InterLACE Slide Presentation Instructions

*Presenting at Nova Southeastern University's **InterLACE Research Showcase** allows NSU faculty, clinicians, students, and research staff to present their interdisciplinary and/or interprofessional collaborative research in an oral presentation format.*

Instructions:

- InterLACE slide presentations will be a **maximum of 15 minutes** with additional time for questions
- Presenters typically utilize a PowerPoint (PPT) slideshow to organize their presentation, and presenters are encouraged to use the "InterLACE Slide Presentation Template" located in the **Materials & Guides** dropdown here.
- For tips and advice on conducting the oral presentation to accompany your slides, please consult the "InterLACE Slide Presentation Guide" located in the **Materials & Guides** dropdown here.

InterLACE 2024

InterLACE Slide Presentation Instructions

Presenters should consider including the following main sections (example slide # indicated below):

- Title slide (Slide 1)
- Description of the interdisciplinary/interprofessional research team (Slide 2)
- Introduction/background (Slides 3-7)
- Methods (Slides 8-9)
- Results (Slides 10-12)
- Conclusions/future goals (Slides 13-15)
- Acknowledgements (Slide 16)
- References (Slide 17)

Presenters can add/remove sections as they see fit, or reorganize this example presentation order, as long as the presentation does not exceed a maximum of 15 minutes (a good plan for a 15-minute presentation is 1 minute per slide, 15 content slides max.)

InterLACE 2024

NSU Florida

InterLACE 2024

Outcompeting Cancer's "Don't Eat Me" Signal to Promote Immune Clearance

Jordan Merritt, Ph.D.[1]; Lisa Alvarez, M.D.[2]; & Sandra Joe, Ph.D.[3]

[1]Division of Research & Economic Development, [2]College of Medicine, [3]College of Science

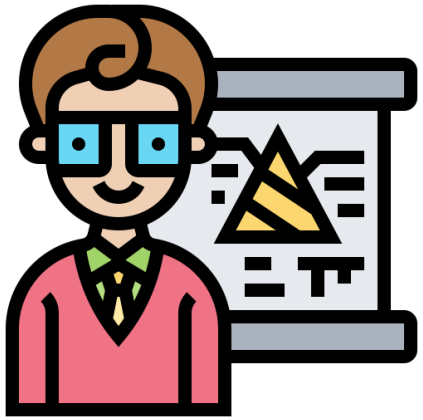
Nova Southeastern University

Research Team

- The ***interdisciplinary & interprofessional*** research team included a diverse set of experts (students, lab techs, clinicians, and research scientists) from multiple departments in the College of Science and Medicine, with skills in:
  - Biochemistry
  - Medicine
  - Morphology
  - Biology

Dr. Sandra Joe
Biochemistry

Dr. Lisa Alvarez
Medicine & morphology

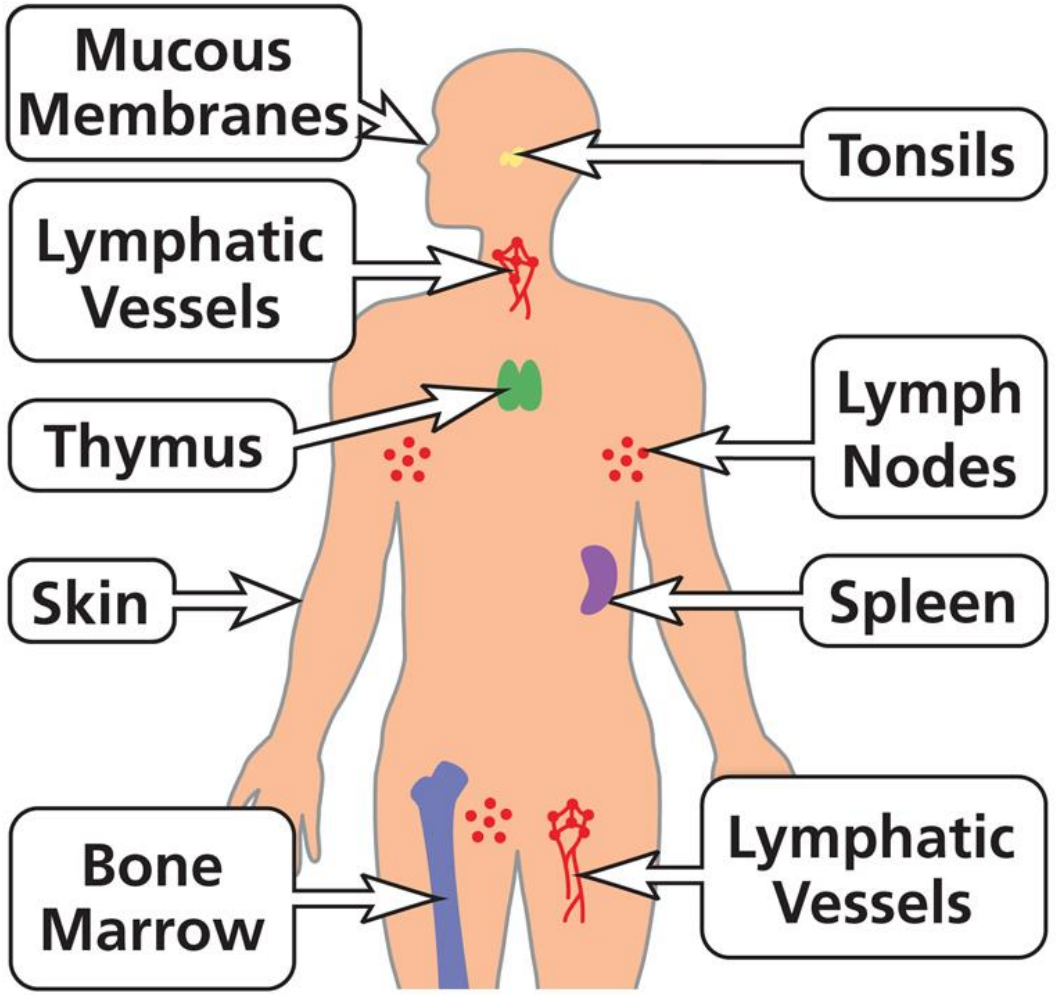


Dr. Jordan Merritt
Biology

InterLACE 2024

The Immune System

- Function: to prevent and eliminate infection
- Subsystems:
  - Physical barriers
    - Skin, mucus membranes
  - Innate immune system
    - Non-specific
    - 1st line of defense
  - Adaptive immune system
    - Specific, learned
    - Immune memory



[Immune System]. Clinical Info NIH: HIV/AIDS Glossary. https://clinicalinfo.hiv.gov/en/glossary/immune-system

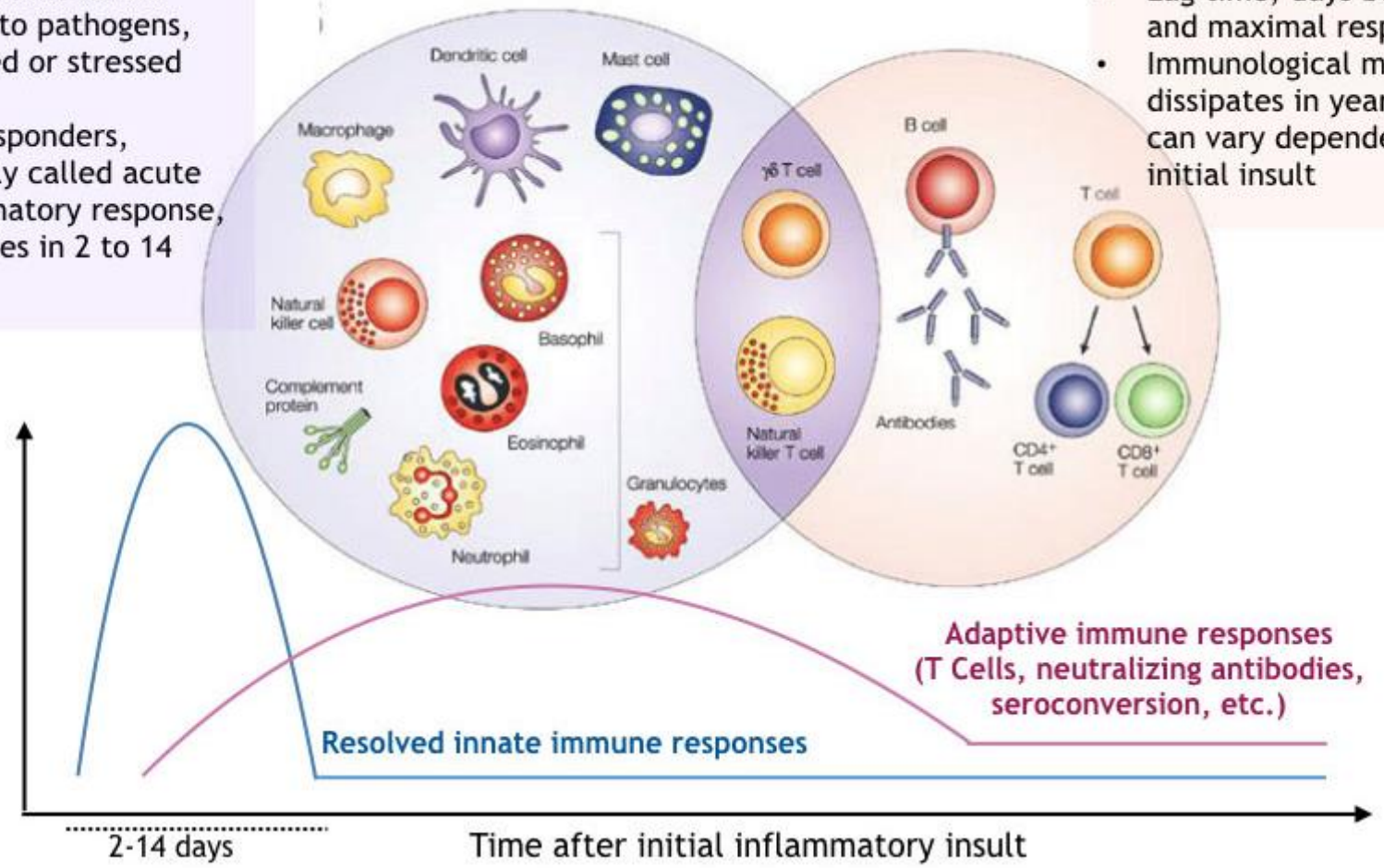
The Immune Response

Innate

- Non-Specific alarm system to pathogens, damaged or stressed cells
- First responders, clinically called acute inflammatory response, dissipates in 2 to 14 days

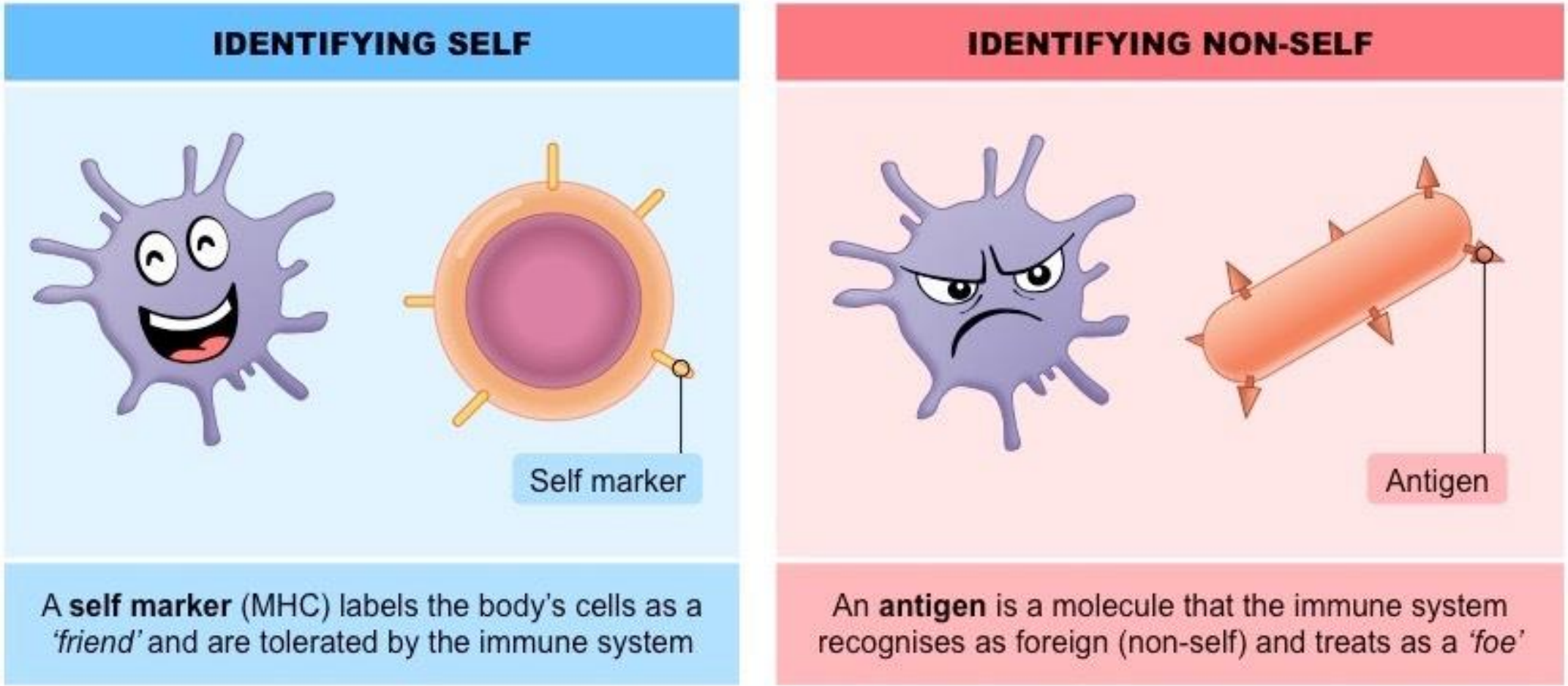
Adaptive

- Lag time, days between exposure and maximal response
- Immunological memory, response dissipates in years not days, and can vary dependent upon the initial insult



[Immune Response]. Biotech Support Group. https://www.biotechsupportgroup.com/re-imagining-proteomics-for-developing-precision-medicine-b-s/319.htm

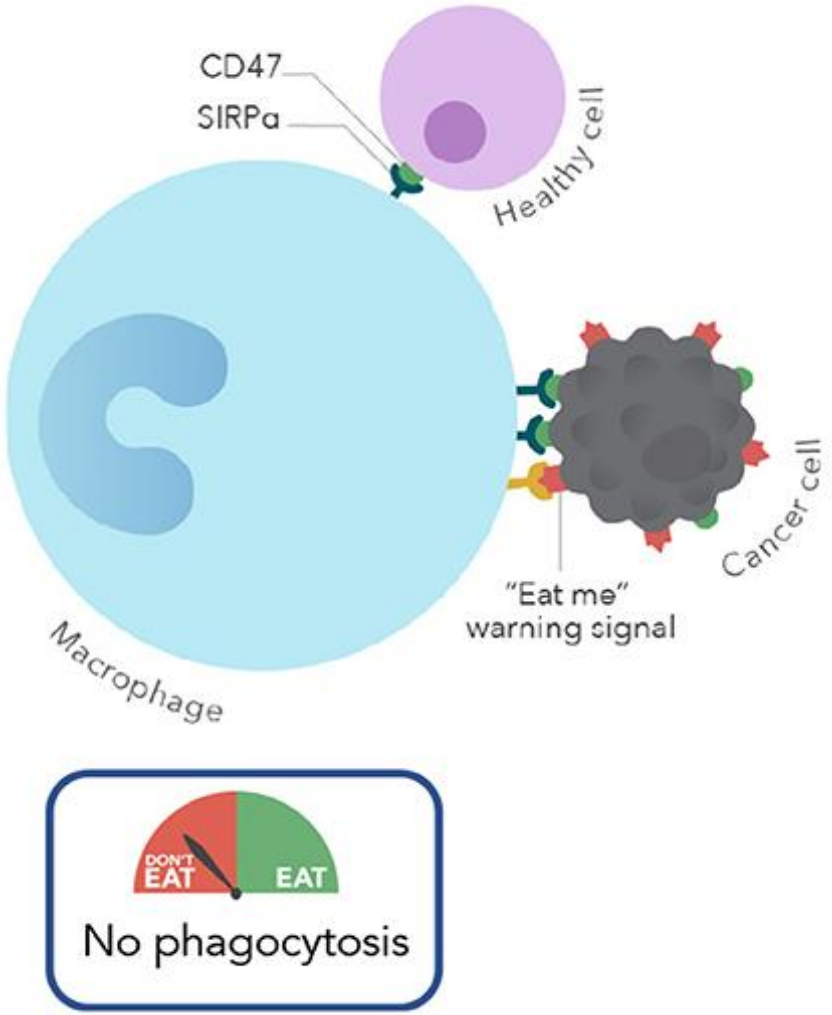
Foreign Antigen vs Self Antigen



[Self vs Non-Self]. BioNinja. https://ib.bioninja.com.au/higher-level/topic-11-animal-physiology/111-antibody-production-and/self-versus-non-self.html

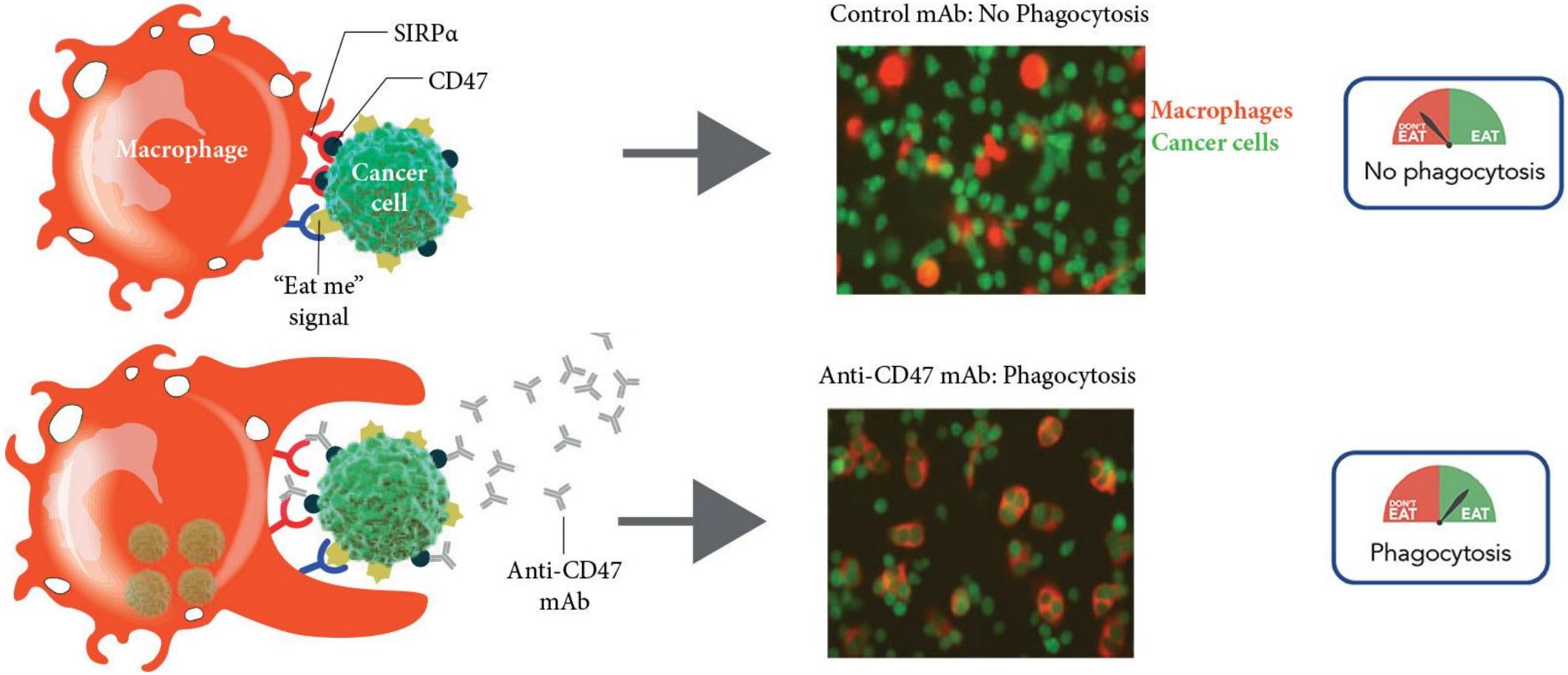
Self Receptor Signaling

- Phagocyte “Don’t eat me” signals
  - CD47 on healthy cell
  - Signal-regulatory protein alpha (SIRPα) on phagocyte
- Strong signaling
  - Overriding
  - Exploited by cancer cells
    - Abnormal self-cell growth
    - Hijacks the cell machinery: upregulate CD47 on the cell’s surface
    - Hides from immune clearance



Chao, M.P. et al. (2020). Therapeutic Targeting of the Macrophage Immune Checkpoint CD47 in Myeloid Malignancies. *Frontiers in Oncology*. *22*(January 2020). https://doi.org/10.3389/fonc.2019.01380

Targeting the “Don’t Eat Me” Signal



Can we outcompete the CD47-SIRPα interaction using soluble reagents?

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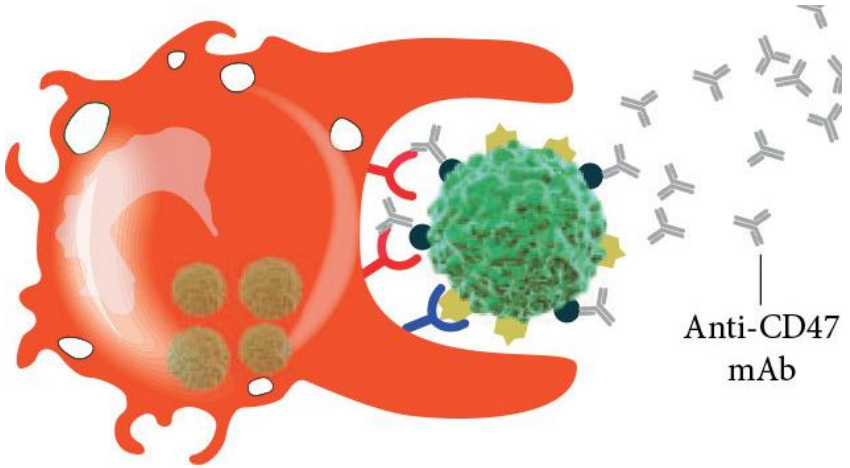
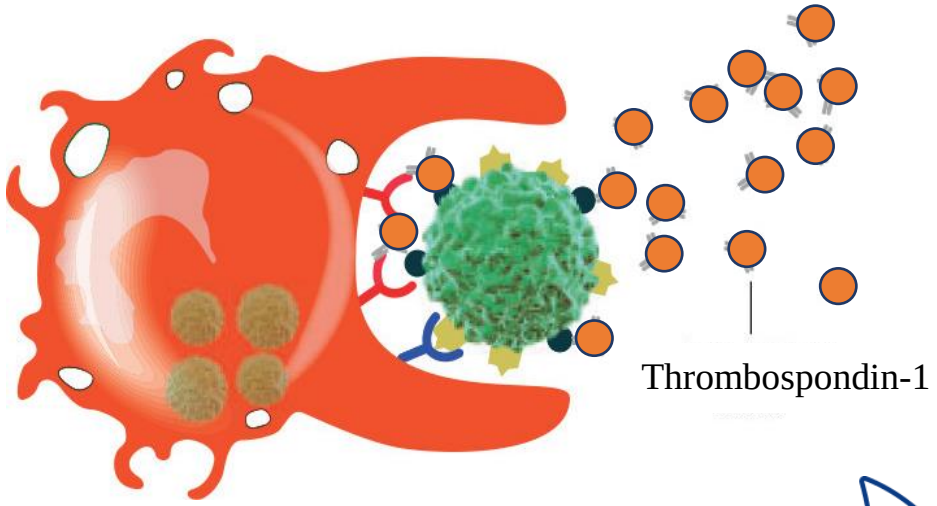
Takimoto, C.H. et al. (2019). The Macrophage “Do not eat me” Signal, CD47, is a Clinically Validated Cancer Immunotherapy Target. *Annals of Oncology. 30*(3). 486-489. https://doi.org/10.1093/annonc/mdz006

Methodology: Experimental Groups

- Interact innate immune cells (THP-1) with breast cancer cells (MCF-7)
  1. Differentiate THP-1 monocyte to M0 macrophage with PMA
  2. Label THP-1 macrophage with anti-CD14-PE monoclonal antibody
  3. Label MCF-7 cells with CellTracker Deep Red
  4. Co-culture cells together
- Experimental Groups
  1. THP-1 M0 macrophage
  2. THP-1/MCF-7 co-culture
  3. THP-1/MCF-7 co-culture + anti-CD47 monoclonal antibodies
     - Block CD47-SIRPα interaction
  4. THP-1/MCF-7 co-culture + Thrombospondin-1
     - Outcompete CD47-SIRPα interaction with known CD47 ligand





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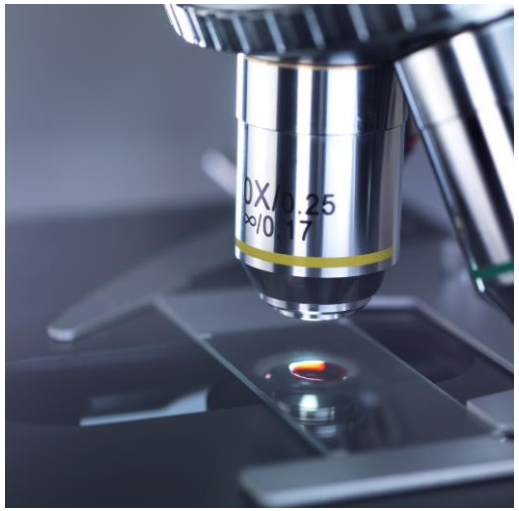
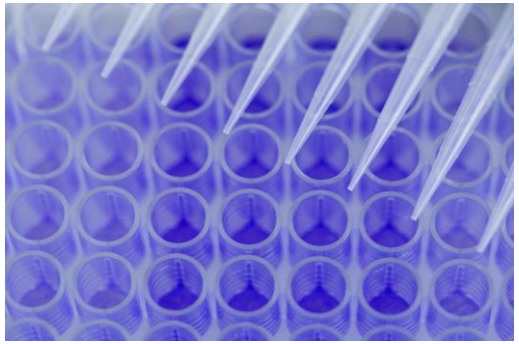
Methodology: Immune Response Assays

- Visualize immune response
  - Confocal Microscope to detect fluorescent labels
  - Looking for co-localization of fluorescent signal and/or internalization of MCF-7 via z-stack
- Quantify and characterize immune response
  - Enzyme Linked Immunosorbent Assay (ELISA) to detect immune byproducts created
  - Quantify production of pro-inflammatory cytokine TNFα





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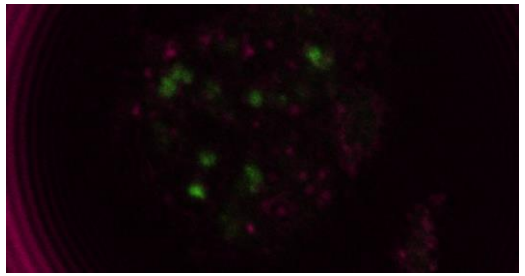
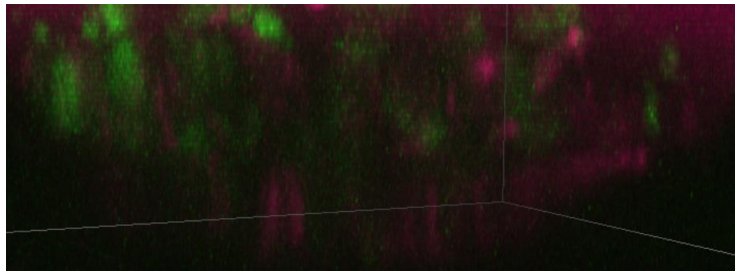
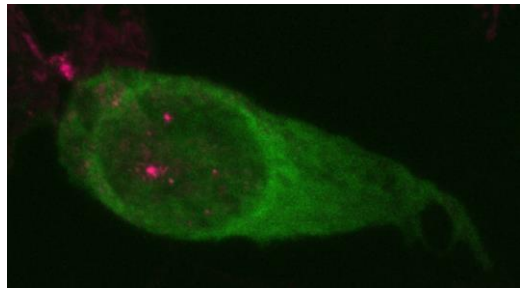
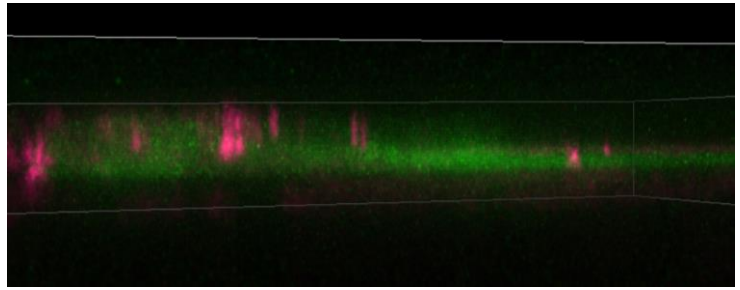
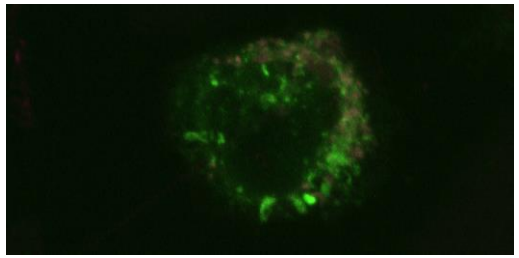
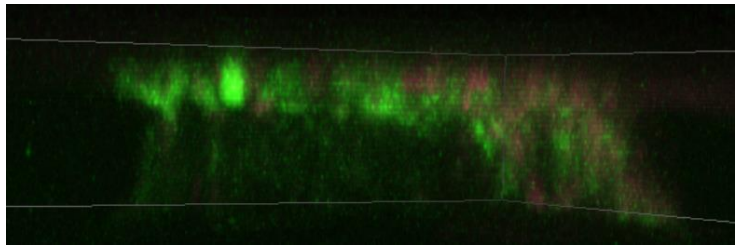
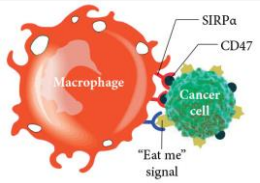
Results: Confocal Microscopy

	Top View	Side View
THP-1/MCF-7 Co-culture (Control)		
THP-1/MCF-7 Co-culture + Anti-CD47 antibody		
THP-1/MCF-7 Co-culture + Thrombospondin-1		

Results: Fluorescent Image Analysis

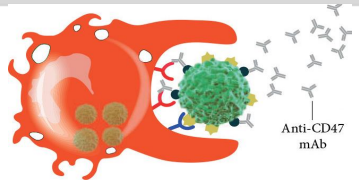
THP-1/MCF-7 coculture

- Rounded THP-1 macrophage
- Disbursed red dye
- MCF-7 cells not clearly inside-outside macrophage



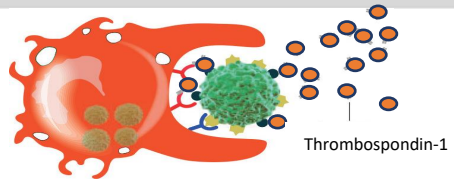
THP-1/MCF-7 coculture + anti-CD47

- Flat THP-1 macrophage
- Clearly labeled MCF-7 cells
- MCF-7 cells appear to be internalized



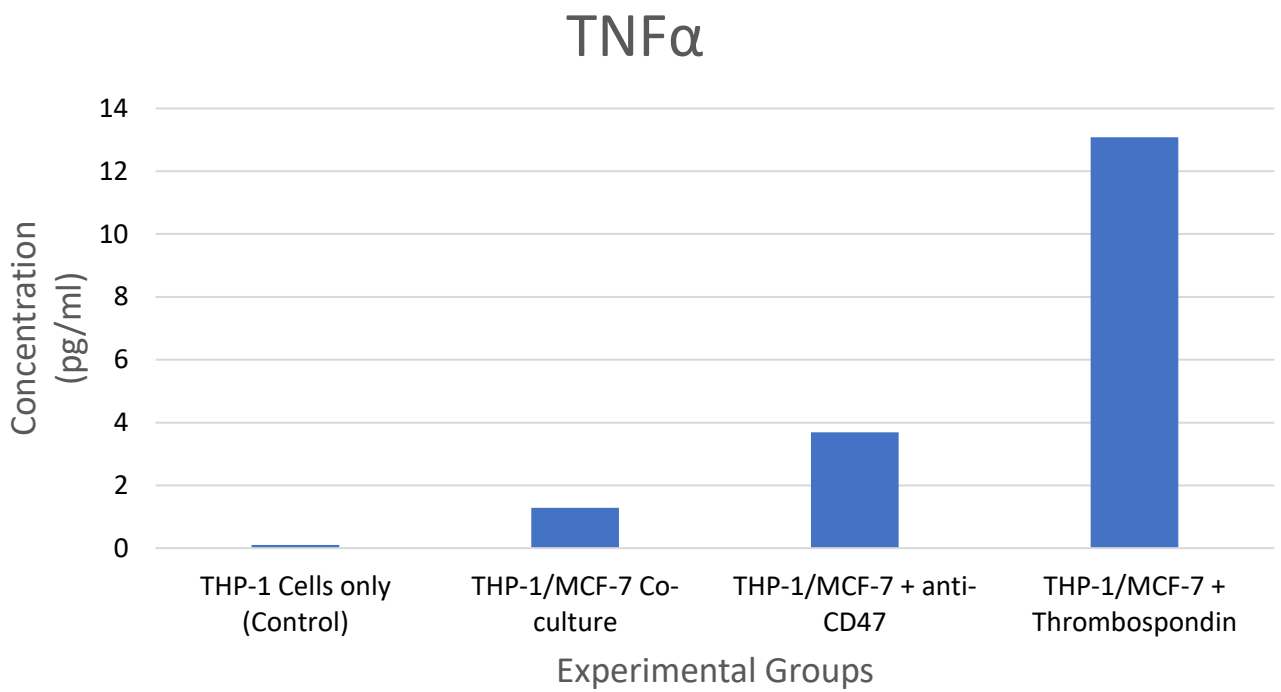
THP-1/MCF-7 coculture + Thrombospondin-1

- Rounded THP-1 macrophage
- Disbursed red dye
- MCF-7 cells not clearly inside-outside macrophage



Takimoto, C.H. et al. (2019). The Macrophage "Do not eat me" Signal, CD47, is a Clinically Validated Cancer Immunotherapy Target. *Annals of Oncology*. *30*(3). 486-489. https://doi.org/10.1093/annonc/mdz006

Results: ELISA for TNFα

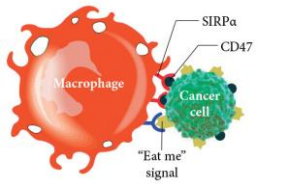


TNFα	Concentration (pg/ml)	Absorbance (OD)
THP-1 Cells only (Control)	0.10465	0.0951
THP-1/MCF-7 Co-culture	1.289645	0.1152
THP-1/MCF-7 + anti-CD47	3.687839	0.1440
THP-1/MCF-7 + Thrombospondin	13.07613	0.2835
within range of 0pg/ml		

Conclusion: Summary

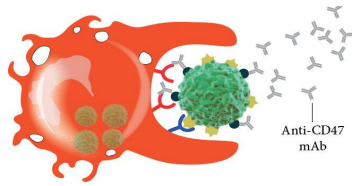
THP-1/MCF-7 coculture

- MCF-7 cells not clearly inside-outside macrophage
- Slight increase in TNFα production



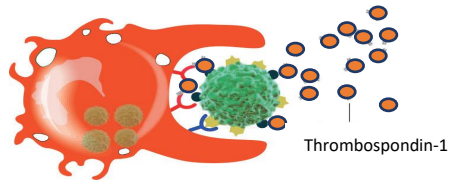
THP-1/MCF-7 coculture + anti-CD47

- MCF-7 cells appear to be internalized
- Moderate increase in TNFα production



THP-1/MCF-7 coculture + Thrombospondin-1

- MCF-7 cells not clearly inside-outside macrophage
- Large increase in TNFα production



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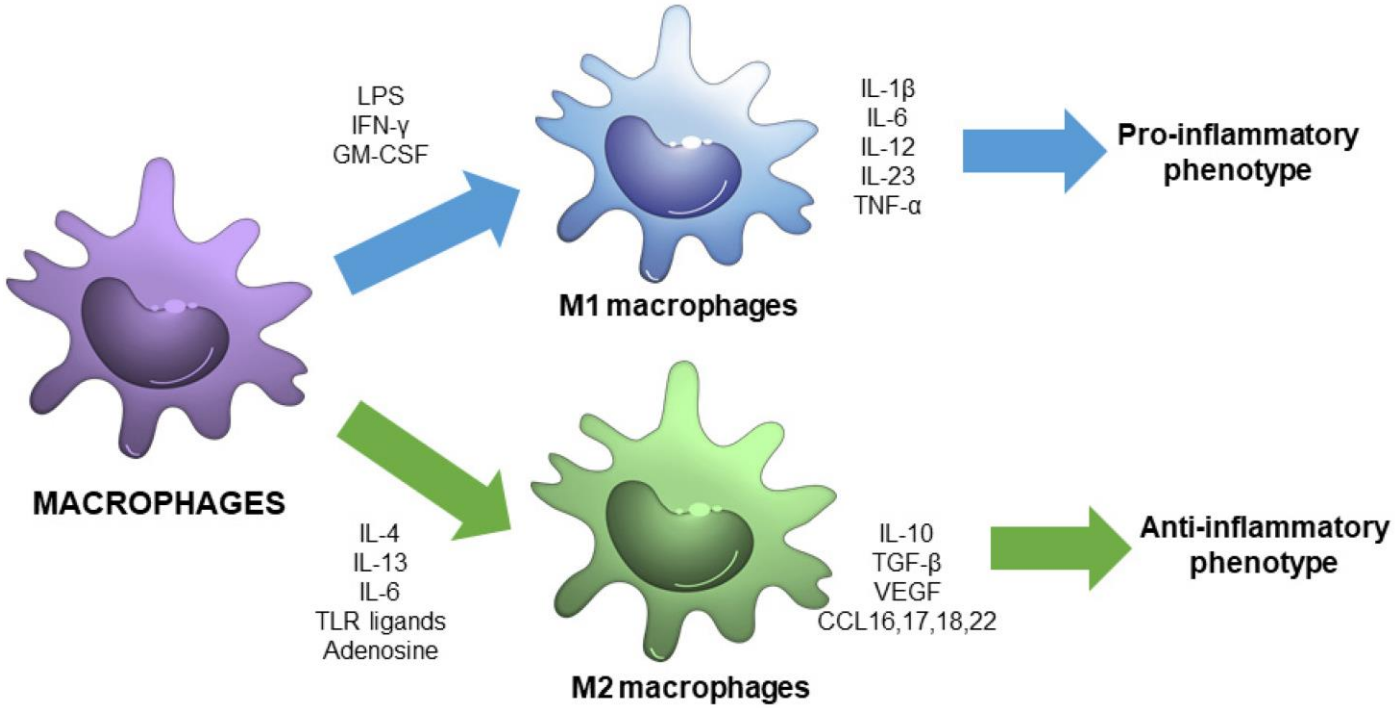
Conclusion: Reflections

- Further optimization is needed to determine if soluble reagents can outcompete the CD47-SIRPα interaction
- **Areas to improve**
  - Optimize co-incubation time
  - Utilize whole cell label instead of surface molecule label (THP-1 cells)
  - Use less culture media to condense immune byproducts for ELISA analysis

Conclusion: Future Studies

- **Future Studies**
  - Try other known CD47 ligands (Integrin αVβ3)
  - Use activated THP-1 cells (M1 and M2)



Perez, S., Rius-Pere, S. (2022). Macrophage Polarization and Reprogramming in cute Inflammation: A Redox Perspective. *Antioxidants. 11*(7). 1394. https://doi.org/10.3390/antiox11071394

Acknowledgements

Thank you….

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- Heather Butler, Graduate Teaching Assistant
- FAU Wilkes Honors College
- FAU Jupiter Life Science Initiative
- NSU Division of Research and Economic Development

References

1. [Immune System]. Clinical Info NIH: HIV/AIDS Glossary. https://clinicalinfo.hiv.gov/en/glossary/immune-system
2. Overview of the Immune System. (2013). National Institute of Allergy and Infectious Diseases. https://www.niaid.nih.gov/research/immune-system-overview
3. Parham, P. (2009) Elements of the immune system and their roles in defense. The Immune System. 3rd edition. Chapter 1. Garland Science.
4. [Immune Response]. Biotech Support Group. https://www.biotechsupportgroup.com/re-imagining-proteomics-for-developing-precision-medicine-b-s/319.htm
5. Timonina, I. Phagocytosis [Online Image]. Shutterstock. https://www.shutterstock.com/image-vector/innate-immunity-adaptive-specific-phagocytosis-infographics-566823208
6. [Self vs Non-Self]. BioNinja. https://ib.bioninja.com.au/higher-level/topic-11-animal-physiology/111-antibody-production-and/self-versus-non-self.html
7. Chao, M.P., Takimoto, C.H., Feng, D.D., McKenna, K., Gip, P., Liu, J., Volkmer, J., Weissman, I.L., Majeti, R. (2020). Therapeutic Targeting of the Macrophage Immune Checkpoint CD47 in Myeloid Malignancies. *Frontiers in Oncology*. *22*(January 2020). https://doi.org/10.3389/fonc.2019.01380
8. Takimoto, C.H., Chao, M.P., Gibbs, C., McCamish, M.A., Liu, J., Chen, J.Y., Majeti, R., Weissman, I.L. (2019). The Macrophage “Do not eat me” Signal, CD47, is a Clinically Validated Cancer Immunotherapy Target. *Annals of Oncology*. *30*(3). 486-489. https://doi.org/10.1093/annonc/mdz006
9. Perez, S., Rius-Pere, S. (2022). Macrophage Polarization and Reprogramming in cute Inflammation: A Redox Perspective. *Antioxidants*. *11*(7). 1394. https://doi.org/10.3390/antiox11071394