Current FDA thinking on the use of Non-Clinical Tools in Immunogenicity Risk Assessments: Possibilities and Challenges

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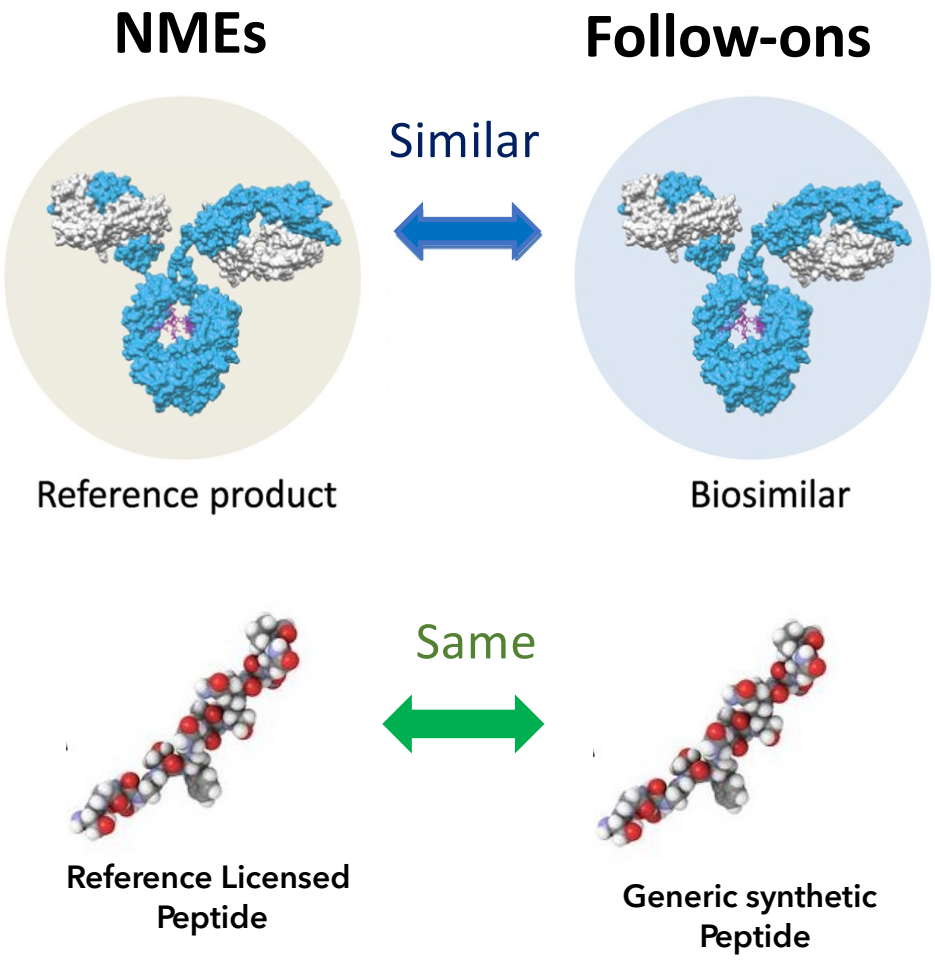
Disclaimer:
The opinions and views expressed herein are my own and not necessarily reflective of those of the FDA or current policy and herein should not be used in place of regulations, published FDA guidance, or discussions with the Agency

EIP - Portugal 2023


CDER's Office of Pharmaceutical Quality
FDA
A quality product of any kind consistently meets the expectations of the user – drugs are no different.
Patients expect safe and effective medicine with every dose they take.
Pharmaceutical quality is assuring *every* dose is safe and effective, free of contamination and defects.
It is what gives patients confidence in their *next* dose of medicine.

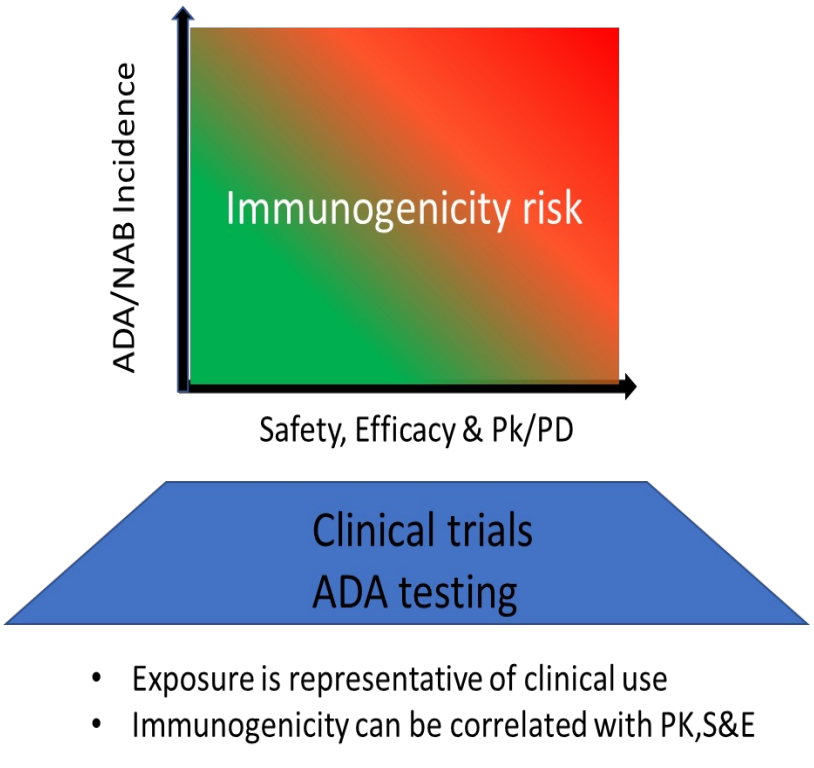
Talk layout:

- Immunogenicity risk & CQAs
- Immunogenicity risk of NMEs
- Immunogenicity risk of “follow-on” peptides, oligonucleotides, and proteins
- FDA’s experience with Immunogenicity risk of synthetic generic peptides
- Case studies
- Lessons learned and knowledge and technical gaps



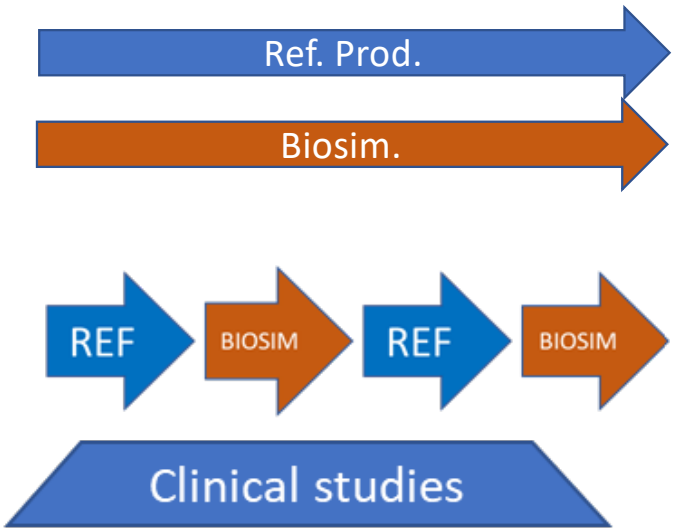
Current FDA thinking on Predicting Immunogenicity Risk for NMEs (peptides, proteins, oligonucleotides…)

- Predicting whether a product will induce an immune response, in what subset of patients, and how the immune response will impact the clinical outcome, remain some of the most challenging questions in the development and regulation of novel therapeutic proteins.
- Currently immunogenicity cannot be predicted from product structure and formulation, or from animal modeling therefore **clinical studies are needed to assess product immunogenicity and its clinical consequences.**



Immunogenicity Risk of Follow-on Products:

- Generics (505(j): No clinical trials for safety or immunogenicity
- 505(b)(2) and Biosimilars: Descriptive comparative parallel arm safety and immunogenicity studies.
  - Binding and neutralizing antibodies
  - Incidence and titer
  - Rates of antibodies formation and persistence



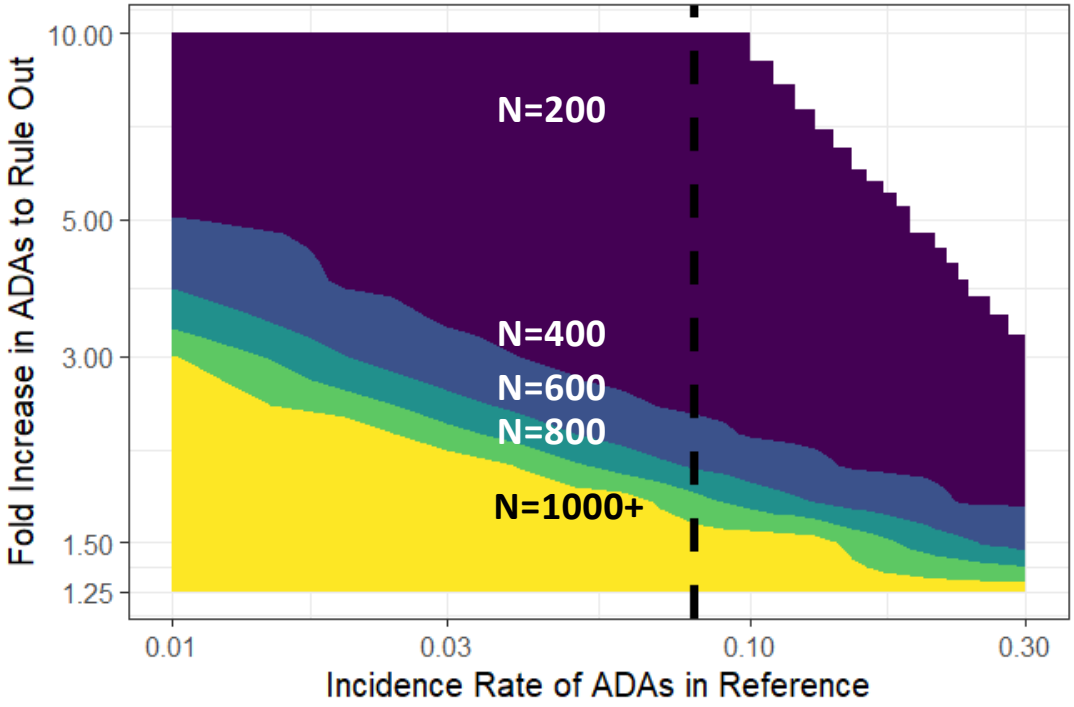
No difference in incidence of ADA or severity of clinical impact

Limitations:

- Size and length (abbreviated)
  - Delayed onset of product immunogenicity
  - Low frequency/high clinical impact immunogenicity.
  - Switching studies rely on changes in PK (not powered for safety)
- Cost (time, risk, $)

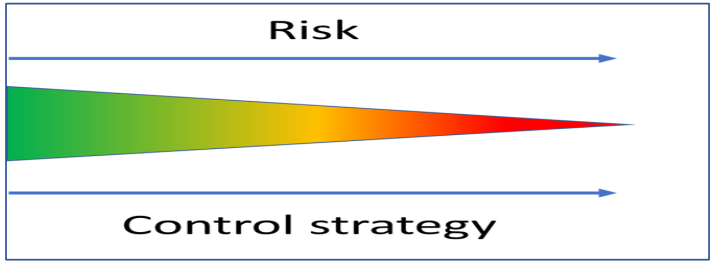
Anti-Drug Antibodies as an Endpoint –for an abbreviated clinical program

Number of Subjects (randomized 1:1) needed establish No increase (alpha = 0.1, power = 80%, one sided test)



Design depends on goal:

- Exclude a pre-specified difference in incidence of ADAs?
- Exclude catastrophic impact on clinical safety outcomes associated with immunogenicity?
- Exclude a pre-specified difference in efficacy and demonstrate similar safety?
  - ➢ Discuss with Agency



Can non-clinical tools inform an immunogenicity risk assessment for follow on products?

Immunogenicity risk assessment of follow-on products

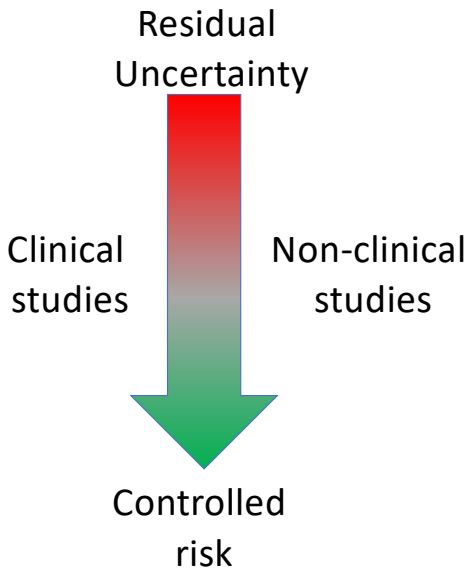
Risk = Probability X Consequences

- Population ✓
- Treatment ✓
- Product

- Safety ✓
- Efficacy ✓

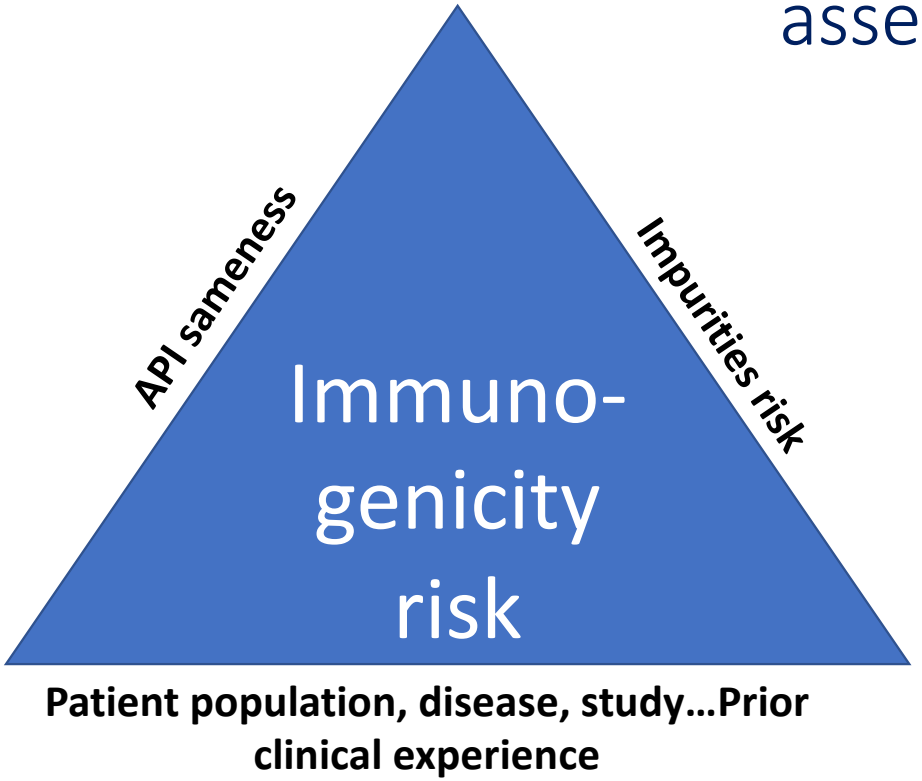
If API is highly similar, then most residual uncertainty is due to <u>impurities</u>

- Product-related impurities
- Process-related Impurities



Product and process-related impurities can elicit immune response or have adjuvant activity inducing or augmenting local/systemic inflammatory response and/or anti-drug antibodies

Assessment of impurities to inform immunogenicity risk assessment



Immunogenicity-Related Considerations for Low Molecular Weight Heparin
Guidance for Industry 2016

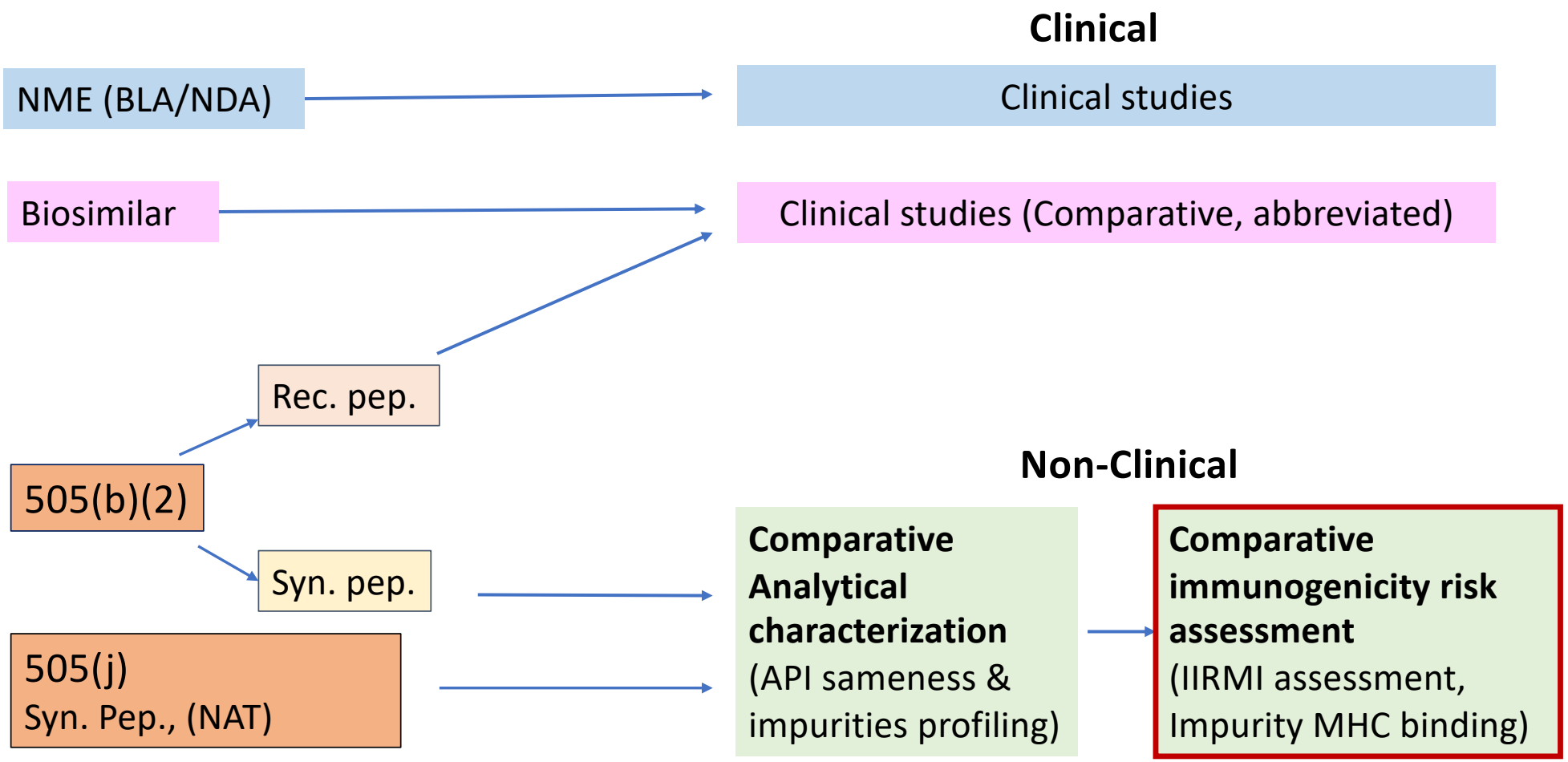
ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin
Guidance for Industry
2021

- glucagon, liraglutide, nesiritide, teriparatide, and teduglutide.

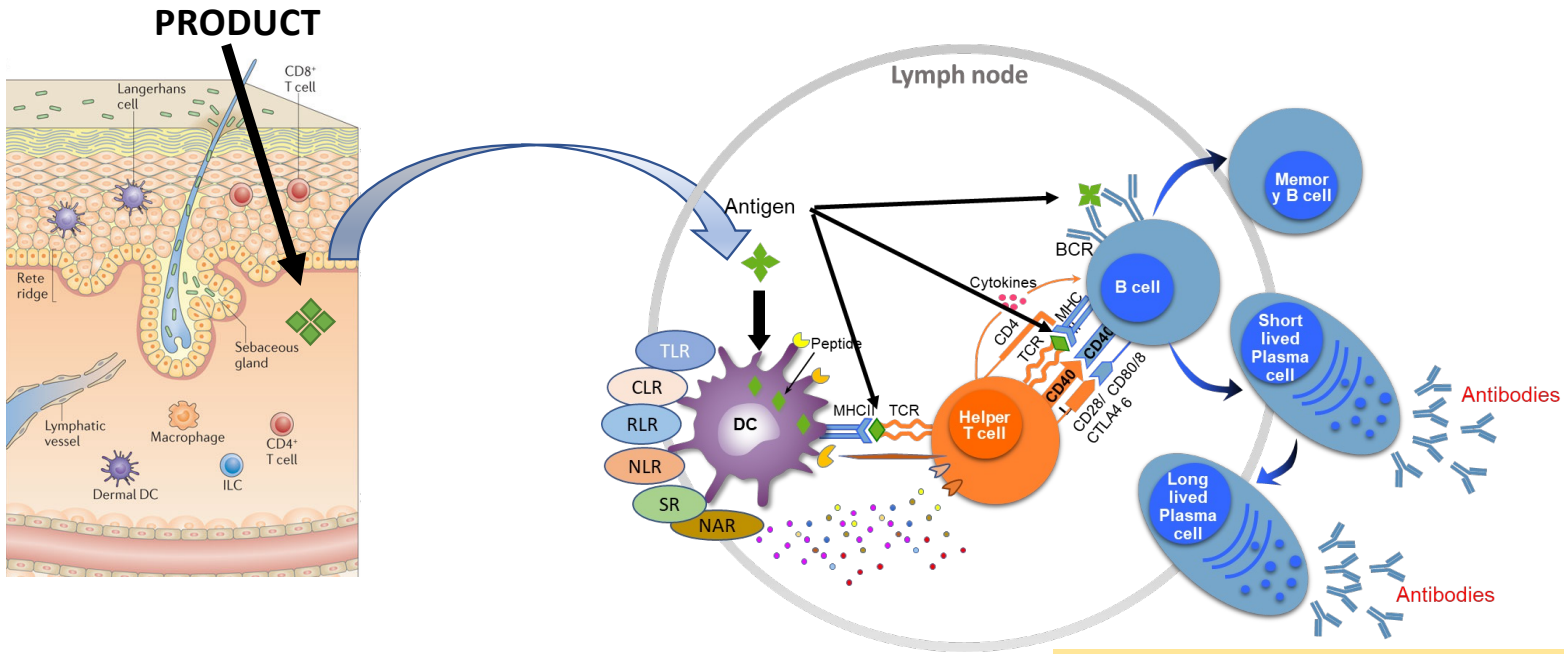
"For a synthetic peptide that is intended to be a "duplicate" of a previously approved peptide of rDNA origin, a determination of whether an application for the synthetic peptide should be submitted as an ANDA depends largely on *its impurity profile as compared to the impurity profile for the peptide of rDNA origin*."

https://www.fda.gov/files/drugs/published/Immunogenicity-Related-Considerations-for-Low-Molecular-Weight-Heparin-Guidance-for-Industry.pdfhttps://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

Immunogenicity Assessments Across Regulatory Pathways

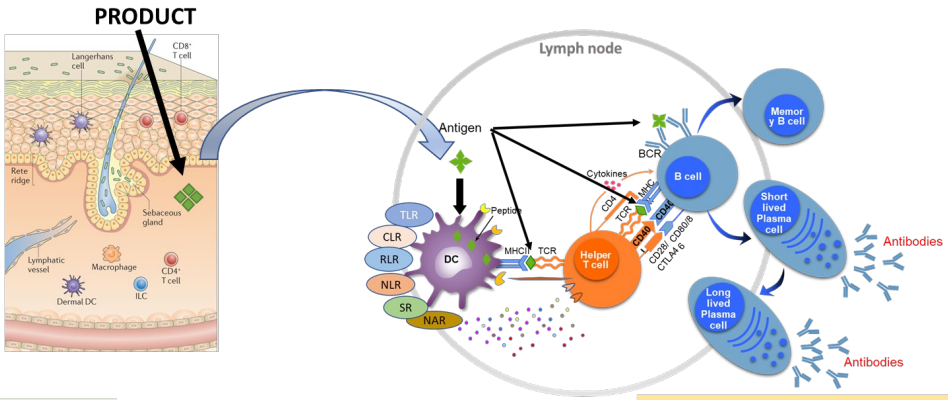


APCs and Thelper Cells are the Lynchpin in Generating Immune Responses



Process–related impurities	Methods that examine innate immune activation by IIRMI In vitro (IIRMI, Ag uptake, DC maturation)
New or increased Product – related impurities (>0.1%)	Methods that assess binding to MHC In silico In vitro (MHC binding, MAPPs) Methods that assess T cell activation In vitro (DC-T cell) In vivo (humanized mice)

APCs and Thelper Cells are the Lynchpin in Generating Immune Responses



Biologics framework:
Inform the immunogenicity risk assessment prior to the clinical use.

- Target selection
- Deimmunization of therapeutic candidates

Process–related impurities	Methods that examine innate immune activation by IIRMI In vitro (IIRMI, Ag uptake, DC maturation)
New or increased Product – related impurities (>0.1%)	Methods that assess binding to MHC In silico In vitro (MHC binding, MAPPs) Methods that assess T cell activation In vitro (DC-T cell) In vivo (humanized mice)

Research Method → Fit for Purpose Method →

- Sensitivity (LOD, LOQ)
- Specificity
- Precision
- Linearity and range
- System suitability controls
- Acceptance criteria
- Documentation
- Traceability
- …

Predicting new T cell epitopes: In silico tools

- Advantages:
  - High throughput,
  - Covers multiple MHC including rare types
  - Disruption of Treg sequences
  - Potential impurities

- Current limitations:
  - Primary sequence. No unnatural or modified amino acids
  - HLA DR, but not DP, DQ
  - Strength of data supporting different MHC differs
  - Proprietary computational algorithms

In silico assessments of MHC binding are informative but not sufficient: Orthogonal approaches to assess MHC binding are needed.

In vitro assays to assess MHC binding and naïve T cell responses:

- Multiple formats:
  - MAPPs
  - MHC binding
  - DC:T cell assays

  - Low throughput and complex
  - High donor-donor variability
  - Low frequency of naïve T cells (age-dependent)
  - Difficult to validate

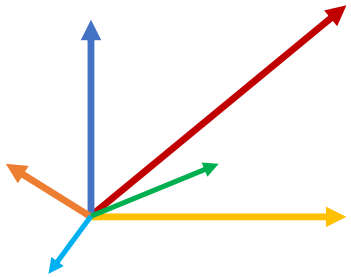
- Provide clear experimental design and culture conditions
  - Justify method used (size, MHC, target population)
  - Culture conditions & concentration of the product used
  - Confirm APC activation and presentation (MAPPs assay, DC activation markers)
  - Readout selection (proliferation, cytokines, cell markers)
  - Suitability controls that confirm sensitivity for naïve T cell responses

Common problems in DC:T cells assays

- Number of screened T cells is too low to detect responder naïve T cells (~1-10/1,000,000 ag-specific naïve T cells)
- Peptide concentrations is too low to elicit response (<0.1uM).
- Inadequate suitability controls: LPS, PHA, KLH can be used to ensure the presence of live APC and responsive cells in the culture but are not recommended as suitability controls for naïve T cell responses to specific antigens.
- MHC tested not high risk per in silico.

In Vitro Assays to characterize Innate Immune Response Modulating Impurities (IIRMI)

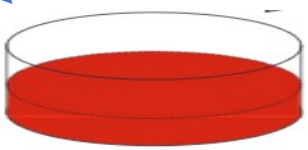
- ➢ *IIRMI can increase local reactogenicity and immunogenicity*
- ➢ *Trace level different IIRMI can synergize*
- • *IIRMI assays use biomarkers to detect biological differences caused by different impurities capable of inducing local inflammation and/or act like adjuvants.*
- • *IIRMI assays should detect broad array of potential IIRMI, including DAMPs*
- • *IIRMI assays do not ID impurities*



Polumuri et al, 2018

PBMC
Whole blood
Reporter Cell lines

DP



Innate immune activation

- NFkB activation
- Ag uptake
- Cytokine production
- Cell surface marker expression
- mRNA expression patterns

Critical Attributes for Assays for Innate Immune Response Modulating Impurities: Platform

Cell line		Origin	Commercial Availability
PBMC/ Whole blood	Proliferation/ Cytokines	Human M∅, DC, MΘ, and Ly's	Yes
Dendritic cells activation	Activation markers	Fresh or frozen Human DC	Yes
THP-1, MM6, Ramos	NFkB, Cytokines	Human cell lines	Yes
RAW-BLUE	NFkB	Mouse macrophages	Yes
Single Receptor line*	Single Receptor line	e.g. Human embryonic kidney (HEK-TLRX)	Yes

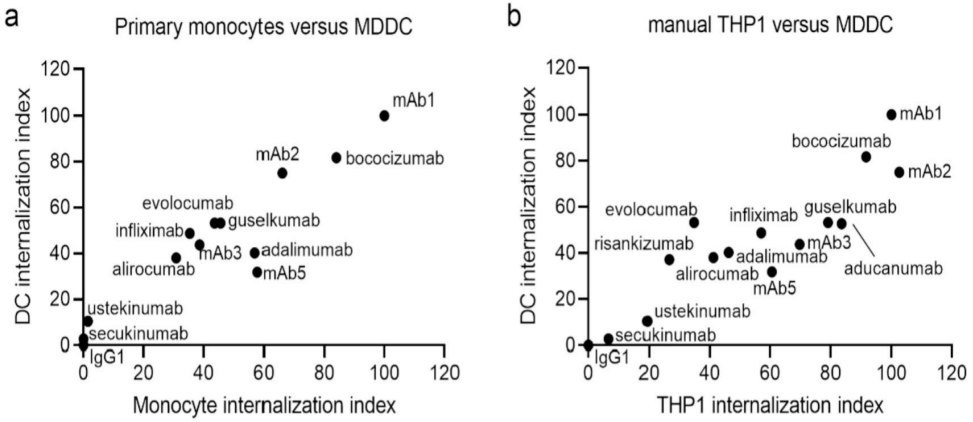
* ID impurity type

Critical Attributes for Assays for Innate Immune Response Modulating Impurities: Platform

Cell line		Origin	Commercial Availability	
PBMC/ Whole blood	Proliferation/ Cytokines	Human M∅, DC, MΘ, and Ly's	Yes	✓ Broad Rec. array • Low throughput • Availability & variability • Few DC, no ILC in PB • APC in PBMC ≠ Tissue
Dendritic cells activation	Activation markers	Fresh or frozen Human DC	Yes	
THP-1, MM6, Ramos*	NFkB, Cytokines	Human cell lines	Yes	✓ Reproducible • Limited Receptor Repertoire • (NFKB – centric) • No aggregate response
RAW-BLUE*	NFkB	Mouse macrophages	Yes	
Single Receptor line	Single Receptor line	e.g. Human embryonic kidney (HEK-TLRX)	Yes	

* Potentially not sufficient on their own. Consult Agency

IIRMI Assay Platforms:



	cell	LPS	mAb1*	mAb2*	mAb3*	mAb4	mAb5	bococizumab
IL1β	MDDC	+++	+++	-	-	-	-	-
	Monos	+++	+++	-	-	-	-	++
	THP1	+++	++	-	-	-	-	-
IL-6	MDDC	+++	+++	++	-	-	-	+++
	Monos	+++	+++	-	-	-	-	++
	THP1	+++	-	-	-	-	-	-

Comparability study of monocyte derived dendritic cells, primary monocytes, and THP1 cells for innate immune responses.

Wen Y, Wang X, Cahya S, Anderson P, Velasquez C, Torres C, Ferrante A, Kaliyaperumal A.
J Immunol Methods. 2021

Cell lines are higher throughput but less sensitive to IIRMI differences.

- Use of a single cell line is discouraged
- Acceptability of cell lines as a testing platform depends on product risk.

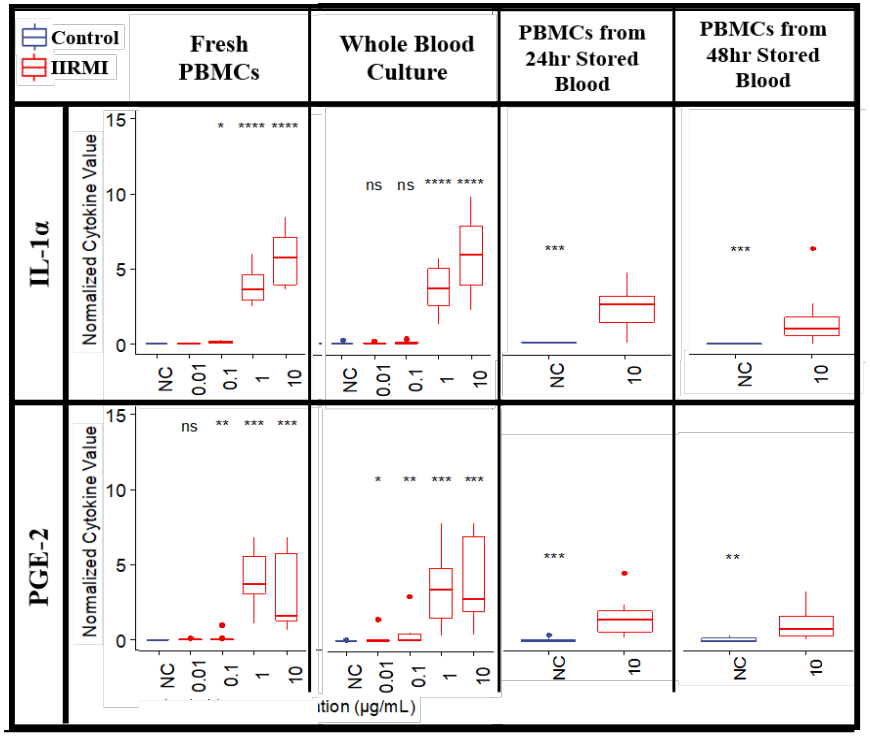
Critical Attributes for Assays for Innate Immune Response Modulating Impurities: Fit for Purpose

- **Sensitivity** (LOD, LOQ), Drug Tolerance, Specificity, Precision, Accuracy (ICH Q2(R2)

- **Suitability controls** (Neg., Low, High PC). Demonstrate consistent sensitivity to low levels of a variety of innate immune response modulators capable of triggering diverse innate immune pathways.
    - Establish acceptance criteria for controls
    - Demonstrate signal recovery
    - Account for all dilutions to determine assay sensitivity
- **Result interpretation**
    - Multiparametric semi-quantitative assessment of different paths of innate immune activation rather than positive/negative.
- **Data traceability and controls**

IIRMI Critical assay attributes (1):

- Cell Platform:
  - ❑1ry cells (WB/PBMC/DC)
    - **>20 donors**
    - Donors acceptance/selection criteria (healthy vs target)
    - Sample processing (Fresh vs frozen)
      - Cell viability (pre and post assay - APC).
  - ❑Cell lines:
    - Passage number
    - Confluency
- Culture conditions
  - Cells/well, culture time, media, etc.
  - Matrix interference (e.g. formulation)
- Drug concentration in well



Holley et al, 2021

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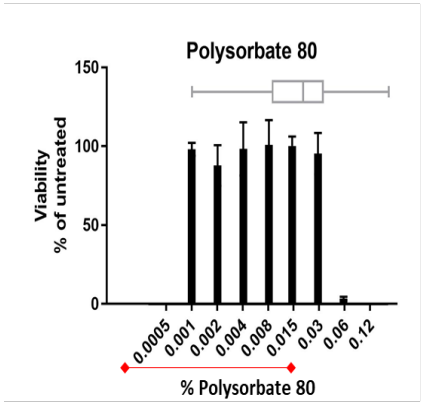

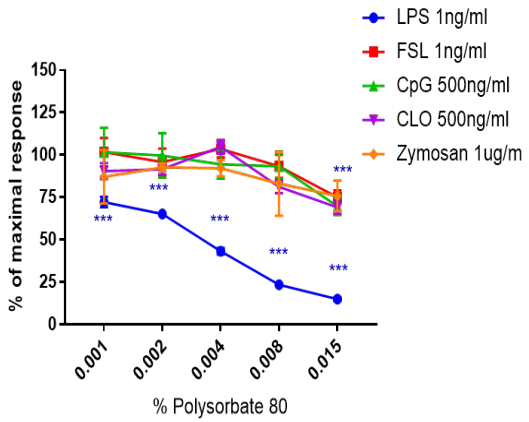

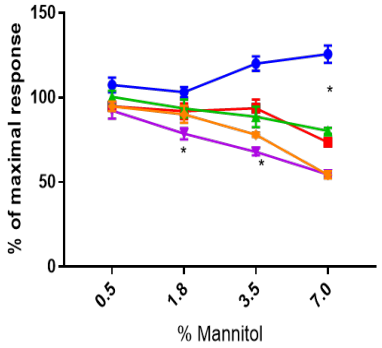

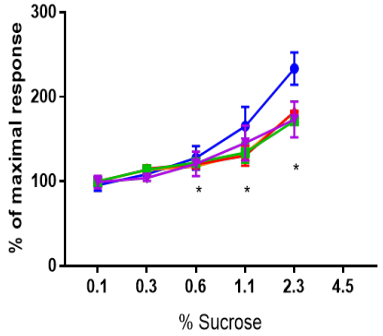

RAW cells

Thacker et al, 2021

IIRMI Critical assay attributes (1):

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    - Donors acceptance/selection criteria (healthy vs target)
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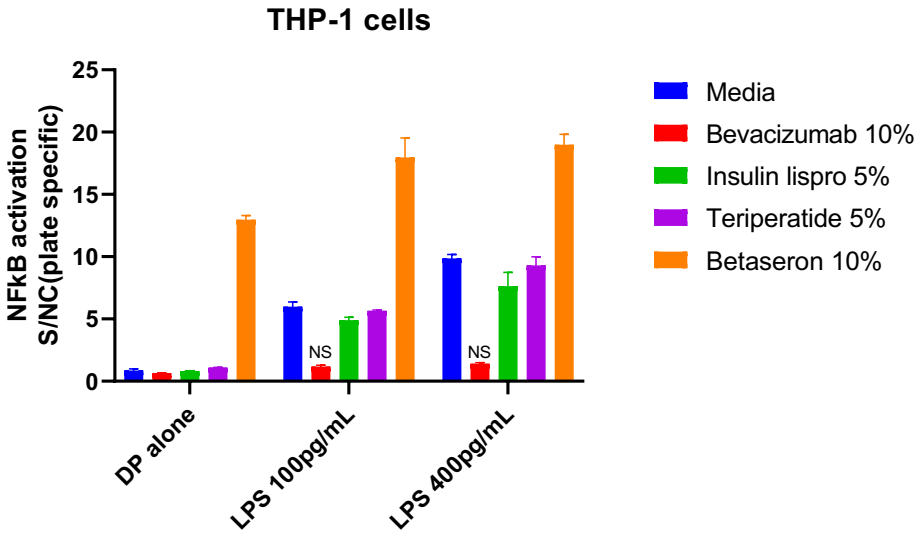
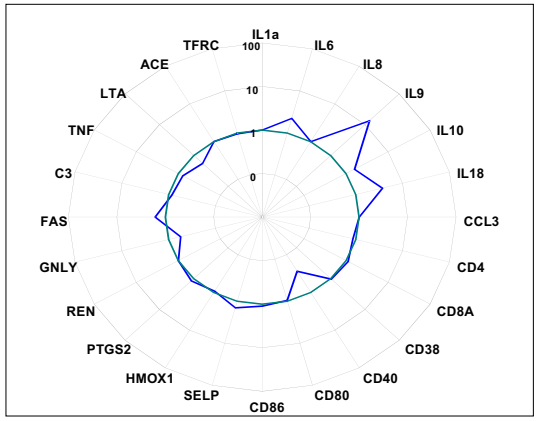


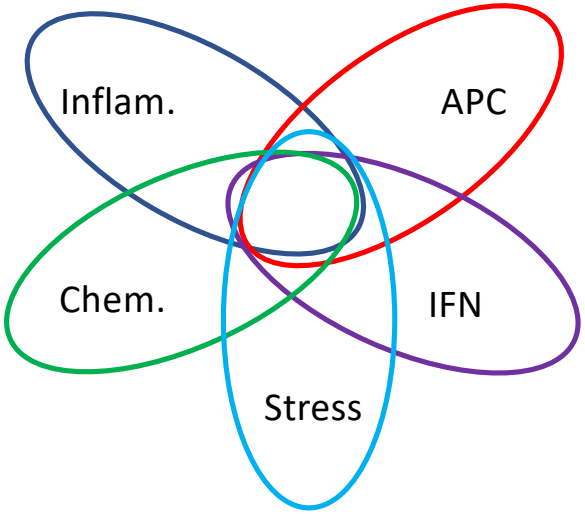
- ➢ Confirm response to trace levels of multiple PRR agonists in presence of DP
  - ➢ Examine changes to cell viability and metabolic activity
- ➢ Understand the impact of product formulation on IIRMI detection
- ➢ Optimize matrix vs dilution

IIRMI Assay Readout:

- NFKB activation in reporter cell lines (THP-1, RAW-Blue etc.)
- DC activation (CD11c, CD86, HLA)
- APC Ag uptake
- Cytokine expression (e.g. IL-1α, MIP-1α, IP-10, MCP-1, IL-6, IL-8, and PGE-2)
- Gene expression(mRNA): Single gene vs **Expression patterns**

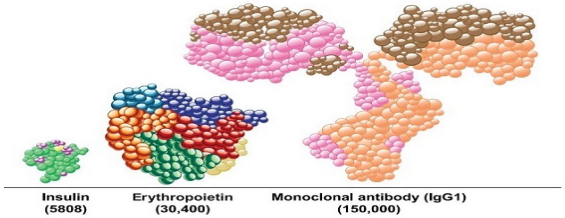


Comprehensive multiparameter assessment are preferred since impurities can trigger different innate immune pathways capable of increasing immunogenicity risk



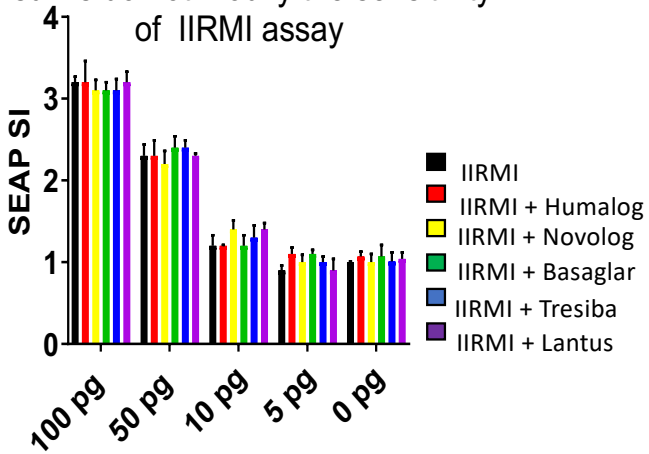
Case studies

Case study I: Assessing IIRMI in biosimilar insulins



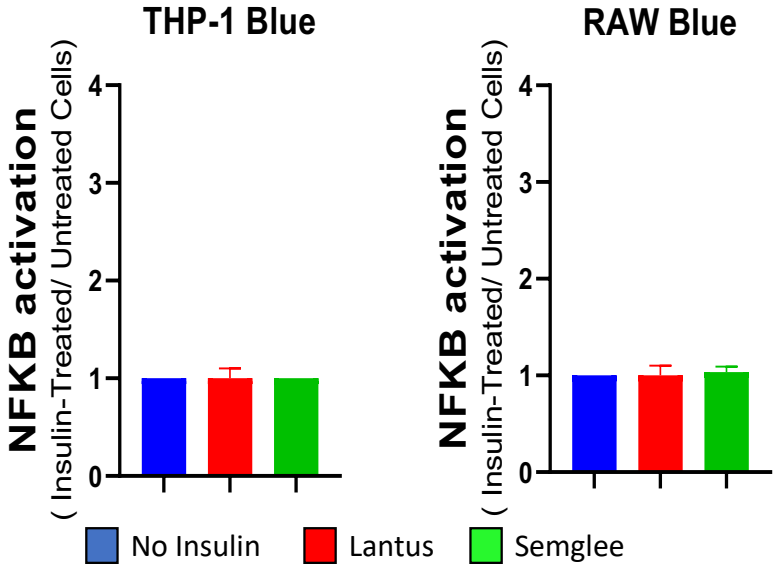
Formulation contains
M-Cresol
Polysorbate

Insulins do not modify the sensitivity of IIRMI assay



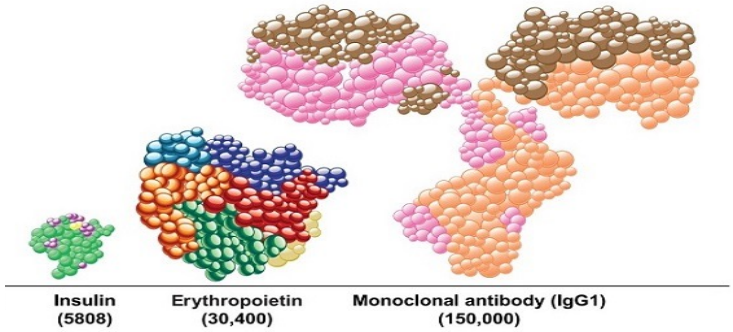
DP does not mask TLR or NLR agonists

- Strategy: IIRMI assay using Reporter cell lines



No difference in NFKB Lantus (e.coli) and Semglee (picchia) in THP-1 or RAW cells

Case study I: Assessing IIRMI in biosimilar insulins



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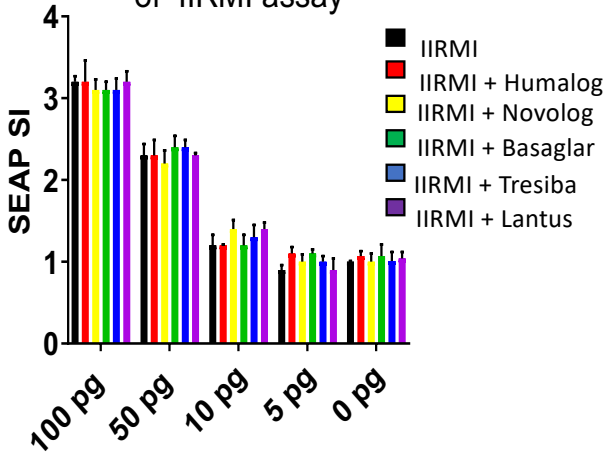

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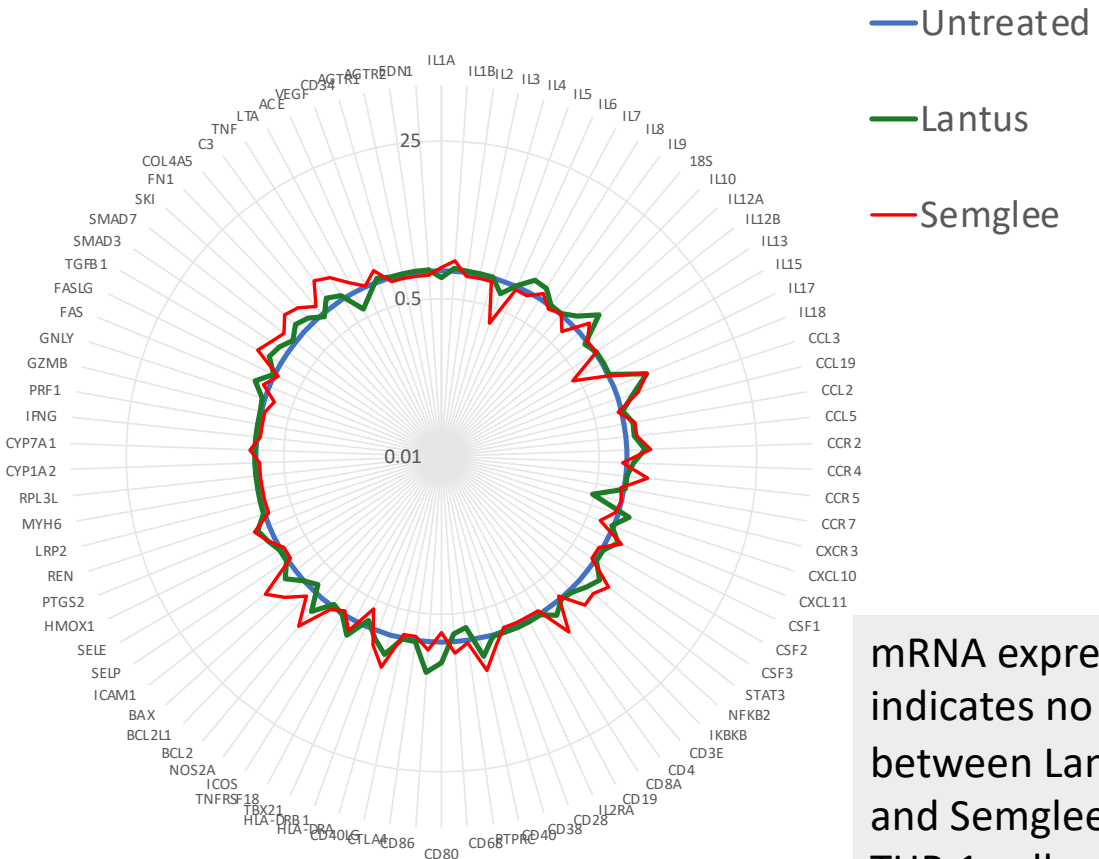
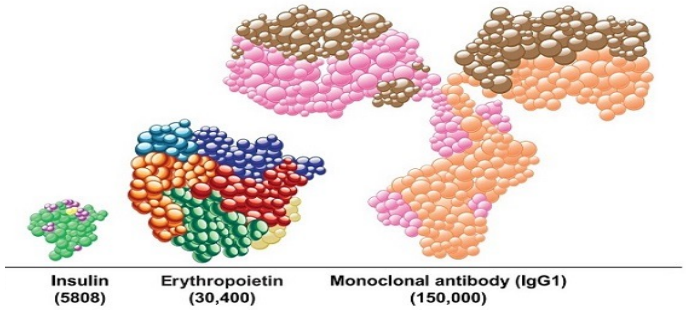
- Strategy: IIRMI assay using THP-1 cells



mRNA expression pattern indicates no difference between Lantus (e.coli) and Semglee (picchia) in THP-1 cells

Case study I: Assessing IIRMI in biosimilar insulins

- Strategy: IIRMI assay using THP-1 cells



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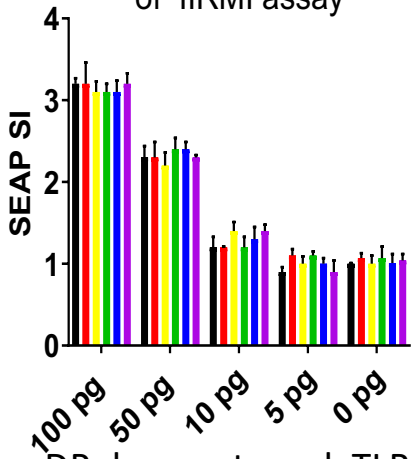
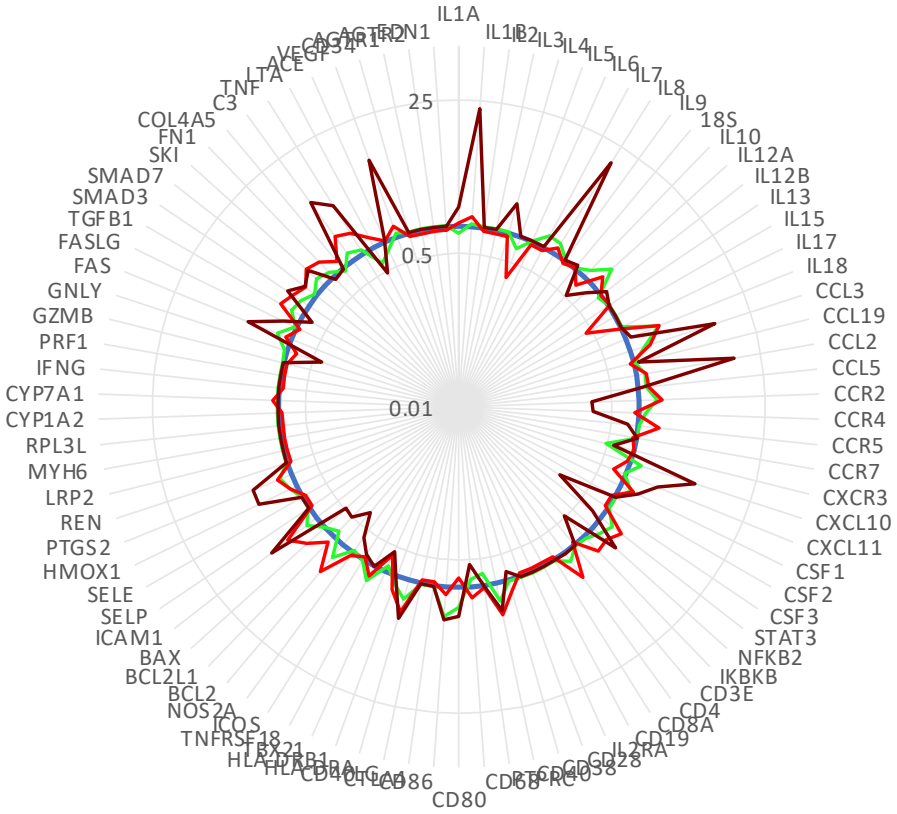


DP does not mask TLR or NLR agonists

Insulin spiked with LPS



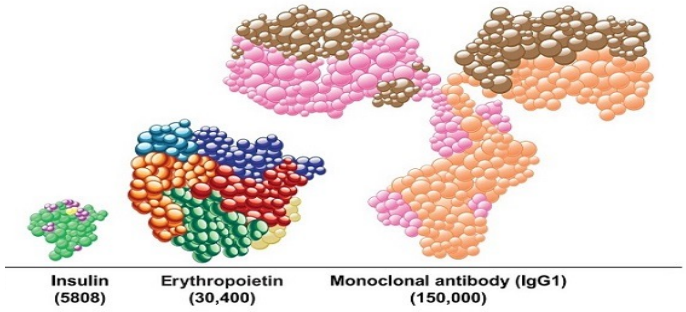
- Untreated
- Lantus
- Semglee
- Semglee + LPS (100 pg/ml)

Similar results with trace levels of TLR2, TLR7, TLR5 ag.

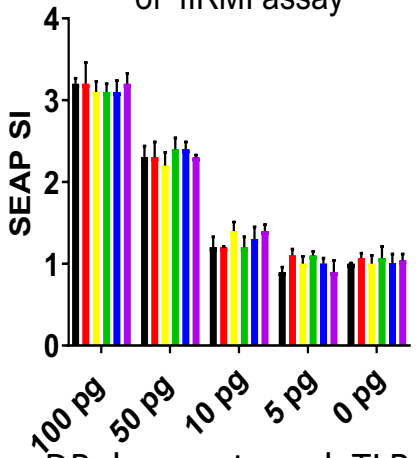
IIRMI spiked into product confirmed sensitivity and breadth of IIRMI response

Case study I: Assessing IIRMI in biosimilar insulins

- Strategy: IIRMI assay using fresh PBMC

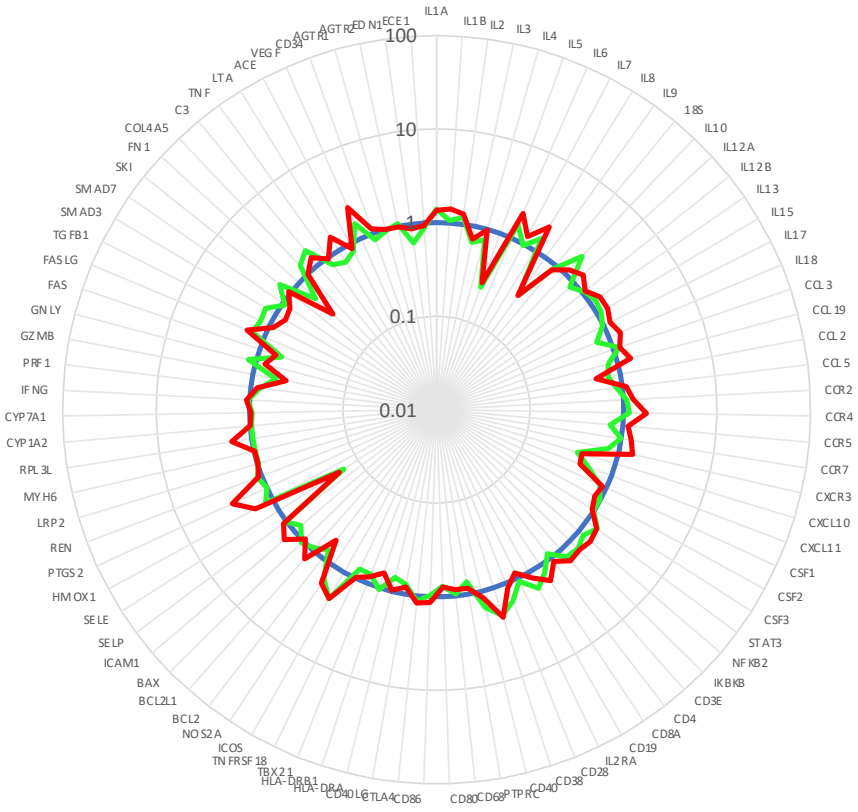


Insulins do not modify the sensitivity of IIRMI assay



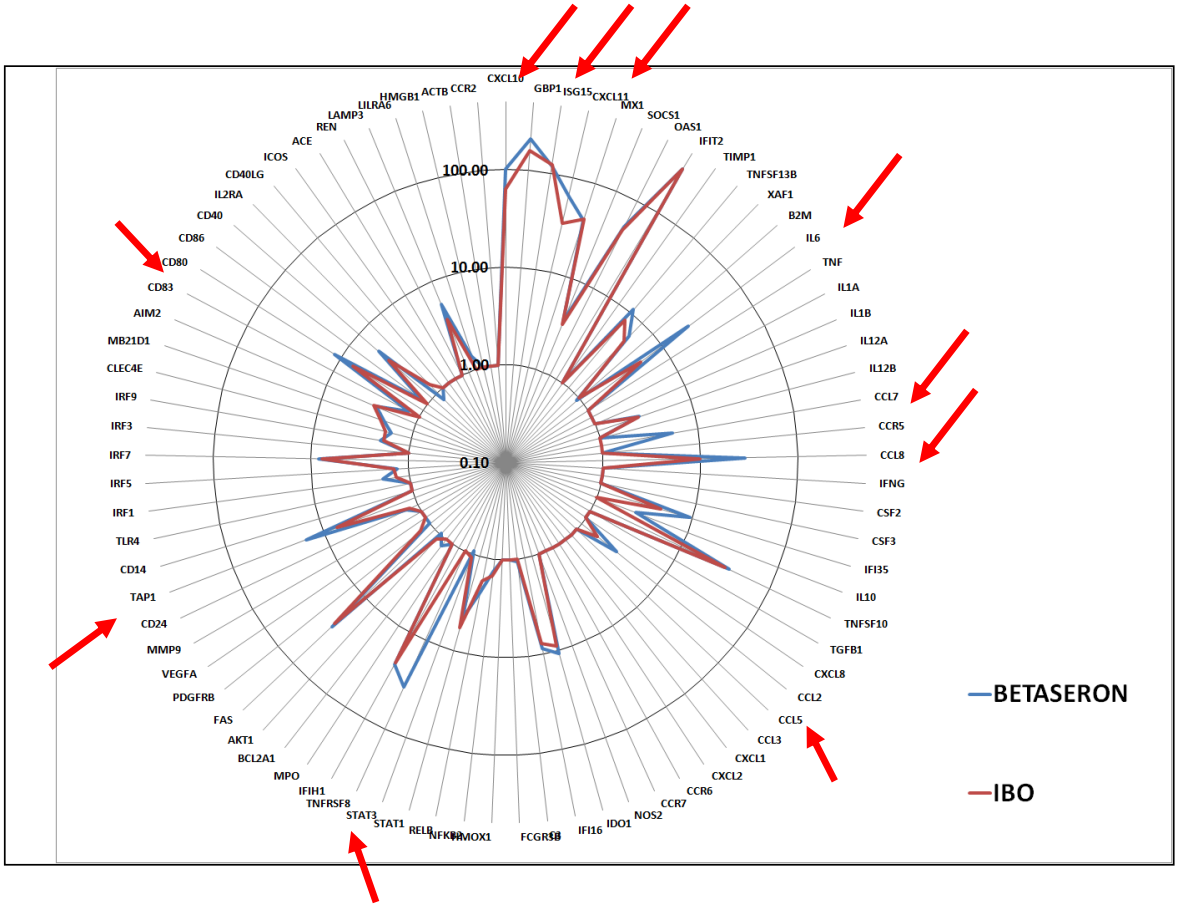
DP does not mask TLR or NLR agonists

Response from 20 healthy donors



Gene expression pattern elicited by Lantus and Semglee in PBMC from 22 healthy donors confirmed Cell-line-based assessment: **No increased risk due to IIRMI.**

Case Study II: Distinct gene expression by immune modulator and its biosimilar candidate



No difference:

Il1 α

Il1β

Ccl3 (Mip1α)

Ifnγ

Differences:

Il6

Ccl8

Tnfrsf8

Ccl2

Ccr7

Cd80

Cd40

Tap1

Lamp3

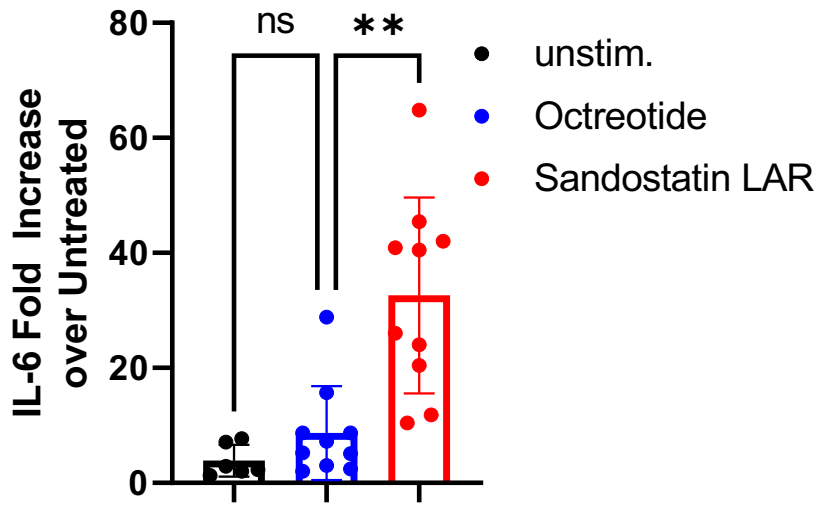
Multiparameter IIRMI assessment indicates significant difference between Betaseron and biosimilar candidate in MM6 cells.

- Reduced inflammatory signal in biosimilar was acceptable

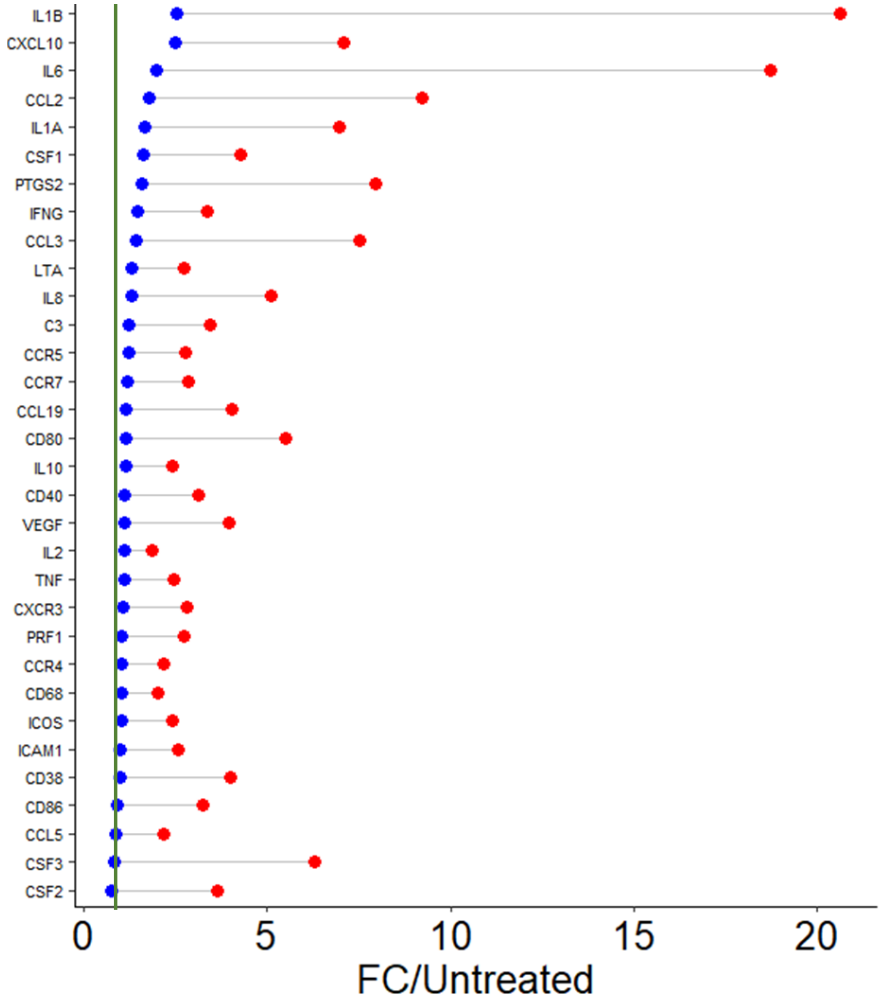
Mufarrege et al. 2019
Haile et al, 2017

Case Study III: Generic Octreotide

Clinical trials for Octreotide: 8 amino acids
Oc. Acetate (monomer): <1%
Oc. Sandostatin LAR (in PLGA microparticles): 25%



Octreotide Acetate vs Octreotide SLR



Multiple gene can differentiate OC. acetate from OC. LAR

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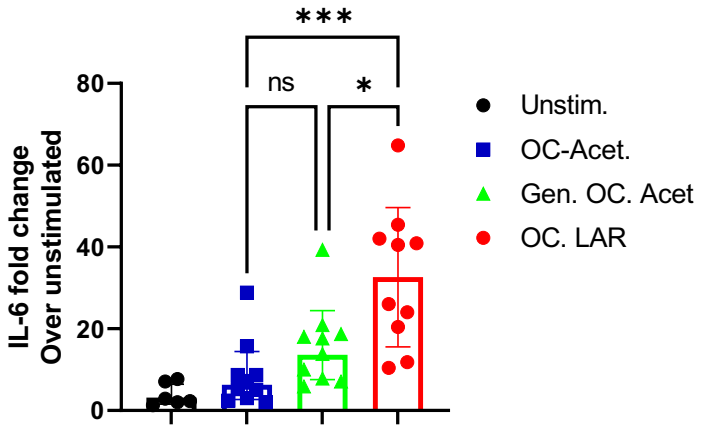
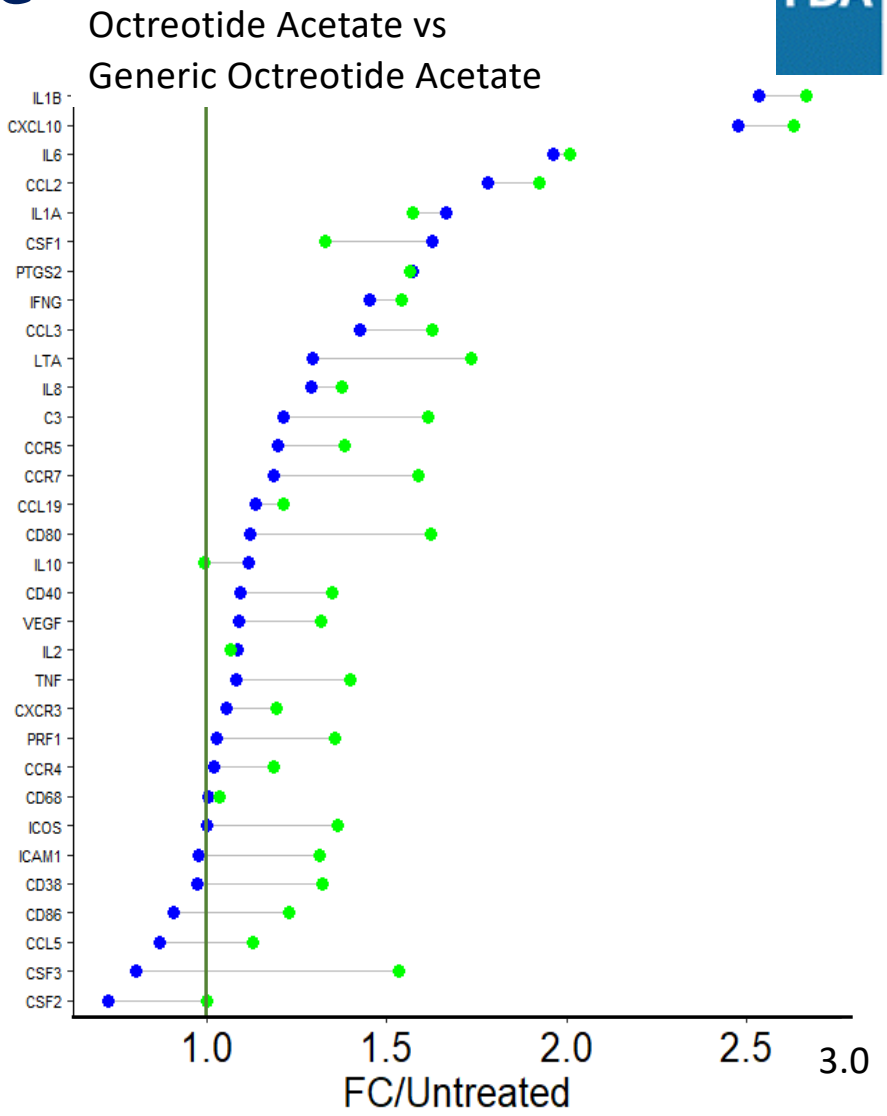
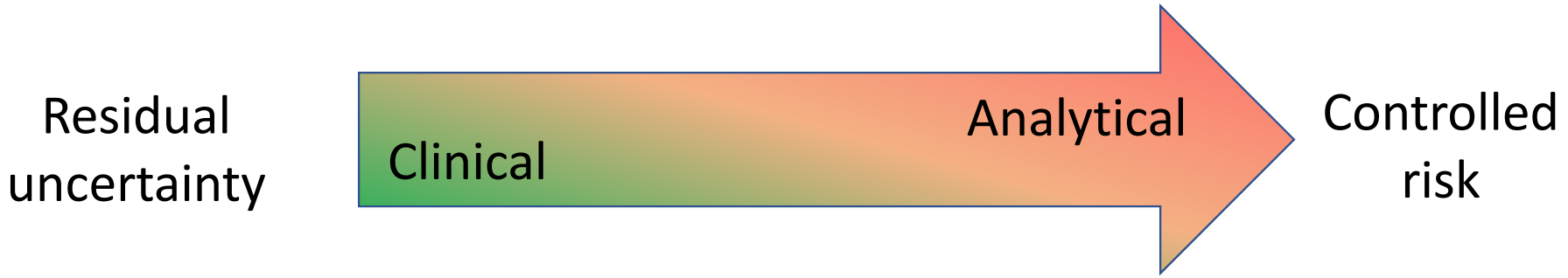


Integrated data needed to assess whether there is a difference between RLD and generic OC acetate. → Discuss with the agency your proposed strategy



Current FDA thinking on the use of Non-Clinical Tools in Immunogenicity Risk Assessments:

- Product and process related impurities can impact on product quality, safety, immunogenicity, and efficacy and thus they should be within an appropriate limit, range, or distribution to ensure desired product quality
- Assessing the risk of product and process related impurities is not sufficient to determine the immunogenicity risk of a new product but can support a risk assessment of “relative” immunogenicity risk as compared to the product that was used in clinical trials.
- Innate Immune Response Modulating Impurities assays can be useful tools in assessing process-related impurities capable of eliciting an innate immune or inflammatory response.



Regulatory submission of IIRMI studies:

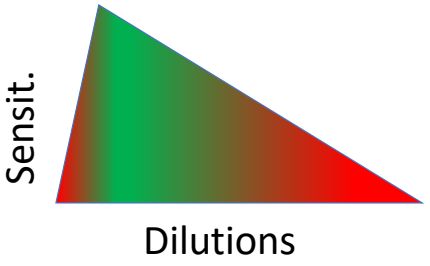
Provide:

- *Assay SOP including:*
  - *Cell isolation method or passage number*
  - *Relevant cell recovery and viability*
  - *Final concentration of cells and DP in the well and any DP manipulations.*
  - *Numerical results (Excel table containing all responses by donor or cell line)*
- *Studies demonstrating assay is fit for purpose: sensitivity (LOD & LOQ), linearity, precision, etc.*

  Recommend confirming assay sensitivity to multiple PRR agonists by spiking product prior to any DP manipulation or dilution.
  - *Study results with a Table linking individual measurements with cell recovery and viability as well as the corresponding suitability controls confirming the responsiveness of each donor or cell line run.*

Common deficiencies for IIRMI assays:

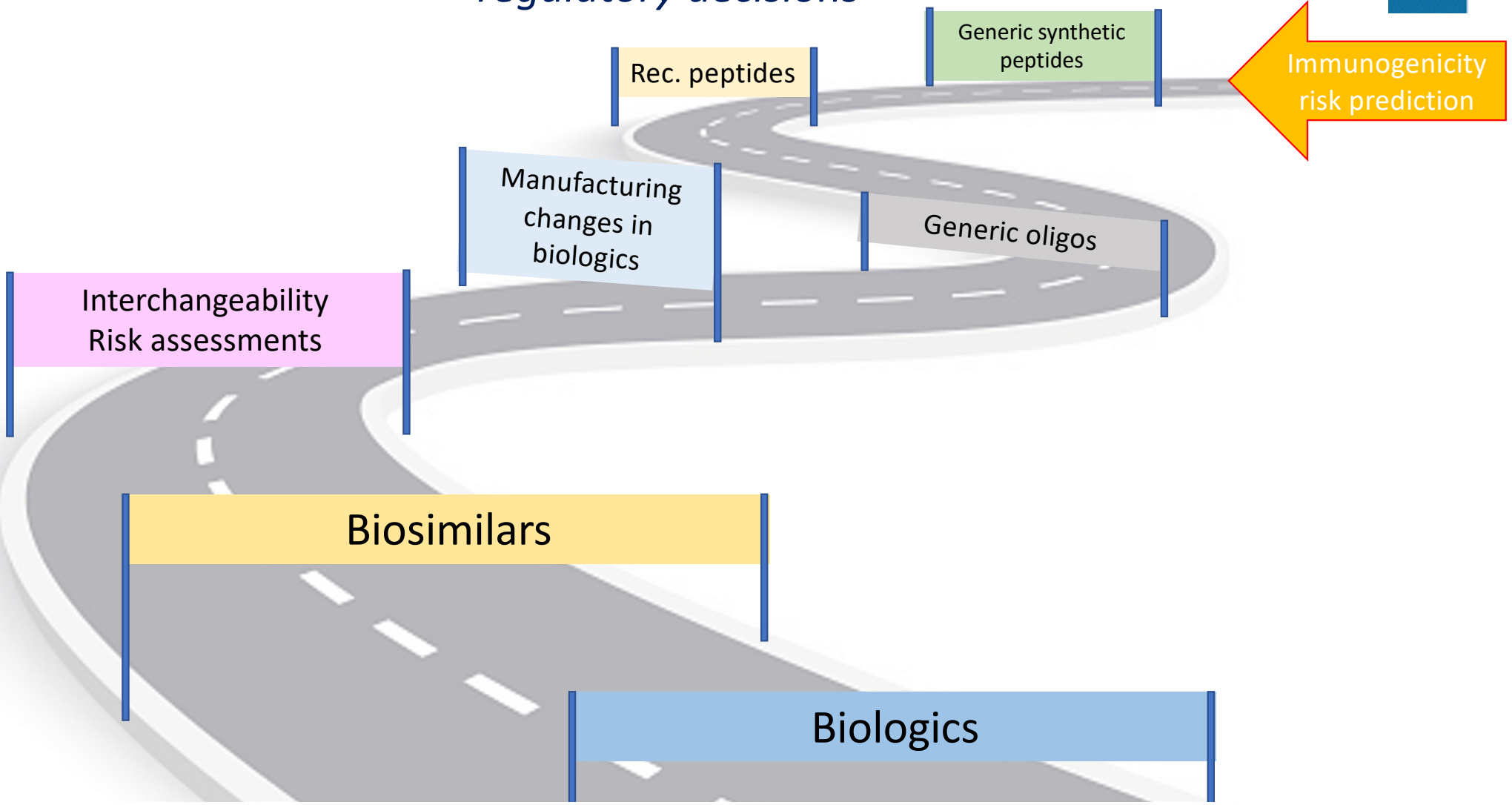
- *Inadequate assay (sensitivity or breadth).*
- *Inadequate demonstration of fit for purpose:*
  - *Number of donors, donor selection criteria, cell numbers, duration and culture conditions used for the assay*
  - *Inappropriate suitability controls (negative, low (confirming LOD) and high positive controls)*
- *Inadequate number, selection, or information of DP batches (e.g. dates of manufacturing, expiry and testing, DS lot used etc.)*
- *Excessive DP dilution leading to loss of sensitivity. In general, highest concentration of minimally manipulated DP that does not decrease cell viability or metabolic activity needs to be tested in the assay. Calculations on the sensitivity of the assay should account for all dilutions and manipulations of the samples during the testing process.*



IIRMI assays to assess immunogenicity risk : Next steps

- Identify most useful testing platforms and readouts
- Gain additional experience with complex drug products and cell substrate-associated impurities (e.g. HCP & other remnants of cell culture)
- Develop additional statistical tools & models to integrate orthogonal data
- Develop additional models and information to better correlate biological response differences with clinical outcomes
- Develop validated standards to benchmark assays across sponsors

Assays that inform immunogenicity risk assessment may allow for better regulatory decisions



Acknowledgements

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- Joey Balsamo

Parting thought: Absence of evidence is not the same as evidence of absence… To use in vitro assay data to inform immunogenicity risk, assays have to be fit for purpose and the clinical correlation of the differences we see understood!