NCI Alliance for Nanotechnology in Cancer

Understanding In vitro Innate Immune Responses to Teriparatide and Innate Immunity Modulating Impurities

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https://ncl.cancer.gov

Frederick National Laboratory for Cancer Research

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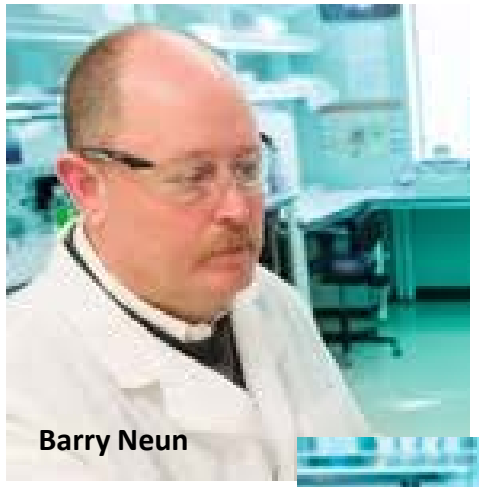
Presentation outline

- NCL overview
- Team
- In vitro innate immune responses to RLD
  - Complement activation
  - Leukocyte proliferation
  - Cytokines
- Take Home Messages

- Nanotechnology Characterization Lab (NCL) is part of the Frederick National Laboratory for Cancer Research located at the Advanced Technology Research Facility (ATRF) in Frederick, Maryland

- The goal of NCL collaboration with the FDA was to evaluate in vitro responses to innate immunity modulating impurities (IIMIs), reference listed drugs (RLD) and their generic counterparts


Edward Cedrone


Barry Neun


Tim Potter


Duncan Donohue


Eric Pang

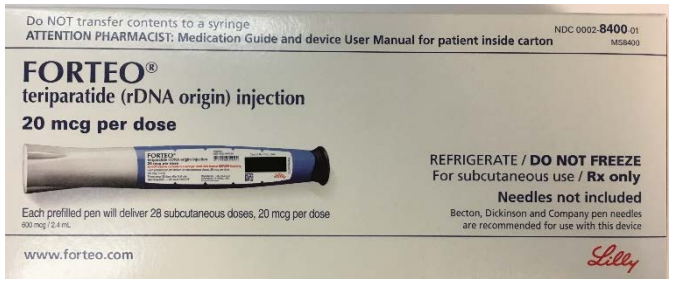

Claire Holley

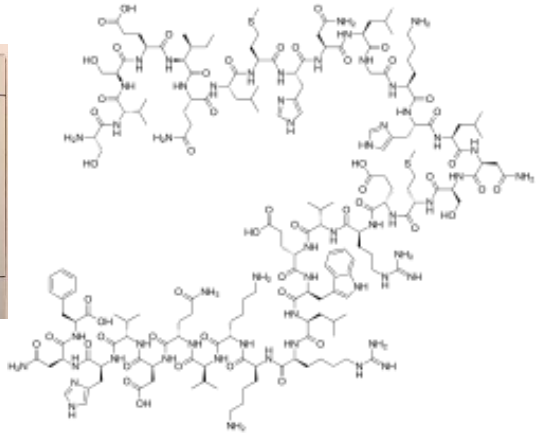

Daniela Verthelyi

Models and assays

- **Teriparatide** is a form of parathyroid hormone consisting of the first N-terminus 34 amino acids (the bioactive portion of the hormone).
- It is approved for osteoporosis indication and is also used off-label to speed fracture healing.
- Recombinant teriparatide is sold by Eli Lilly under the brand name **Forteo**

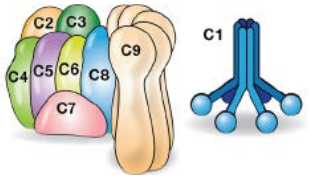
Model RLD

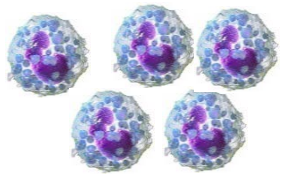


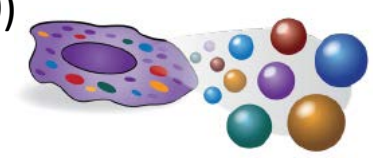


Assays

1. Complement activation (NCL ITA-5)
2. Leukocyte Proliferation (NCL ITA-6)
3. Cytokines (NCL ITA-10)







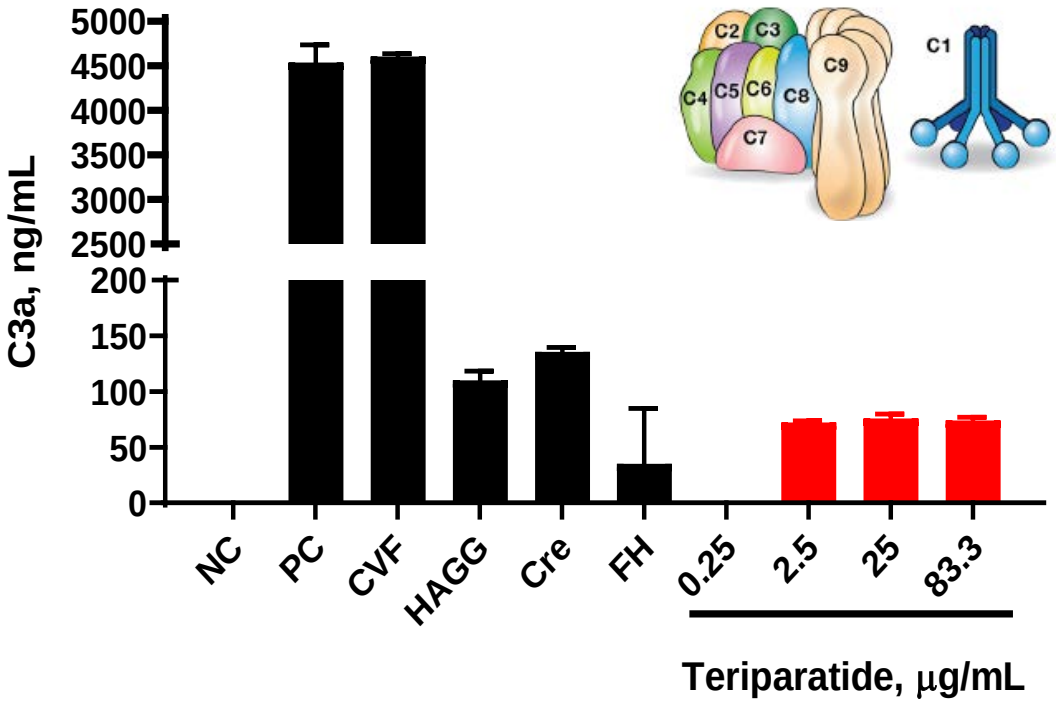
Detailed protocols are available at https://ncl.cancer.gov/resources/assay-cascade-protocols

Model IIMIs

IIMI#	Reagent	Invivogen Cat #
1	Ultrapure flagellin from B. Subtilis	tlrl-pbsfla
2	FSL-1	tlrl-fsl
3	ODN 2006 Class B	tlrl-2006
4	Poly(I:C) HMW	tlrl-pic
5	Poly(I:C) LMW	tlrl-picw
6	Zymosan	tlrl-zyn
7	CLO75	TLR-c75
8	MDP	TLR-mdp
9	ODN2216	Tlrl-2216
10	E. Coli O111:B4 LPS	tlrl-3pelps

Complement activation

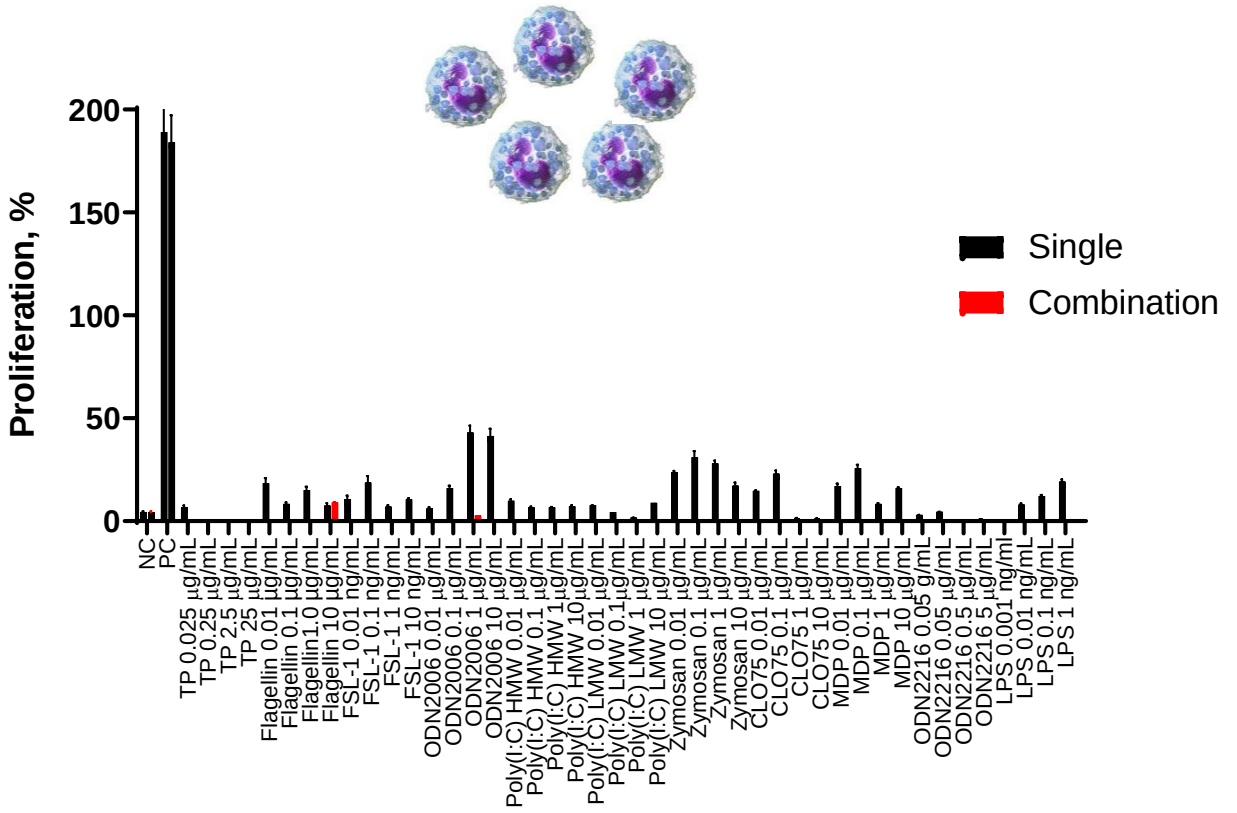
- The complement activation assay is known to have good in vitro-in vitro correlation and can be predictive of complement activation related pseudoallergy (CARPA) to various types of drug products
- Activation of the complement system is also known to contribute to vaccine efficacy due to the role of complement in the maturation of dendritic cells (DC) and activation of T-cells and B-cells
- Complement activation by a drug product in a fashion similar to vaccine efficacy may contribute to undesirable immunogenicity and anti-drug antibody (ADA) response to the drug product



- **Complement activation assay could be used to compare this RLD to its generic version, provided a difference in the formulation is expected, or to other formulations of the same API**
- **Complement activation assay is not expected to pick up potential differences in low levels of IIMI contamination between RLD and generic products because concentrations of IIMI required to trigger complement activation are usually high**
- **This method was not chosen for subsequent studies**

Leukocyte Proliferation

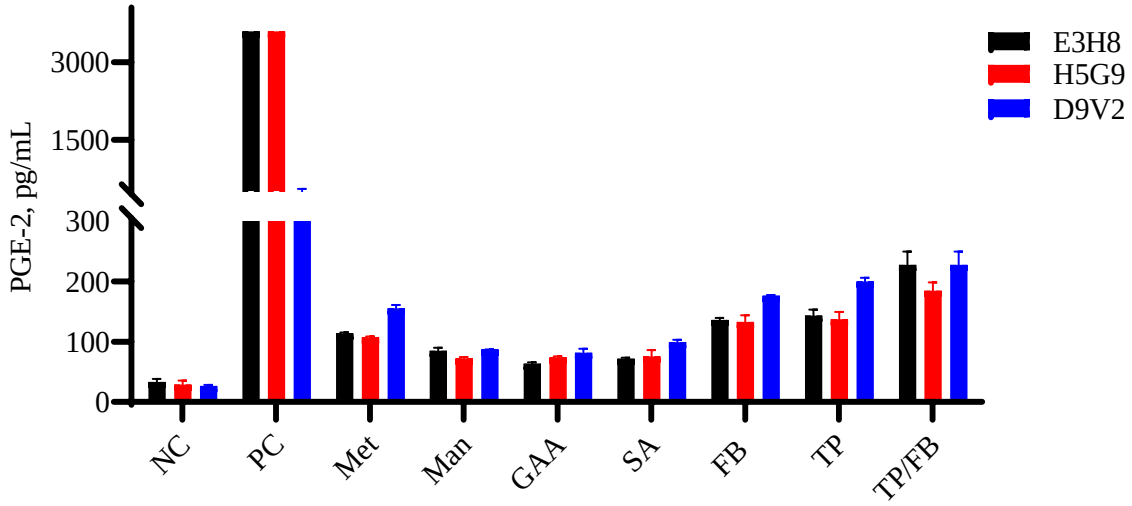
- The proliferation of leukocytes in response to mitogens or antigens is an indication of immune response activation
- An in vitro assay utilizing PBMC was used to assess the proliferation of leukocytes in response to TP and IIMIs
- Low level of proliferation was detected in all tested samples and is likely attributed to the cytokine response as described later in this presentation

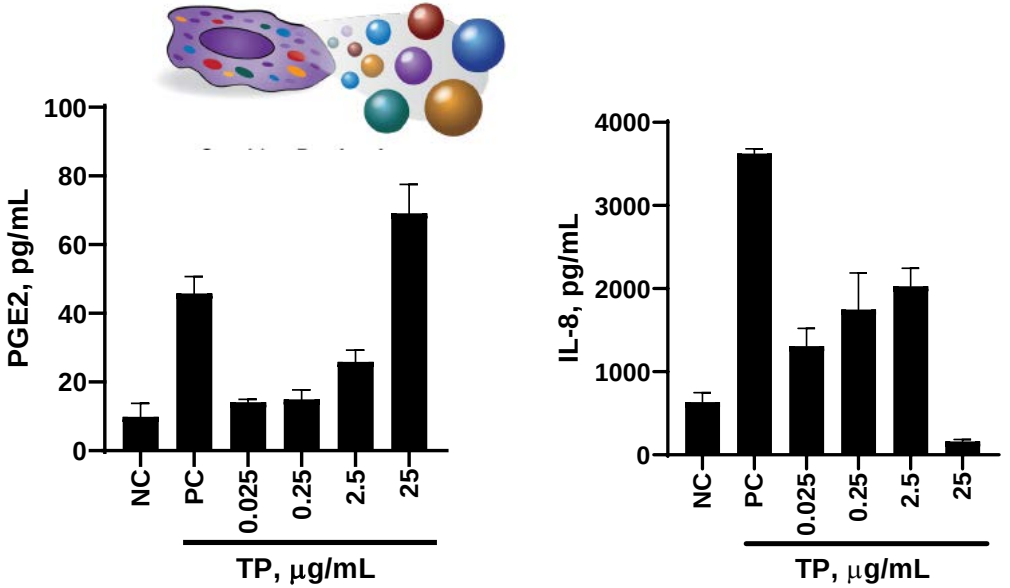


- **Since the magnitude of proliferation in response to IIMIs was low, and the combination of IIMI and TP resulted in complete suppression of IIMI-mediated proliferation, this method was not chosen for subsequent studies.**

Cytokines

- We used a 16-plex panel that included the following cytokines: type I interferon (IFNα), type II interferon (IFNγ), type III interferon (IFNλ), interleukins (IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-17), tumor necrosis factor alpha (TNFα), interferon-gamma inducible protein (IP-10), prostaglandin E2 (PGE2), macrophage inflammatory protein (MIP-1α), and monocyte chemoattractant protein (MCP-1).
- The study was performed using peripheral blood mononuclear cells isolated from 10 healthy donors

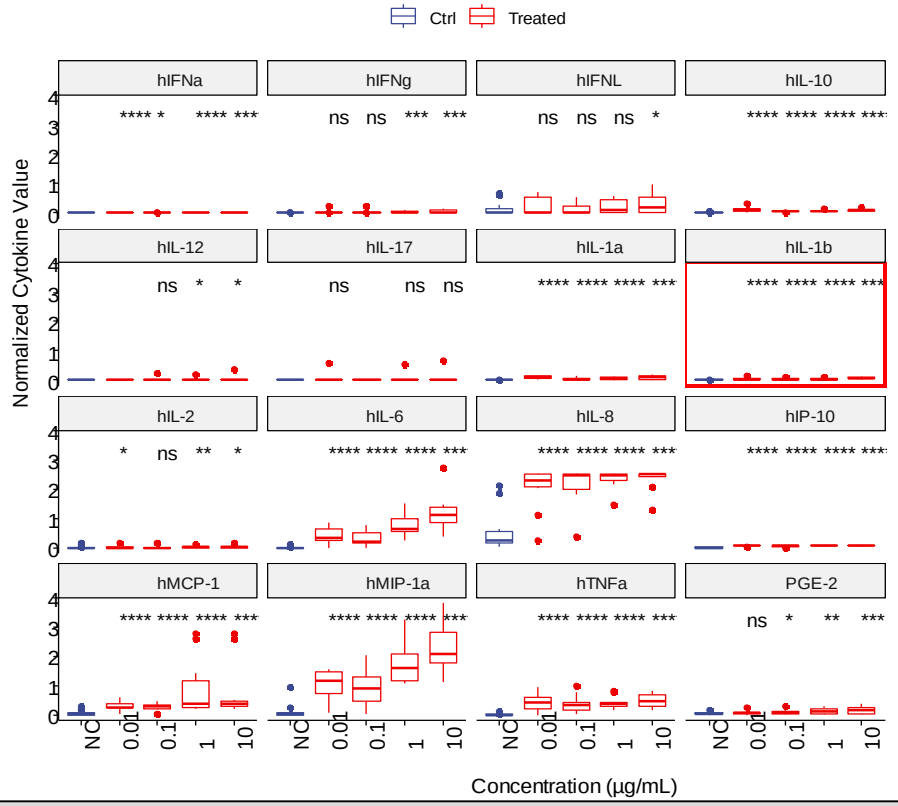


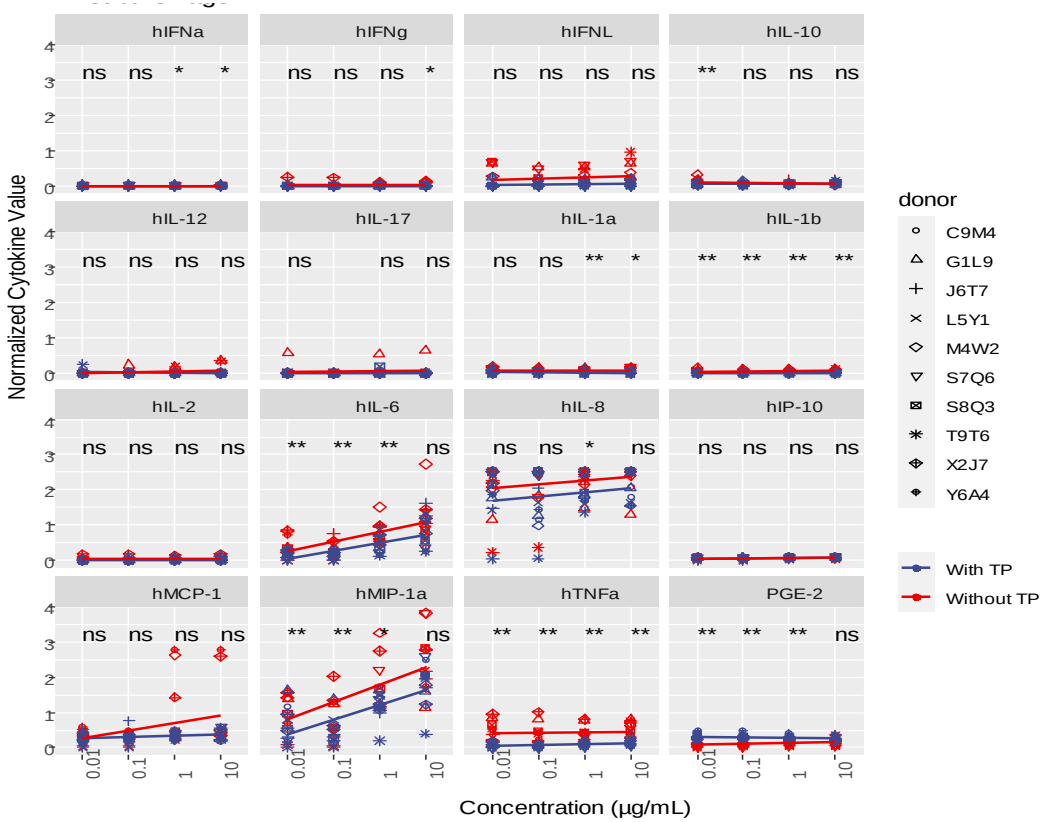


Teriparatide product induces PGE2 and IL-8

- **Induction of these cytokines is due to the formulation buffer and not API**
- **All components of formulation buffer contribute to the induction of cytokine response**

IIMIs

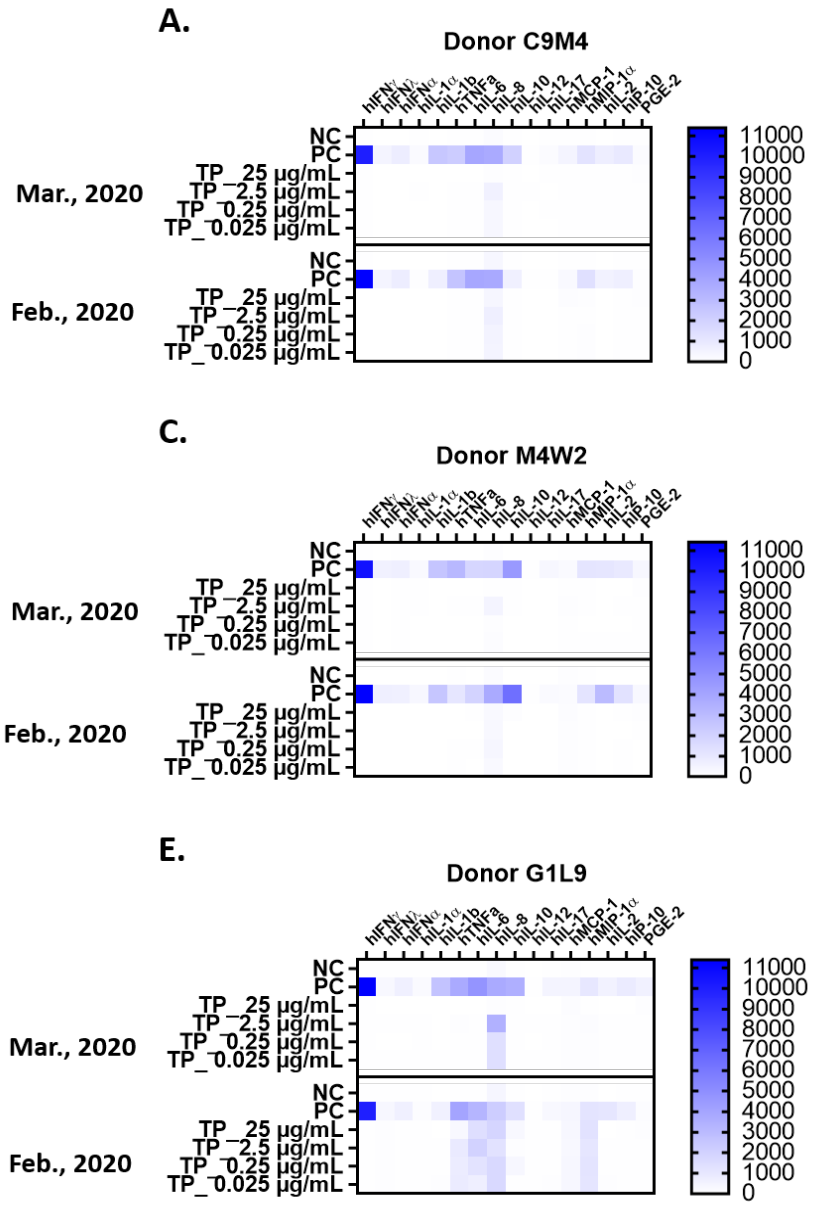




- **IIMIs induced a broad and often overlapping cytokine response consistent with the current knowledge of their cognate pattern recognition receptors and relevant signal transduction pathways**
- **Three cytokines would provide at least one positive result for all 10 IIMIs and potentially could be used by users who do not have access to more than 3-plex cytokine detection panel:**
  - *panel1 (IL-1α , IP-10, and IL-8) ; panel2 (MIP-1α, IP-10, and IL-8)*
  - *panel 3 (IL-1α, MCP-1 and IL-8); panel 4 (MIP-1α, MCP-1 and IL-8)*
  - *panel 5 (IL-1α, MCP-1 and IL-6); panel 6 (MIP-1α, MCP-1 and IL-6)*

- **Teriparatide formulation suppressed IIMI-mediated cytokine responses**
- **This effect was also due to the formulation buffer**

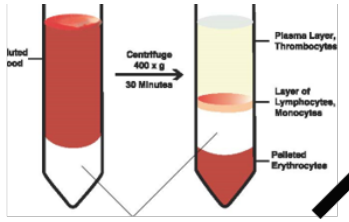
Genetic Variability



- PBMC from some donors demonstrated more robust (i.e., higher magnitude) responses to TP than cultures from other healthy donors
- It could be inherent to the donor's genotype or a consequence of variability of sample handling
- Control experiment in which donors were called for the second blood donation one month after the original experiment
- The results were compared between the two experiments

- **The quality of the response remained the same**
- **Genetic background of donors that donate their blood for in vitro experiments, is an important factor determining the PBMC response to individual IIMIs**
- **Day-to-day variability in phlebotomy and handling of whole blood and PBMC may result in quantitative differences (i.e., may influence the magnitude of the responses in the cells from the given donor)**
- **Such variability does not change the overall conclusion of the study**

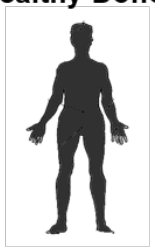
Assay logistics



Ficoll

Day 1

Healthy Donor(s)

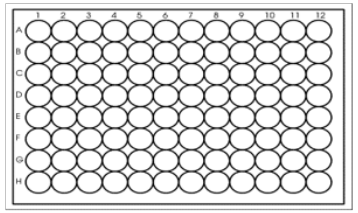


Fresh Whole Blood Collection

1. Fresh PBMC isolation
2. Freshly Isolated PBMCs cultured 24 hrs (37°C)
3. Freshly Isolated PBMCs cryopreserved (-80°C)
4. Whole Blood Culture
5. Whole Blood Storage 24 hrs (4°C)
6. Whole Blood Storage 48 hrs (4°C)

PBMC isolation

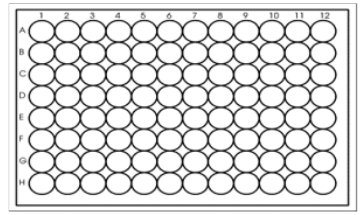
Day 2*



24 hr incubation
- Controls (NC, PC)
- IIMIs
- TP
- IIMIs + TP

Day 3*

- Centrifuge to pellet PBMCs
- Collect culture supernatants
- Analyze fresh or save for later in -80°C freezer

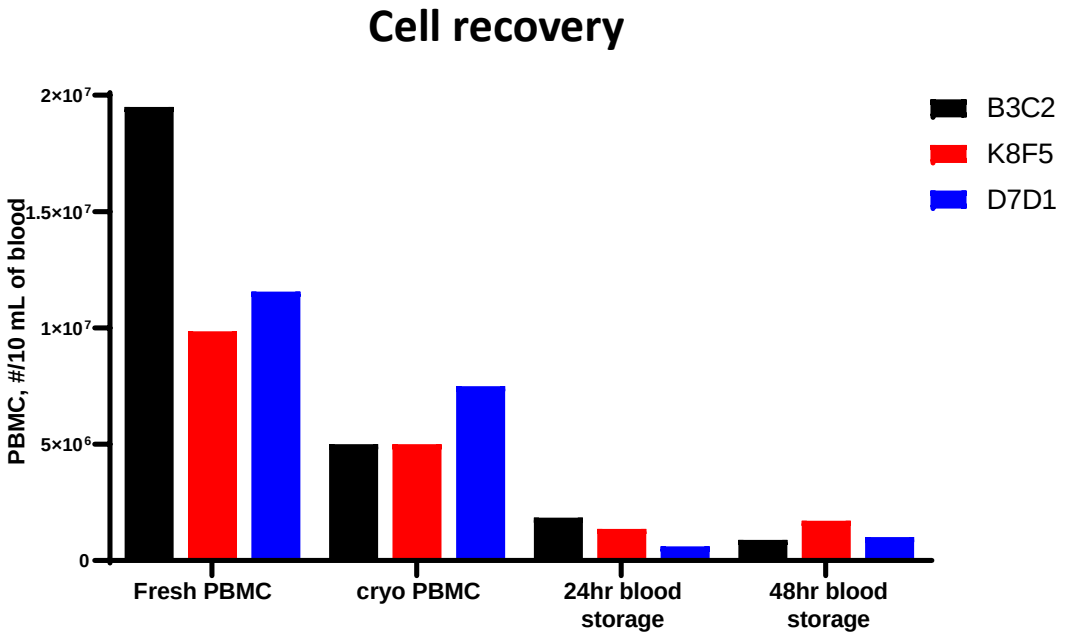


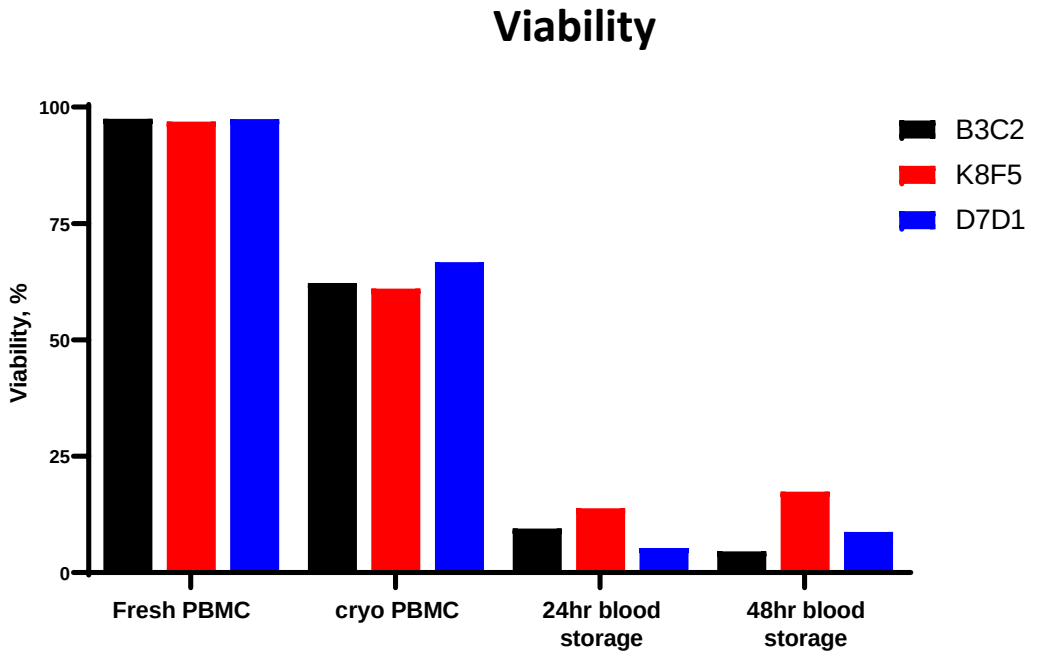
Chemiluminescence Multiplex ELISA (Quansys)

Cytokines Analyzed (7-plex):
IL-1α, MIP-1α, IP-10, MCP-1, IL-8, IL-6, PGE-2

When fresh blood is not available, what is the best source of PBMC for cytokine assay?

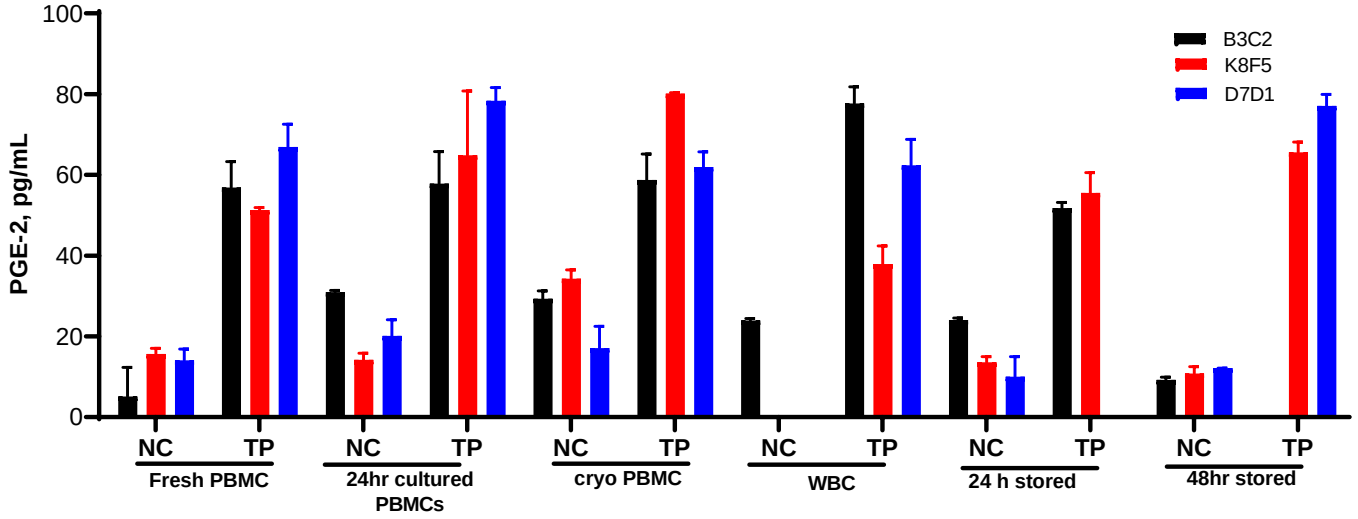
PBMC source and handling



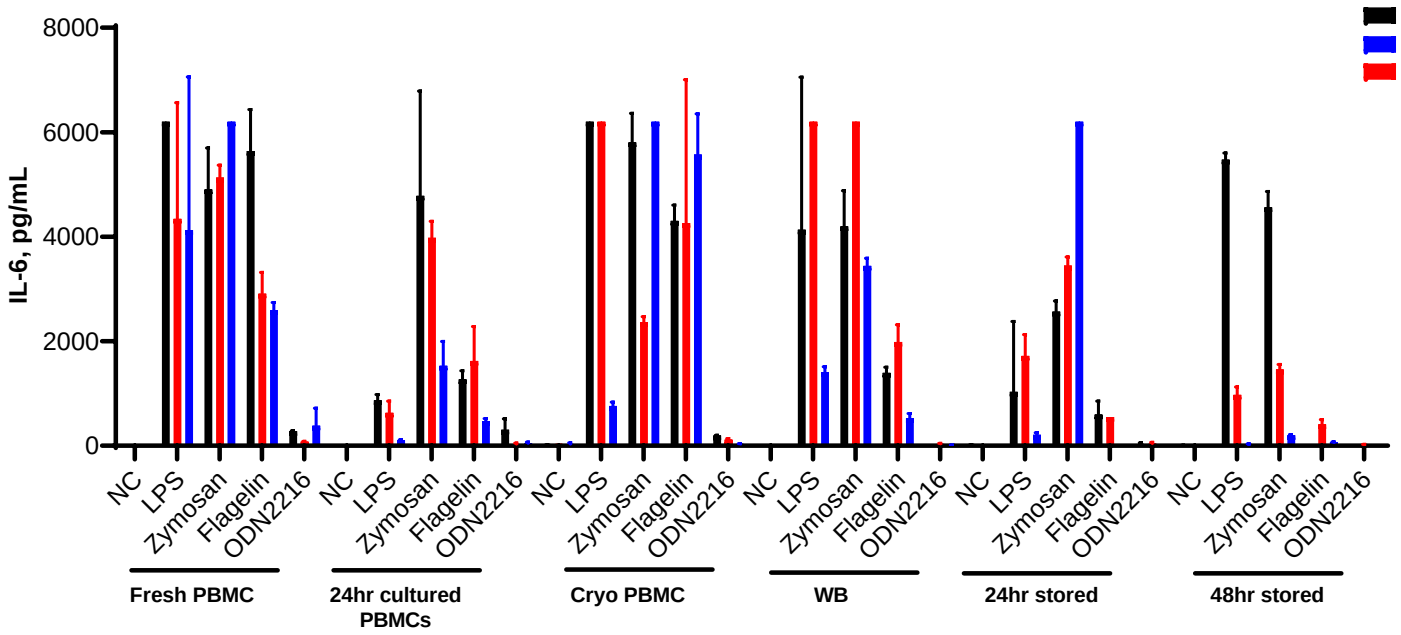


- The best cell recovery and viability were observed when fresh blood was used to isolate PBMC
- Cryopreserved PBMC isolated from fresh blood was the second best choice
- Refrigeration of whole blood for 24 and 48 hours results in dramatic loss in both cell recovery and viability
- Are these two parameters sufficient to judge the quality of cells?

PBMC source and handling (continues…)



Handling and storage conditions may affect cytokine response to RLD



Handling and storage conditions may affect cytokine response to IIMIs

Take Home Messages

- Cytokine assay is preferred when differences in IIMIs levels are of interest
- Signature cytokines may be different for different products
- Broad cytokine panels are preferred but can be narrowed when information about signature cytokine(s) for the given RLD is available
- PBMCs and blood handling and storage conditions may influence results
- PBMCs isolated from fresh blood are preferred
- Assay validation is important and should include evaluation of signature cytokines, assay sensitivity to IIMIs, and assays logistics (blood handling procedures and conditions)


Thank you for
your attention!