Assessing impurities to inform peptide immunogenicity risk: developing informative studies

SBIA 2022: Advancing Generic Drug Development:
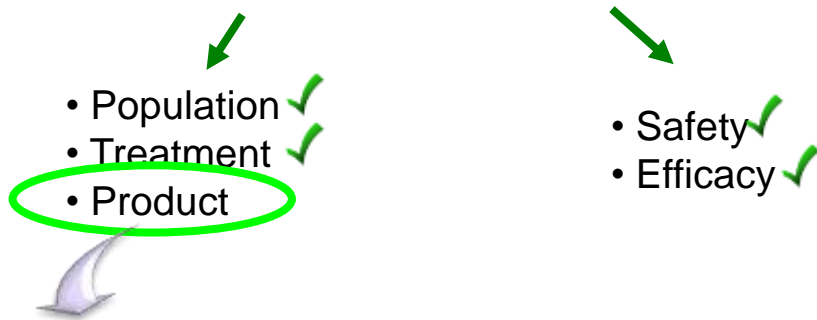*Translating Science to Approval*
*Day (1), Session (1A): (*Peptide Immunogenicity Risk and Impurity Assessment Considerations *)*

Daniela Verthelyi, M.D., Ph.D.
Laboratory of Innate Immunity
Division of Biologics Research and Review III
OBP, OPQ, CDER, FDA
9/20/2022

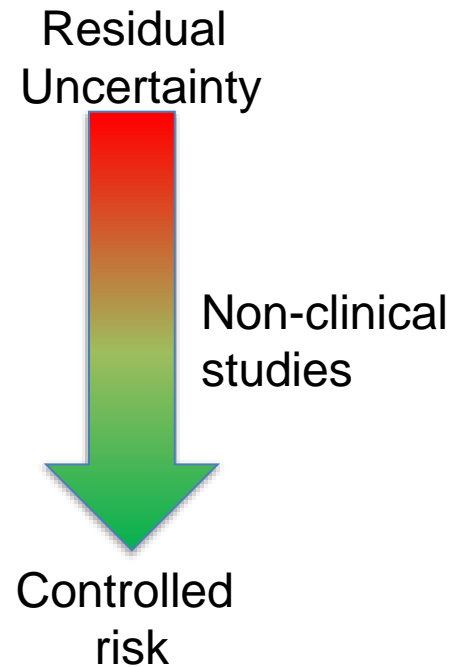# Immunogenicity risk assessment of generic peptide products

FDA

Risk = Probability X Consequences

- Population ✓
- Treatment ✓
- (Product)

- Safety ✓
- Efficacy ✓

Residual Uncertainty

Non-clinical studies

Controlled risk

If API is same as RLD, then most residual uncertainty is due to <u>impurities</u>

Impurities can impact on immunogenicity and thus be considered as CQAs

➢ Process-related Impurities → Innate immune activation
➢ Peptide-related impurities → MHC binding/ T cell activation

FDA Guidance on Synthetic Generic Peptides Referencing NDA Peptides of rDNA Origin

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

Guidance for Industry

"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."

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

➢ Peptide-related impurities

➢ Process-related impurities

➢ Aggregates

Risk assessment of impurities

Prior clinical experience

Immunogenicity risk assessment

Same Active Product ingredient

https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf
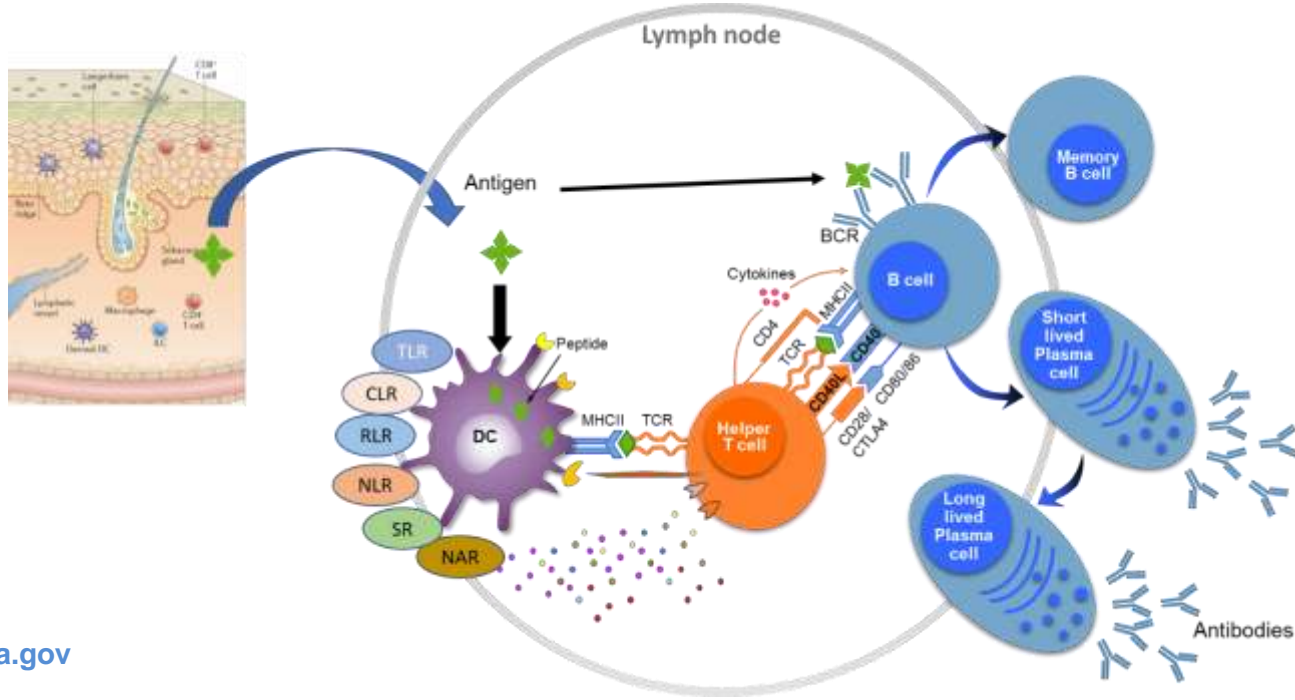
APCs and T~helper~ Cells are the Lynchpin in Generating Immune Responses to Therapeutics Peptides

"Immunogenicity is antigenicity in the context of an inflammatory milieu resulting in a successful humoral response".

Modified from Krishna and Nadler, 2016

Impuritiy assessment

Process-Related impurities

- Innate immune response modulating impurities

- In vitro

- DP (can be supplemented w/DS lots)

Peptide-Related impurities

- Focuses on impurities >0.1%

- New or present in higher amounts

- Orthogonal in silico & In vitro assays

- MHC binding capacity

- Degree of tolerance

- T cell stimulating ability

- In vitro: Purified impurities

In Vitro Assays for Innate Immune Responses

FDA

PBMC
Whole blood
Reporter Cell lines

DP ← Suitability controls (TLR agonists)

Incubate →
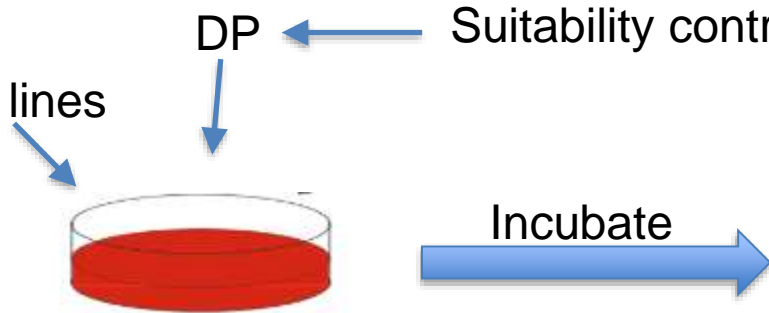
- NFkB activation
- Cytokine production
- Cell marker expression
- mRNA expression patterns

- *IIRMI assays are designed to detect biological differences in immune markers.*
- *Increased biological response suggest increased risk of clinical differences…*
- *For synthetic peptides, the expectation is no IIRMI… Highly sensitive assays are critical to demonstrate "absence" of IIRMI.*

In Vitro Assays for Innate Immune Response Modulating Impurities (IIRMI)

Cell line		Origin	Commercial Availability	
PBMC/ Whole blood	Proliferation / Cytokines	Human M∅, DC, M⊖, and Ly's	Yes	⎫ Availability & variability
Dendritic cells activation	Activation markers	Fresh or frozen Human DC	Yes	⎬ Low throughput
THP-1, MM6, Ramos	NFkB, Cytokines	Human cell lines	Yes	⎫ Limited Receptor Repertoire (NFKB –centric)*
RAW-BLUE	NFkB	Mouse macrophages	Yes	
Single Receptor line	NFkB	e.g. Human embryonic kidney	yes	⎭

* Potentially now sufficient on their own. Consult Agency

IIRMI Critical assay attributes (1):

- Cell Platform:
  - ❑ 1ry cells (WB/PBMC/DC)
    - Number & qualification of donors (healthy vs target)
    - Sample processing (Fresh vs frozen)
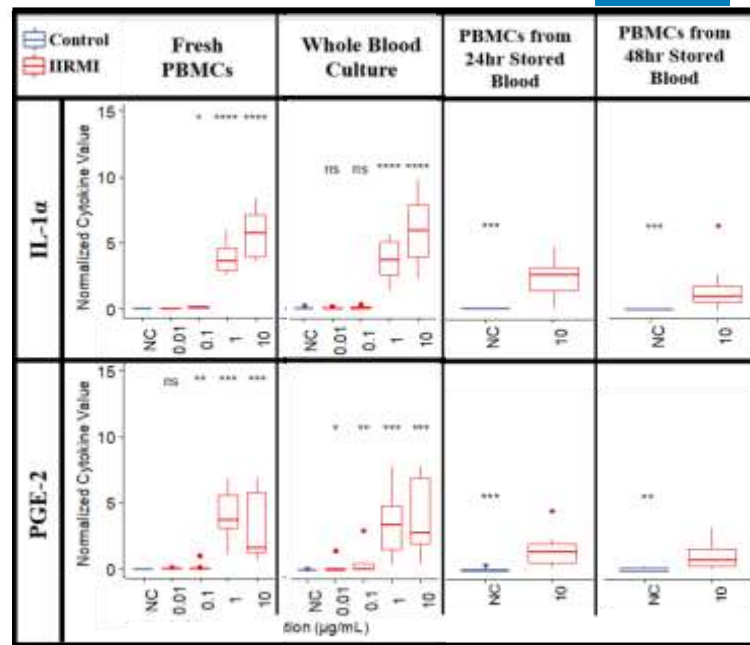   - Cell viability (pre and post assay).
      - Percent viable APCs
  - ❑ Cell lines:
    - Multiple to increase receptor coverage
    - Limited sensitivity to product aggregates
    - Depending on product risk

- Culture conditions
  - Cells/well, culture time, media, etc.
  - Matrix interference (e.g. formulation)

- Drug concentration in well

Holley et al, 2021

Thacker et al. Detection of innate immune response modulating impurities (IIRMI) in therapeutic peptides and proteins: Impact of excipients. Front. Immunol., 06 September 2022

IIRMI Critical assay attributes (2):

FDA

Demonstrate assay is suitable:
- Sensitivity, Drug Tolerance, Specificity, Precision, Robustness, Accuracy (ICH Q2(R2)
  - Cell number, viability and composition
  - Cell passage
  - Drug formulation

- 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.
- Demonstrate signal recovery
- Account for all dilutions to determine assay sensitivity
- Result interpretation
  - Multiparametric quantitative assessment of different paths of innate immune activation rather than positive/negative.

IIRMI Assay Readout:

➢ NFKB activation in reporter cell lines (THP-1, RAW-Blue etc.)

➢ DC activation (CD11c, CD86, HLA)

➢ Cytokine expression (e.g. IL-1α, MIP-1α, IP-10, MCP-1, IL-6, IL-8, and PGE-2)

➢ Gene expression patterns (mRNA)

Comprehensive multiparameter assessment are preferred since impurities can trigger different innate immune pathways capable of increasing immunogenicity risk
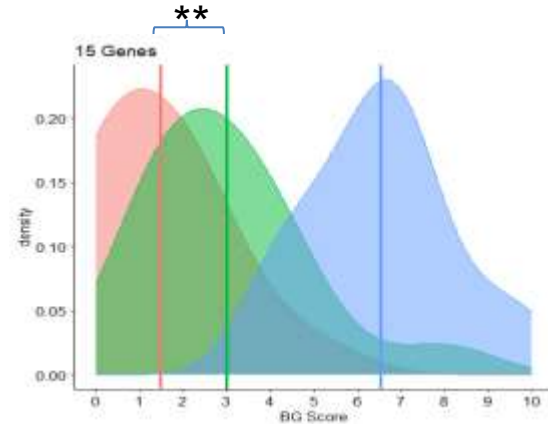
Composite analysis of the gene expression profile uncovers differences between products

Assay sensitivity and suitability controls:

Receptor	Ligand	In vivo (NHP)	PBMC	THP1	MM6	RAW-Blue
TLR2	Pam3CSK4	10 pg	1ng/mL	100pg/mL	500 pg/mL	500 pg/mL
TLR3	Poly I:C	1ng	100ng/mL	1µg/mL	<LLOD	<LLOD
TLR4	Endotoxin	10pg	10pg/mL	10pg/mL	10pg/mL	100pg/mL
TLR5	Flagellin	1ug	5µg/mL	5µg/mL	<LLOD	<LLOD
TLR2-6	FSL-1	10ng	100pg/mL	100pg/mL	100pg/mL	100pg/mL
TLR7	Imiquimod	10ng	100ng/mL	<LLOD	<LLOD	100ng/mL
TLR7-8	CLO75	10ng				
TLR9	CpG ODN	5µg	100ng/mL	<LLOD	<LLOD	60ng/mL
Dectin1	Betaglucan/zymosan	10ng	1ng/mL	10ng/mL	10ng/mL	1µg/mL
NOD2	MDP	1ng	1ng/mL	<LLOD	10µg/mL	<LLOD

*Not all purified PRR ligands are created equal… need to characterize controls
- Spike product prior to any manipulation of DP and demonstrate signal recovery to establish sensitivity
- Include suitability controls in all plates

11

Haile et al, 2015

IIRMI assay characterization and results:

Provide:

- *Assay SOP including:*

  - *Cell isolation method or Passage number*

  - *Final concentration of DP in the well and any DP manipulations.*

- *Studies demonstrating sensitivity (LOD & LOQ), linearity, precision, etc. Assays should be fit for purpose. Recommend confirming assay sensitivity by spiking product prior to any DP manipulation.*

# IIRMI assay characterization and results:

Provide:

- *Results should include data from:*

  - *At least 3 batches of DP at release and at end of shelf life (≠ DS, ≠ manuf. campaigns).*

  - *Relevant cell recovery and viability.*

  - *Data from suitability controls confirming the responsiveness of each donor or cell line run.*

  - *Data from each run confirming assay sensitivity.*

 - *Numerical results, not positive/negative (Excel table containing all responses by donor or cell line)*

Common deficiencies for IIRMI assays:

FDA

➤ **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.

Sensit.

Dilutions
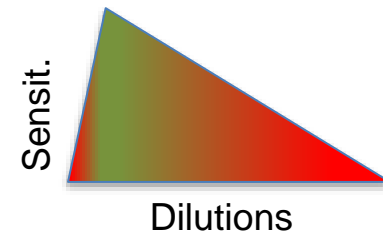
Peptide –Related impurities
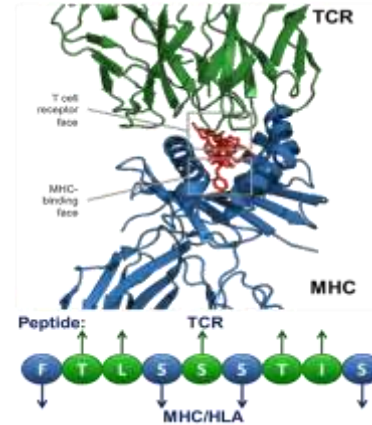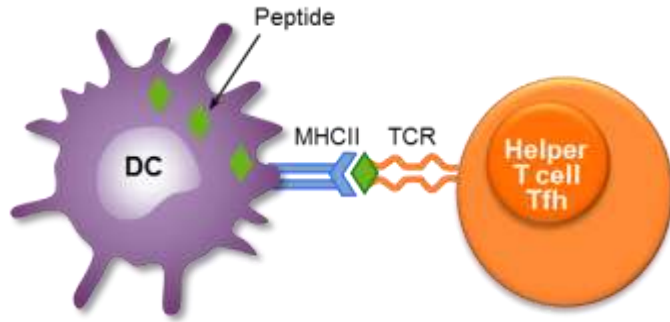
		Half-life	ADA	
Taspoglutide	HA$_{1B}$EGTFTSDVSSYLEGQAAKEFIAWLVKA$_{1B}$R	7d	55% ADA$^+$	→ SAE and anaphylaxis
Semiglutide	HA$_{1B}$EGTFTSDVSSYLEGQAAKEFIAWLVRGRG	7d	<5% ADA$^+$	

2xEOG-γGlu-C18diacid

*A$_{1B}$ aminoisobutyric acid(reduced peptide degradation)   Red indicates AA different from Hu GLP1

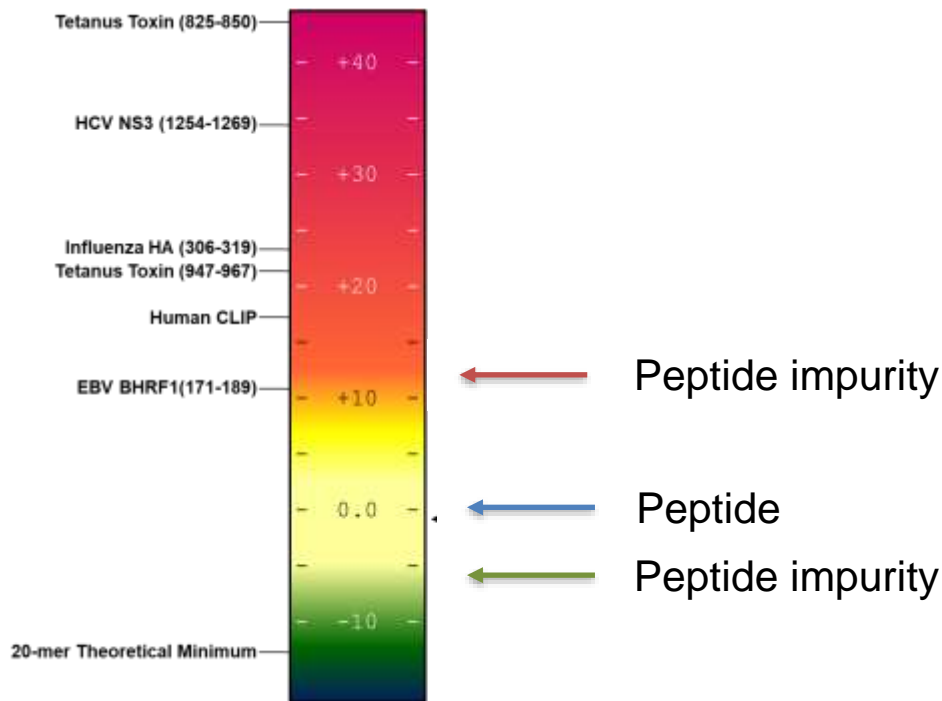# Immunogenicity risk of product-related impurities: In silico tools

FDA

• Evaluate the risk of peptide impurities

Strengths:
> High throughput,
> Covers multiple MHC-DR
> Potential impurities
> Neoepitopes or tolerogenic sequence disruption

Weaknesses:
> 1ry sequence
> HLA DR, DQ& DP
> No unnat. amino acids or modif.
> No B cell epitopes

Tetanus Toxin (825-850)
+40
HCV NS3 (1254-1269)
+30
Influenza HA (306-319)
Tetanus Toxin (947-967)
+20
Human CLIP
EBV BHRF1(171-189)
+10
← Peptide impurity
0.0
← Peptide
← Peptide impurity
-10
20-mer Theoretical Minimum

Immunogenicity risk of product-related impurities: In vitro tools

- ➢ Affinity of peptide impurities for MHC

- ➢ Reactive naive T cells (DC-T cell assays)

- ➢ **Binding and/or T cell activation relative to API**

  - ❑ Multiple readouts:
    - ➢ Proliferation
    - ➢ Cell surface markers
    - ➢ mRNA expression
    - ➢ Protein expression

  - ❑ Suitability controls
    - ❑ Naïve vs memory responses.
    - ❑ KLH, PPD… can confirm live APCs & responsive T cells
    - ➢ Peptide of similar length and general structure containing promiscuous T cell epitopes to confirm ability of assay to detect naïve T cells

  - ➢ High donor-donor variability
  - ➢ Low frequency of naïve T cells

Peptide-related impurities assay characterization and results:

FDA

Provide:

- *SOP & supporting data*
  - *Cell preparation and culture conditions (sample size, MHC coverage and targeting)*
  - *Final concentration of peptide(s) in the well and qualification performed on the peptide impurity preparations used in the assays.*
  - *Readout selection (proliferation, cytokines, cell markers)*
  - *Suitability controls: selection criteria and justification. DC:T cell assay → naïve T cell responses*
  - *Demonstration that assays are fit for purpose: sensitivity (LOD & LOQ), linearity, precision, etc.).*

Peptide-related impurities assay qualification and results:

Provide:

- *Results should include data from:*

  - *Cell recovery and viability.*

  - *Data from suitability controls confirming the responsiveness of each donor.*

  - *Numerical results, not positive/negative (Excel table).*

Common deficiencies 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) (Jenkins and Moon, 2012)).

➢ 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 to support the sensitivity of an assay to detect innate immune response in the presence of the product or to detect the presence of naïve T cells to specific antigens.

➢ Peptide concentrations is too low to elicit response (<0.1uM).

Summary:

- For candidate generics shown to have the same API as the RLD, product and process related impurities can impact on immunogenicity

- In silico and cell-based methods may help assess the summative effect of different impurities to inform risk.

- Assays should be carefully developed and fit for purpose to provide data that informs the immunogenicity risk

Acknowledgements

- IRC
- Eric Pang
- Cameron Smith
- Susan Kirshner
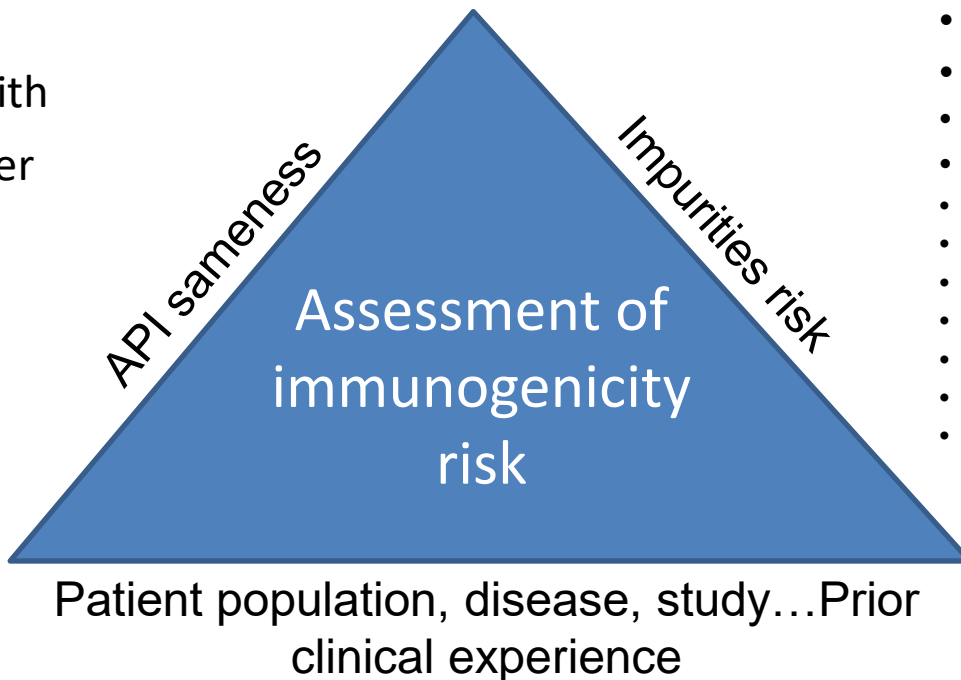
The Innate Immunity Lab:
- Mohanraj Manangeeswaran
- Seth Thacker
- Cheng Her
- Logan Baker
- Ha-Na Lee
- Derek Ireland
- Eduardo Mufarrege
- Lydia Haile
- Vivian Wang
- Montserrat Puig
- Swamy Polumuri

API sameness

Impurities risk

Assessment of immunogenicity risk

Patient population, disease, study…Prior clinical experience

Challenge Question #1

An IIRMI assay SOP should include information on:

A.   Cell platform

B.   Final concentration of DP in the well and any DP manipulations.

C.   A description of the suitability controls included on each run

D.   All of the above

Challenge Question #2

Suitability controls in DC:T cells assays should:

A.   Distinguish between CD4 and CD8 T cells

B.   Be very sensitive to innate immune response modulating impurities

C.   Confirm that the assay detects naive T cell responses

D.   Confirm that the assay detects memory T cell responses

Parting thoughts...

Absence of evidence is not the same as evidence of absence … unless the assays are really good.