SRI International

Cytokine storm potential of siRNA in human PBMCs and DCs

Toxicology & Applied Pharmacology

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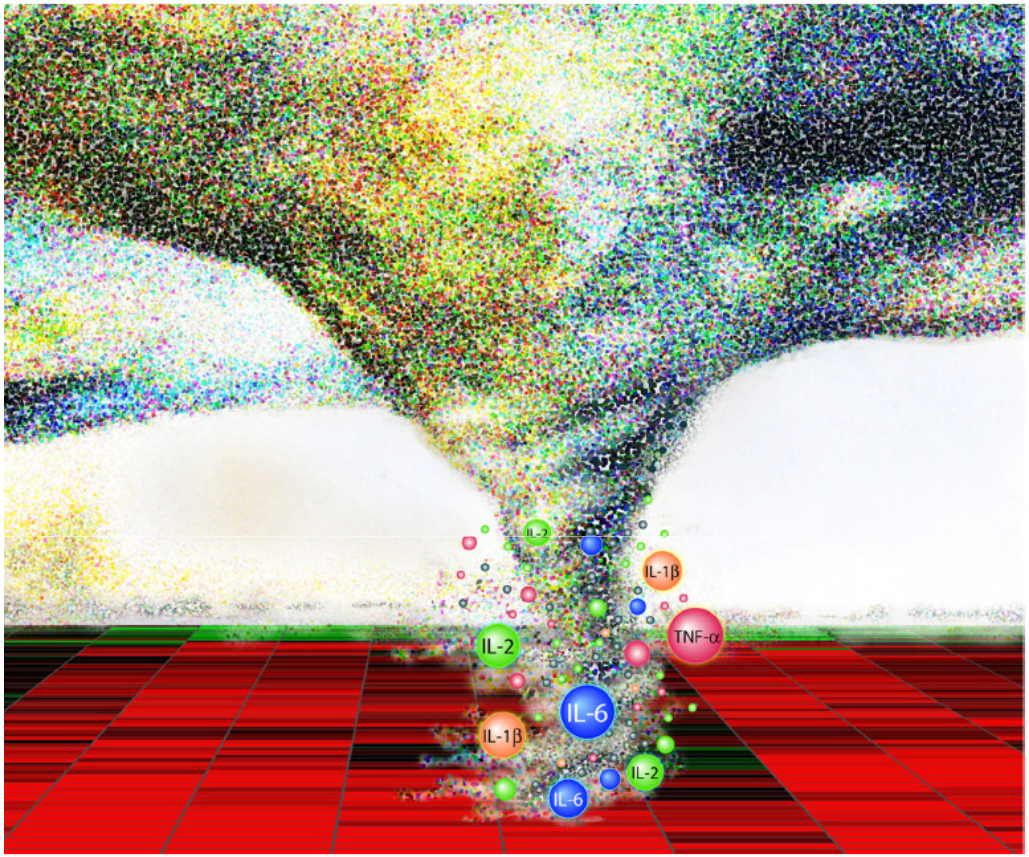
Overview

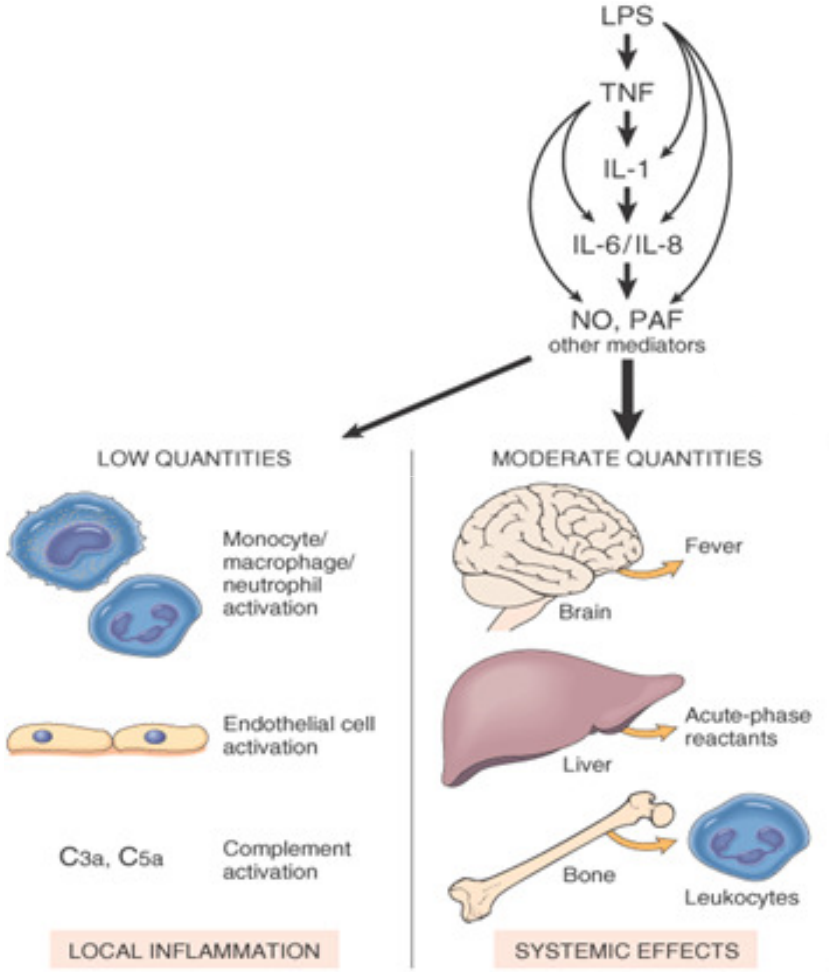
- Introduction
- Clinical Example
- Regulatory Guidance
- siRNA compounds
- Conclusions



Tisoncik, et al. *Microbiol Molec Biol Rev*. 2012. 76: 16-32.

Cytokine Storm

Normal vs Pathogenic Immune Response

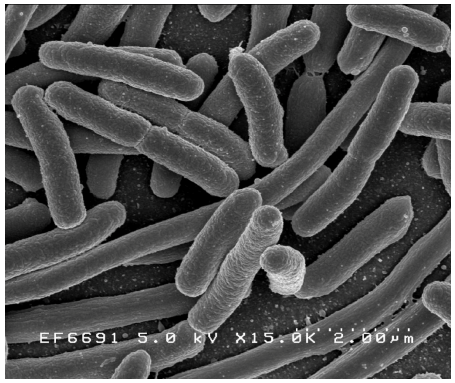


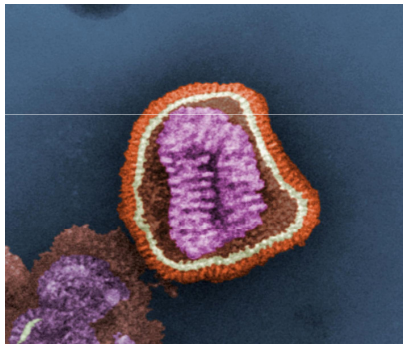
Abbas et al. Pathologic Basis of Disease. 7th ed. Elsevier. 2005.

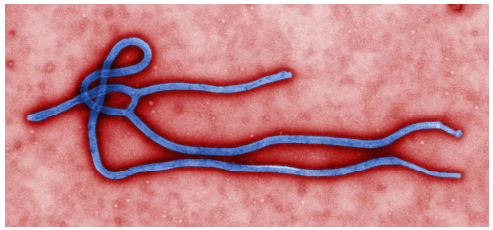
Cytokine Storm

Causes

- Infection (viral, bacterial, protozoa)
  - Bacterial sepsis
  - Influenza
  - Ebola
- Immunomodulatory agents
  - Superantigens
  - TLR adjuvants
  - mAbs



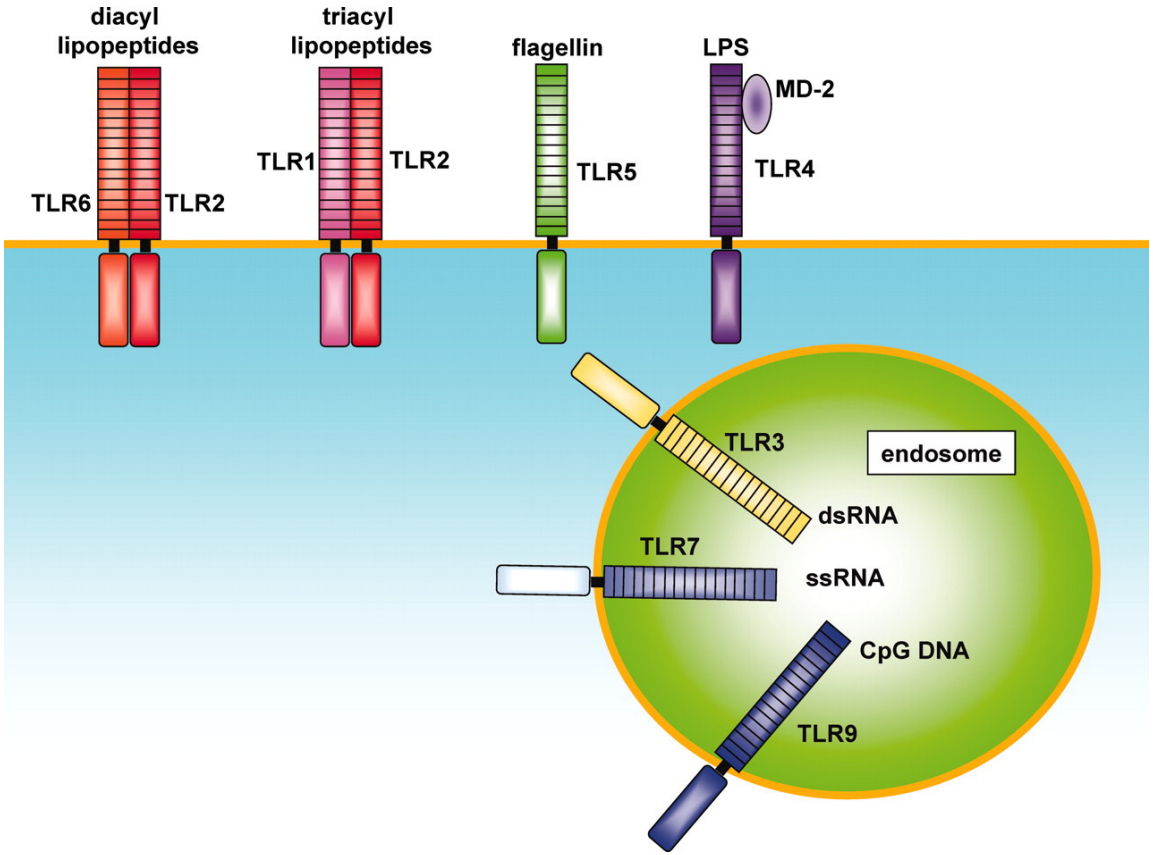




Images: Wikimedia

Cytokine Storm

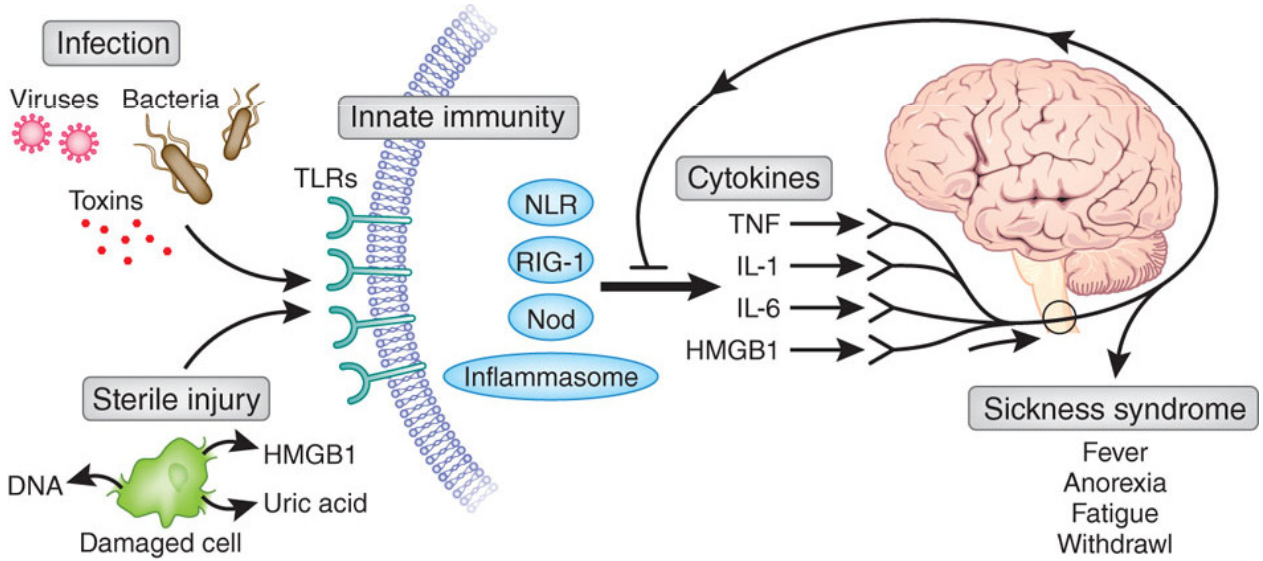
Description



Takeda K and Akira S. *Int. Immunol*. 2005. 17:1-14

Cytokine Storm
Characteristics

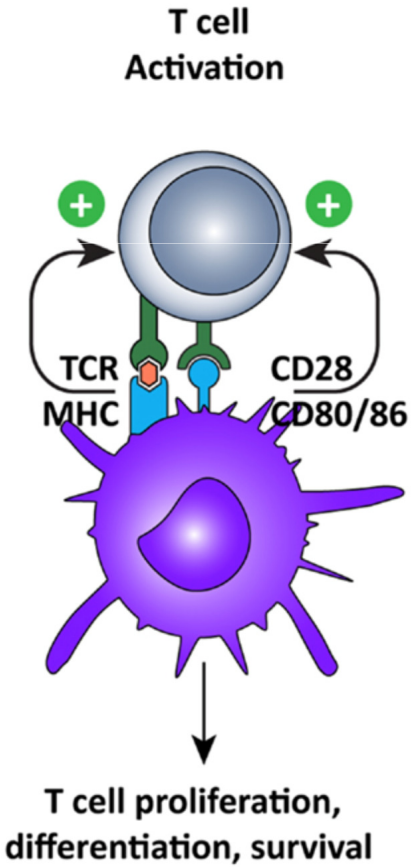
- Systemic inflammation
  - Increased endothelial permeability and capillary leakage
  - Fever
  - Hypotension and tachycardia
  - Fibrosis
- Clinical results
  - Edema
  - Hemorrhage
  - Organ damage
  - Death



Tracey. *Nature Immunol*. 2010. 11:561-564.

TGN1412
Dangers of Immunomodulators Revealed

- Monoclonal anti-CD28 Ab (superagonist)
- Intended for treatment of autoimmune disease by activating Treg



Vasaturo et al. *Front Immunol*. 2013. 4:1-14.

TGN1412
Unexpected Outcome

- Performed preclinical safety studies in cynomolgus and rhesus monkeys
  - CD28 sequence homology nearly 100%
- No toxicity observed at 50 mg/kg
- Approval granted for Phase I clinical trial at 0.1 mg/kg
- Trial began March 2006
- 8 healthy volunteers (including 2 placebo)
  - Males aged 19-34
  - TGN1412 administered by i.v. infusion
- Symptoms began within 1 hour in all 6 people
  - Initial symptoms: Headache, vomiting, erythema
  - Progressive symptoms: Fever (39.5 to 40° C), hypotension, tachycardia, respiratory failure, multiple organ failure
  - 4 to 21 days in ICU
  - Increased cancer risk

TGN1412
Lessons learned

- Effector memory T cells demonstrated in vitro to play an important role
  - Cynomolgus and Rhesus monkey effector memory T cells do not express CD28
- In vitro cytokine release from PBMC required immobilized TGN1412
- Need to measure multiple cytokines because in vitro response may not be identical to in vivo
- Small increases in cytokines should not be dismissed
- **More cautious approach to clinical trials when potential for cytokine storm exists**

Cytokine storm

Regulatory guidance

Guidance for Industry

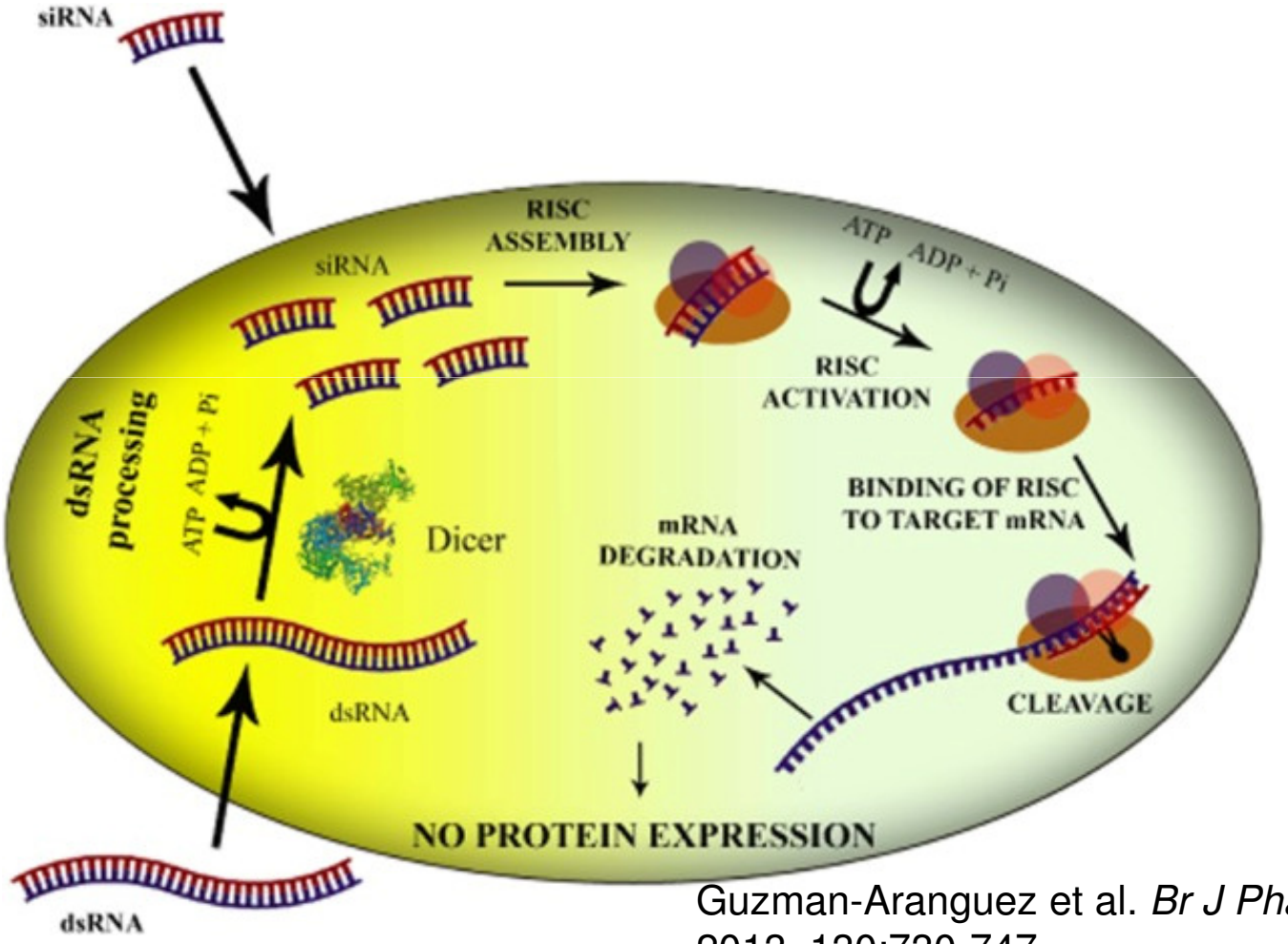
Immunogenicity Assessment for Therapeutic Protein Products

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

August 2014
Clinical/Medical

Cytokine storm
Regulatory guidance

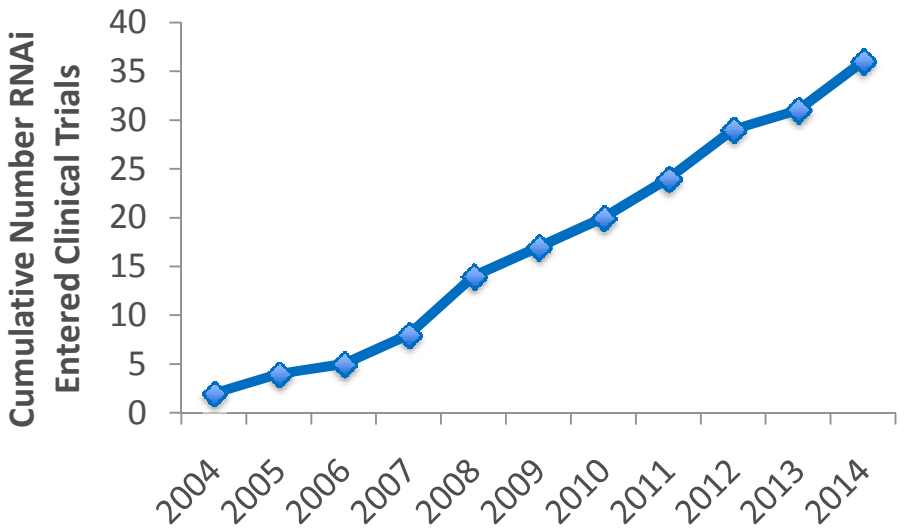
- When risk of cytokine storm, start with lower dose than traditional calculations and infuse more slowly
- Animal studies useful only if compound is pharmacologically active
- Test a broad panel of cytokines
  - Minimally IL-2, IL-6, TNF-α and IFN-γ
- In vitro studies with human whole blood or PBMC
  - Cytokines, proliferation or other signs of activation
  - Signs of activation should indicate potential for cytokine storm even if no indication in preclinical studies



Guzman-Aranguez et al. *Br J Pharmacol*. 2013. 130:730-747.

siRNA Therapeutics
Background

- Interest in RNAi therapeutics has grown significantly in the past decade
- None have made it to market
- A few studies have been terminated

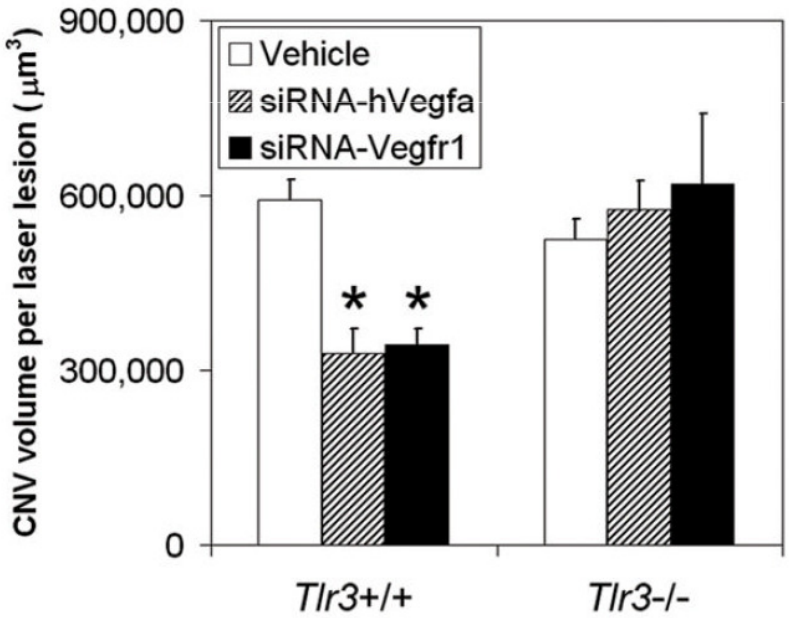


Data source: clinicaltrials.gov

siRNA Therapeutics
Lesson learned

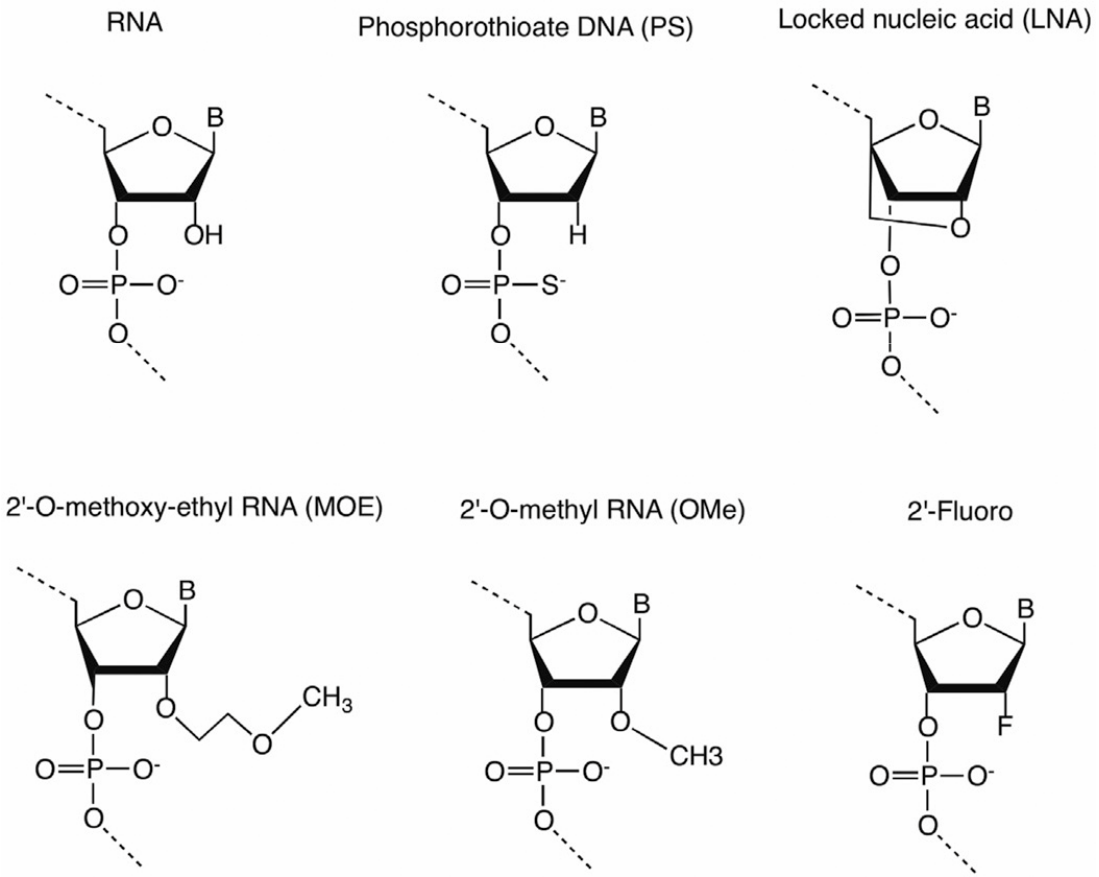
- AGN211745 (Allergan)
  - siRNA specific for VEGF for treatment of age-related macular degeneration
  - Reached Phase II trial before termination
- Anti-angiogenic effect not sequence specific
  - TLR3
  - IFN-γ and IL-12

Kleinman et al. *Nature*. 2008. 452:591-597



siRNA Therapeutics
Modifications

- Modifications increase stability and reduce immunogenicity



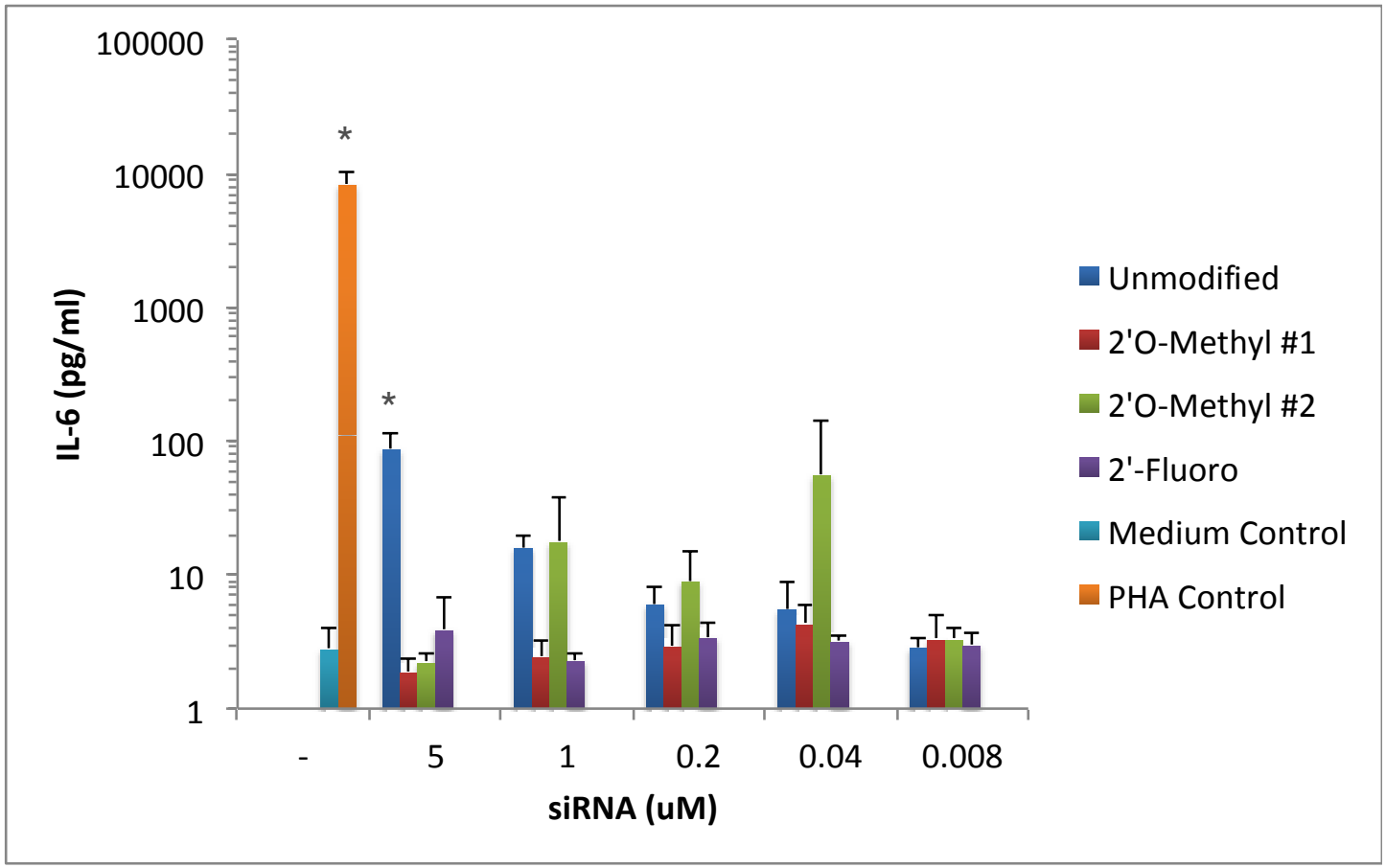
Burnett and Rossi. *Chem Biol*. 2012. 19: 60–71.

siRNA Evaluation
Methods

- Topical anti-viral siRNAs
  - Unmodified
  - 2'-O-Methyl
  - 2'-Fluoro
- In vitro PBMC assay
  - Ficoll separation of PBMC isolated from healthy donors (n=3)
  - Incubation for 24 hr with siRNAs or controls
  - Cytokine panel measured by Luminex
- In vitro DC assay
  - CD14+ monocytes isolated from PBMC
  - Incubation for 5 days with IL-4 and GM-CSF to generate immature DCs
  - Incubation and cytokine analysis as above

siRNA Evaluation

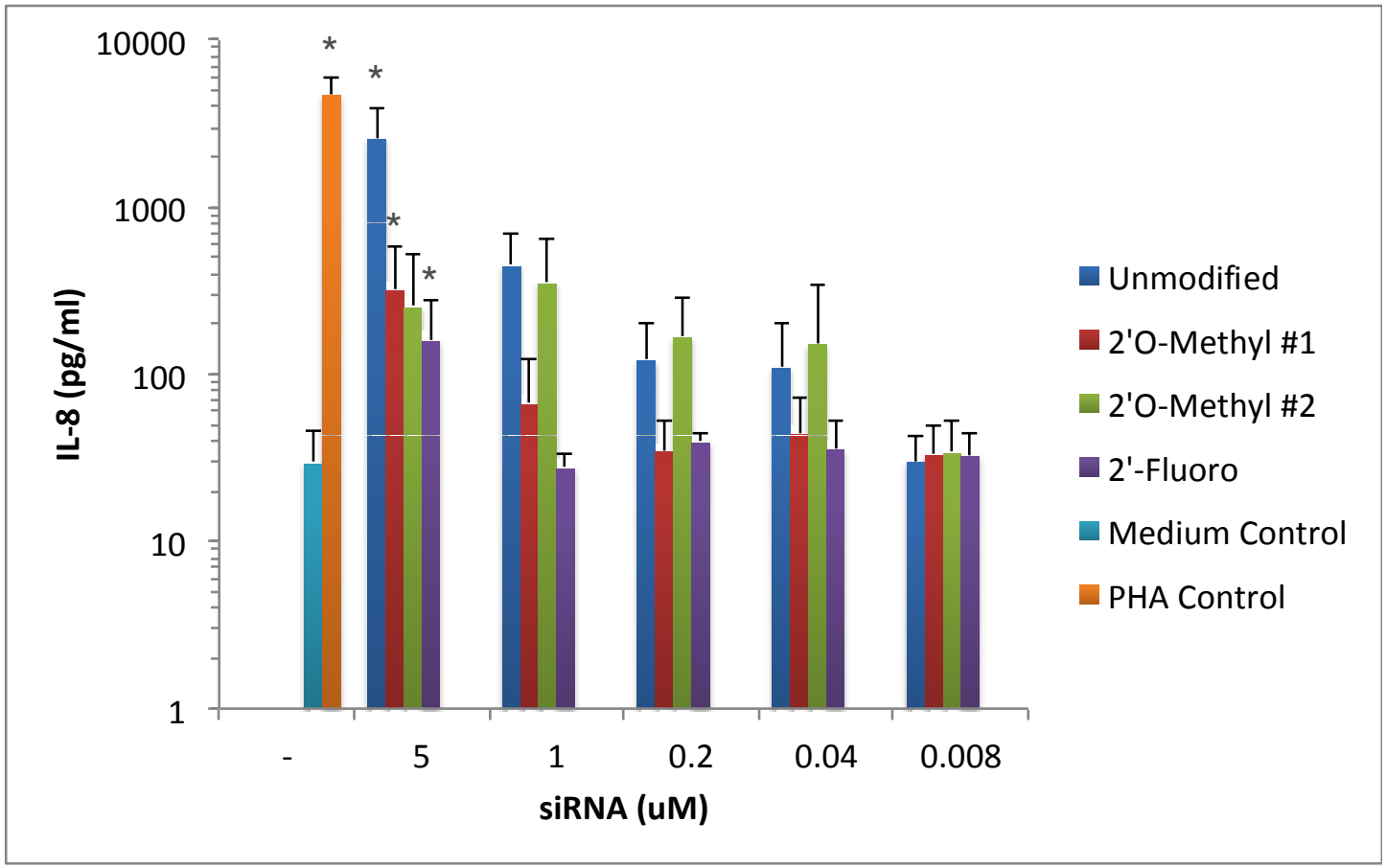
Results



*Denotes statistically significant increase compared to medium control ($p<0.05$)

siRNA Evaluation

Results



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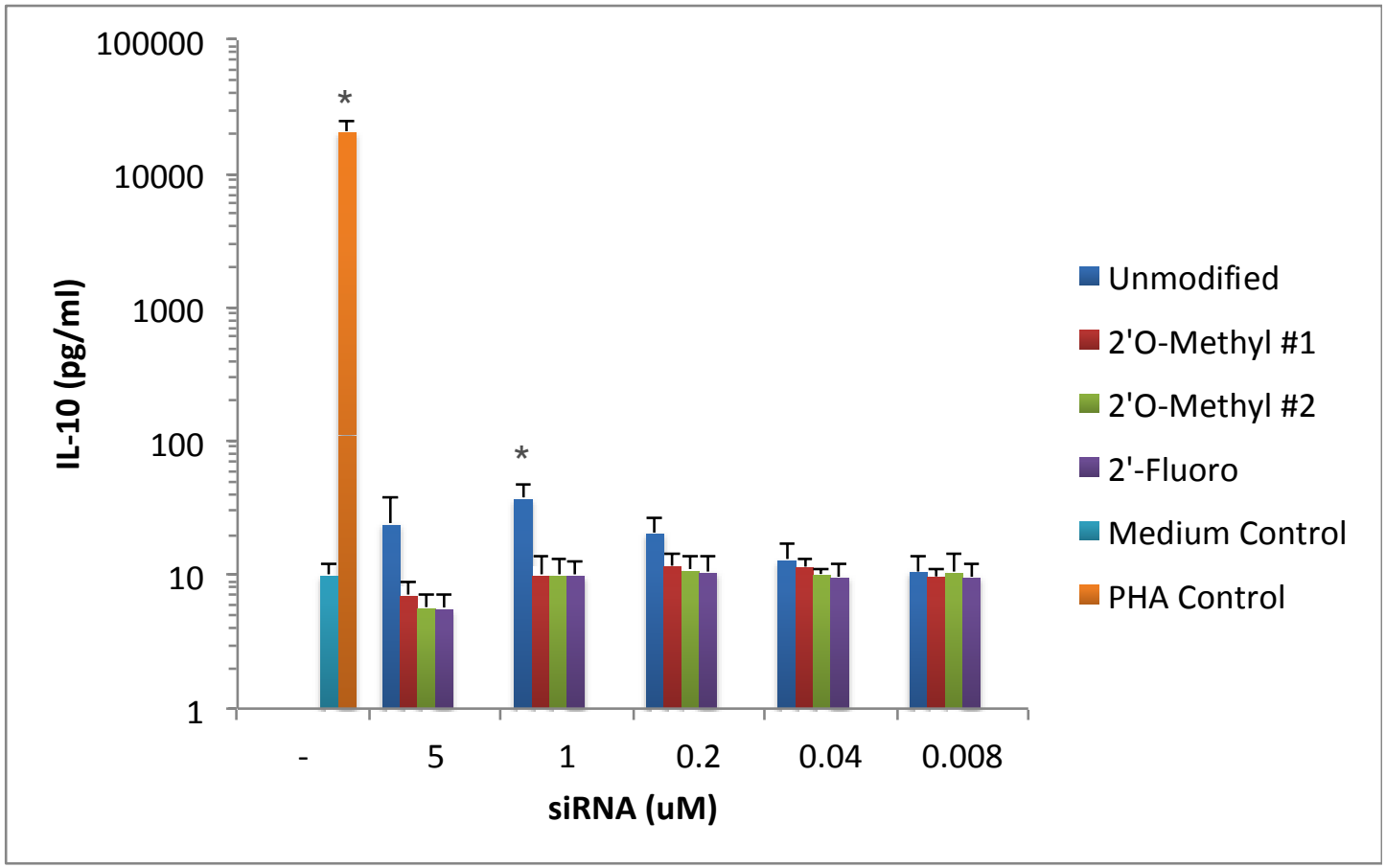
siRNA Evaluation

Results



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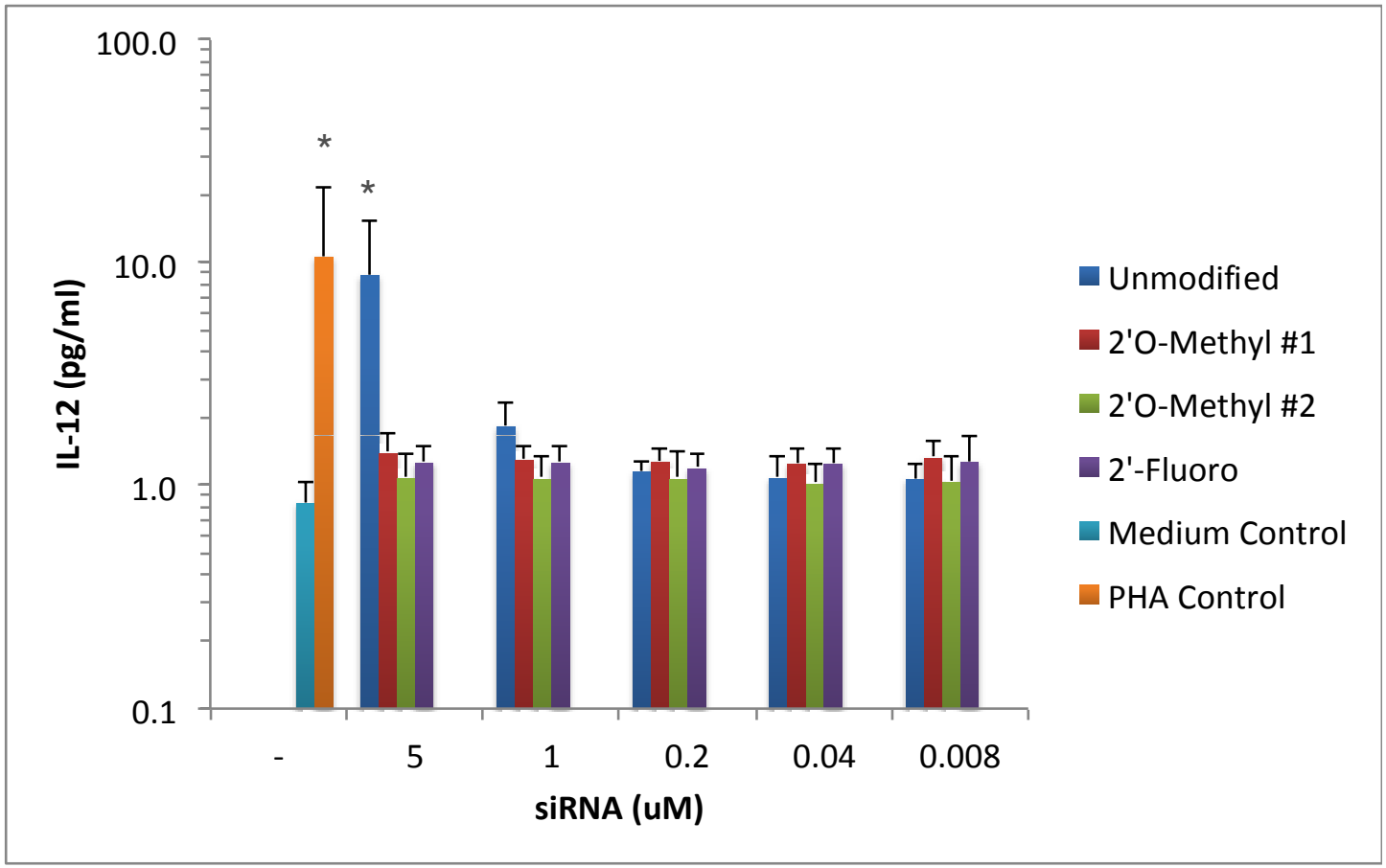
siRNA Evaluation

Results



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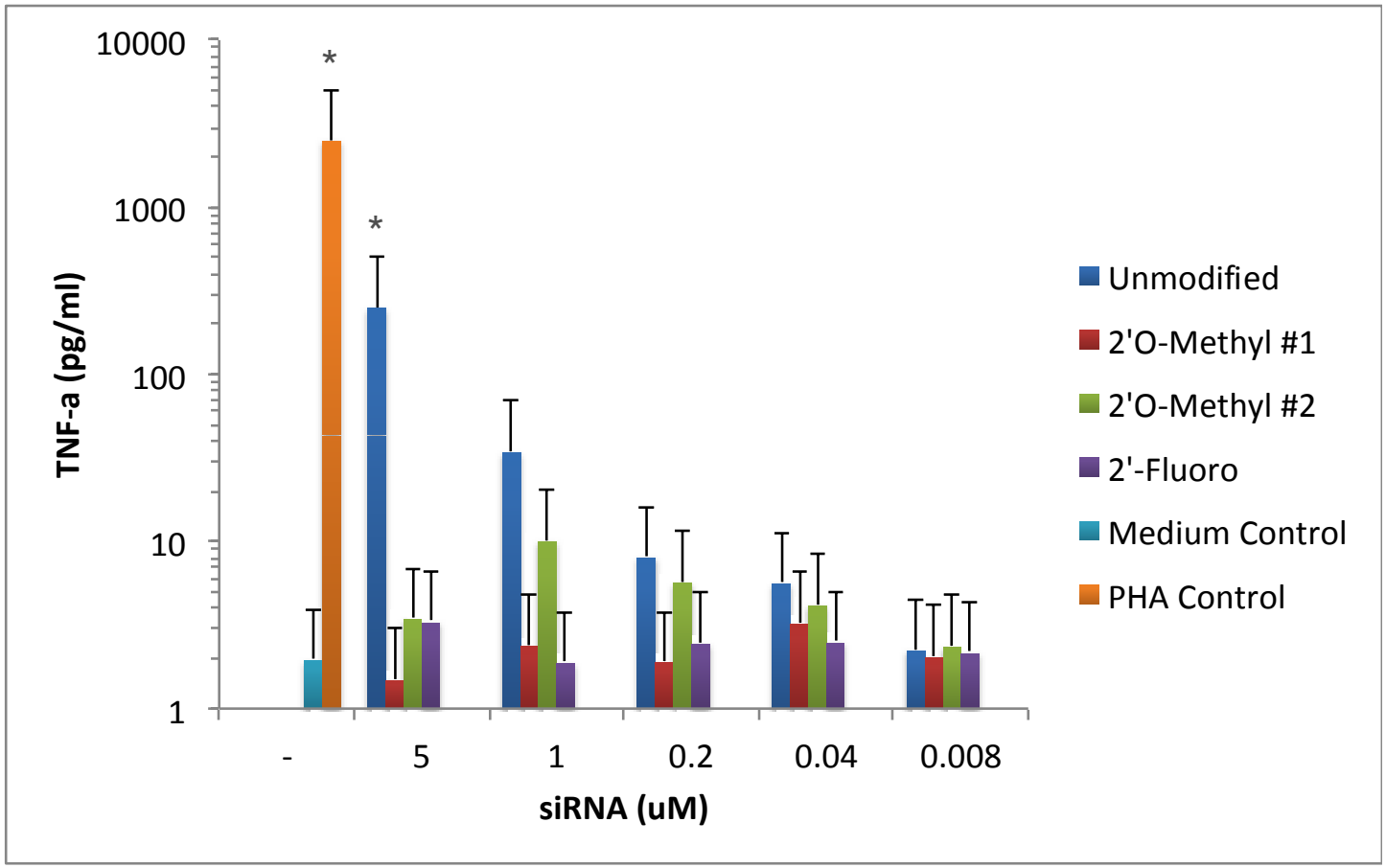
siRNA Evaluation

Results



*Denotes statistically significant increase compared to medium control ($p<0.05$)

siRNA Evaluation
Cytokines (PBMC)

Cytokine(s)	Unmodified siRNA	2'O-Methyl (#1)	2'O-Methyl (#2)	2'-Fluoro
IL-6				
IL-8				
IL-10				
IL-12				
TNF-α				
IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-13, GM-CSF				

siRNA Evaluation
Cytokines (DC)

Cytokine(s)	Unmodified siRNA	2'O-Methyl (#1)	2'O-Methyl (#2)	2'-Fluoro
IL-6				
IL-8				
IL-10				
IL-12				
TNF-α				
IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-13, GM-CSF				

Cytokine Storm
Conclusions and Considerations

- Unmodified siRNA induced several cytokines in human PBMC
  - IL-6, IL-8, IL-10, IL-12, TNF-α
- Cytokine profile improved with siRNA modification
- No cytokines in monocyte-derived DC
  - IFN-α not part of panel
  - TLR3 expression also found on subsets of T, B, and NK cells
- Considerations
  - Larger number of donors to increase statistical power
  - PBMC vs whole blood
  - Cellular composition of blood differs from tissues

Acknowledgements

Jon Mirsalis, PhD

Nahoko Dunlap

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