

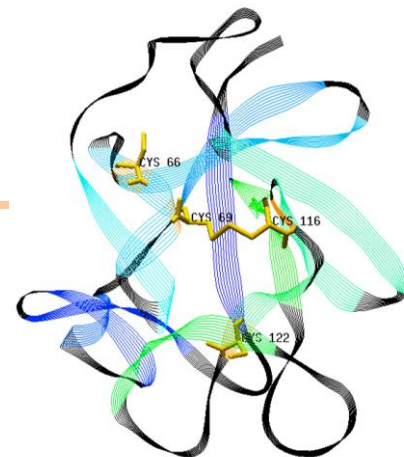
Anakinra

Technology from the group of Rahul Bhambure
at **CSIR-National Chemical Laboratory, Pune, India**

Match Maker/ Biosimilars / 31 Aug 2021/DrBhambure CSIR-NCL

About Anakinra

Anakinra is a **recombinant, nonglycosylated form** of the **human interleukin-1 receptor antagonist (IL-1Ra)**, that can reduce the activity of interleukin-1, a key driver of inflammation in autoimmune and autoinflammatory diseases.



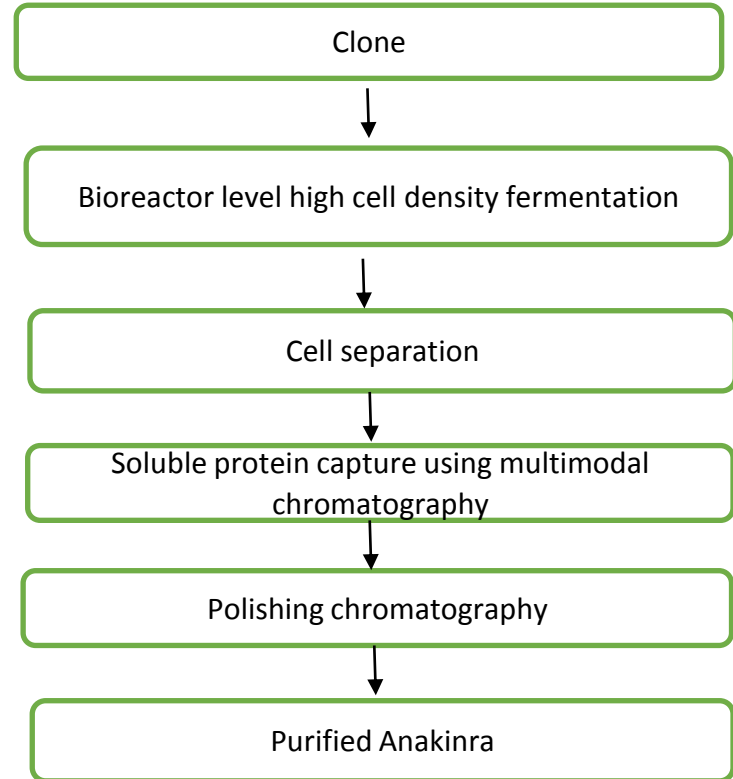
- **Originator / reference product: Kineret** is marketed by Swedish Orphan Biovitrum, approved by the USFDA in 2001 and by EMA in 2002. The **original patent on Anakinra expired in 2008.**
- **Indications:** Used in rheumatoid arthritis as a **second in line treatment** to a Disease Modifying Anti Rheumatic Drug (**DMARD**), **Stills disease** (a rare form of rheumatoid conditions), **Neonatal-onset multi-system inflammatory disease**, Cryopyrin-associated periodic syndromes (CAPS), Familial Mediterranean fever, another inherited periodic fever syndrome

The Opportunity: Why you should be interested?

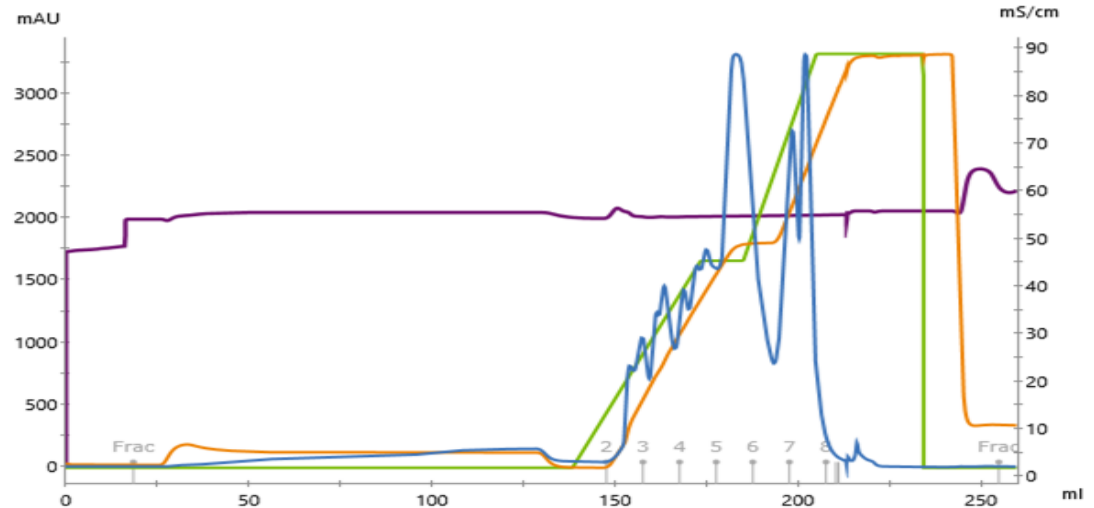
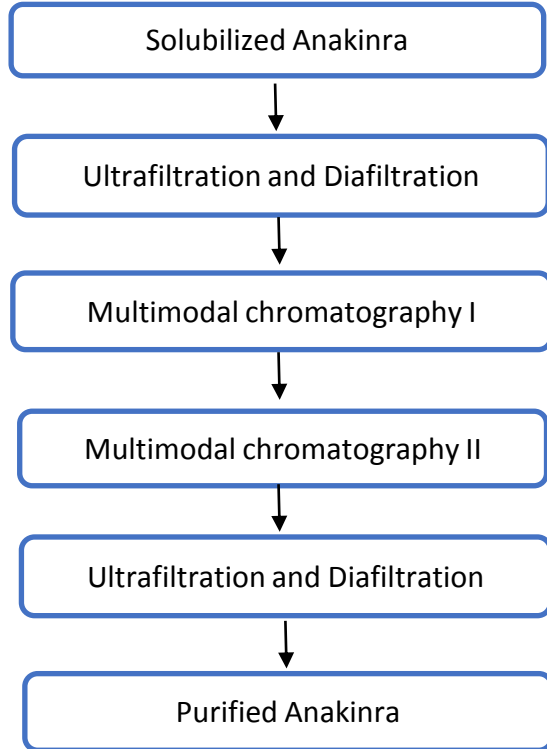
- **Market interesting:**
 - Nearly **4% of the world's population** is affected by one of more than 80 different autoimmune diseases, **rheumatoid arthritis** being one of the **most common**. (Source: [NSCF](#))
 - **Global prevalence** of rheumatoid arthritis is between **0.24-1%**, varies considerably around the globe (Source: [NCBI](#))
 - Cost of manufacturing Anakinra (a 2nd in line drug for RA) is 1/10th that of Rituximab.
- **New indications/ application:**
 - Originator company SOBI state that the interest in Kineret remains strong with more utility being tested out
 - As a treatment for COVID-19-induced SARS (severe acute respiratory syndrome) and CSS (cytokine storm syndrome) was featured in prestigious publications such as **The Lancet Rheumatology**. EMA has started **review** of Anakinra for **treatment of COVID 19 in adult** patients as on July 2021.
 - Expanded scope with studies underway: **Familial Mediterranean fever, Deficiency of interleukin-1 receptor antagonist (DIRA), Moderate to severe COVID treatment, Psoriasis**
- **Industry not yet crowded:** Very few companies seem to be working on developing biosimilars of the molecule.
- **Cost still high:** \$ 1194 (for 4.69 ml) and \$ 3811 (for 18.76ml)
- **Opportunities for process innovations to reduce costs**

The Technology Offering

- Clone, upstream and downstream process for producing biosimilar Anakinra
- Soluble expression of Anakinra eliminating in-vitro refolding step
- Purification process involving novel multimodal chromatographic purification steps > 2X improvement in productivity
- Time and cost effective expression avoiding in-vitro refolding of protein
- Soluble protein expression > 1gm/L of fermentation broth



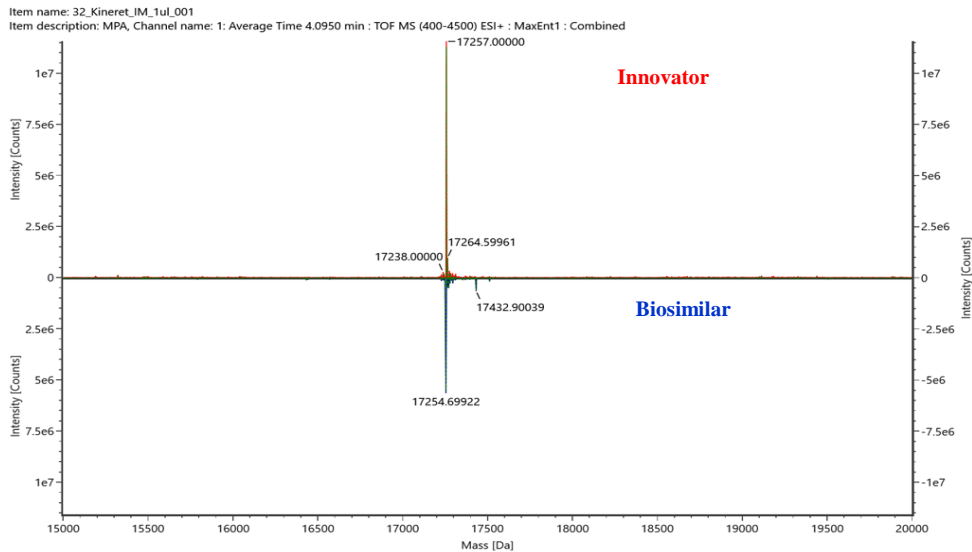
Downstream process platform



Multimodal chromatography
I

Designed a novel multimodal chromatography based purification platform for Anakinra downstream processing

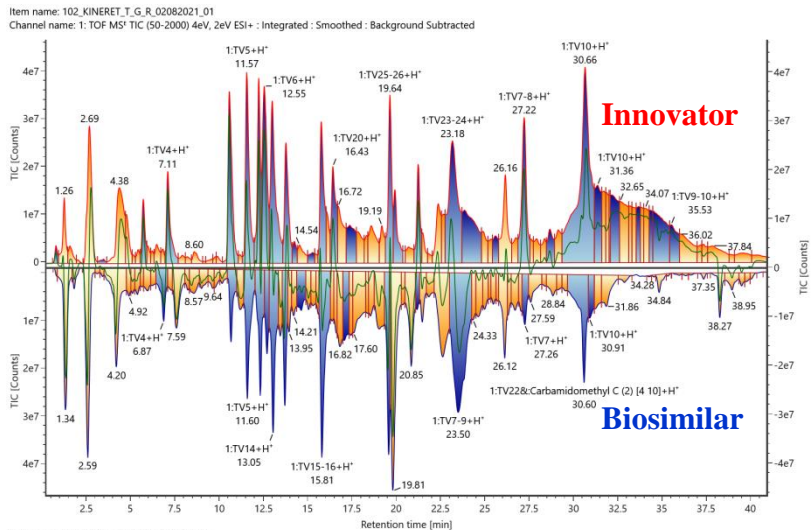
Biosimilarity- Intact mass analysis



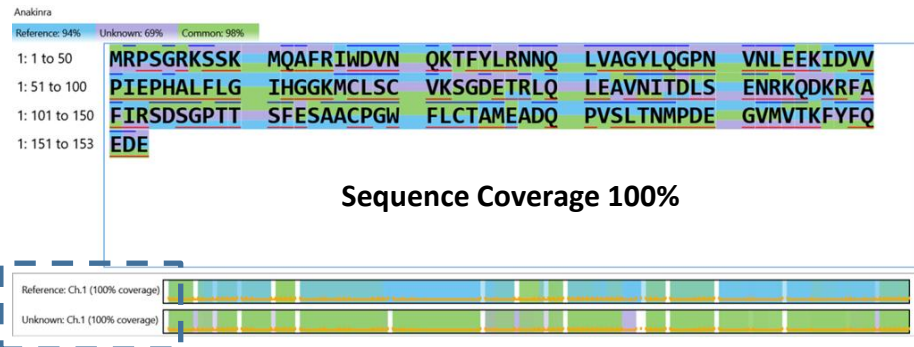
Item name: 116_Anakinra_IM_29052021_01
Item description: , Channel name: 1: Average Time 4.0951 min : TOF MS (400-4500) ESI+ : MaxEnt1 : Combined

Sample	Observed mass (Da)	Expected mass (Da)	Mass error (Da)
Anakinra_NCL	17254.6712	17255.4267	-0.7555
KinereT	17256.9708	17255.4267	1.5441

Biosimilarity – Peptide fingerprinting

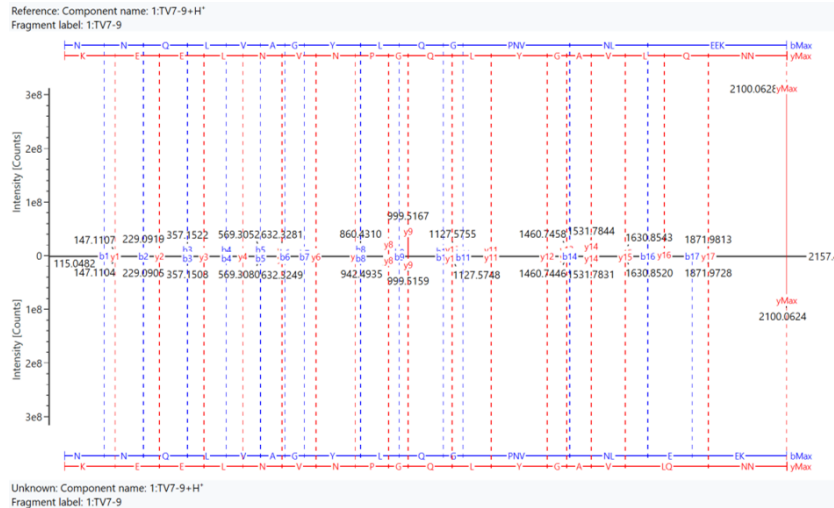


Item name: 30_Anakinra_R_T_G_11062021_01
Channel name: 1: TOF MS¹ TIC (50-2000) 4eV, 2eV ESI+ : Integrated : Smoothed : Background Subtracted



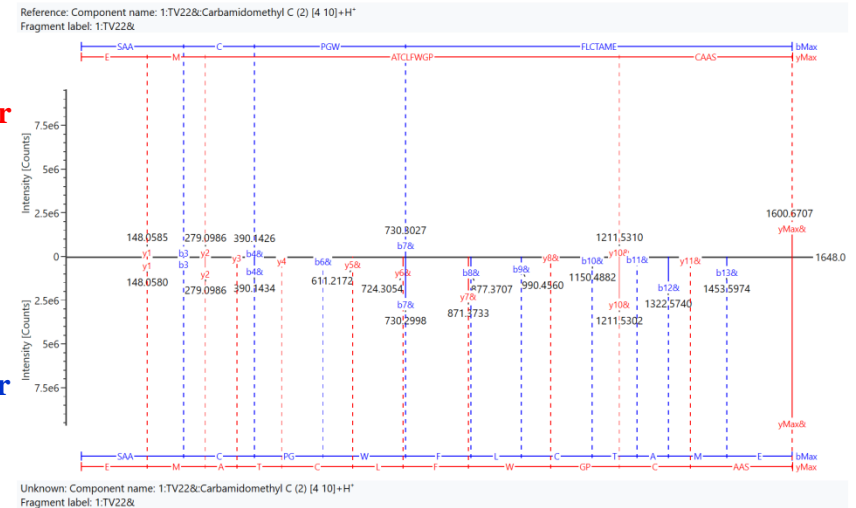
Biosimilarity – Peptide fingerprinting

Sequence confirmation at MS²



Innovator

Biosimilar



Summary of Biosimilarity Analysis

Test	Test performed at CSIR-NCL
Molecular weight	SDS- PAGE, MALDI-TOF, SEC, ESI-MS/MS
Secondary structure	CD Spectroscopy & Fluorescence Spectroscopy
Carbohydrate content and details of component	Not applicable for this molecule
Aggregate quantification	MALDI-TOF and SEC analysis
HCP quantification	ELISA based assay < 100 ppm in DS
Residual DNA	Picogreen assay < 10 ng/dose in DS
Amino acid sequence	LC-MS/MS
Disulfide bond mapping	LC-MS/MS
Pyrogenic testing	Not applicable for work at CSIR-NCL

- Completed all the biosimilarity analysis required for RCGM submission
- Good agreement between an innovator and developed biosimilar protein

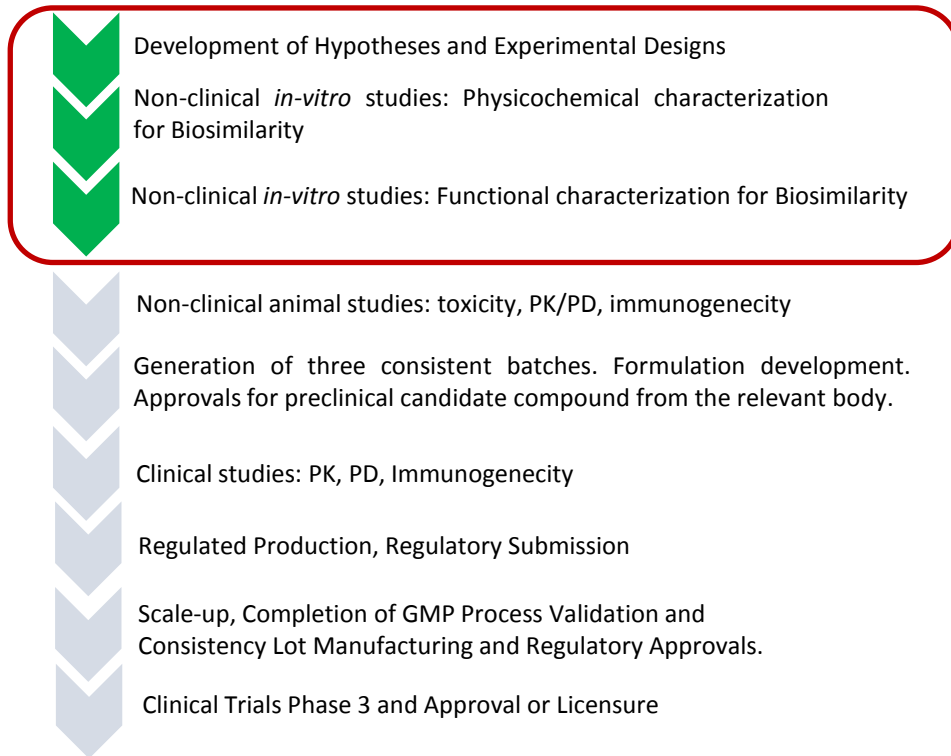
Current Status of Technology

Stage of Development

- Protein expressed at 10 L scale reactor

Key process parameters

- Yield of **Anakinra_NCL was determined to be 1.1 ± 0.20 g/L**



Next steps

Bioprocess Engg Group at CSIR-NCL is keen to forge industry partnerships for

- ◆ Advancing the biosimilar technologies presented today through *in vivo* and clinical studies.

Seeking Industrial partners interested in:

- ❖ Licensing technology knowhow with patents
- ❖ Joint development, technology advancement and scale-up projects
- ❖ Sponsored projects for process development for other biopharmaceuticals
- ❖ Industry projects utilizing expertise, capabilities and facilities with the group
- ❖ Consulting projects

Bioprocess Engineering Group



Dr Rahul Bhambure

Senior Scientist
Chemical Engineering and Process
Development Division,
CSIR-NCL, Pune, India

Recognitions:

DST Early Career Research Award

Past affiliations:

University of Delaware, IIT Delhi, ICT
Mumbai

Expertise:

Biochemical engineering; Bioprocess development;
Biopharmaceutical manufacturing (upstream and downstream);
Applied protein biophysics

Fact file of Dr Bhambure's Lab:

- More than 10 years of experience in the field of biosimilars
- Current team strength: 6
- Well equipped labs and analytical facilities including continuous processing platform for monoclonal antibody therapeutics, high resolution and high definition mass spectrometer



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