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

Trastuzumab, Ranibizumab & Others

Technology from the group of **Anurag Rathore**
at **Indian Institute of Technology, Delhi, India**

TechEx.in Case Manager:
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Outline

- About **Indian Institute of Technology, Delhi**
- About **Center of Excellence Biopharmaceutical Technnology**
- Technology 1: Trastuzumab
- Technology 2: Ranibizumab
- Other Capabilities and Offerings
- The next steps.

About Indian Institute of Technology, Delhi

Center of Excellence Biopharmaceutical Technology

Indian Institute of Technology Delhi (an institute of eminence) is India's premier institute (amongst top 3 IITs in India) for Centres of Excellence for training, research and development in science, engineering and technology.

Research and development @ IITD: The Indian Institute of Technology Delhi lays a strong emphasis on sponsored research and industrial interaction. The Institute is actively involved in collaborative research programmes with international organizations and takes consultancy projects too (> 1000 sponsored research projects executed, > 200 industry partners, > 300 faculties collaborated) . For more information: https://ird.iitd.ac.in/content/ird-activities.

Institute has demonstrated history of collaborative research, sponsored research,co-development projects and consultancy projects.

Dr Anurag Rathore's Group

Center of Excellence
Biopharmaceutical Technology

Lead Scientist: Prof Anurag Rathore

EXPERIENCE

Academic:
- **Current affiliations : Coordinator**, DBT CBT, **Professor,** Deptt of Chem Engg, **Dean,** Corporate Relations at IITD
- **Past affiliations**: UCLA, Washington Univ, & Yale University

Past Industry affiliations: Amgen Inc. & Pfizer Biologics

Expertise: Continuous processing, Stability of biotech therapeutics, Analytical and functional characterization of biosimilars, Scientific and regulatory issues of biosimilars

Agilent Thought Leader Award 2020

Fact file of Prof Rathore's Lab:

- **Authored more than 700** publications in his areas of expertise.
- Current Team strength:
  - 20 PhD students
  - 20 Post-doctoral
  - 10+ SRF/JRF
- **13 unique patent families** (Filed internationally)
- State-of-the-art **bioprocess development till 10 L scale, analytical and functional characterization facilities**

Partners: Tech transfer, Collaborations and Consultancy projects

FDA, CDSCO, NIST National Institute of Standards and Technology, TATA CONSULTANCY SERVICES, ABB, Ansys, Agilent Technologies, J&J, Biocon, Waters, Department of Biotechnology Government of India, GE, SANOFI, SUN PHARMA, APPLIED MATERIALS, DSM, PALL, ThermoFisher SCIENTIFIC, Dr.Reddy's, sartorius stedim biotech

Technology Development at CBT

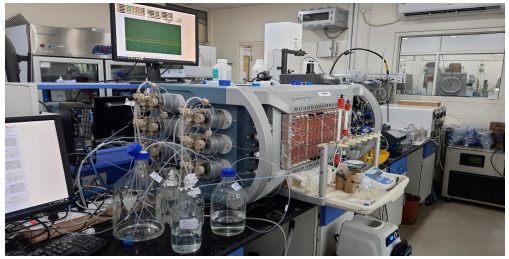
Know-how/ methods transferred:

- An **innovative CFIR** for heat transfer for manufacturing of a heat labile API to a major Indian pharma.
- **Multivariate data analysis** (MVDA) model for evaluating comparability of biotech processes and products **for a major Indian biopharma.**
- Developed a **process analytical technology (PAT)** based control scheme for a process chromatography column for a **major Indian biopharma.**
- Process development for removal of **f-met impurity in GCSF for a major Indian biopharma.**

Patents filed:

- System and method to control a **continuous biopharmaceutical manufacturing**, 2021
- Surge tank based system for **automated operation and control of continuous biopharmaceutical manufacturing**, 2020
- A system for real time monitoring of protein and excipients, 2020
- Fingerprinting Biotherapeutics with FTIR Spectroscopy, 2019
- Bioprocess Performance **Enhancing Strains Of Escherichia coli**, 2019
- A process for **preparation of pegylated therapeutic proteins**, 2020.
- A system for monitoring and control of chromatography, 2019
- Process for **producing recombinant peptides**, 2018
- Method for monitoring of foulants present on chromatographic resins using fluorescence probe, 2016
- An innovative coiled flow inverted reactor for continuous refolding of denatured recombinant proteins, 2015
- A process for **purification of recombinant granulocyte colony stimulating factor**, 2012

Glimpses of state-of-the-art facility at Center of Excellence Biopharmaceutical Technology



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Technology 1: Trastuzumab

WHO Essential medicine

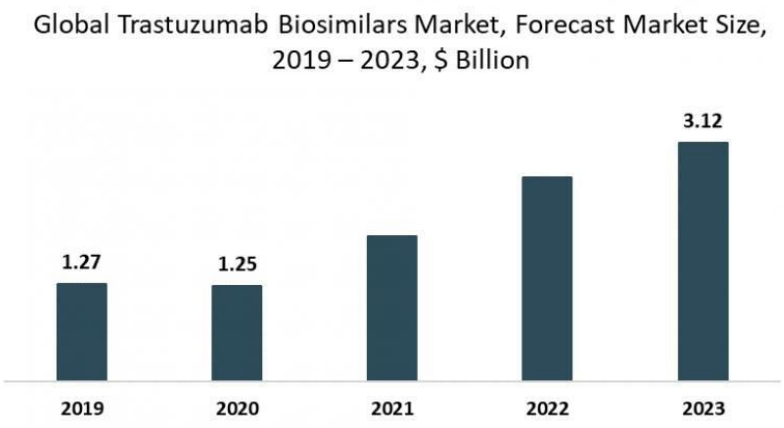
About Trastuzumab

Trastuzumab is a **monoclonal anti-human epidermal growth factor receptor 2** protein antibody.

- **Originator / reference product:** The originator product, Roche's Herceptin (trastuzumab) was approved by the US Food and Drug Administration (FDA) in September 1998 and by the European Medicines Agency (EMA) in August 2000. **Patent expired in US in June 2019** and in **Europe in July 2014**. (Source: GaBI Online)

- **Indications:** Treatment of **HER2 overexpressing breast cancer** and **HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma**

Market and Industry Overview



Source: The Business Research Company

Market:

Global trastuzumab biosimilars market is expected to grow from $1.22 billion in 2020 to **$1.4 billion in 2021** at a compound annual growth rate (CAGR) of 14.8% and is expected to reach **$4.25 billion in 2025 at a CAGR of 32%.** (Source: The Business Research Company)

Industry players:

- **Global:** Biocon/ Mylan (Ogivri, 2017), Celltrion (Hermuza, 2018), Samsung Bioepis (Ontruzant, 2019), Pfizer (Trazimera, 2019), Amgen (Kanjinti, 2019)

- **India:** Biocon, Cadila Healthcare, Reliance Lifesciences, Dr Reddy's, Lupin

The Opportunity: Why you should be interested?

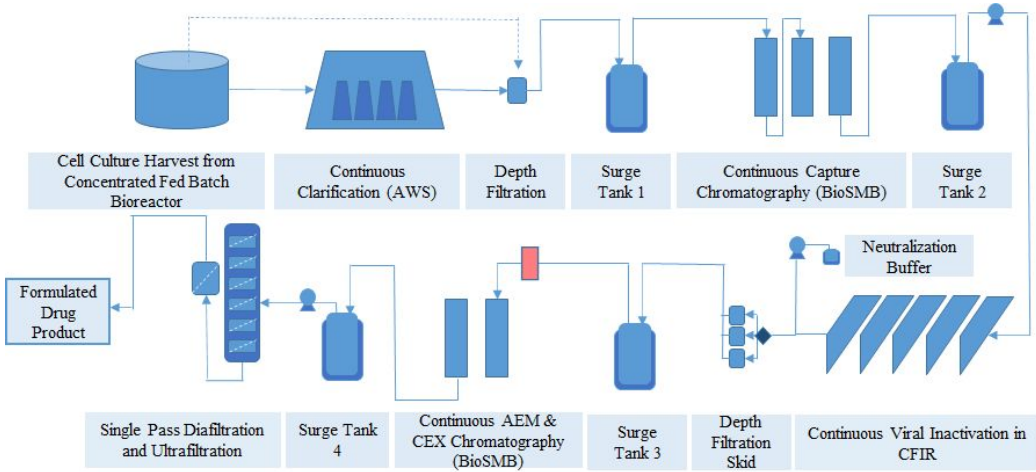
- **Market interesting:**
  - According to the World Health Organization (WHO), in 2018, the **new breast cancer cases** registered were around **2.09 million**, and **stomach (gastric) cancer cases** were around **1.03 million**.(Source: Cancer Network)
  - According to Cancer India, breast cancer is the most common cancer in women in India and accounts for 14% of the cancers in women.
- **Cost still high: Annual cost of treatment**
  - North America*
    - Adjuvant breast cancer settings per patient : $49,915
    - Metastatic breast cancer settings per patient : $28,350
  - India*
    - $15,000 per patient.
  - **Due to prohibitively high cost of the therapy, a large group of patients do not have the opportunity to receive trastuzumab.**
- **Opportunities for process innovations to reduce costs: Novel continuous processing platform** results in **reduction in cost** of manufacturing **by 70% for clinical** and **35% for commercial production**

* Ref: The cost burden of trastuzumab and bevacizumab therapy for solid tumours in Canada, *Curr Oncol* 2008 Jun;15(3):136-42.

The Technology Offering – Trastuzumab Biosimilar

Key highlights of the offering:

- Novel use of CFIR for viral clearance: Allows us to do **viral clearance continuously**
- Novel continuous processing platform**:** Results in **reduction** in **cost of manufacturing** by 70% for clinical and 35% for commercial production



Parameters	Responses
Product Titer	2-2.3 g protein /L reactor
Purification yield (including refold)	90 %

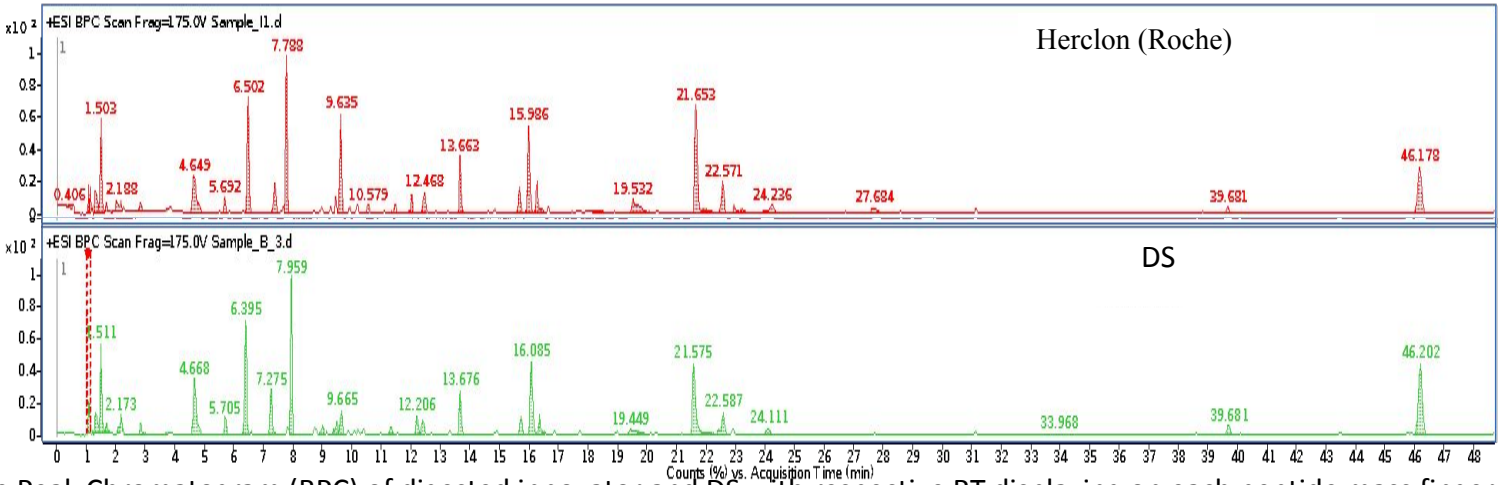
Relevant publications:

- Complete or periodic continuity in continuous manufacturing platforms for production of monoclonal antibodies? *Biotechnology Journal* (2021) 2000524
- Economic assessment of continuous processing for manufacturing of biotherapeutics, *Biotechnology Progress* (2021) 37 (2), e3108

Selected Data
Biosimilarity - Intact mass analysis

The Total Ion Chromatogram (TIC) represents the deconvoluted spectra in comparison of intact analysis between innovator and drug substance (DS). **Confirms the correct molecular mass of trastuzumab.**

Selected Data
Biosimilarity - Peptide mapping fingerprinting

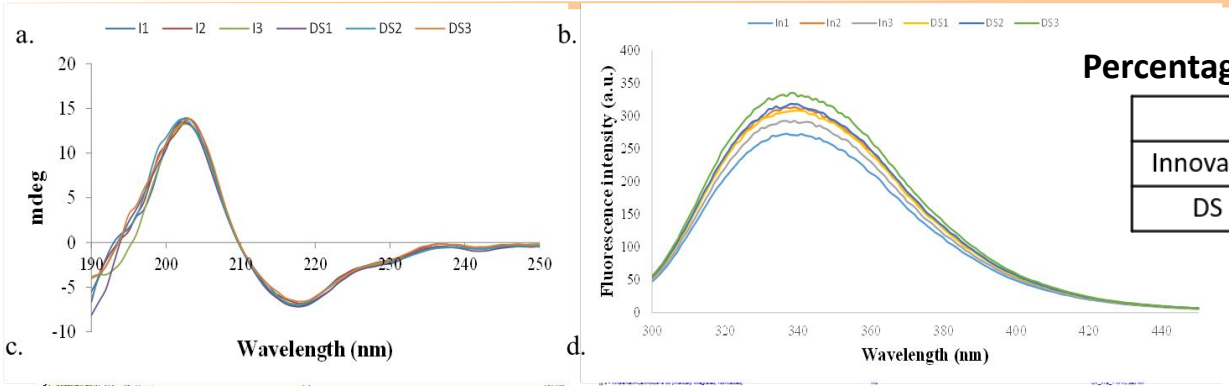


Base Peak Chromatogram (BPC) of digested innovator and DS with respective RT displaying on each peptide mass fingerprint.

Sequence similarity to *in-silico* trastuzumab sequence

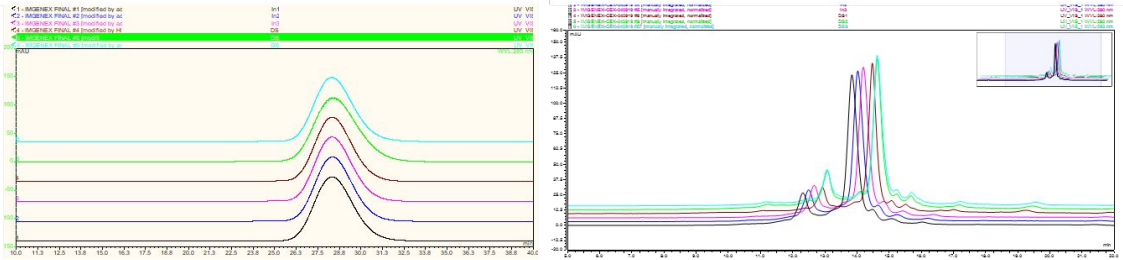
Sample	Chain A (%)	Chain B (%)
Herclon (Roche)	98.76	99.7
DS	99.05	99.85

Selected Data
Biosimilarity – Physicochemical characterization



Percentage of variants of Herclon (Roche) vs DS

	Acidic	Main	Basic
Innovator	28.00±1.15	59.86±1.44	13.15±1.65
DS	29.09±1.33	54.50±0.23	16.40±1.11



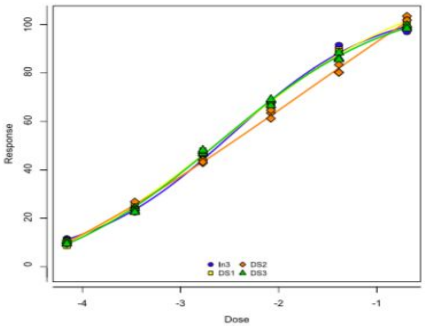
Confirms a) **Identical secondary structure** (Far-UV CD spectra), b) **Identical tertiary structure** (fluorescence spectra), c) **Similar aggregation** (purity ~ 99%), d) **Similar charge variant profile**

Selected Data
Biosimilarity – Functional characterization

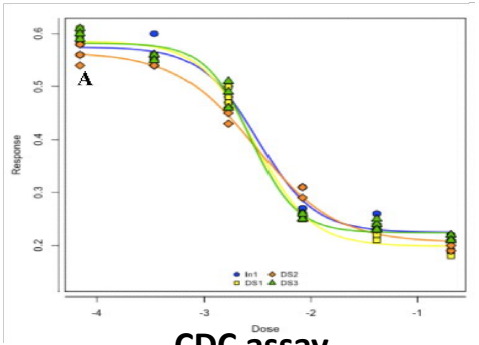
Binding kinetics (SPR) of DS of Trastuzumab and Herclon (Roche) to Recombinant human FcRn and FcγRIIIa

Name of the Sample	FcRn KD (M)	FcγRIIIa KD (M)	Binding activity
Innovator	4.28E-08	1.33E-07	No difference
DS	2.19E-08	1.76E-07	

Confirms similar binding activity



ADCC assay



CDC assay

The relative potency values of DS of Trastuzumab in case of Herclon (Roche) (In) taken as standard by using ADCC and CDC assay

Name of Samples	Estimated potency (ADCC) (in IU/ml)	Estimated potency (CDC) (in IU/ml)	System suitability test (linearity and parallelism)
DS	1.02	1.05	Passed
DS2	0.97	1.11	Passed
DS3	1	1.04	Passed

Summary of Biosimilarity analysis vs. Herclon (Roche)

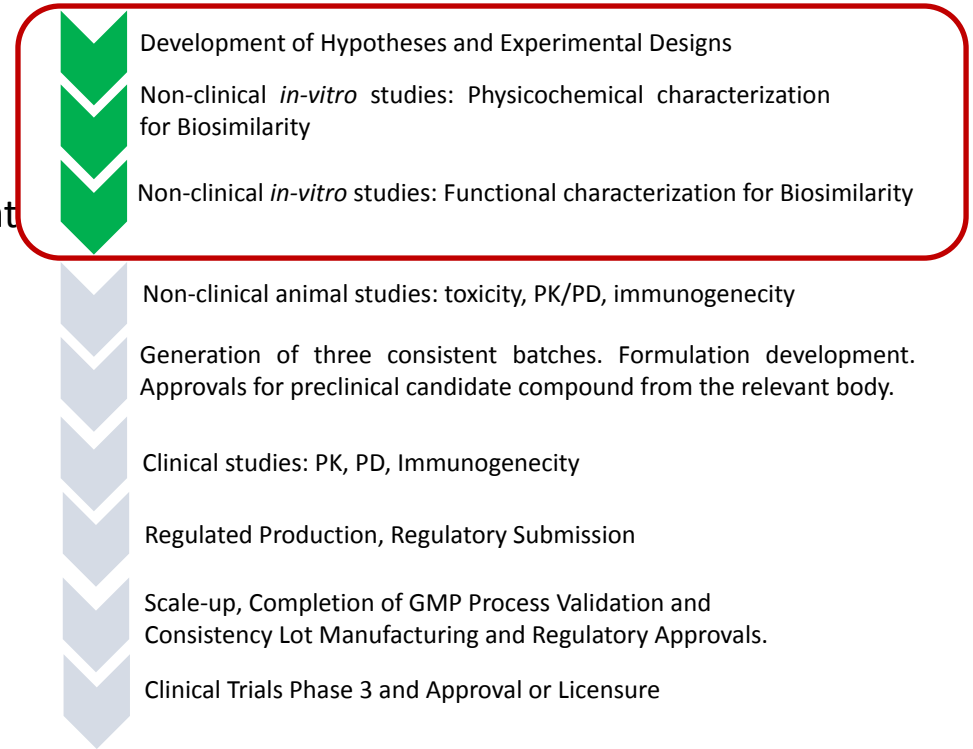
	CQA	Characterization	Status	Acceptance criteria
Physiochemical characterization	Purity	Tricine PAGE	Reduced: Two major bands were observed at 50 kDa and 25 kDa Non-reduced: 150 kDa band was observed	Comparable
		RP-HPLC	Similar profile to innovator	Comparable
	Size heterogenity	SEC	~ 99% purity	Sum of aggregates NMT 2.0%
	Charge variant	CEX	Acidic: 30.30%, Main: 53.48%, Basic: 15.32%	Acidic: NMT 35.0%, Basic: NMT 15.0%
	Intact mass analysis	LC-MS	Identical profile of DS to the innovator profile	
	Reduced mass analysis	LC-MS		
	Amino acid Sequence (Primary sequence)	Peptide Mapping by Mass Spectrometry	Identical profile of DS to the innovator profile and similarity to *in-silico* sequence ~99%	
	Disulfide linkage	LC-MS	Identical to innovator	Comparable
	Secondary/ tertiary structure analysis	CD/Fluorescence spectroscopy	Identical to innovator	Comparable
	Glycan profile	InstantPC Labelling	Identical to innovator	Comparable
Functional characterization	Binding kinetics	SPR	Similar binding affinity compared to innovator	Comparable
				Comparable
	ADCC	Cell-based assay	Similar ADCC and CDC activity compared to innovator	Comparable
	CDC			

Current Status of Technology and Path Ahead

Clone: In collaboration with Imgenex India Pvt Ltd

Stage of Development

- Upstream and downstream process development complete
- Process has been demonstrated up to 10L bioreactor
- Titer of **2.0-2.3 g/L in 10L bioreactor**
- Purification yield of 63 ± 2 %
- Analytical and functional similarity to innovator molecule has been established
- **Cost of manufacturing** lower by 70% for clinical and 35% for commercial production



Technology 2: Ranibizumab

About Ranibizumab

Ranibizumab is a **recombinant humanized** monoclonal antibody and **VEGF-A antagonist**

- **Originator / reference product:** Lucentis, was marketed by Genentech (Roche)/Novartis, approved by the USFDA in June 2006 and by EMA in Jan 2007. The patents on Lucentis **expired** in the **US in June 2020** and will expire in **Europe in 2022.** (Source: GaBI Online)

- **Indications:** Used in treatment of neovascular (wet) **age-related macular degeneration (wAMD),** Macular edema following retinal vein occlusion (RVO), Diabetic macular edema (DME), Diabetic retinopathy (DR) and Myopic choroidal neovascularization (mCNV).

Market and Industry Overview

Market:

The global age-related macular degeneration (AMD) market stood at $ 1.58 billion in 2020 and is projected to reach **$ 2.64 billion by 2026, growing at CAGR of 8.93%** between 2021 and 2026. (Source: EMR)

Industry players:

- **Global:** Genentech, Novartis
- **India:** Intas

The Opportunity: Why you should be interested?

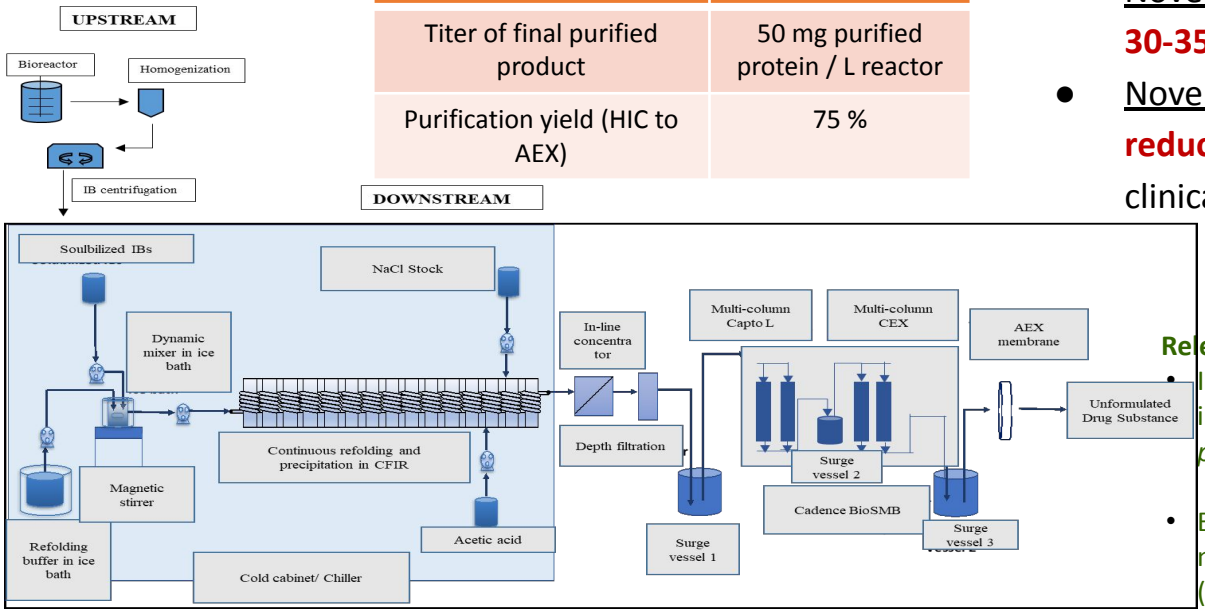
- **Market interesting**: **AMD Affects nearly 8.7% of the worldwide population,** and the numbers are projected to increase to around 196 million in 2020. Projected number of people with the disease is around **196 million in 2020, increasing to 288 million in 2040**. (Source: All About Vision)
- **Cost still high:** Approximately**, 51% of the patients on VEGF therapy dropout of therapy** after initial injections. The most common reason is non-affordability of the injection followed by no improvement in vision. (Source: The Indian Express).

Price point Global	**Price point India**
• Razumab: 2.3mg Injection @ ~ $ 270 • Lucentis: 0.5 mg injection @ ~$ 1120	• Razumab: injection $130 • Lucentis (Branded Accentrix): injection $320

- **Industry not yet crowded: 1st ever Biosimilar of Ranibizumab**- 'Razumab' launched by Intas Pharma in 2015. Few players globally.
- **New indications:** A 2021 survey of Indian vitreoretinal specialists showed progressive trend favouring ranibizumab-biosimilar over bevacizumab-biosimilar.
- **Opportunities for process innovations to reduce costs: Novel continuous processing platform** results in reduction in Cost of Manufacturing **by 80% for clinical** and **75% for commercial production**.

The Technology Offering – Ranibizumab Biosimilar

Parameters	Responses
Titer of final purified product	50 mg purified protein / L reactor
Purification yield (HIC to AEX)	75 %



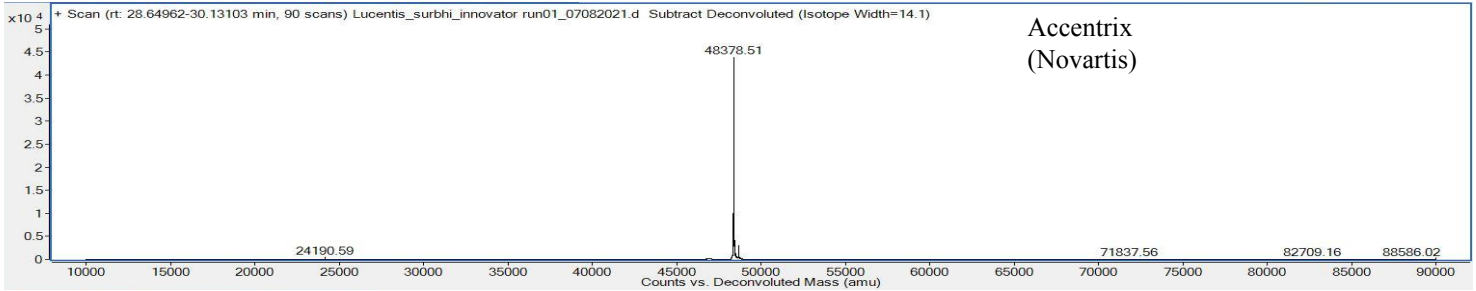
Key Highlights of the Offering

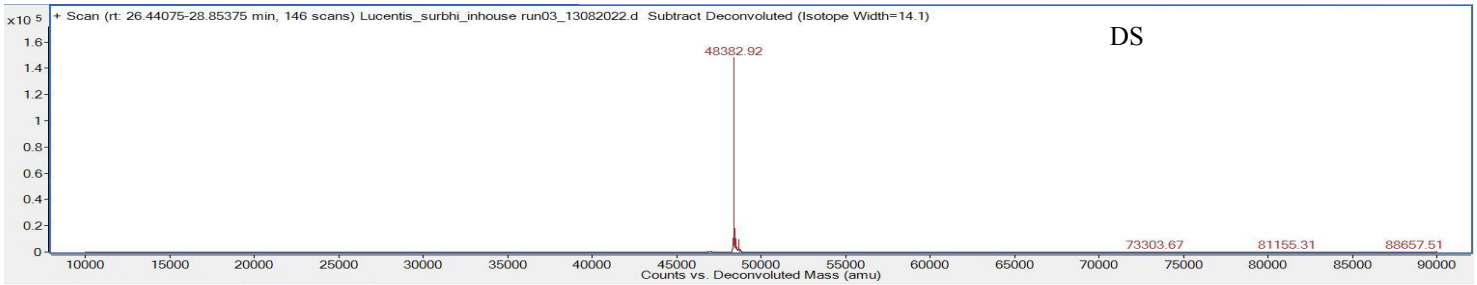
- Novel refolding process: Refolding yield of **30-35% vs the industry standard of 15%.**
- Novel continuous processing platform: Results in **reduction in cost of manufacturing** by 80% for clinical and 75% for commercial production.

Relevant publications:

- Integrated continuous processing of proteins expressed as inclusion bodies: GCSF as a case study, *Biotechnology progress* (2017) 33 (4), 998-1009
- Economic assessment of continuous processing for manufacturing of biotherapeutics, *Biotechnology Progress* (2021) 37 (2), e3108

Selected Data
Biosimilarity - Intact mass analysis

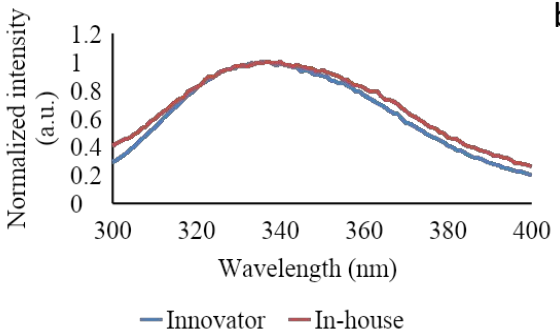




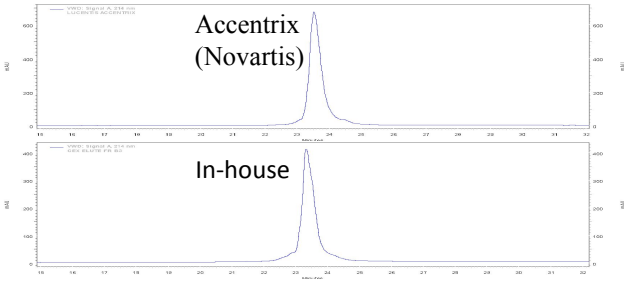
The Total Ion Chromatogram (TIC) represents the deconvoluted spectra in comparison of intact analysis between innovator and drug substance (DS). **Confirms the correct molecular mass of Ranibizumab.**

Selected Data
Biosimilarity - Physicochemical characterization.

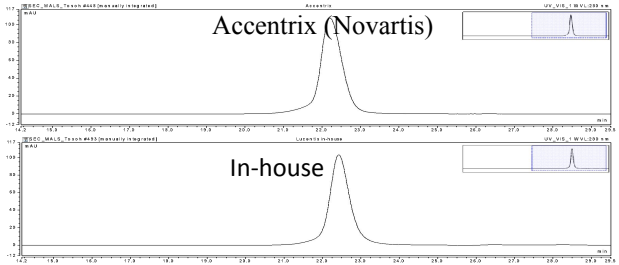
a.



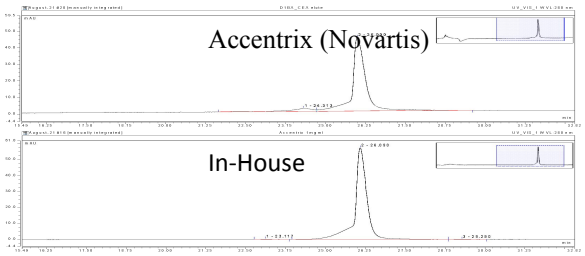
b.



c.



d.



Percentage of variants of innovator vs DS

Sample	Acidic	Main	Basic
Accentrix (Novartis)	0.12	99.8	0.08
In-house	2.35	97.65	0

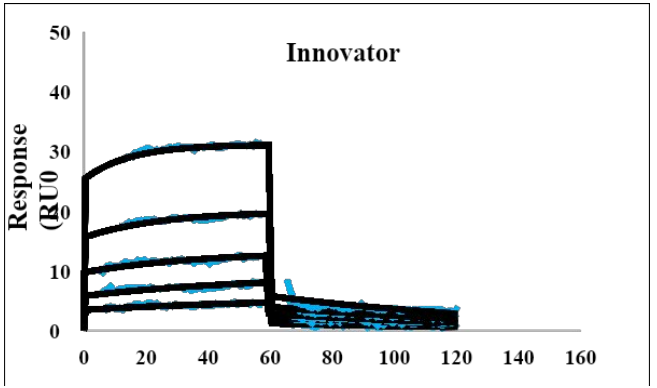
Confirms a) **Identical tertiary structure** (fluorescence spectra), b) **Similar purity** (RP HPLC ~ 99%), c) **Similar aggregation** (purity ~ 99%), d) **Similar charge variant profile**

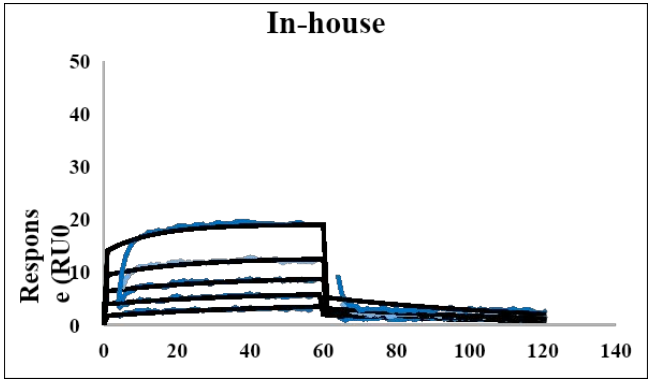
Selected Data
Biosimilarity – Functional characterization

Binding kinetics (SPR) of DS of Lucentis and Accentrix (Novartis)

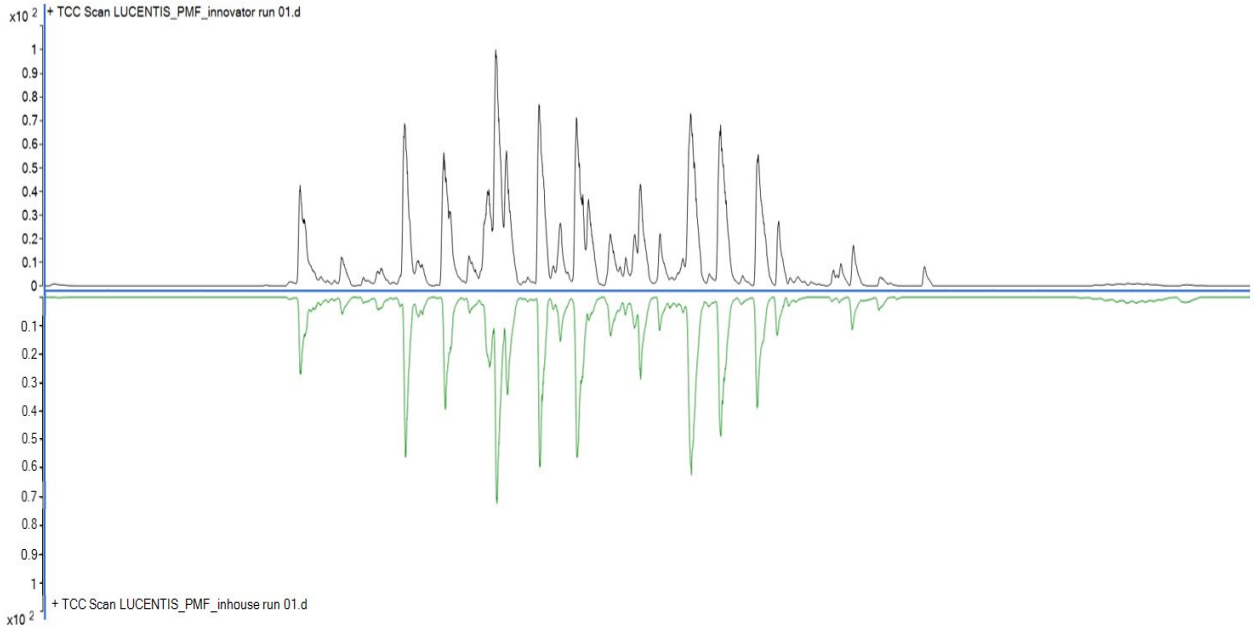
Name of the Sample	ka	kd	KD (M)	Binding activity
Accentrix (Novartis)	4.28E-08	1.33E-07	1.01E-08	No difference
DS	2.19E-08	1.76E-07	1.27E-08	

Confirms similar binding activity





Selected Data
Biosimilarity – Peptide Mapping



Sample	% coverage	Standard deviation *
Innovator	94.49	± 2.36
In house	92.7	± 1.91

*No significant difference between sequence coverage of innovator and in-house sample of Lucentis when compared with *in silico* sequence

Summary of Biosimilarity analysis vs. Accentrix (Novartis)

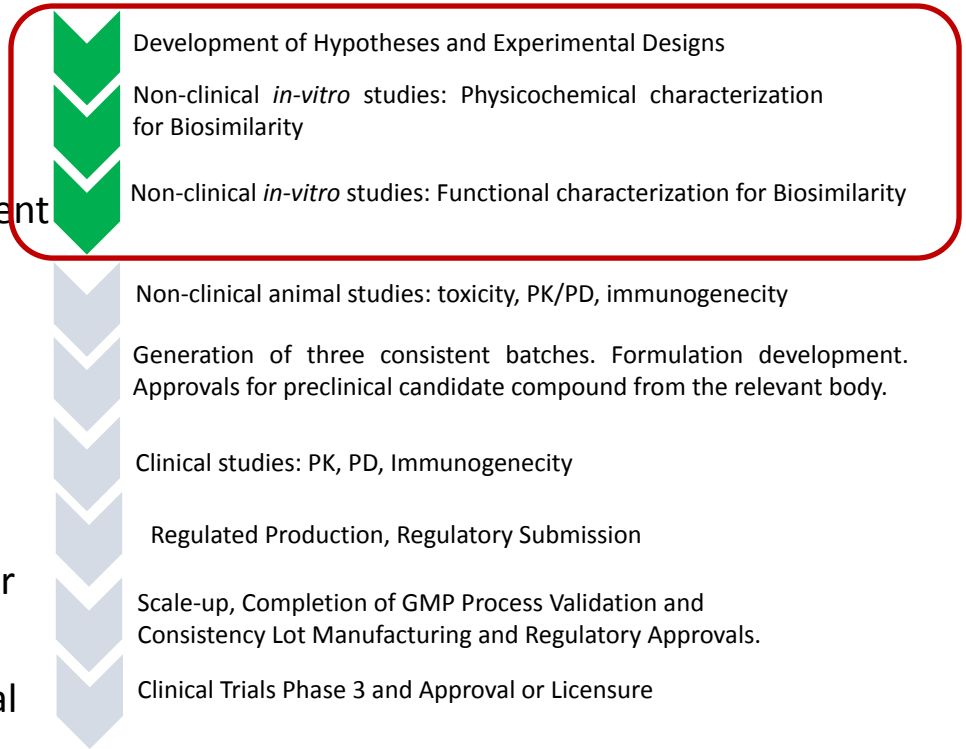
	CQA	Characterization	Status
Physiochemical characterization	Purity	RP-HPLC	Similar profile to Innovator
	Size heterogenity	SEC	~ 99% purity
	Charge variant	CEX	Acidic : 2.35, Main : 97.65
	Intact mass analysis	LC-MS	Identical profile
	Reduced mass analysis	LC-MS	Pending
	Amino acid Sequence (Primary sequence)	Peptide Mapping by Mass Spectrometry	Pending
	Disulfide linkage	LC-MS	Pending
	Secondary/ tertiary structure analysis	CD/Fluorescence spectroscopy	CD - Pending/Tertiary structure identical to Innovator
Functional characterization	Binding kinetics	SPR	Similar binding affinity compared to innovator
	HUVEC anti-proliferation assay	Cell-based assay	Pending

Current Status of Technology and Path Ahead

Clone: Purchased from Thermo Scientific

Stage of Development

- Upstream and downstream process development complete
- Process has been demonstrated up to 5L bioreactor
- Titer of **50 mg/L reactor**
- Purification yield of 23 ± 2 %
- Analytical and functional similarity to innovator molecule has been established
- **Cost of manufacturing** lower by 80% for clinical and 75% for commercial production



Other Capabilities and Offerings

Molecules under development

Molecule	Disease indication	Stage of development
Insulin Lispro	Type 1 and 2 Diabetes Mellitus	Process development in progress
GCSF	Chemotherapy-induced neutropenia	Process development complete. Analytical and functional characterization ongoing.
Peg-GCSF	Chemotherapy-induced neutropenia	
Asparaginase	Acute Lymphocytic Leukemia	Process development in progress
Human Serum Albumin (in Pichia pastoris)	Replace lost fluid and help restore blood volume in trauma, burns and surgery patients.	Process development in progress
Symlin	Type 1 and 2 Diabetes Mellitus as an adjunct to insulin	Process development in progress

R&D & Technical Capabilities – End to End Infrastructure and Expertise

Bacterial Cell Culture

Fermenters (1 L, 5 L, 10 L)
Biochemistry Analyser YSI
Ultrasonic Processor Cell Disruption
Laminar Flow
Incubator Shaker
Refrigerated Centrifuge- 2 No.
-20°C Deep Freezer- 2 No.

Mammalian Cell Culture

Bioreactor (1L, 10 L)
Biosafe Cabinet Laminar Flow- 2 No.
Inverted Microscope
Cell Imaging System (Cytel)
CO_2 Incubator Shaker
Refrigerated Centrifuge
Liquid Nitrogen Container

Downstream Processing

BioSMB Continuous Chromatography
Akta Purifier- 2 No.
Akta Avant- 2 No.
Akta Cross Flow
TFF Assembly
Cold Cabinet
Refrigerators- 3 No.
-80°C Deep Freezer
pH Meter- 2 No.
Conductivity Meter
Digital Weighing Balance
Water Purification System

Analytical Instruments

CD (Circular Dichroism)
FTIR (Fourier-transform infrared spectroscopy)
Fluorescence Spectroscopy
UV Vis Spectroscopy- 2 No.
Lyophilizer/Speed vac
NIR Analyser (Near Infrared)
SEC MALS (Multi Angle Light Scattering)
1D, 2D Electrophoresis/IEF
Densitometer
BLI (Biolayer Interferometry)
SPR (Surface Plasmon Resonance)
ELSD Detector
HPLC (High Performance Liquid Chromatography)- 3 No.
UPLC (Ultra Performance Liquid Chromatography)- 3 No.
ESI TOF MS (Mass Spectroscopy)
CE (Capillary Electrophoresis)
DLS (Dynamic Light Scattering)
ITC (Isothermal Titration Calorimetry)
TEM (Transmission Electron Microscopy)

Only lab in India to have a end to end continuous process infrastructure and platforms for both microbial and mammalian derived proteins.

Next steps - Interest in Technologies

CBT team has demonstrated capabilities in **clone development, upstream and downstream processing, formulation.** The next step would be:

- Collaborate with companies interested in licensing and taking the biosimilars to the market
- Co-development of other biosimilars

Seeking Industrial partners interested in:

- Licensing technology knowhow with patents
- Sponsoring further technology advancement and scale-up
- Utilizing the R&D skills for other projects
- Collaborative development/ bidding for joint projects
- Licensing of patents

TechEx.in
Tech Transfer Hub

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nbm
NATIONAL BIOPHARMA MISSION
innovate in India for inclusiveness (i3)

VENTURE CENTER

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