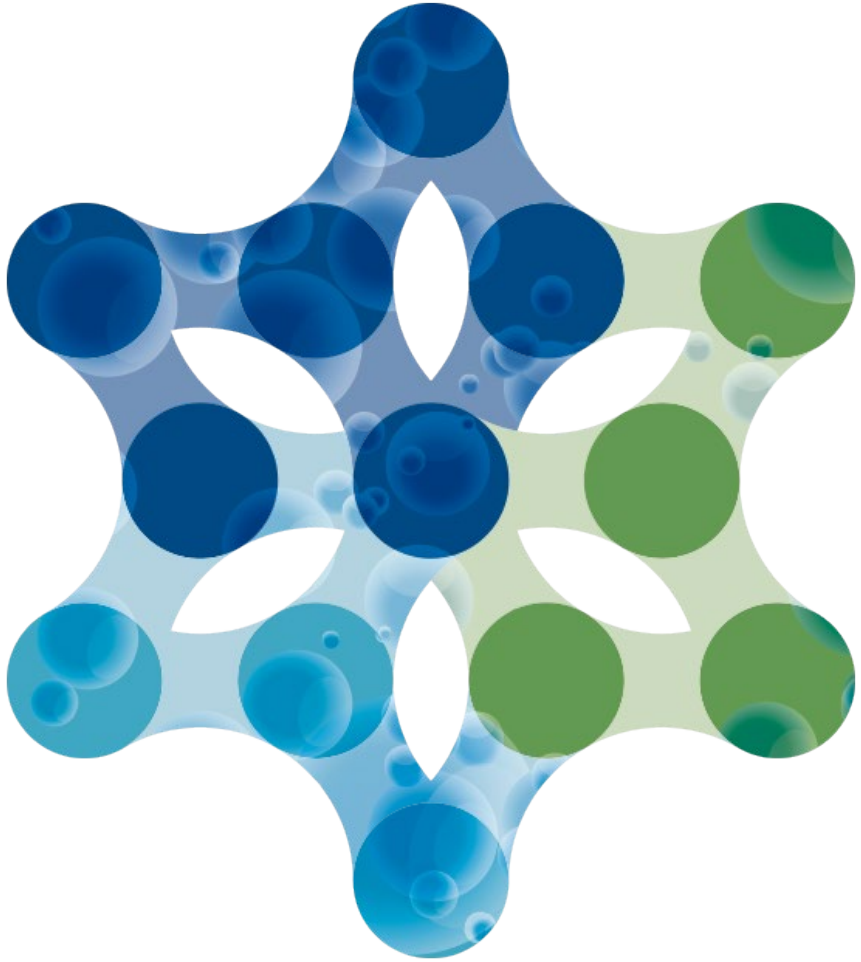
Challenges in Immunogenicity Risk Assessment for Complex Active Ingredients (like Peptide Related Drug Products)



Mr. Manoj Kumar Pananchukunnath
Biocon Limited, India

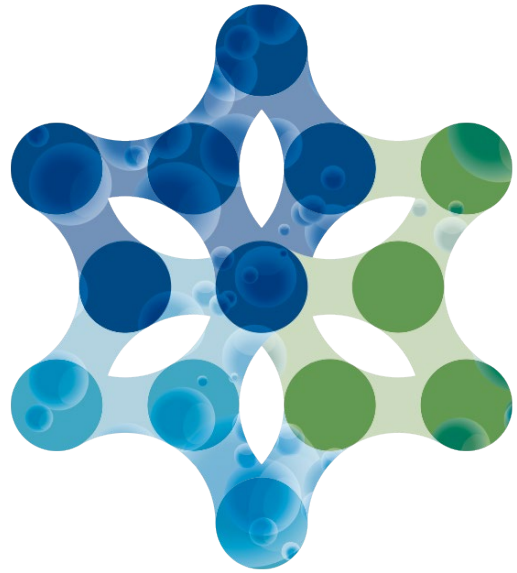
June-2025

Presentation Disclaimer

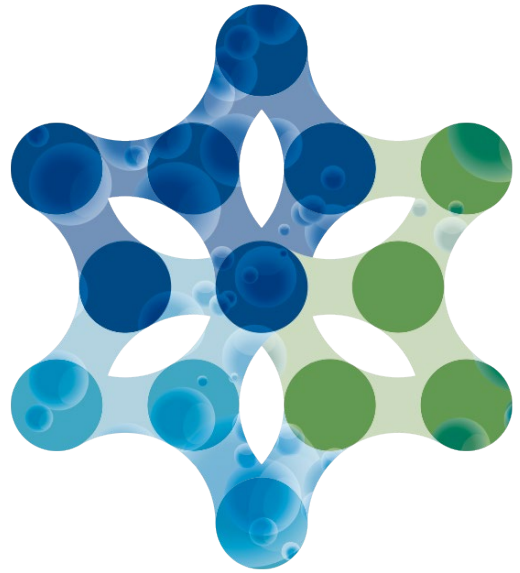
- This presentation is for educational purposes only.
- These slides are not intended for wider distribution outside the intended purpose without speaker approval.
- These slides are based on publicly available information (including data relating to non-Biocon products or approaches) and wherever applicable references have been provided.
- The views presented are the views of the presenter, not necessarily those of Biocon.
- The content of this slide deck is accurate to the best of the presenter's knowledge at the time of presentation.

Agenda

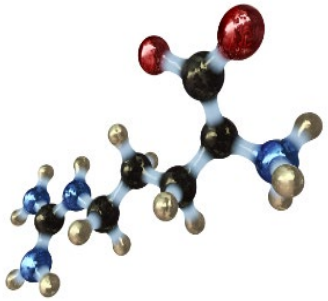
- Challenges in Immunogenicity
- Comparison of Different Regulatory Guidances
- Examples of Different Requirements
- Potential Harmonization Opportunities



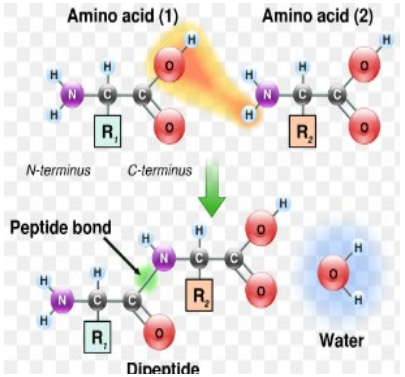
Challenges in Immunogenicity



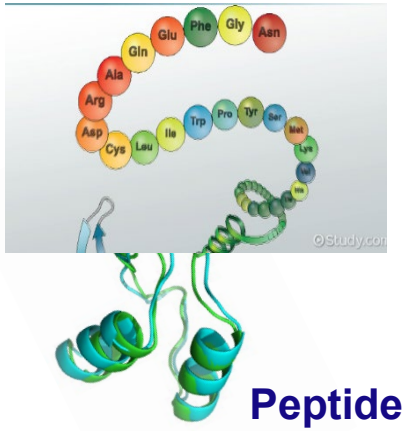
Peptides and Related Immunogenicity



Amino acid



Peptide Bond

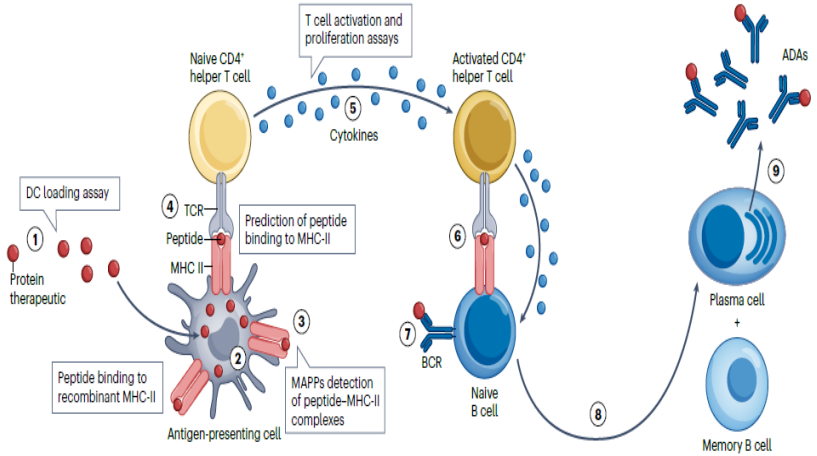


Peptide

Peptide structure related impurities

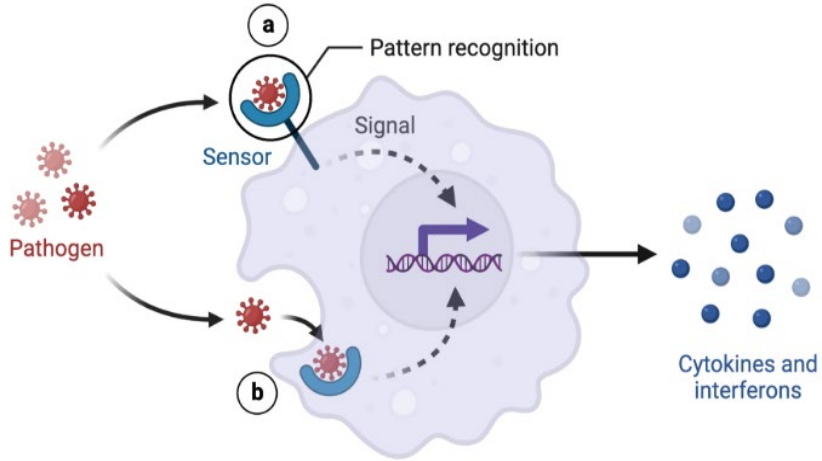
Process related impurities

Peptide structure related impurities (Individual impurities): Adaptive Immunogenicity



https://doi.org/10.1038/s41573-024-01051-x

Process related impurities (Drug Product): Innate Immunogenicity



https://app.biorender.com/biorender-templates/figures/all/t-5f17711942594f00af7e311a-innate-immunity
https://doi.org/10.1038/s41573-024-01051-x

Immunogenicity Assessment of Generic Peptides

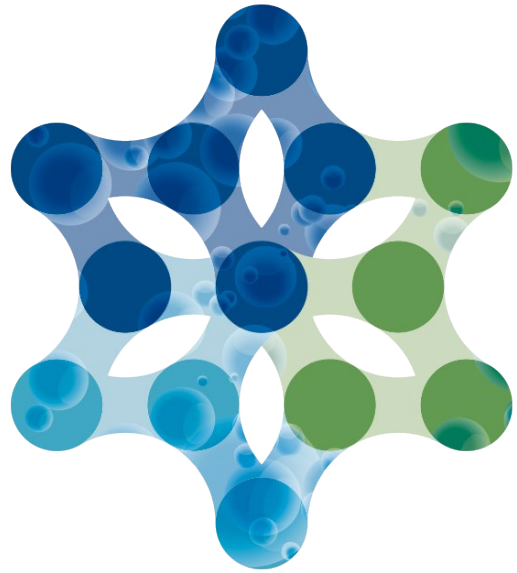
- **Immunogenicity - the ability of a molecule to induce an immune response**
  - Generation antibodies
  - Neutralizing antibodies reduce drug efficacy
  - Cross-reactivity with endogenous non-redundant proteins
  - Hypersensitivity responses - unwanted side effects
- **Immunogenicity of reference drugs is assessed through elaborate preclinical studies and clinical studies**
- **A generic peptide drug relies on the safety and efficacy assessment of reference drug (ensuring immunogenicity risk for the generic drug is largely mitigated)**
- **Residual immunogenicity risk due to different impurities arising from different manufacturing process**
  - Process-related (host cell proteins, leachable, extractables, microbial contaminant)
  - Peptide structure-related (impurities related to the API peptide, such as deletion, addition)

What are suitable approaches to assess this residual immunogenicity risk for a well defined, high purity peptide products of relatively small size as compared to Proteins?

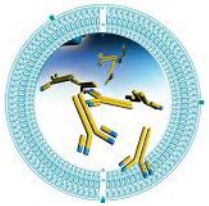
Why Generic Peptides Pose Unique Challenges ?

1. Synthetic Peptide API vs rDNA-based Peptide API. Science Vs Pathway??
2. Manufacturing variability (e.g. Manufacturing process, impurities)
3. Small changes in sequence of formulation
4. Immunogenicity study design and rationale

Comparison of Different Regulatory Guidances



Guidelines: Comparison on Generic Peptide Impurities & Immunogenicity



US FDA/Canada	VS	EMA/UK/India etc.
New Impurity with more than 0.5% is not acceptable	**Impurity**	No explicit threshold; Any levels if appropriately justified are acceptable.
Tiered approach: In silico, in vitro and in vivo assays	**Immunogenicity**	Risk-based approach: In silico, Invitro-assays may include PK data
ANDA: Requires demonstration of comparable impurity profile and immunogenicity risk. (Difficult path to get approval)	**Regulatory**	**Article 10 (1) generic application:** Requires demonstration of comparable impurity profile and immunogenicity risk. (Navigable path).

Considerations on Peptide Impurities & Immunogenicity

US FDA/Canada		EMA/UK/India/etc.
Identification threshold: 0.10 %	VS	▪ Reporting threshold: 0.1%, ▪ Identification threshold: 0.5% ▪ Qualification threshold: 1.0%.
▪ New impurity limit NMT 0.5% with Immunogenicity study. ▪ Common impurity higher than RLD- Immunogenicity shall be assessed.		▪ New impurity not concern on immunogenicity due to lower size ▪ Total Impurities should be ≤ RLD ▪ EP – General monographs limits are applicable.
Peptide structure related impurities- Adaptative immunogenicity study: **Expectation of additional data controls.**		Adaptative immunogenicity study: Accepted
Manufacturing process related impurities- Innate immunogenicity study : **Expectation of additional data controls.**		Innate immunogenicity study: Accepted

Is it reasonable expectation to have harmonization for the study design?

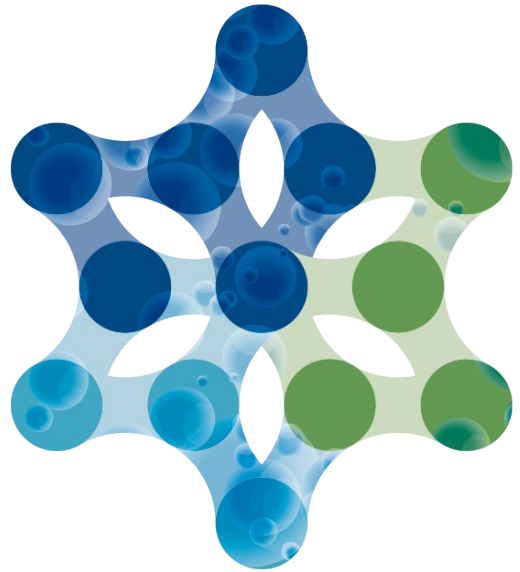
Innate Study for Process Related Impurities: Cytokine Screening Assay

Method	Attribute	EMA/UK/India etc.	US/Canada
Design	**Data acquisition**: FLEXMap 3D®	✓	✓
	Measured cytokines: IL-6, IL-8, IL-10, IFN-γ and TNF-α Established LQCs and HQCs of each cytokine	✓	✓
Sensitivity	**Positive control**: Pokeweed mitogen (PWM)	✓	Not accepted
	Clinical controls: Lemtrada® and Erbitux®	✓	Not accepted
	Specific PRR mechanism-based impurity (IIRMIs*) controls. *IIRMIs -Innate immune response modulating impurities	Accepted without any additional expectations	Additional expectations
	Spiking study: IIRMIs spiking into test product	Accepted without any additional expectations	Additional expectations

Adaptive Study for Peptide Structure Related Impurities: T cell Proliferation Assay

Method	Attribute	EMA/UK/India etc.	US/Canada
Design	**Mode of measuring**: T cell proliferation assay ([3H]-Thymidine uptake) on days 5-8	✓	✓
	Empirical threshold SI (Stimulation index): ≥1.9	✓	✓
Sensitivity	**Positive control**: Neo-antigen KLH (3414 AAs)	✓	Not accepted
	Low clinical control: Herceptin® (1328 AAs)	✓	Not accepted
	High immunogenicity control: CEFT-peptide pool (9-21 AAs)	✓	Not accepted
	Specific control: Peptide with similar length and general structure (XX AAs)	Accepted without any additional expectations	Additional expectations

Examples of Different Requirements



Innate Study: Specific PRR Mechanism-Based Impurity (IIRMIs) - Controls & Rationale

- ✓ Each cytokine LQC and HQC are established.
- ✓ Used controls are very well induced to release cytokines above established LQCs.
- ✓ Both EMA and US FDA noted that Process-related impurities from the cell construct (e.g. host cell protein (HCP), DNA) are not as concern for synthetic APIs.

Design ??

Cytokines as measured through all kind of PRR-based mechanism is accepted by EU/UK/India etc.

- ➢ **Why are additional PRR based controls (IIMRIs) required?**

Innate study: Harmonization of Suitability Controls

#	Control sample name	Cytokine responses ≥ LQCs (EMA/UK/India etc.)	Cytokine responses ≥ LQCs (US)
Clinical control -Low frequency (Unknown mechanism)	Erbitux	✓	✓
Clinical control -High frequency (Unknown mechanism)	Lemtrada	✓	✓
Positive control (Multiple TLR mechanism)	PWM	✓	✓
Specific PRR mechanism-based impurity (IIRMIs) controls	Zymosan (TLR2/Dectin 1)	Accepted without these controls	✓
	LPS (TLR4)		✓
	Poly IC (TLR3)		✓
	R848 (TLR8)		✓
	MDP (NOD2)		✓
All IIRMIs spiked in test product*	Spiked at different concentrations	Accepted without these controls	✓

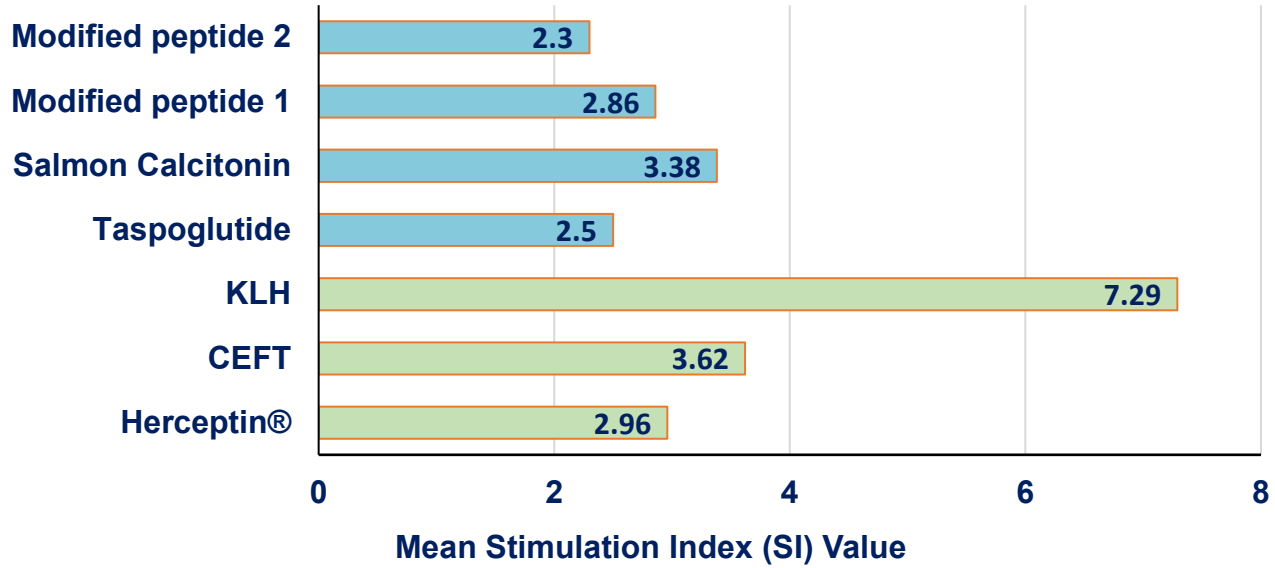
Erbitux, Lemtrada and PWM appear to be sufficient as method sensitivity controls as evidenced by acceptance by various agencies.

Do we need additional IIRMIs controls?

Adaptive Study: Harmonization of Suitability Controls

EdU (5-ethynyl-2′-deoxyuridine) Incorporation Assay

Naïve T cell Proliferation Assay (Day 6 & 8)



Additional expectations:

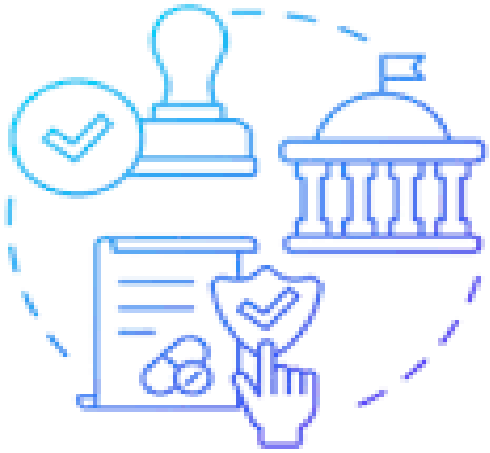
- **Modified peptide 1: (31 AAs)**
  - Two AA residue substitution.
- **Modified peptide 2: (32 AAs)**
  - Two AA residue substitution and one AA insertion.
- **Completely different AA sequence peptide:**
  E.g.,
  - Salmon calcitonin (32 AAs).
  - Tasopoglutide (30 AAs).

Observations :

- Naïve CD4+ T cells responses of all controls shown more than empirical threshold 2.0.
- KLH is shown higher Naïve T cell responses.
- KLH & Herceptin can be used as suitability controls.

Is a peptide control (with similar length and structure) required as suitability control?

Standardization of Immunogenicity Risk Assessment



REGULATORY AGENCIES

standardization

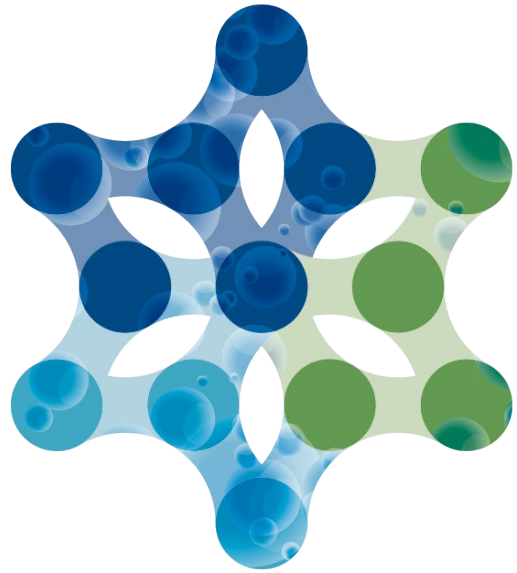
- No universally accepted Protocols
- Uncertainty in study design and regulatory compliance

•Leads to:
  - Inconsistent data interpretation
  - Development delays
  - Increased costs and regulatory risk
  - Slows down market entry of safe, effective product

- Not clear when in-vitro immunogenicity assessment is needed for some products

Common product-related challenges:
  - Synthesis of highly purified impurities to perform the adaptive immunogenicity assays
  - Excipient interference in the study

Potential Harmonization Opportunities



Harmonization Opportunities

1. **Is immunogenicity assessment required - if peptide related impurity profile is same?**
   - Is adaptive immunogenicity study required?
   - If required, an Adaptive immunogenicity study – may not required at EOSL.

2. **If manufacturing process is not same for DS/DP as RLDs?**
   - An Innate immunogenicity study could suffice if any different process impurities present (e.g.: host cell-based impurities).

3. **Adaptative study: Is the similar chain length peptide control as API is required?**
   - There is acceptance of KLH, Herceptin and CEFT as controls which are adequate to show the proliferation assay.

4. **Innate study: Method sensitivity (LQC & HQC) is established with Cytokine standards.**
   - Since selected concentrations of test samples could be good enough to induce the cytokines release more than LQC, then IIRMIs and their spiking may not be required?

5. **Synthetic API based drug product vs synthetic API based RLDs with similar impurity profile.**
   - If impurity profile is demonstrated to be same is an immunogenicity risk assessment mandated?

6. **rDNA-based drug product vs rDNA-based RLDs**
   - Manufacturing process capability yielding lower impurity profile than RLDs, could an Innate immunogenicity assessment be adequate.

7. **Synthetic API based drug product Vs rDNA based RLD**
   - Synthetic process yielding lower impurity profile than RLDs, will it mandate immunogenicity risk assessment.

Conclusion

- Major scientific evidences are available to harmonize the protocol for synthetic peptides
- Emphasis on cost effective alternative approaches (In silico models with acceptable limits)
- Future need: More specific regulatory guidance for peptide immunogenicity assessment

Acknowledgments

Mr. Anil Sachdeva
Head – Regulatory Sciences
(API & Formulations)

Dr. Nitin Sopanrao Patil
Head – API R&D Peptides

Mr. Shashi Kant Tiwari
Head – Analytical R&D
(API & Formulations)

Ms. Jaya Patel
Chief of Staff – R&D

Dr. Rajendar Bandari
Lead – Characterization Expert
(API & Formulations)

Thank You!