Scientific Considerations for the Assessment Immunogenicity Risk of Generic Synthetic Peptide Products

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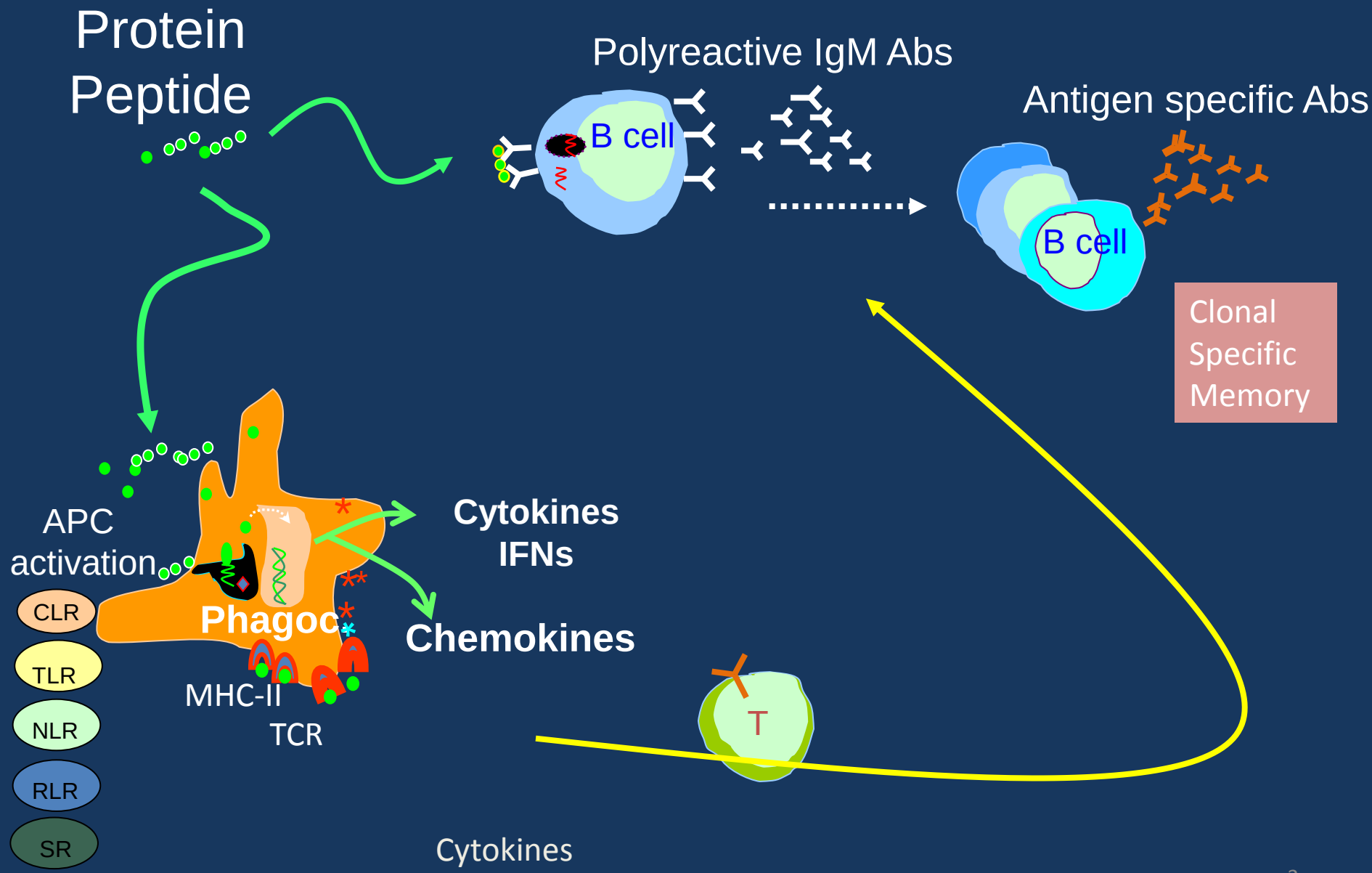
Map to the talk:

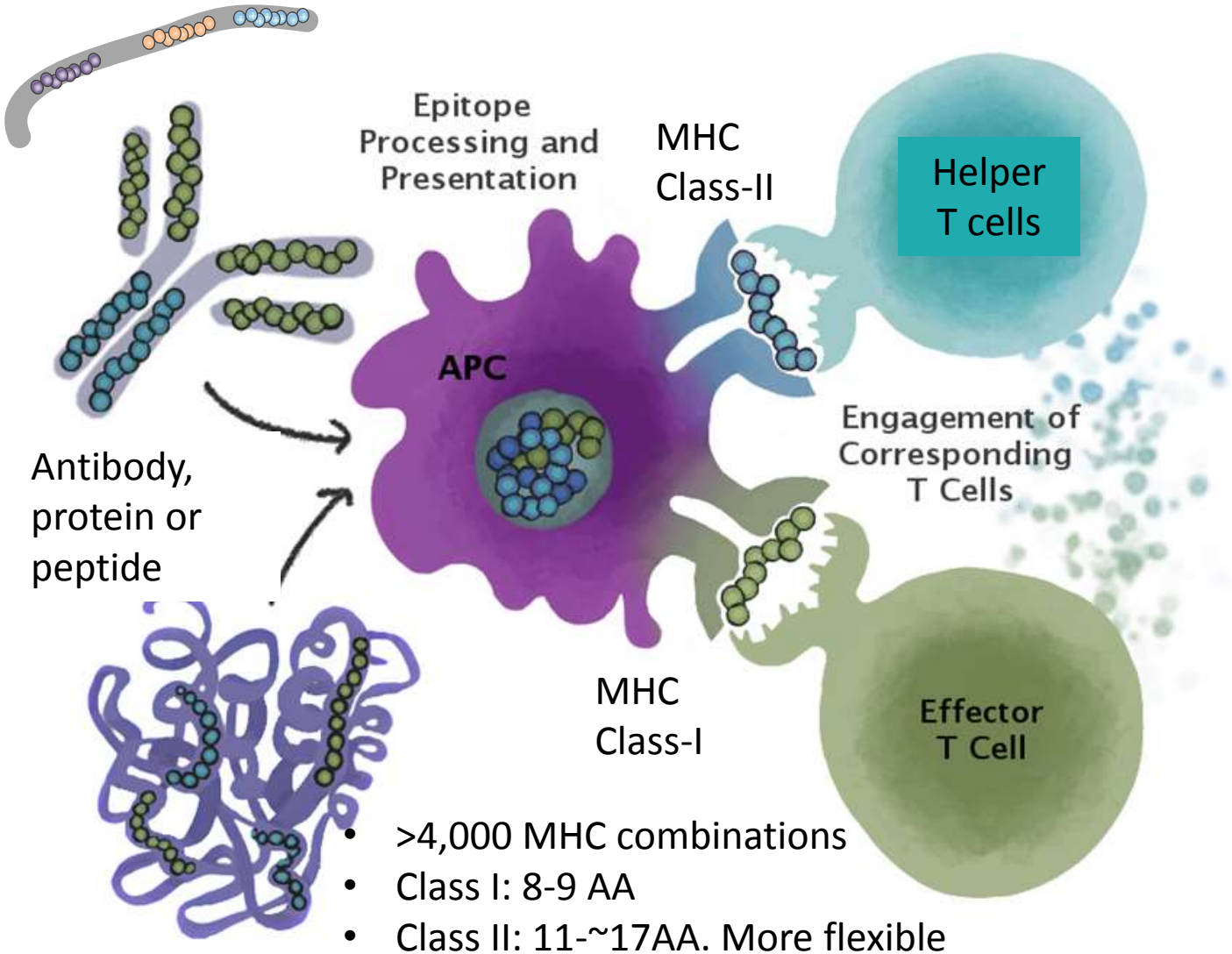
- Immune response: the basics
- Impact on safety and efficacy
- Risk factors for product immunogenicity

  <Big assumption: API sameness>
- Product and process related Impurities
  - T cell epitopes & in silico tools
  - Aggregates
  - Innate immune response modulating impurities (IIRMI); testing methods
- Case studies
  - Note: <Thinking is evolving>

Immune responses



Cognate activation of T cells



- >4,000 MHC combinations
- Class I: 8-9 AA
- Class II: 11-~17AA. More flexible
- T cells w/high affinity for self are deleted / anergic

Immunogenicity Impacts Product Safety and Efficacy

- **None**

Safety

- Hypersensitivity responses: immediate (anaphylaxis, C' activation, bradikinin) and delayed (DTH, serum sickness)
- Deficiency syndrome: cross-reactive neutralization of non-redundant endogenous protein (EPO, TPO)
- Induction of inflammation or autoimmune disease (Heparin)

Efficacy

- Neutralization: Lack or loss of efficacy of product. (IFNβ, enzyme replacement, coagulation factors, GLP-1)
- Change in PK:
  - Accelerated elimination / loss of efficacy
  - Delayed elimination / unexpected toxicities

Proteins, peptides and NDCD

Predicting whether a product will be immunogenic, 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 proteins, peptides, and naturally derived complex drugs (NDCD). Because immunogenicity cannot be predicted from product structure and formulation, clinical studies are needed to assess product immunogenicity and its clinical consequences.

Can advancing techniques allow for assessing the immunogenicity risk of generic synthetic peptides ?

- Defined starting materials
- No significant secondary structure
- No glycosylation
- No host cell proteins

Evaluating Immunogenicity Risk for Generic Synthetic Peptides

Risk = Probability X Consequences

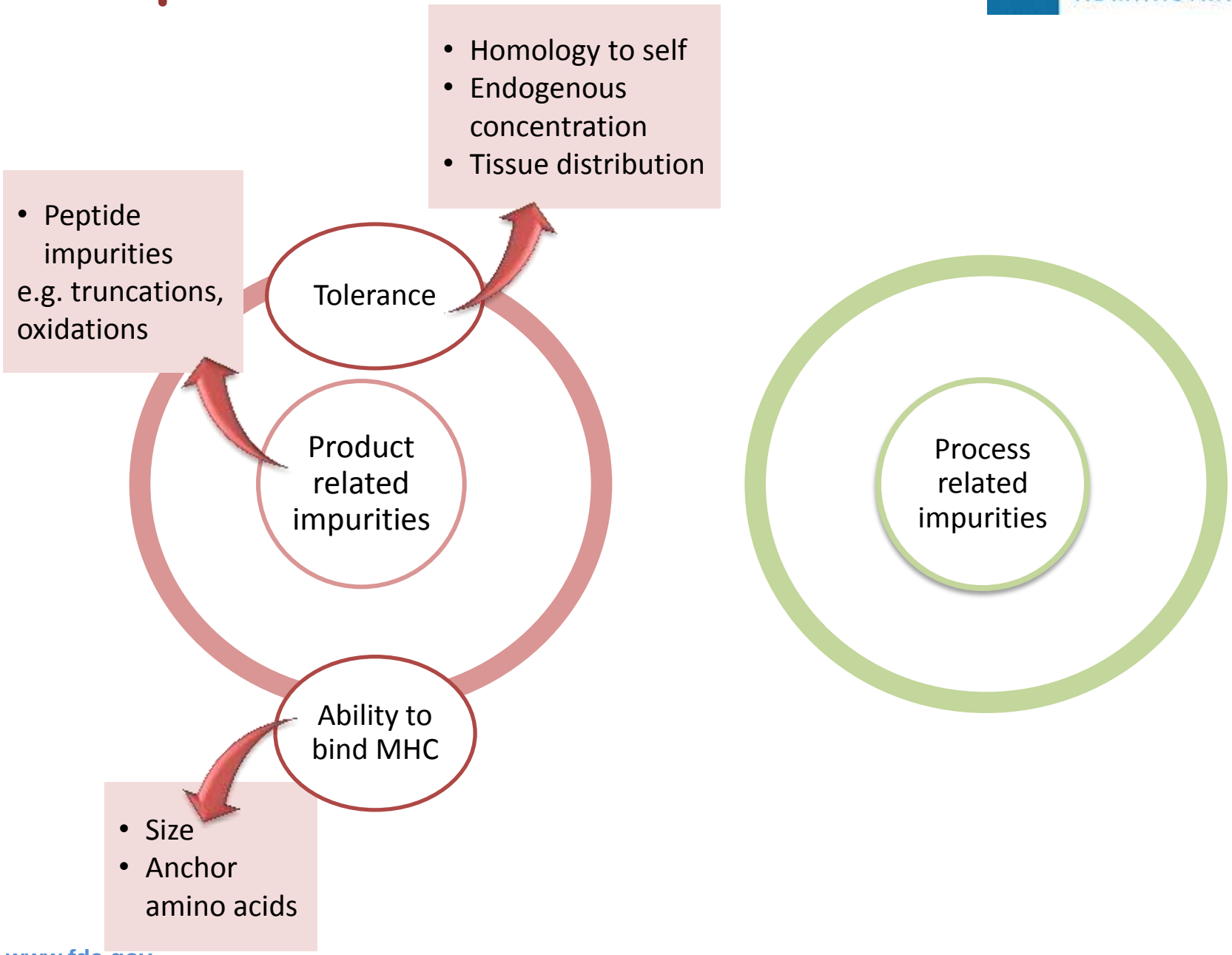
- Population ✓
- Treatment ✓
- Product

- Safety
- Efficacy

Provided:

1. **Clinical shows immunogenicity of RLD is low risk**
2. **Active ingredient sameness**
3. **Residual uncertainty only regarding impurities**

Impurities:

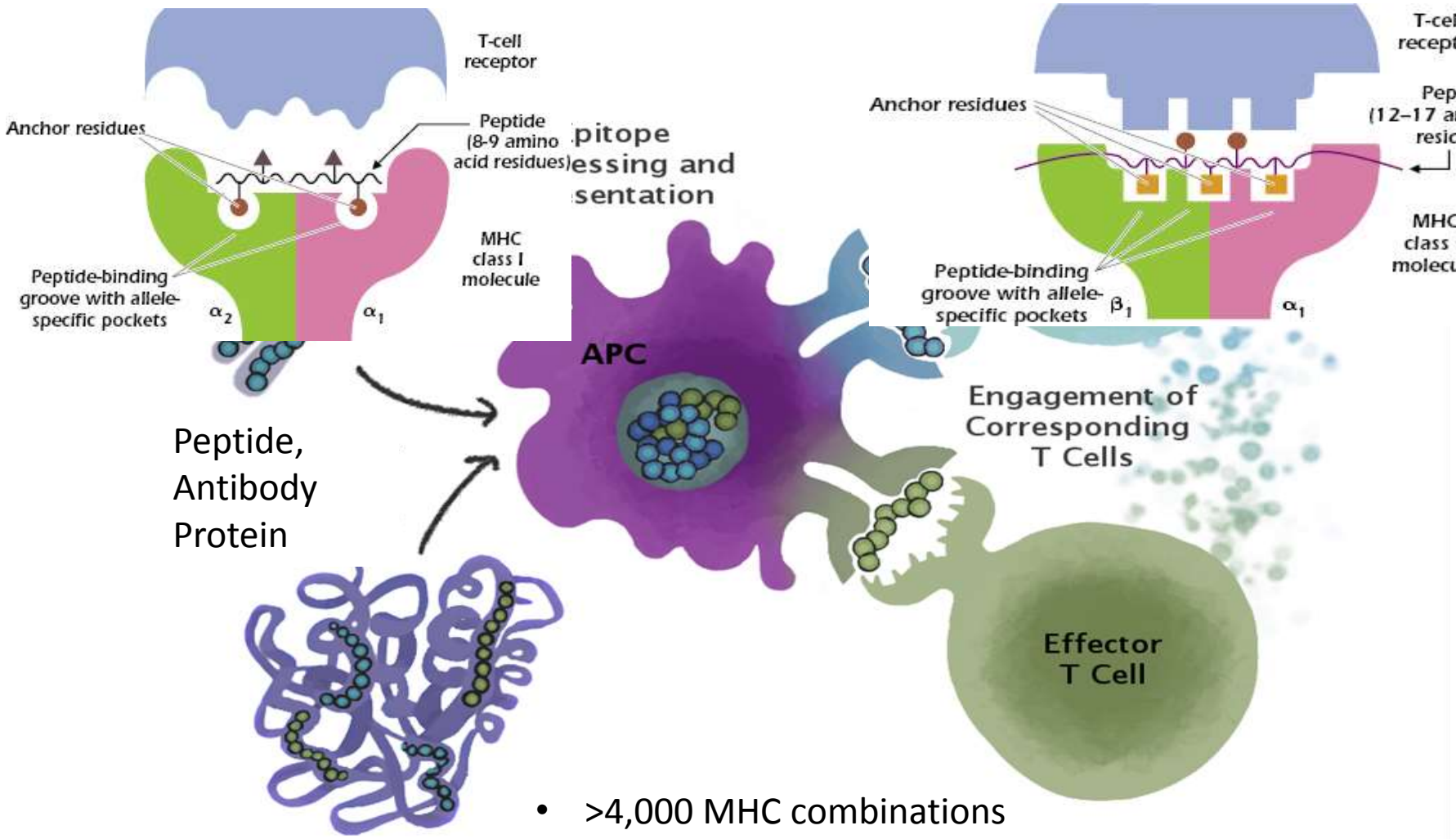

• Homology to self
• Endogenous concentration
• Tissue distribution
• Peptide impurities
e.g. truncations, oxidations
Tolerance
Product related impurities
Ability to bind MHC
• Size
• Anchor amino acids
Process related impurities

Impurities and degradants that may occur during manufacture and storage:

- Denaturation
- Proteolysis
- Deamidation
- Oxidation
- Reduction
- Racemization
- Deletion (incomplete coupling)
- Truncation (missing amino acids)
- Insertion (additional amino acids)
- Incomplete deprotection (attached protective groups)
- Disulphide exchange

Adapted from Ratnaparkhi and Pandya, 2011.

T cells recognize epitopes within MHC



- >4,000 MHC combinations
- Class I: 8-9 AA
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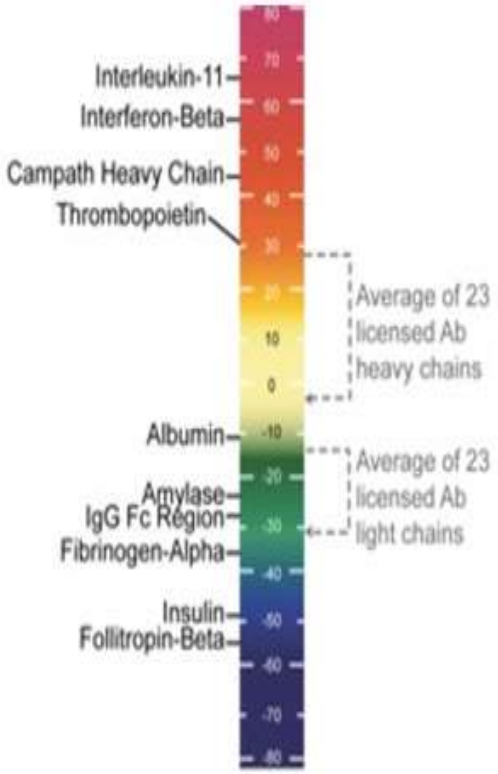
Assess the immunogenicity risk of product-related impurities based on T-cell epitope

- Different in silico tools that predict binding to MHC
- high-throughput screening of partial and complete sequences of proteins/peptides
- identify potentially immunogenic regions
- map individual amino acids that may contribute to the immunogenic potential of the cluster.
- Evaluate the immunogenic potential of T-cell epitope clusters for
  - individuals of different MHC
  - Potential cross-reactivity with non-redundant proteins/peptides



However…

- The genes encoding the MHC class I and class II are highly polymorphic (~4000 combinations of HLA class II α and β subunits).
- The predictive performance for MHC-II remains significantly lower than what can be obtained for MHC-I. One reason for this is that the MHC-II molecule is open at both ends allowing binding of peptides extending out of the groove.
- B cell epitopes are not necessarily linear and thus very hard to predict

Current thinking on product-related impurities in generic synthetic peptides: Immunogenicity risk

U.S. FOOD & DRUG ADMINISTRATION

1) identify any peptide-related impurity that is >0.1%* of DS

2) show that, for each peptide-related impurity *present in both* the proposed generic peptide and the RLD, the level of such impurity in the proposed generic peptide is the same as or lower than that found in the RLD (multiple lots);

3) show that the proposed generic peptide does not contain any new peptide-related impurity that is more than 0.5 %* of the drug substance;

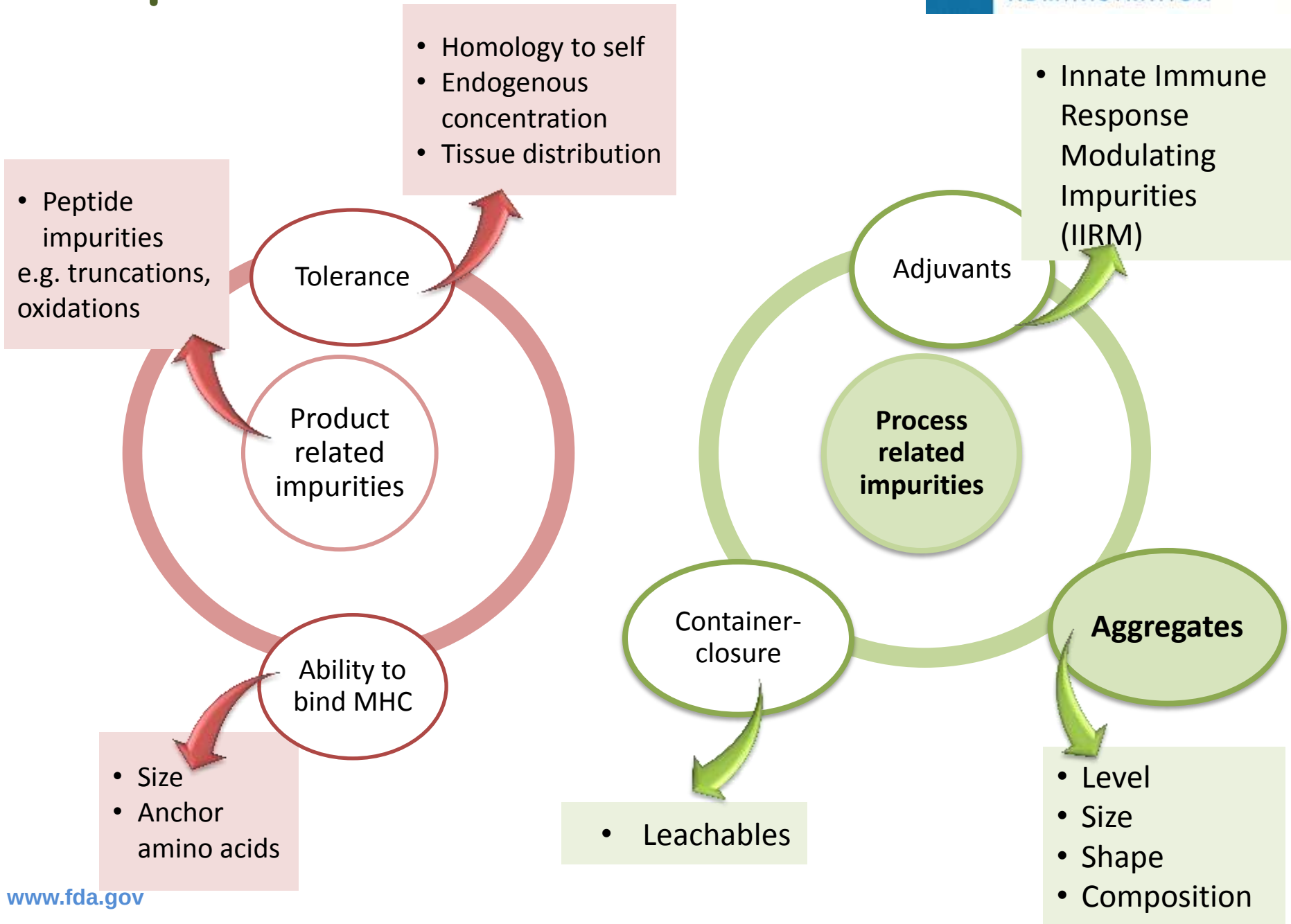
* May be asked to characterize and control at lower levels depending on risk.

Current thinking on product-related impurities in generic peptides: Immunogenicity risk II

4. justify for each new peptide-related impurity of the drug substance why such impurity does not affect the immunogenicity of the proposed generic synthetic peptide (different strategies, e.g. epitope assessment).
5. FDA may recommend additional in vitro or in vivo studies (e.g. animals models) as appropriate
6. If not possible, the different regulatory path may need to be considered.

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

Impurities:


• Homology to self
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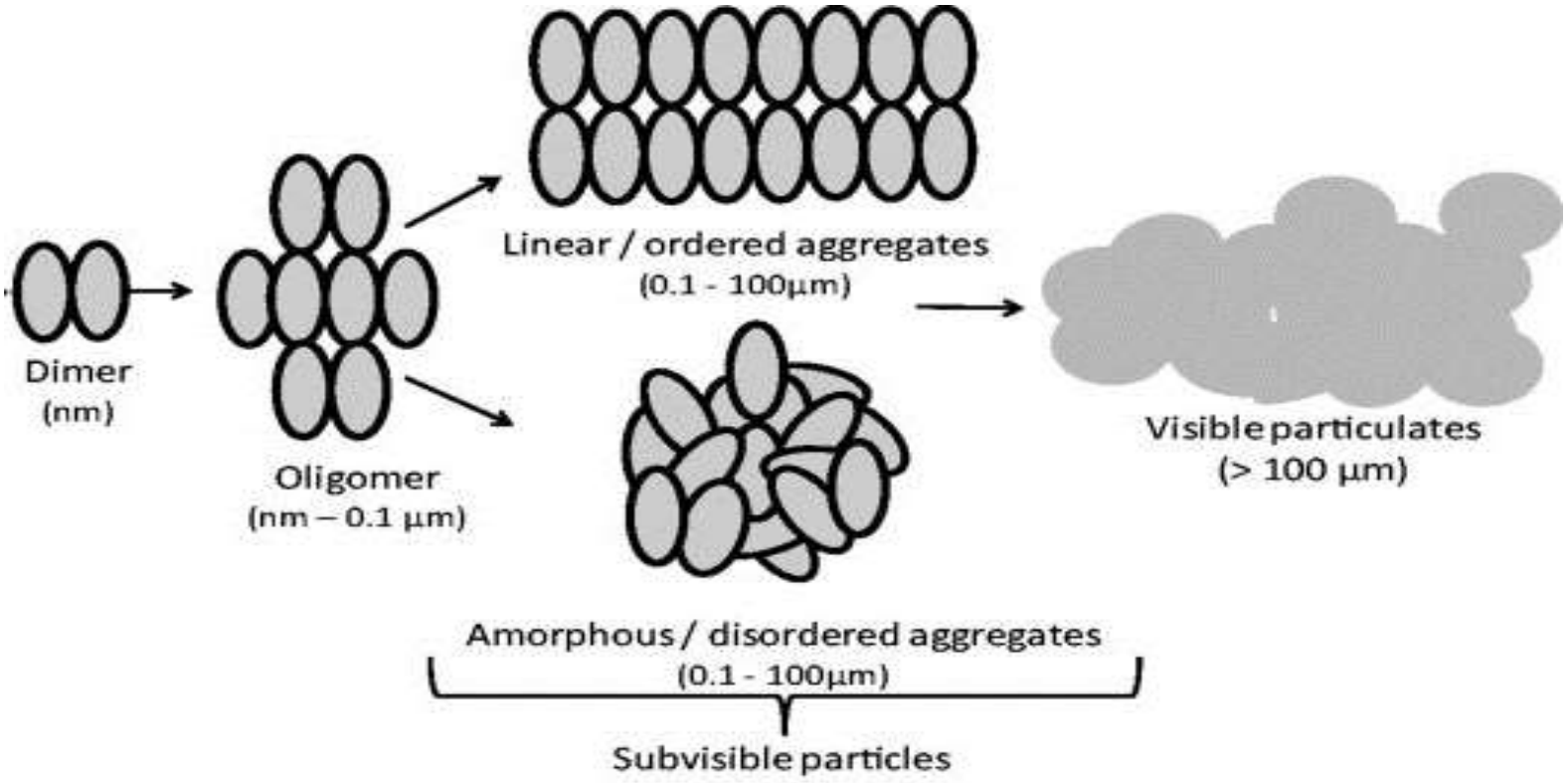
e.g. truncations, oxidations
Tolerance
Product related impurities
Ability to bind MHC
• Size
• Anchor amino acids
• Innate Immune Response Modulating Impurities (IIRM)
Adjuvants
Process related impurities
Container-closure
Aggregates
• Leachables
• Level
• Size
• Shape
• Composition

Aggregates:



- Can range from small (dimers) to large assemblies (subvisible or visible particles).
- Can form during production, storage, shipment, or delivery
- Can nucleate around foreign particles, e.g., steel or rubber particles

Aggregates increase risk of immunogenicity

- Direct crosslinking of B cell Receptor
- Induction of pro-inflammatory cytokines and chemokines favors APC recruitment
- Increased activation and phagocytosis by macrophages and immature Dendritic cells via FcR, Scavenger Receptors
- More efficient antigen presentation
- Little is known about which aggregate species (size, shape, composition) increase/dampen immune response

Current thinking on aggregates:

- Any proposed generic should be characterized at release and in stability to demonstrate that the level and type of aggregates is the same as or lower than that found in the RLD (multiple lots, end of shelf life, orthogonal methods).

Impurities:

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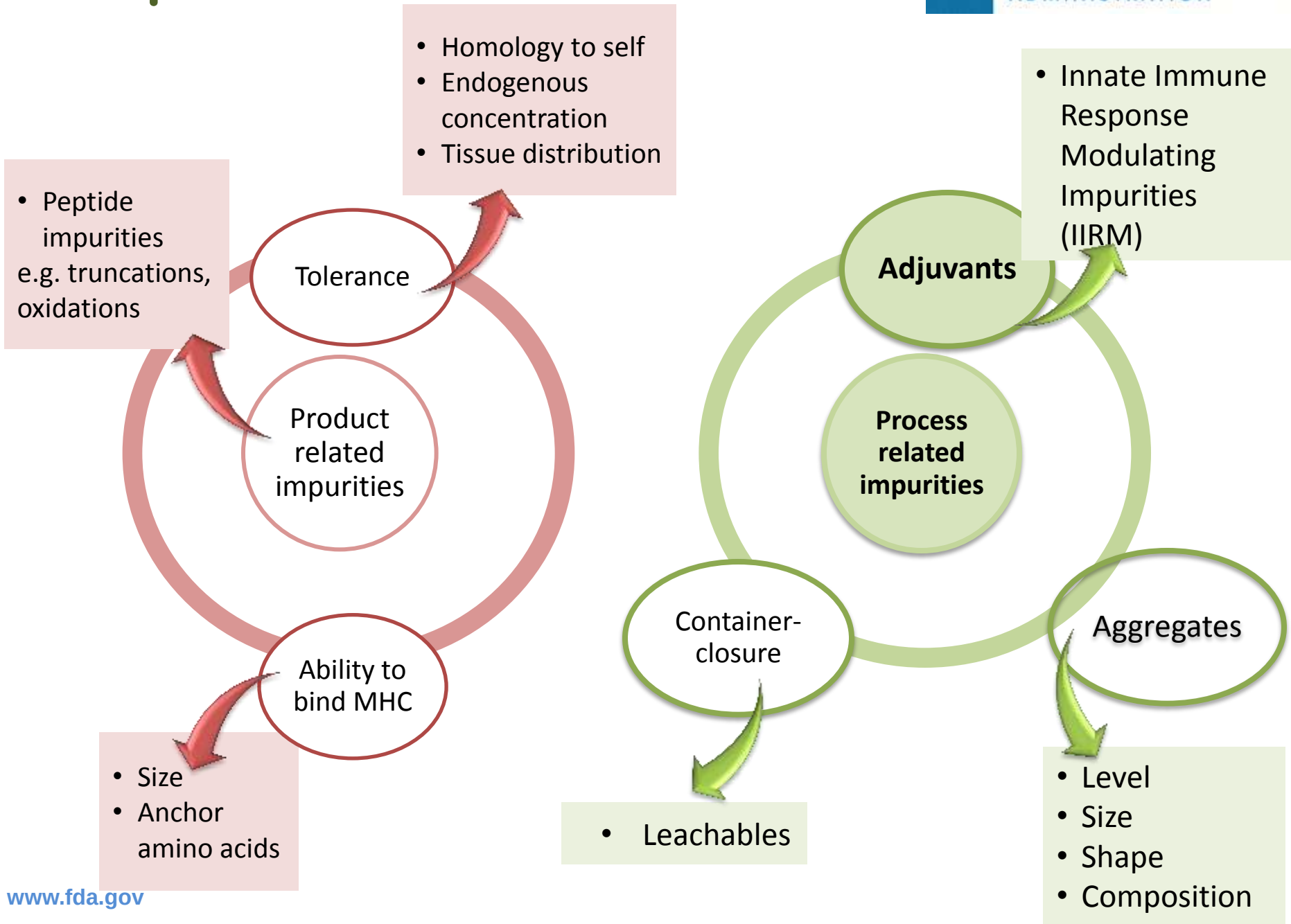
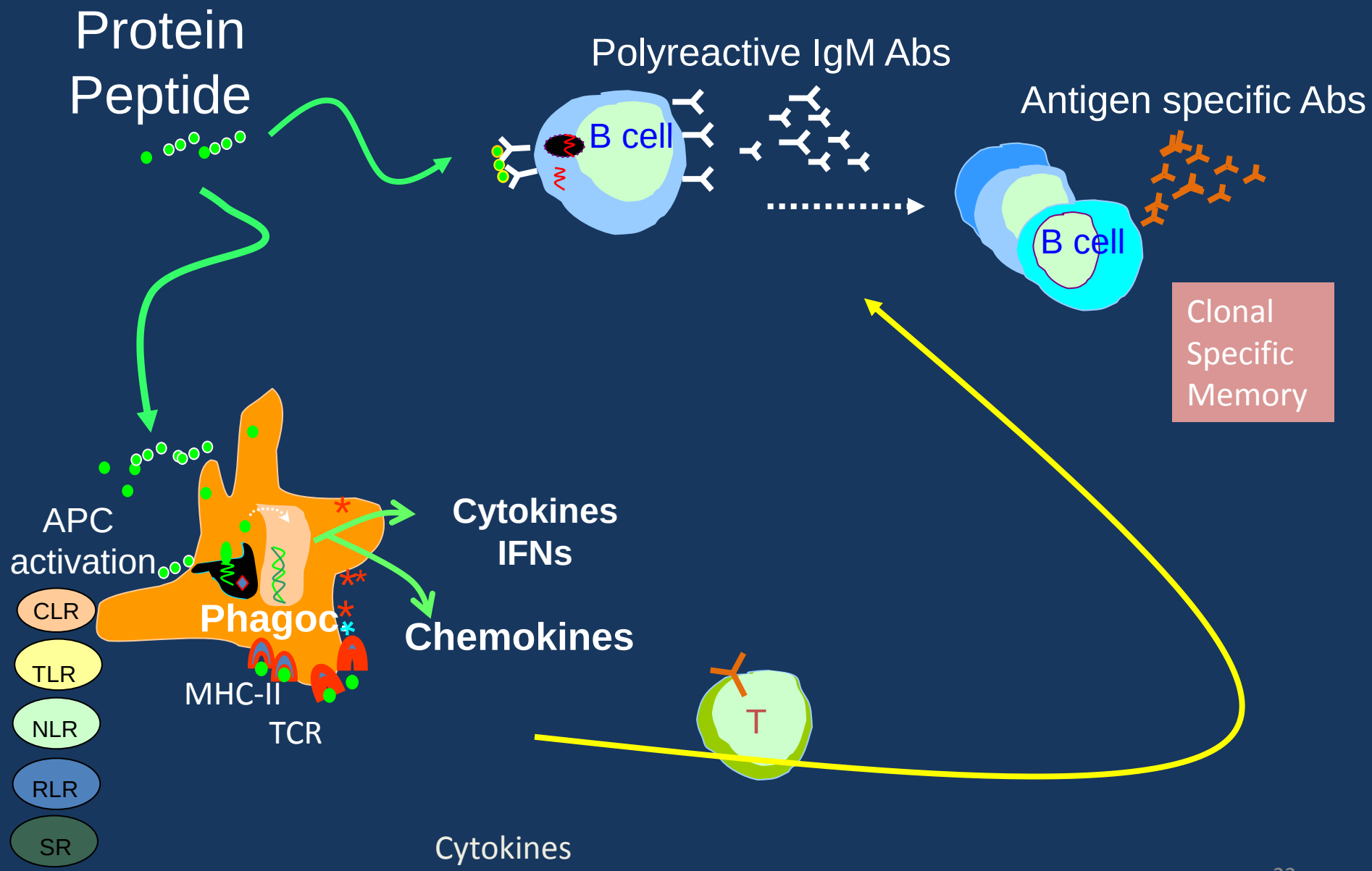
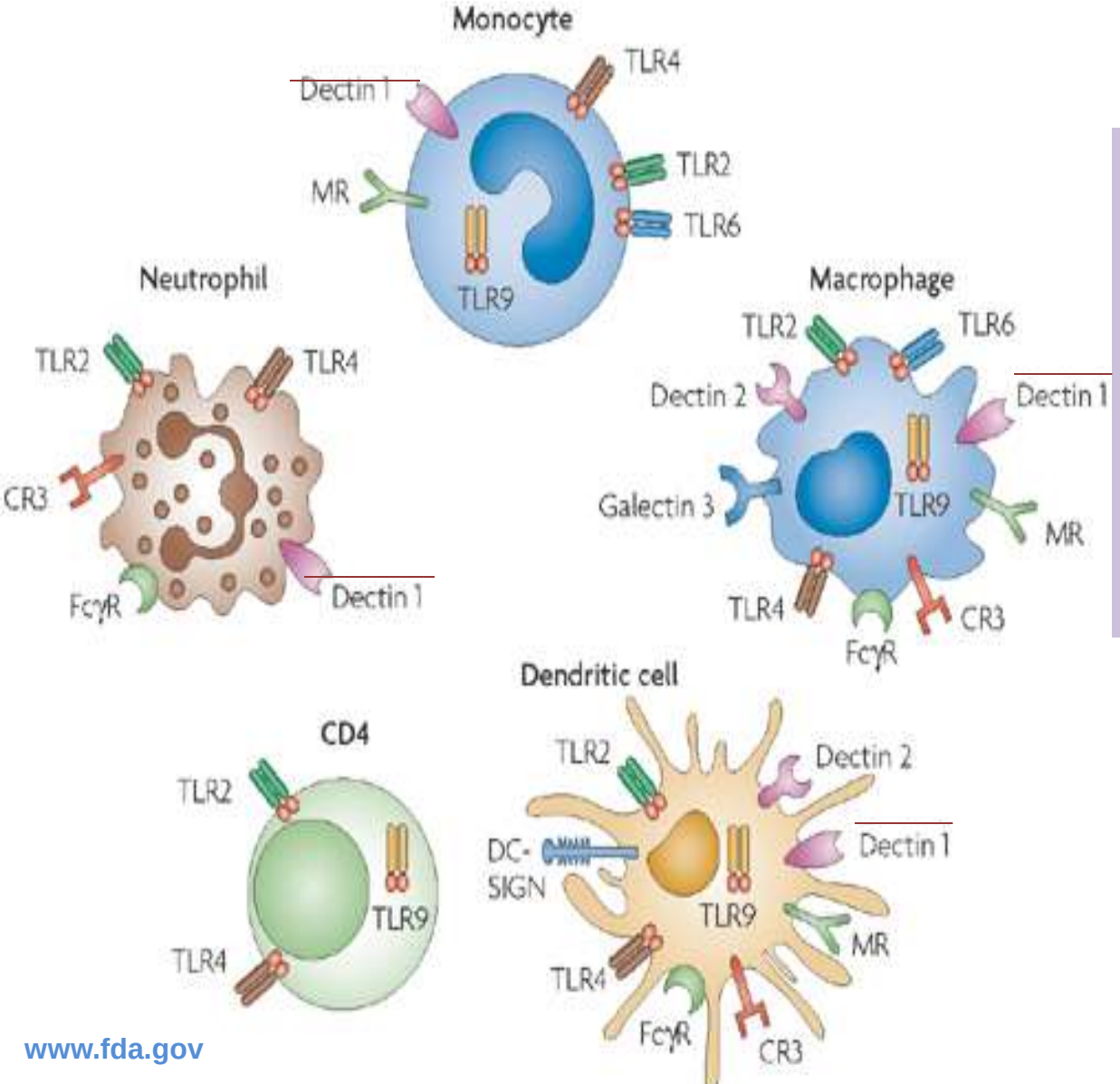


Immune responses



Innate immune receptors (PRR) can recognize process related impurities



- Macrophages and dendritic cells have the most PRR
- Different cells types have different PRR
- Non-immune cells also have PRR

Netea et al 2008

Source of IIRMI

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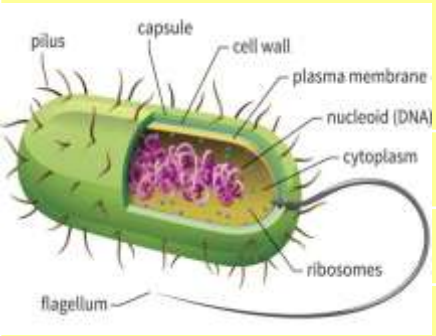
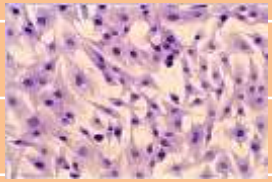
Source	PAMP	TLR	Other PRR
Bacteria	LPS	TLR4	
	Lipoprotein, LTA,PGN	TLR2/1, TLR2/6	NOD1,NOD2,NALP1, NALP3
	Flagellin	TLR5	IPAF,NAIP5
	DNA	TLR9	AIM2
	RNA	TLR7	NALP3
Virus 	DNA	TLR9	AiM2, DAI, IFI16, cGAS
	RNA	TLR3,TLR7,TLR8	RIG-I, MDA5, NALP3
	Structural proteins	TLR4, TLR2	
Yeast 	Zymosan,b-glycan	TLR2/6	Dectin1 NALP3
	Mannan	TLR2,TLR4	MCR
Mammalian cells	HMGB1	TLR2,TLR4,TLR9	RAGE, CD24
	HSP	TLR2,TLR4	CD91,CD24,CD14,CD40
	S100		RAGE
Crystals, inorganic?	Urate	TLR11, TLR2	Scav.R. , NALP3, NALP4
			

Innate immune response modulating impurities (IIRMIs) in generic peptides

Impurities (inorganic, microbial or mammalian) that are recognized by innate immune system receptors can:

- Activate the innate immune system
- Lead to local inflammation
- Facilitate antigen-specific immune response to exogenous proteins.
- Help break tolerance to endogenous peptides/proteins.

Current thinking on innate immune response modulating impurities in synthetic generic peptides:

- Demonstrate that the proposed generic synthetic peptide do not contain impurities or contaminants that produce a greater or distinct stimulation of innate immune activity as compared to the RLD.

Caveats: Only a fraction of possible IIRMI are known & different combinations of IIRMI may synergize in adjuvant activity

- Develop products devoid of impurities
- Develop methods that detect the presence of impurities that can act as adjuvants triggering a local innate immune or inflammatory response

Biomarkers of innate immune activation to identify multiple IIRMI

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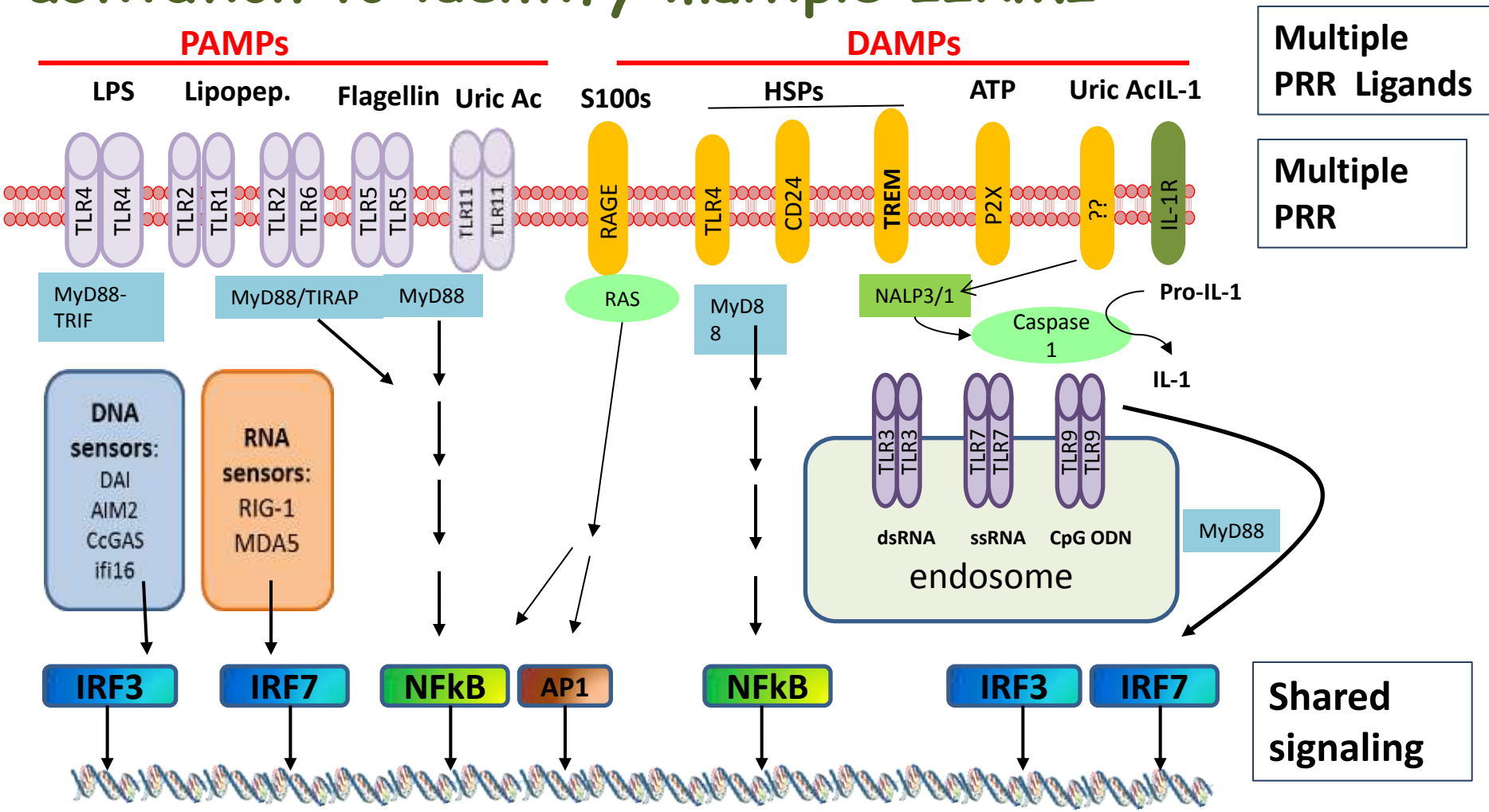


Cytokine, Chemokine, Receptors

Biomarkers of innate immune activation

One possibility is in vitro studies Platform?

PBMC

- Clinically relevant
- Donor to donor variability
- Complexity in obtaining, preparing and storing
- Key cells underrepresented (macrophages, DC, PMN)

Cell lines

- Increased reproducibility
- Control over PRR expression and distribution
- Easier to validate and transfer
  - HEK293-TLR
  - Monocyte/Macrophage

IIRMI: HEK-293-TLR based assay

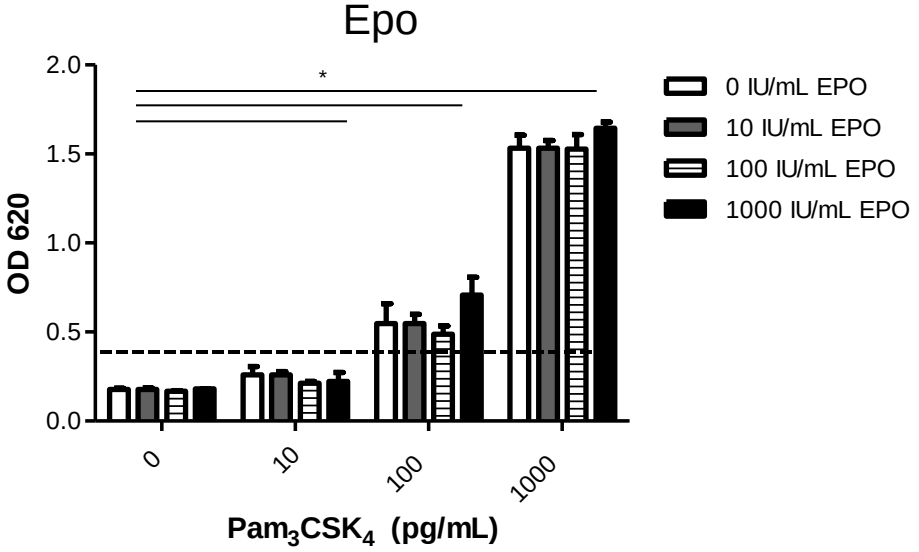
NF-κB-SEAP reporter cell line

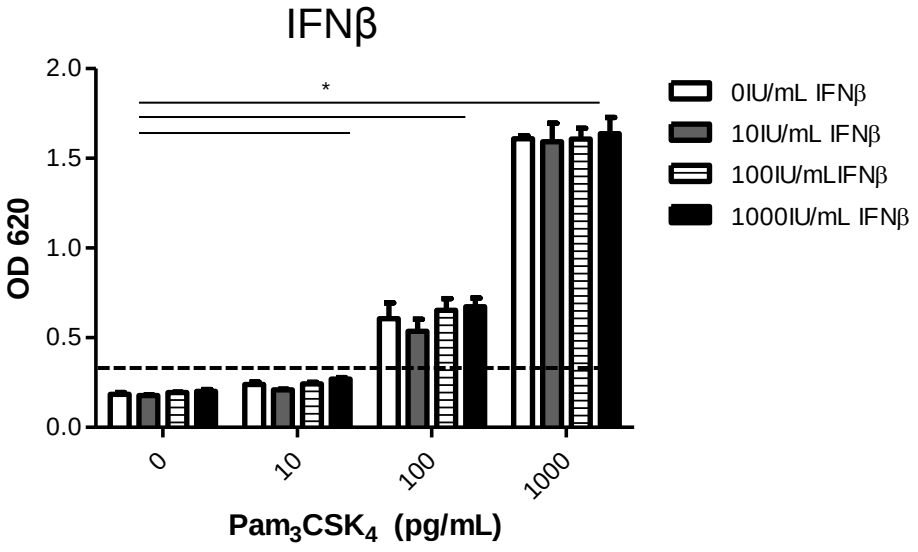
TLR ligand	HEK-BLUE-wt	HEK-BLUE hTLR2	HEK-BLUE hTLR4	HEK-BLUE hTLR5	HEK-BLUE hTLR7	HEK-BLUE hTLR9	PBMC
Pam3CSK4	< LOD	100pg/mL	< LOD	< LOD	< LOD	< LOD	1ng/mL
FSL-1	< LOD	10pg/mL	< LOD	< LOD	< LOD	< LOD	100pg/mL
Endotoxin	< LOD	< LOD	10pg/mL	< LOD	< LOD	< LOD	1pg/mL
Flagellin	10μg/mL	10μg/mL	10μg/mL	10ng/mL	10μg/mL	10μg/mL	5μg/mL
Imiquimod	< LOD	< LOD	< LOD	< LOD	1μg/mL	< LOD	100ng/mL
CLO75	< LOD	< LOD	< LOD	< LOD	1μg/mL	< LOD	100ng/mL
CpG-ODN	< LOD	< LOD	< LOD	< LOD	< LOD	100ng/mL	100ng/mL

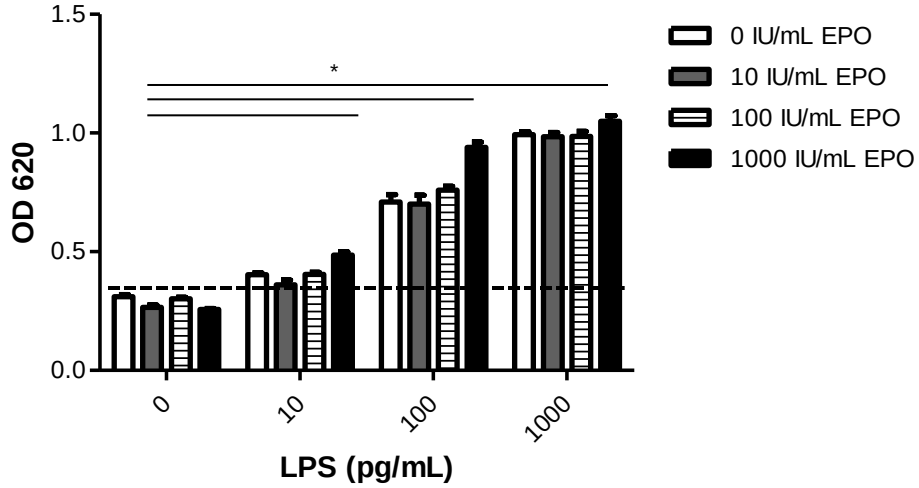
* Purified PRRAgs as model IIRMI

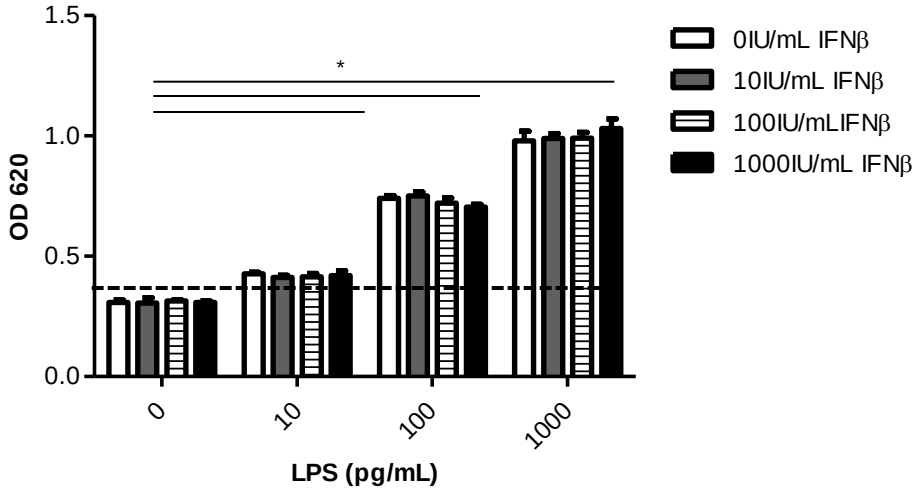
Haile *et al* PLoS One 2015

Cell-based IIRMI detection method using HEK-BLUE-hTLR transfectants









Dose dependent increase in SEAP signal

Utilization of Commercial hTLR Transfected Cell Lines to Detect Impurities

Advantages:

- Cell-line based
- Sensitive, Reproducible, Easily validated
- Result may provide clues as to source of impurities

Disadvantages:

- Limited repertoire of cells
- Requires presumption of impurities

Cell line based assays to detect IIRMIs in products

RAW-BLUE cells (Mouse macrophage cell line)

- NF-κB-SEAP reporter cell line

24h

SEAP

Add Substrate

1-2h

OD 620

MM6 (human macrophage)

24h

mRNA

IL-6/IL-8

THP1 cells (human monocytic)

- TNF-α luciferase reporter cell line

5h

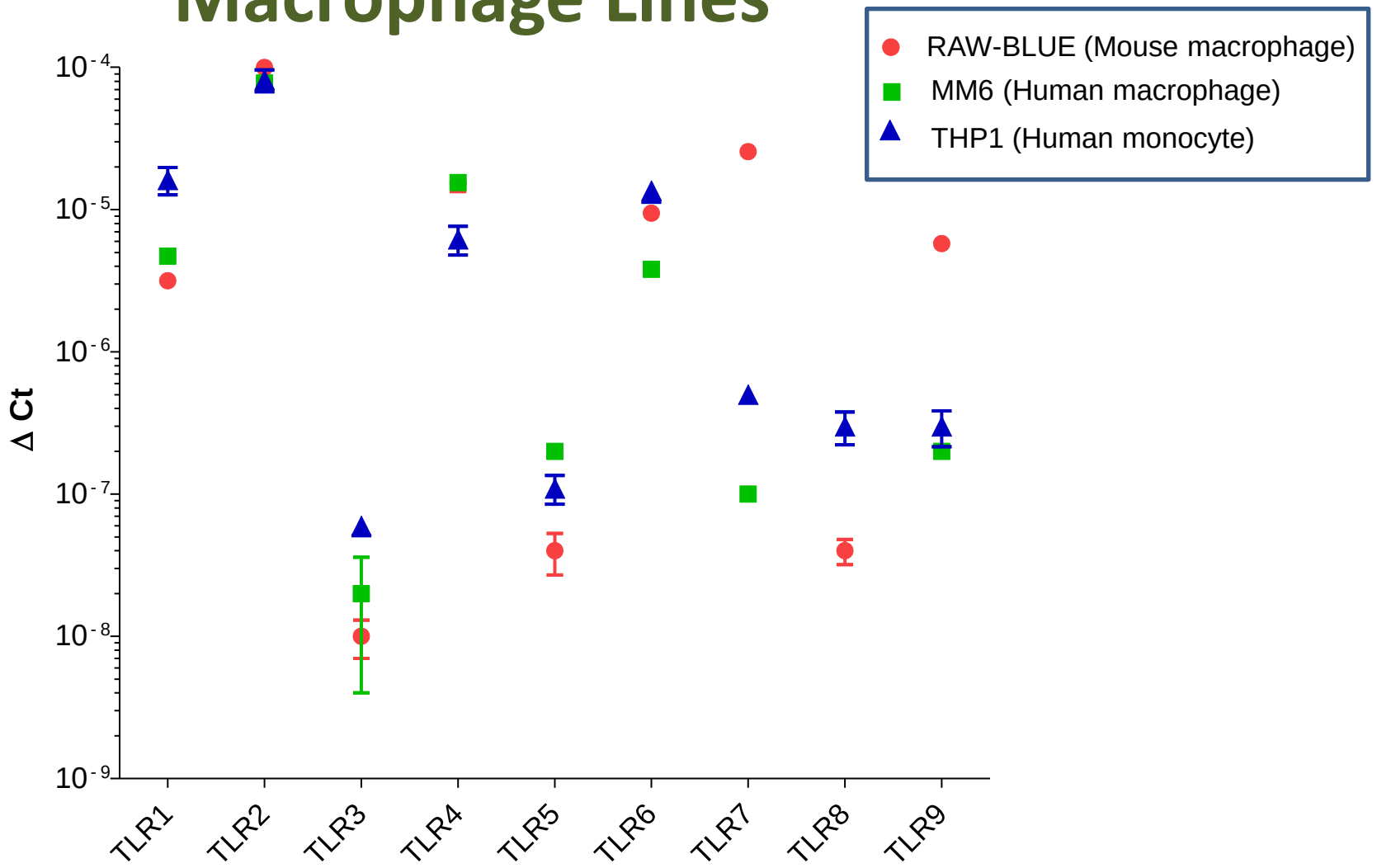
Lyze the cells

Add substrate

Read luminescence

Haile et al PLOS ONE 2015

TLR Expression Profile by Different Macrophage Lines



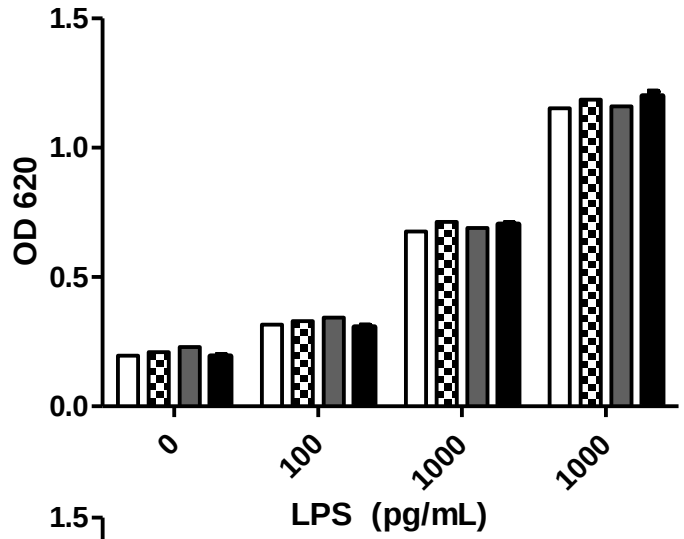
Haile *et al* PLoS One 2015

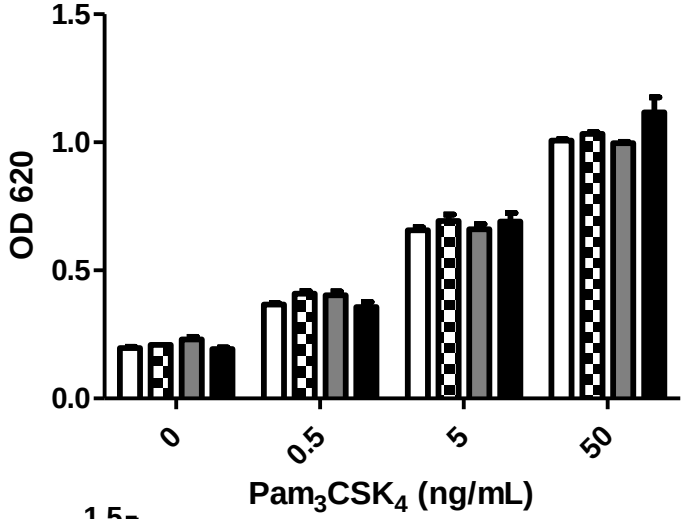
Comparison of LOD for PPR ligands by macrophage cell lines and PBMC

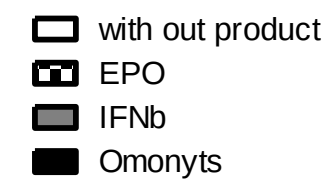
TLR ligand	RAW-BLUE	MM6	THP1
Pam3CSK4	500pg/mL	500pg/mL	100pg/mL
Poly I:C	ND	ND	1μg/mL
Endotoxin	100pg/mL	10pg/mL	10pg/mL
Flagellin	ND	ND	5μg/mL
FSL-1	100pg/mL	100pg/mL	100pg/mL
Imiquimod	100ng/mL	ND	ND
CLO75	50ng/mL	ND	ND
CpG	60ng/mL	ND	ND
Zymosan	1μg/mL	10ng/mL	10ng/mL
MDP	ND	10μg/mL	ND

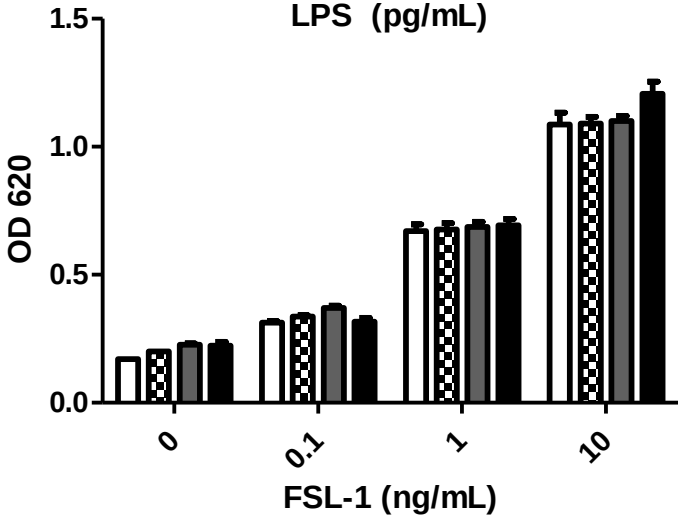
Haile et al PLOS ONE 2015

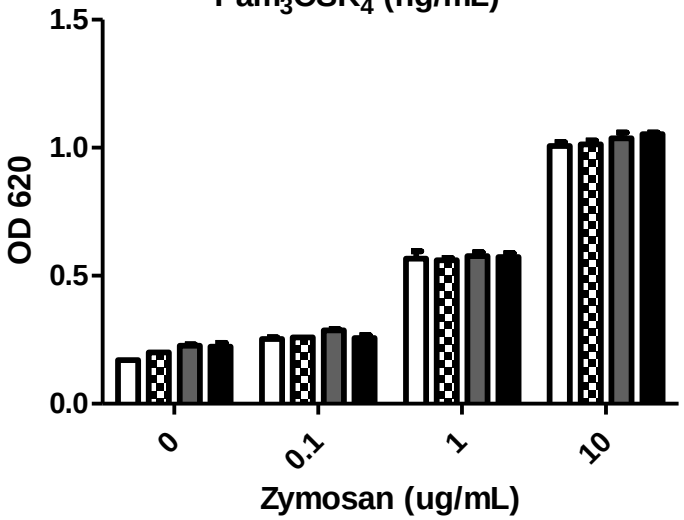
Test products don't impact the detection of impurities by RAW-BLUE cells









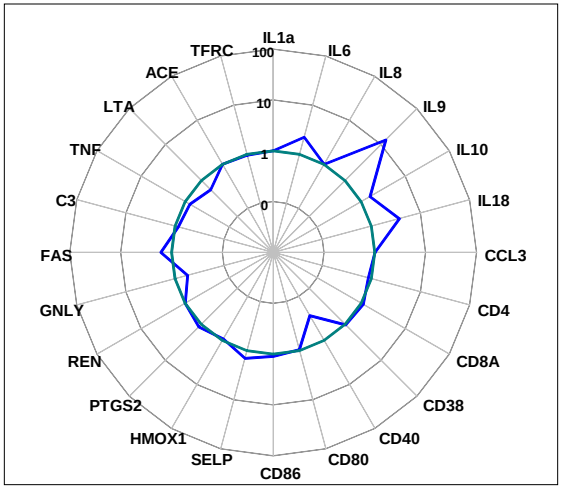


* Immunomodulatory API or preservatives in the formulation may impact the sensitivity of these assays

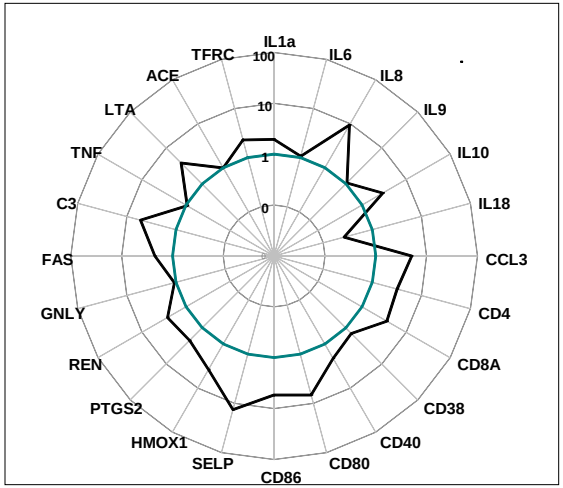
Peptides manufactured on different platforms

Gene expression profile by peptides derived from different source on MM6 cells

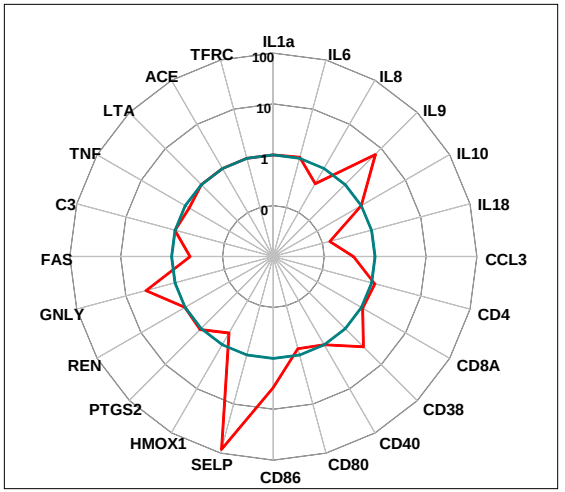
rHu Peptide X



Synthetic peptide X_1



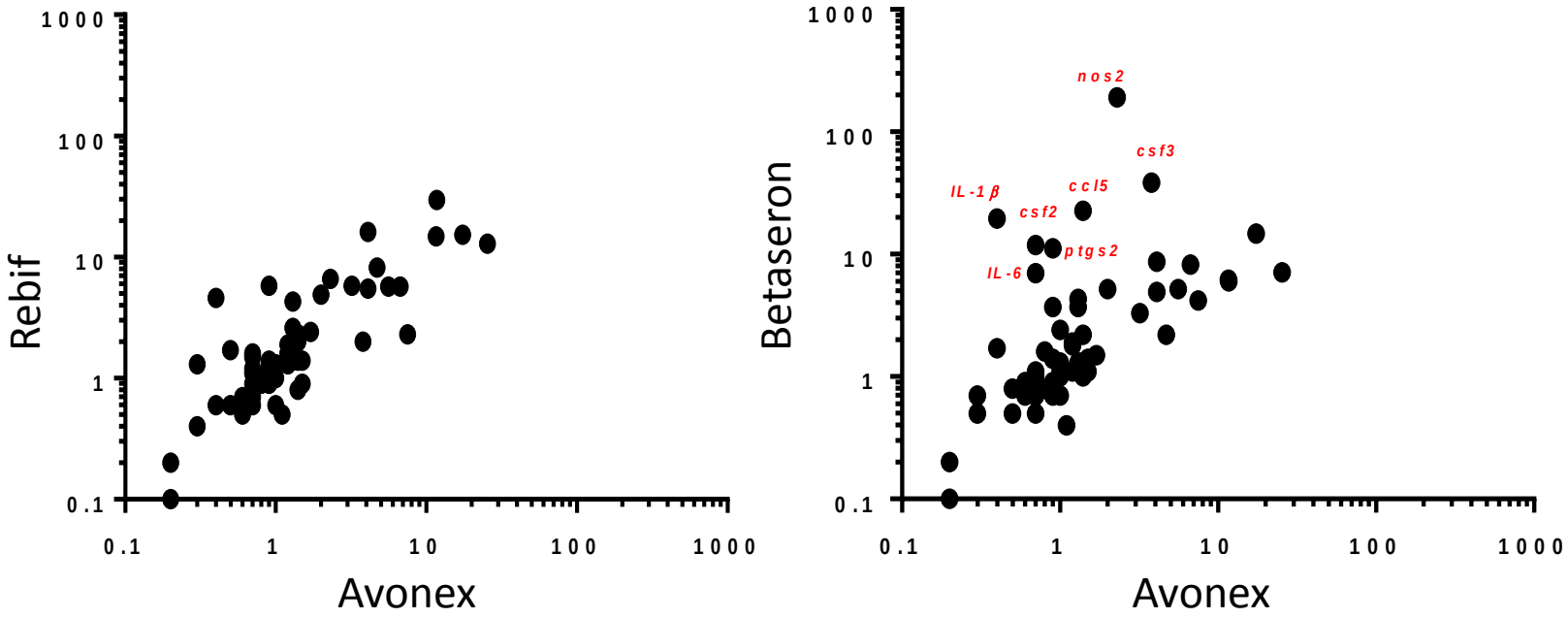
Generic Synthetic Peptide X_2"



- Peptides induce unique gene expression profiles*
  *Caveat: Different preservative in product formulation may interfere with assay.

Comparison of IIRMI signatures: Interferon Beta

Avonex, Betaseron and Rebif are manufactured on different platforms



- Betaseron induced higher levels of pro-inflammatory genes
- Signal is IFNAR-independent
- Signal is TLR2 and TLR4 mediated.

*Cell based assay identifies TLR2 and TLR4 stimulating impurities in Interferon beta Haile et al, Nat. Sci. Rep. 2017

IIRMI Summary

1. IIRMI impact on immunogenicity and thus should be assessed when assessing risk.

2. Cell-line based methods may be useful in detecting a broad spectrum of IIRMI in therapeutic products

3. Products induce predictable profile of immune related gene expression

4. Requires careful validation

Summary

Immunogenicity risk of drug products cannot be predicted from bioanalytical characterization alone, however, depending of the clinical risk, advancing methods may allow a determination that a synthetic generic peptide **does not pose an increased risk of immunogenicity as compared to the RLD**. Such an assessment would be based on

- Sameness of API
- Assessment of the risk of individual product related impurities
- Assessment of Aggregates
- Assessment of Innate Immune Response Modulating Impurities

Thank you

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Swamy Polumuri
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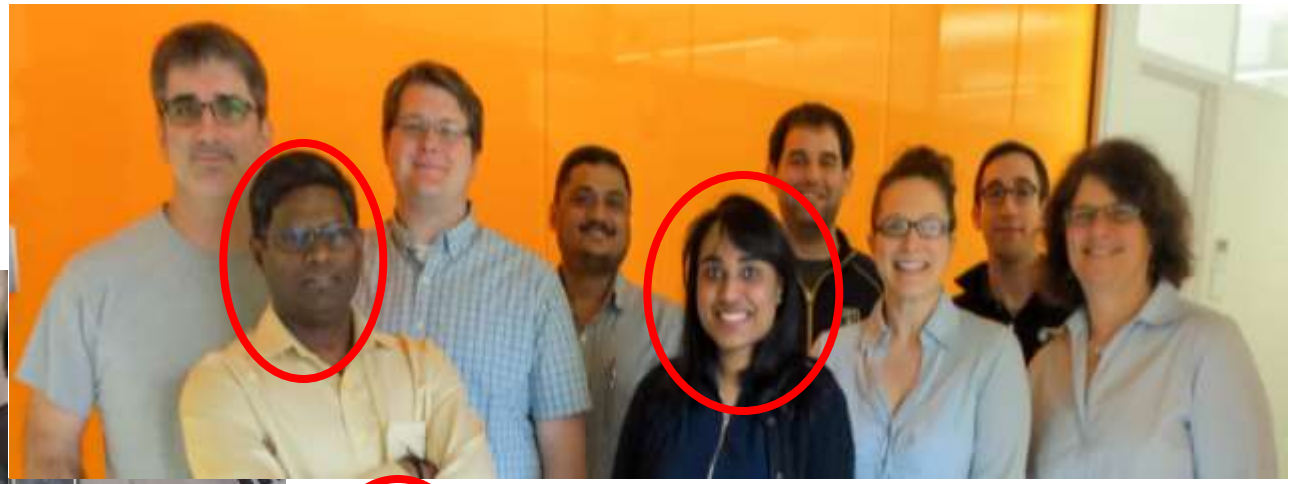
Logan Kelley Baker*

- **Amy Rosenberg**
- **Susan Kirshner**
- **Steven Kozlowski**
- **Emanuela Lacana**



Animal facility:
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