Mastering Immunity

Whole Blood Cytokine Release Assays to Assess the Risk of Innate Immune Activation to Generic Peptide Products

Dr. Jeremy Fry

26 January 2021

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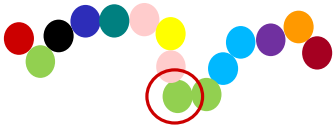
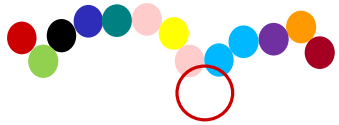
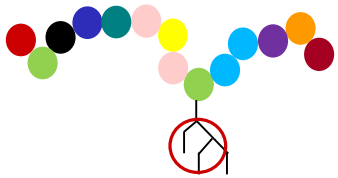
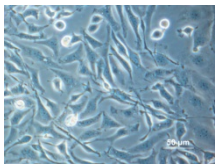
Key Challenges

Generic peptide			
Impurities		**Insertions**	**Impurities arising** …during synthesis? …during storage?
		**Deletions**	
		**Modifications:** oxidation, reduction, methylation, acetylation, glycosylation	
		**Host cell proteins**	
		**Other:** solvents, metals, leaching	

What is the impact of these impurities on immunogenicity?

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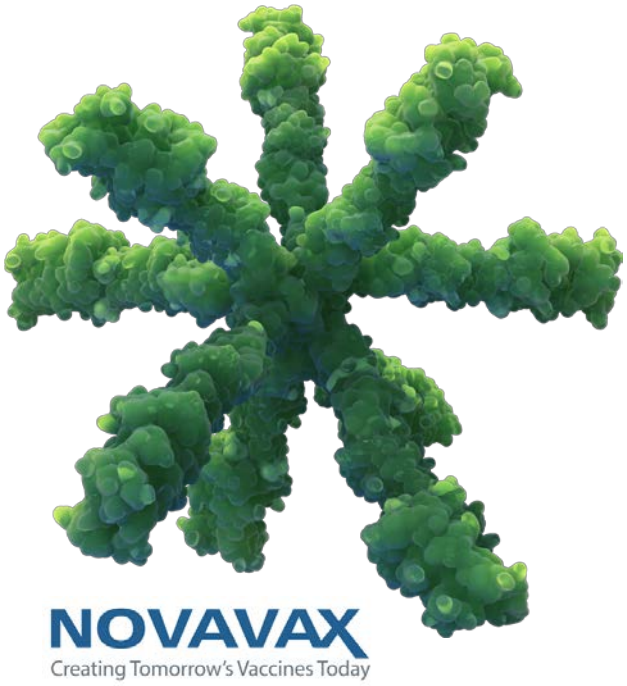
We can learn from vaccines

- Peptides are known to be generally poorly immunogenic
- BUT, when delivered with immuno-stimulatory adjuvants, potent responses can be initiated

Malonis *et al* (2020) DOI:10.1021/acs.chemrev.9b00472



Recombinant Nanoparticle Vaccine

- Examples:
  - link to VLP, nanoparticle, carrier protein, PADRE (pan-DR epitope)
  - in conjunction with poly LCIC (TLR3), imiquimod (TLR7)

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What is the Risk of Clinical Immunogenicity?

Adaptive Immunity

- Impurities may have increased affinity for MHC binding
- Inadvertent incorporation of T cell epitopes
- Helper CD4+ T cell responses ultimately leading to ADA formation

Innate Immunity

- Product aggregation
- Interaction of impurities with pattern recognition receptors (PRRs) leading to activation of adaptive immunity
- Inadvertent adjuvant effect breaking tolerance

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Innate Immunity

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If any generic peptide impurity behaves inadvertently as an *adjuvant*, then there is elevated risk of immunogenicity

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

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002

Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
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October 2017
Generics

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Email: druginfo@fda.hhs.gov
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2017
Generics

"The data should demonstrate that the proposed generic synthetic peptide … does not contain impurities or contaminants that produce a greater or distinct stimulation of innate immune activity as compared to the RLD"

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Selection of Assay Format

Cell lines	PBMCs	Fresh Whole Blood
• Reproducible • Good for mechanistic characterisation: TLR agonists • Easy to procure	• Broad representation of population • Somewhat clinically relevant	• Broad representation of population • Most cell types included • Highly clinically relevant
	• Donor variability	• Donor variability
• Not clinically directly relevant • Limited receptor array • Poor response to aggregates	• Moderately challenging to source • Under-representation of some key cell types due to purification process • Cannot use for mechanistic pathway determination	• Very challenging to source • Cannot use for mechanistic pathway determination

Selection of Assay Format

- Fresh Whole Blood assay takes the whole picture into account
- Perform first to complete broad risk assessment whether any issues
- Cell lines can then be deployed for mechanistic characterisation as required

Fresh Whole Blood
• Broad representation of population • Most cell types included • Highly clinically relevant
• Donor variability
• Very challenging to source • Cannot use for mechanistic pathway determination

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Key Study Design Challenges & Considerations

- By definition any impurities under investigation are at a low level (0.1 – 0.5%)
  - Quantity of impurity available for analysis can be challenging
  - ~5mg of each impurity may be required depending on number of donors and selected test concentrations
  - if provided as solution, for whole blood assays, stock test material should be supplied as 50x the top concentration required
- Max number of test articles that can be tested per donor (blood volume)
- Batch comparison analyses
  - Stability batches
  - Batches of RLD from different geographic regions (i.e. EU vs US)
  - Number of batches to compare
- Most appropriate cytokine panel / flexibility

ProImmune ProStorm® Study Design

- Unmanipulated and undiluted fresh whole blood assay
- ~20-30 healthy donors are specifically recruited for study
- Healthy adult donors are screened to meet a number of key criteria including:
  - being free from symptomatic viral and bacterial infections
  - not taken steroidal for 7 days or non-steroidal anti-inflammatory medication for 3 days before donating
- 50mL blood is drawn into sodium heparin Vacutainers®
- Within 3 hours of draw (typically 1-2 hours), blood samples from each donor are incubated in triplicate with test material at the required range of concentrations

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Typical ProStorm® format for analysis of ANDA

- Assay controls:
  - PBS negative; SEB positive
- Test articles:
  - Impurities (can range from 1 to several depending on manufacturing process)
  - Reference Listed Drug (RLD)
  - Drug Product (DP)
  - Active Pharmaceutical Ingredient (API): DP minus impurities if possible
  - Formulation buffer / excipients control?
  - Add test articles: minimum of 4 doses (depending on drug typically 0.01-100 µg/mL)
- Incubate 24 hours, isolate plasma and quantify **IFNγ, TNFα, IL-2, IL-4, IL-6, IL-8, IL-10** levels by multiplex cytokine immunoassay
  - (*Optional*: IL-1β, IL-3, IL-5, IL-7, IL-9, IL-11, IL-12p70, MIP1α, IP-10, MCP-1)

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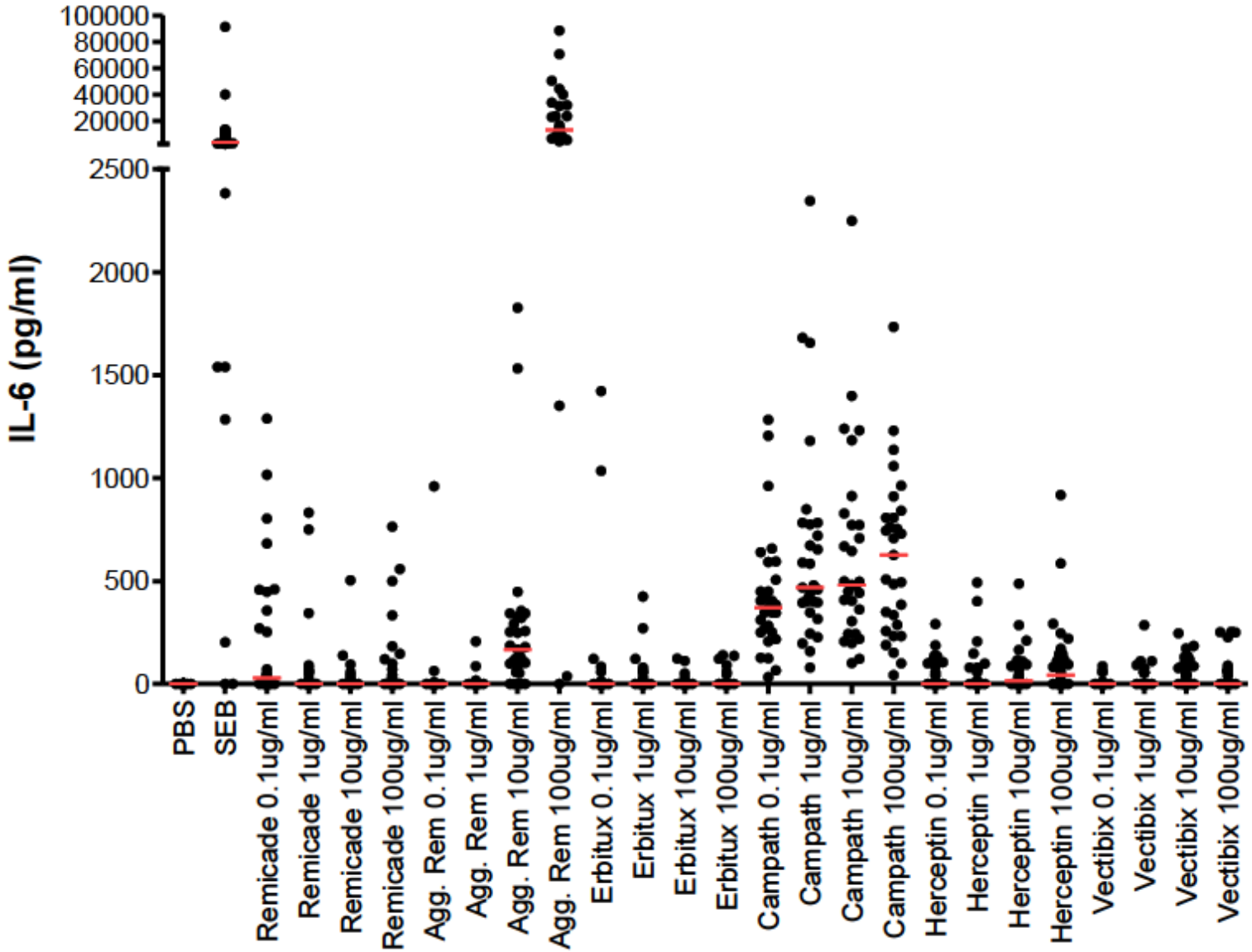
Example: IL-6 Cytokine Response



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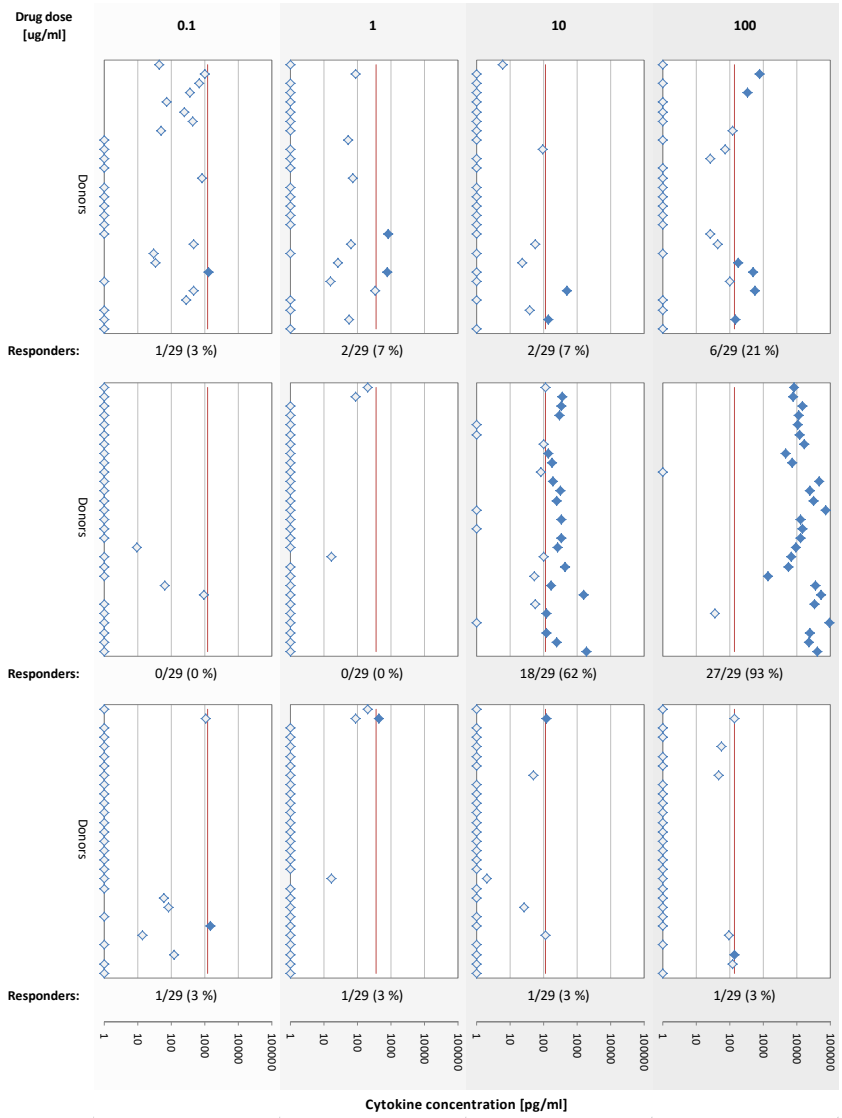
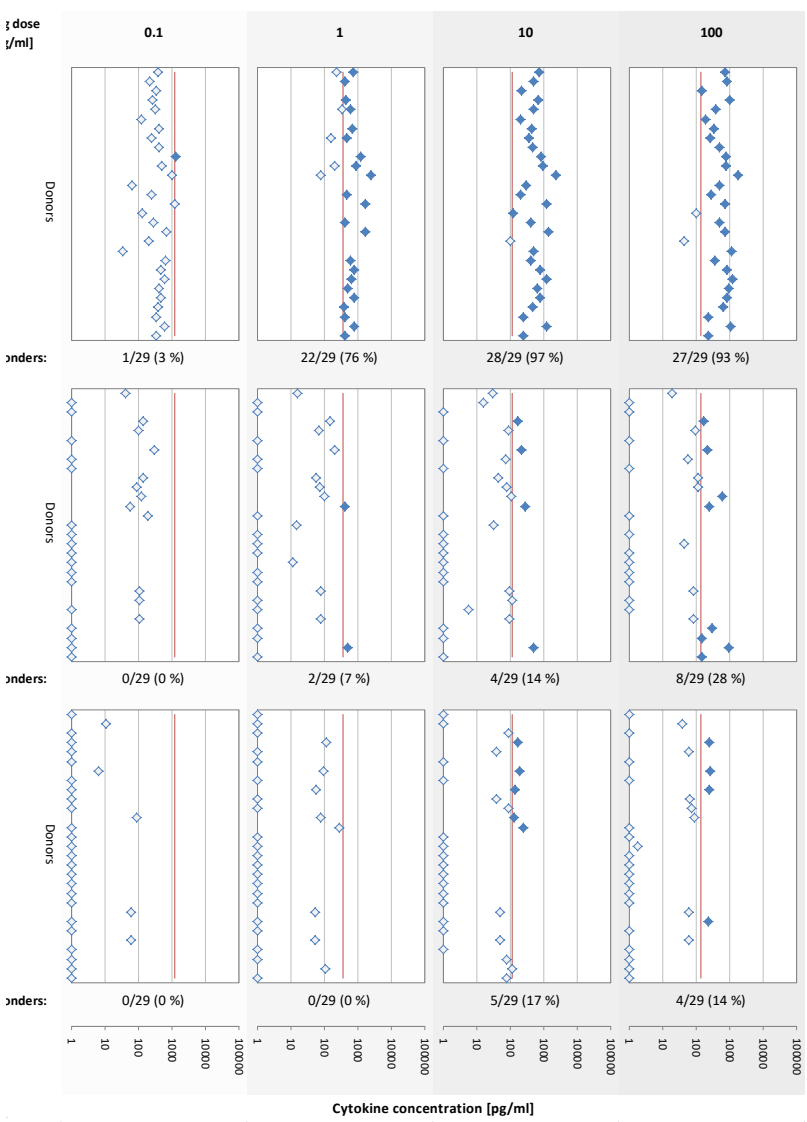
Cytokine Response - IL-6



Cytokine Response - IL-6



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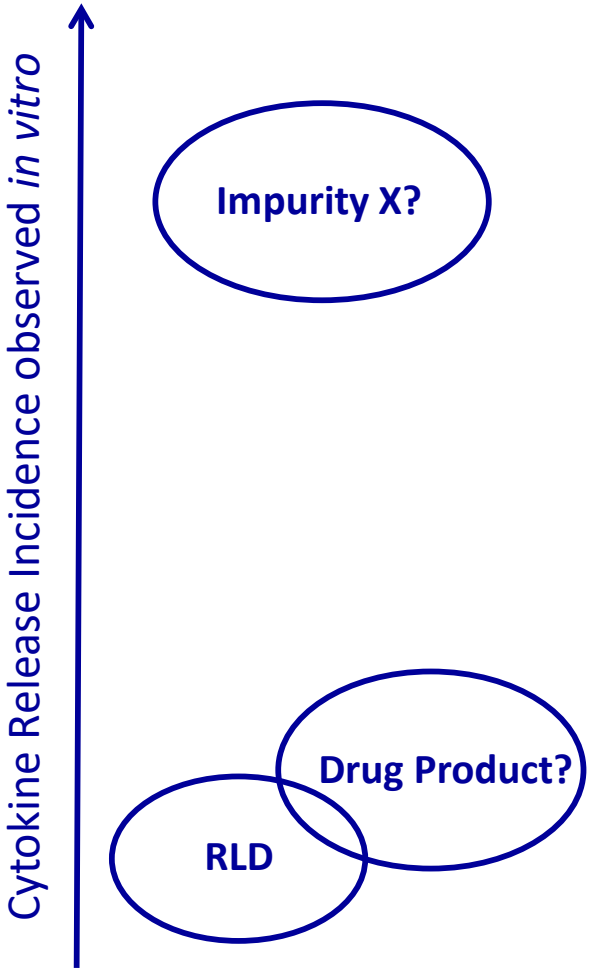
Study analysis



- Cytokine release assay for hazard identification and risk management
- Simple whole blood assay minimizing test system interference
- Most commonly observed responses to impurities by IL-2, IL-6, IL-8, IFNγ and TNFα
- Flexible study design (test article concentrations, cyto/chemo-kines analyzed)
- Rapid project delivery (~4 weeks)

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Summary

- Innate immune receptors can recognize process-related impurities
- Fresh whole blood cytokine assays enable the efficient and robust identification of innate immunogenicity risk
- Additional mechanistic characterization (i.e. specific TLR agonism) using cell lines may be required if risks observed
- ProImmune has extensive experience of analyzing a wide range of these synthetic peptide products of rDNA origin delivering full assay turnaround in just 4 weeks
- Outsourcing to an expert lab overcomes the significant challenges in sourcing fresh blood from a panel of healthy donors

Thank you for your attention!

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