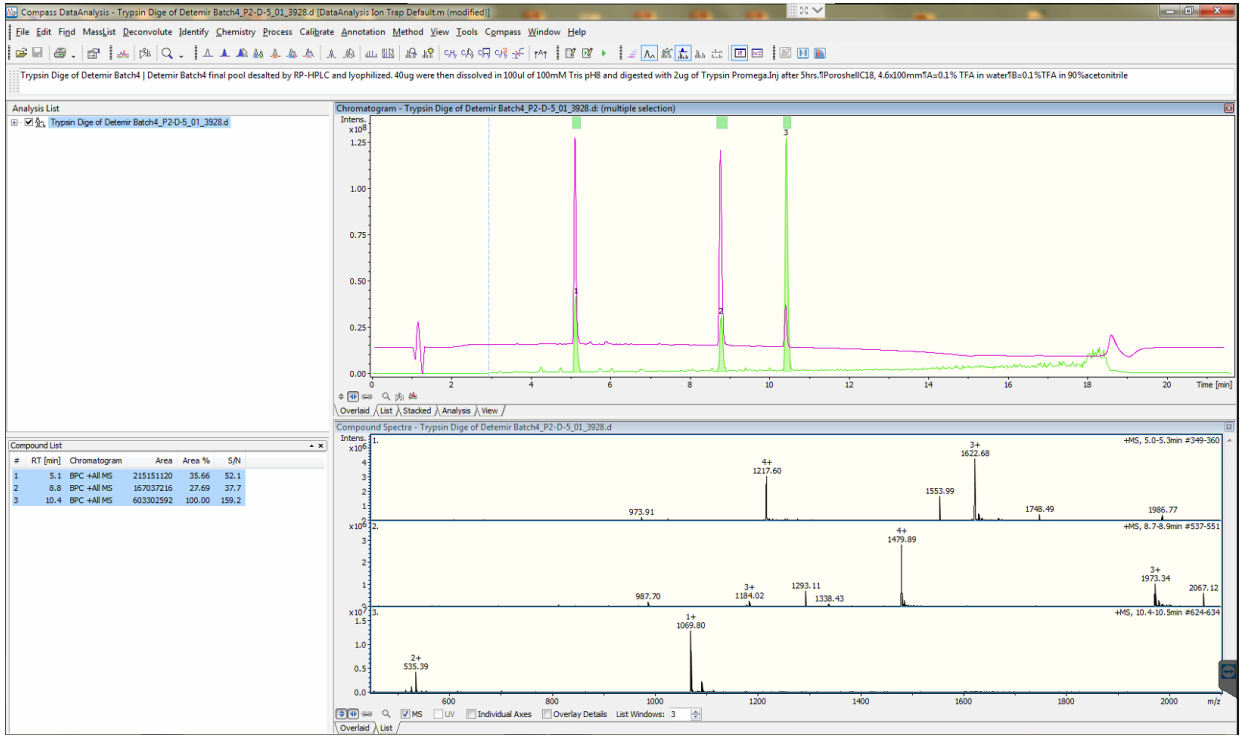




Selected Data: Biosimilarity- Peptide mapping

Biosimilarity - Peptide mapping

Determination of site of fatty acid attachment: experimental peptide mapping (trypsin digestion) **corresponds to the theoretical one and demonstrates the site of fatty acid attachment in B29 Lys.**



Selected Data-Insulin Precursor production and Detemir preparation

Production: 10L fermentation (5 day methanol induction) **yields around 30-40 g of Insulin precursor**.

Purification:

- **Preparation of desb30:** DesB30 by protease digestion of **recombinant Human Insulin Precursor** is performed at 2.3 grams scale with the yield of at least 50%
- **Preparation of linker:** Preparation of Tetradecanoic acid 2,5-dioxo-pyrrolidin-1-yl active ester linker (Myr-OSu) is performed at 10 grams scale with the yield of at least 60%
- **Synthesis and detemir purification:** Attachment of Myr-OSu linker to DesB30 **Human Insulin (1.5 gram scale) is performed to obtain Insulin Detemir**. Preparative RPHPLC separation/purification of **Detemir yields at least 20% in respect to recombinant Human Insulin Precursor.**

Current Status of Technology and Path Ahead

Stage of Development

- Protein expressed in 10L bioreactor.
- Achieved yield of **30-40 g (Insulin precursor) in 10L bioreactor**.

What are we seeking?

Technology ready to transfer on non-exclusive basis. Transferred to different entities from China, South Africa, Bangladesh and Iran so far.

We offer TT package and training in-house or video-based training.

PHASE1

•Scientists from the Company spend **4-6 weeks** in the ICGEB Laboratories gaining hands-on experience in the production of selected technologies OR **video-based train**ing and online technical assistance.

•Supply of **Protocols** describing process for the development of cell lines and complete down and upstream procedures and QC

PHASE 2

•Post training **assistance** to the industrial partner in establishing the process at its own facility

Technology 2: Certolizumab PEGOL

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

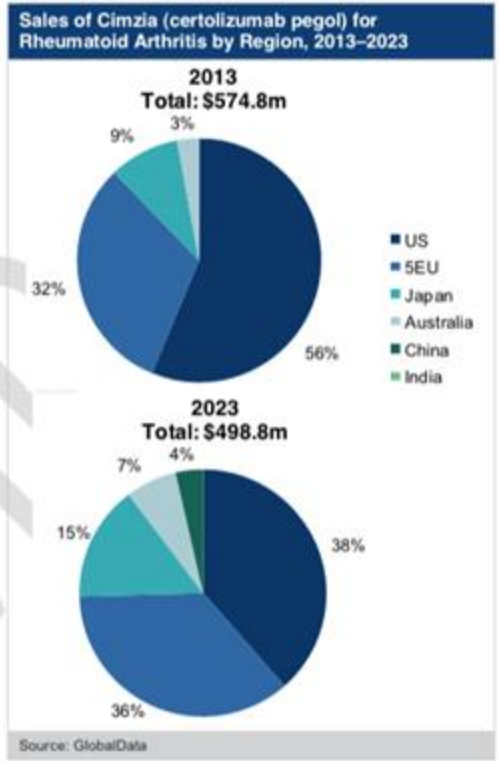
Market:

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

Industry players:

- **Global:** UCB, Pfenex (in the pipeline)

- **India :** None

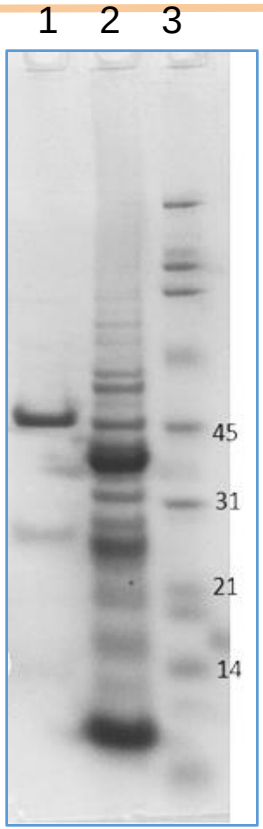


The Opportunity: Why you should be interested?

- **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

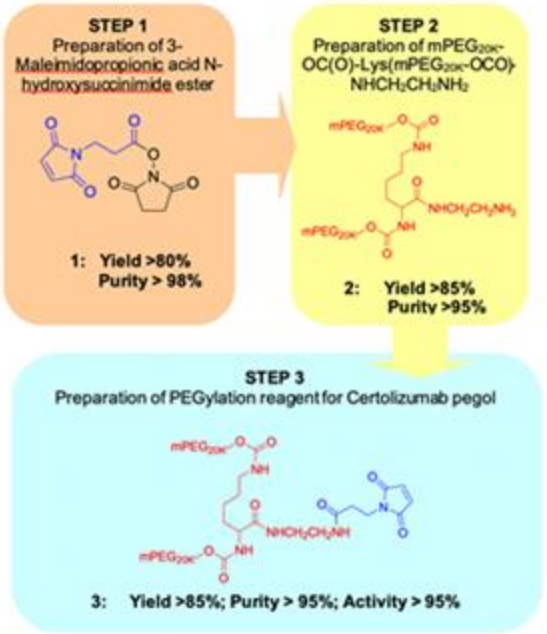


- We produce Certolizumab by fermentation in the periplasm of E.coli in a native state with approx. **yield of 250 mg/L (post-capturing purification step)**

SDS-PAGE analysis of Certolizumab pools:

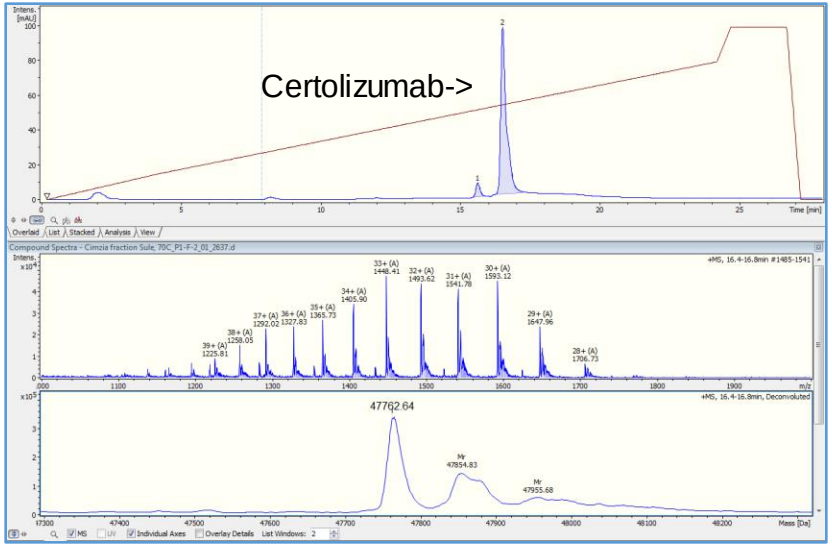
Lane 1: Certolizumab after first chromatography capture step
Lane 2: Flow through from first chromatography capture step
Lane 3: Molecular weight marker

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%.**

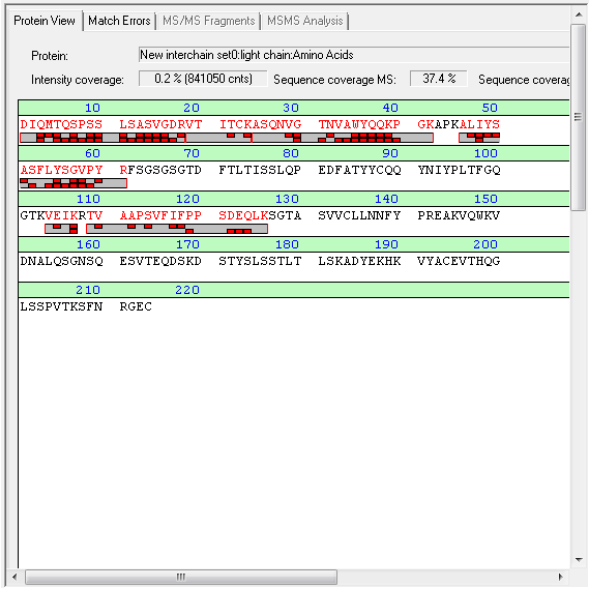
Selected Data: Biosimilarity- Intact mass analysis

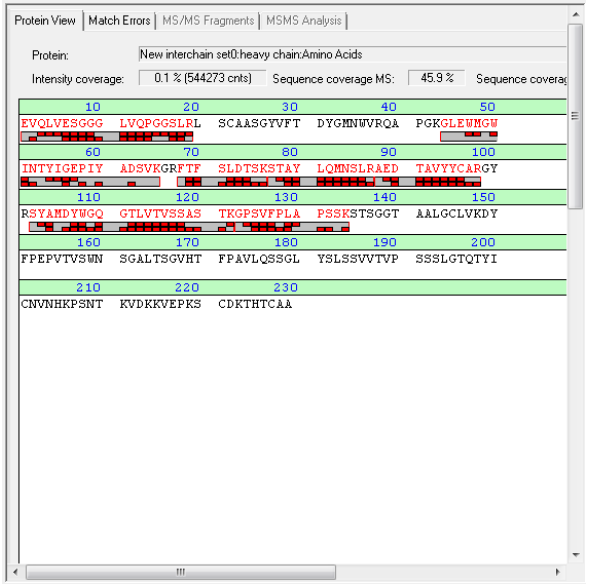


LC-MS (RP-HPLC) of Certolizumab after first chromatography capture step **shows purity >80%.**

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

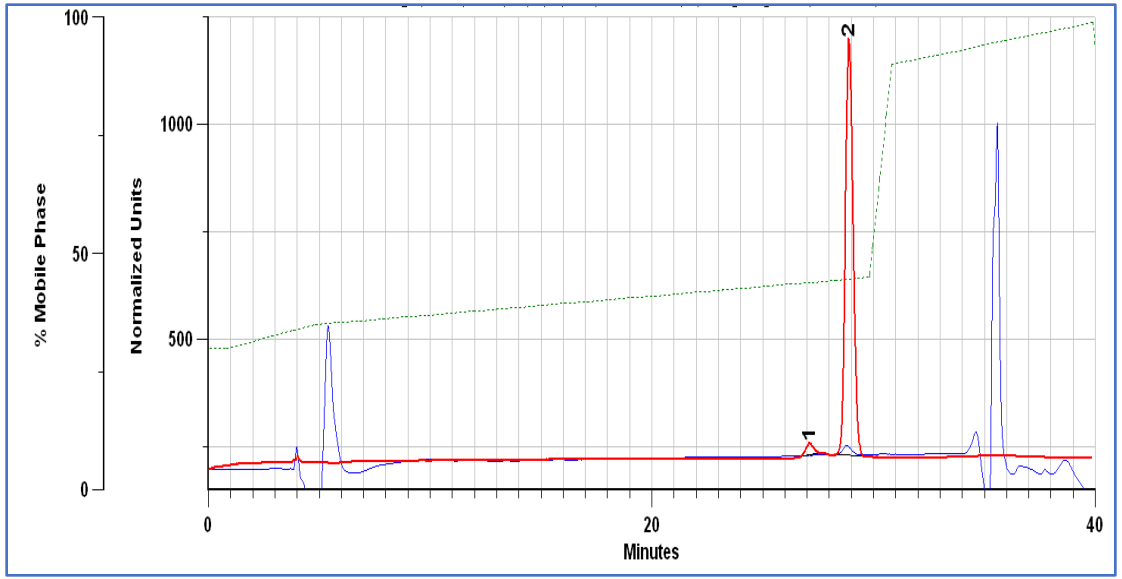




Peptide mapping and MS/MS analysis of protein band from SDS-PAGE:

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

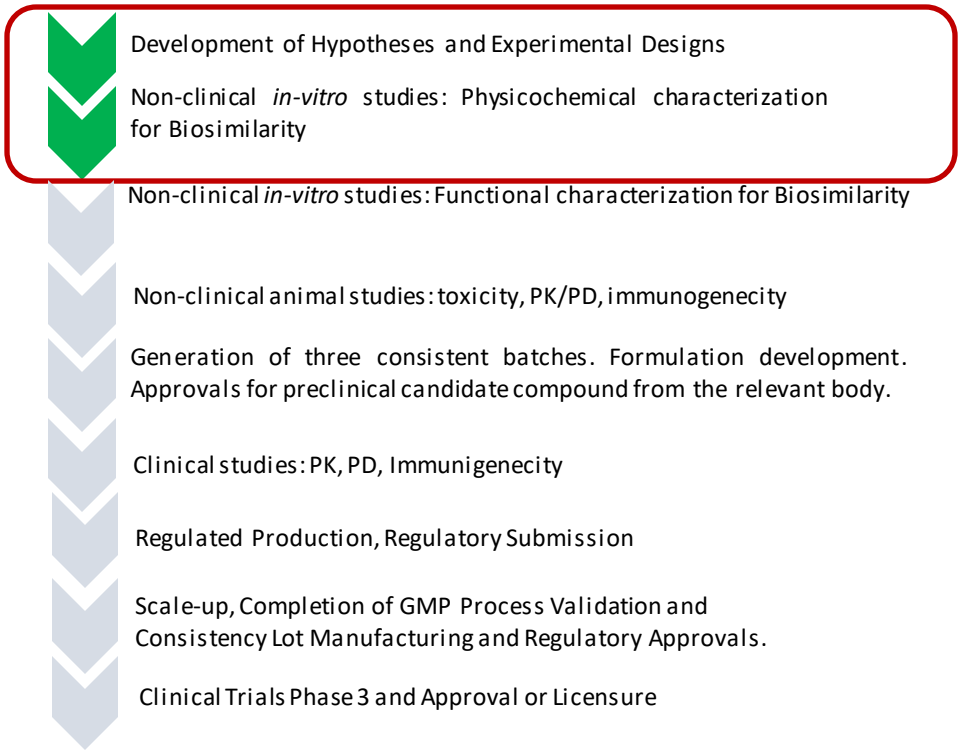


RP-HPLC-UV-ELSD analysis of the PEGylation reagent for Certolizumab: the PEG reagent maleimide activated is reacted with a thiol containing tracer **demonstrating > 97% purity and activity** (peak n.2, red trace is ELSD)

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)**



What are we seeking?

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

Other Capabilities and Offerings

Other technology ready for transfer

BACTERIAL PLATFORM

MOLECULE NAME	EXPRESSION SYSTEM	PRODUCTION MODE	PURE PRODUCT PER BATCH	TOTAL DOSES PER BATCH (dose in mg)
IFNalfa2A	E. coli	Batch (30 L)	5 g	160,000 (0.03)
PEGIFNalfa2a	E. coli	Batch (30 L)	Pure Reagent 8 g Final product 1 g	5500 (0.18)
IFNalfa2b	E. coli	Batch (30 L)	3 g	100,000 (0.03)
GCSF (Filgrastim)	E. coli	Batch (10 L)	3 g	10,000 (0.3)
PEGGCSF	E. coli	Batch (10 L)	Pure Reagent 8 g Final product 2 g	330 (6)

Other technology ready for transfer

MAMMALIAN CELL PLATFORM

MOLECULE NAME	EXPRESSION SYSTEM	PRODUCTION MODE	PURE PRODUCT PER BATCH	TOTAL DOSES PER BATCH (dose in mg)
EPO alpha	CHO	10 Roller bottles (10 L media)	30 mg	300 (0.1)
EPO beta	CHO	Perfusion (RV = 1 L) (15 days / 15 L media)	60 mg	600 (0.1)
PEGEPO	CHO	Roller bottles (10) or Perfusion (15 days)	Pure Reagent 7 g Final product 12 mg	150 (0.075)

Other technology ready for transfer

YEAST PLATFORM

MOLECULE NAME	EXPRESSION SYSTEM	PRODUCTION MODE	PURE PRODUCT PER BATCH	TOTAL DOSES PER BATCH (dose in mg)
Insulin	P. pastoris	Fed-batch (10 L)	10.5 g	3000 (3.5)
Insulin Detemir	P. pastoris	Insuin precursor 2.3 g Reagent 10 g	Pure Reagent 6 g Final product 0.46 g	30 (14.2)
Growth hormone	P. pastoris	Fed-batch (10 L)	2 g	400 (5)

Other R&D/knowhow capabilities available

- Development of technologies for the production of biopharmaceuticals in bacteria, yeast and mammalian cells
- Cell line development for Monoclonal antibodies (e.g. Trastuzumab)
- GMP Quality Controls for biosimilars
- In vivo cell based Bioassays
- HPLC and UPLC analysis using RP, IEX, SEC, HILIC methodologies and detection by UV, Fluorescence, Evaporative Light Scattering (ELSD) or Mass spectrometry detection (MS and MSn)
- Capillary Electrophoresis (CZE and CE-SDS) with PDA detection
- Blitz system (Bio-Layer Interferometry to detect real-time binding interaction between molecules)
- Setup of analytical, semi-preparative and preparative chromatography with low pressure and medium pressure columns using CIEX, AIEX, HI, RP, SEC, IMAC, Protein A (G & L) resins of various types on biochromatography instrumentation
- Peptide synthesis, PEGylation technology and protein chemistry expertise

http://www.icgeb-bdu.org/

State-of-the-art scientific facility

For more information contact:

Case Manager:
Pradnya Aradhye
pradnya@venturecenter.co.in
+91-88050-09010

Lead Scientist:
Dr. Natasa Skoko
skoko@icgeb.org
www.icgeb-bdu.org

TechEx.in is a Regional Tech Transfer Office supported by:

References

- https://www.mordorintelligence.com/industry-reports/global-basal-insulin-long-acting-insulin-market-2012-2024
- https://www.gabionline.net/biosimilars/general/Biosimilars-of-certolizumab-pegol
- https://biosimilarsrr.com/2020/08/14/the-next-generation-biosimilar-race-a-tale-of-two-autoimmune-biologics/
- Gurramkonda C., Polez S., **Skoko N.**, Adnan A., Gäbel T., Chugh D., Swaminathan S., Khanna N., Tisminetzky S. and Rinas U. (2010) "Application of simple fed-batch technique to high-level secretory production of Insulin precursor using Pichia pastoris with subsequent purification and conversion to human insulin." Microbial Cell Factories, 9:31.

- Polez S., Origi D., Zahariev S., Guarnaccia C., Tisminetzky S. G., **Skoko N.,** Baralle M. (2016) "A simplified and efficient process for insulin production in Pichia pastoris", PLos One, 11(12).