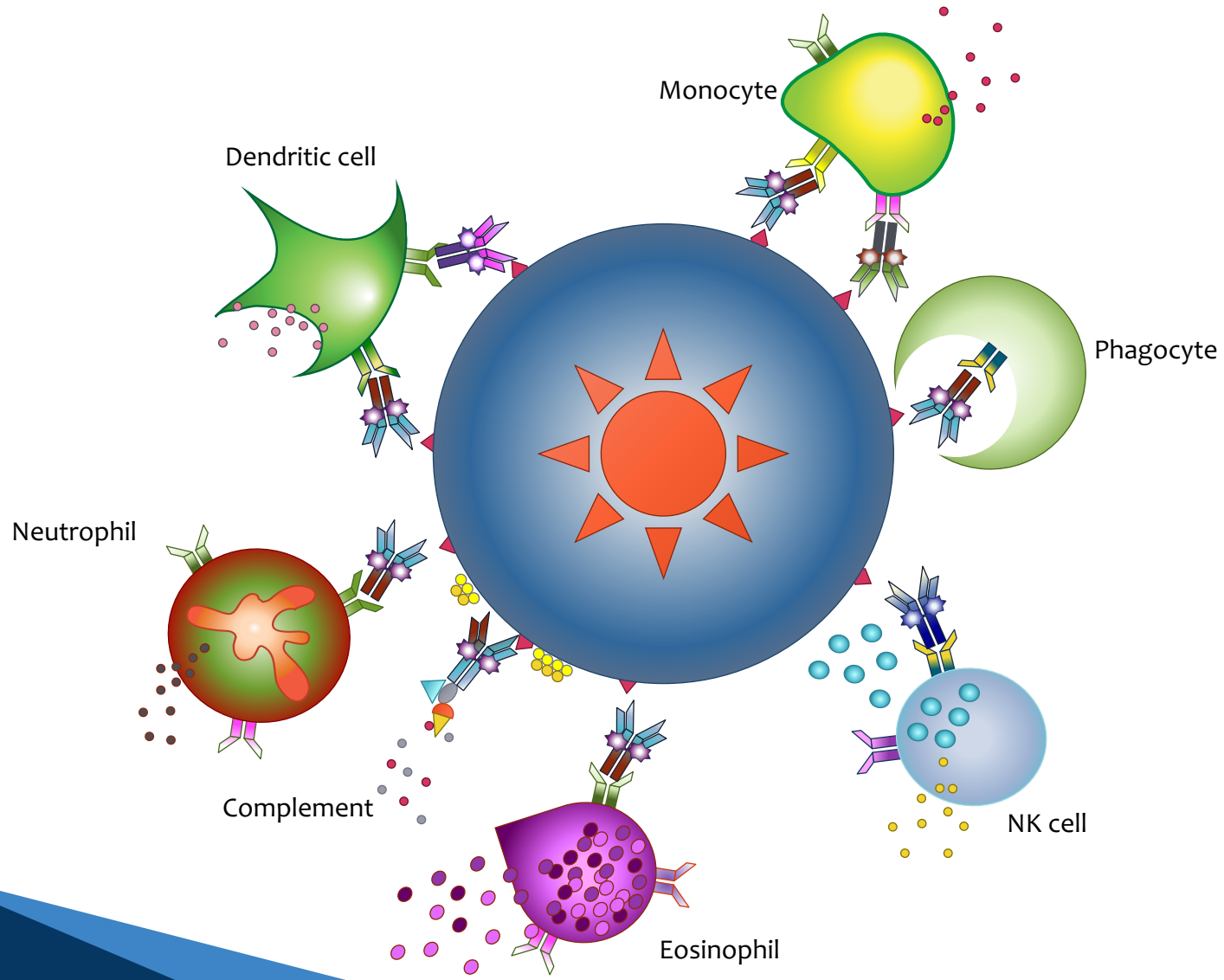
Strategies for Mitigating the Unpredictability of Fc-mediated Functions in Antibody Development

Lenny Moise, PhD
VP, Research
SeromYx Systems

Antibody Society Webinar
09 November 2023

SeromYx SYSTEMS

Antibodies mediate an array of functions beyond binding



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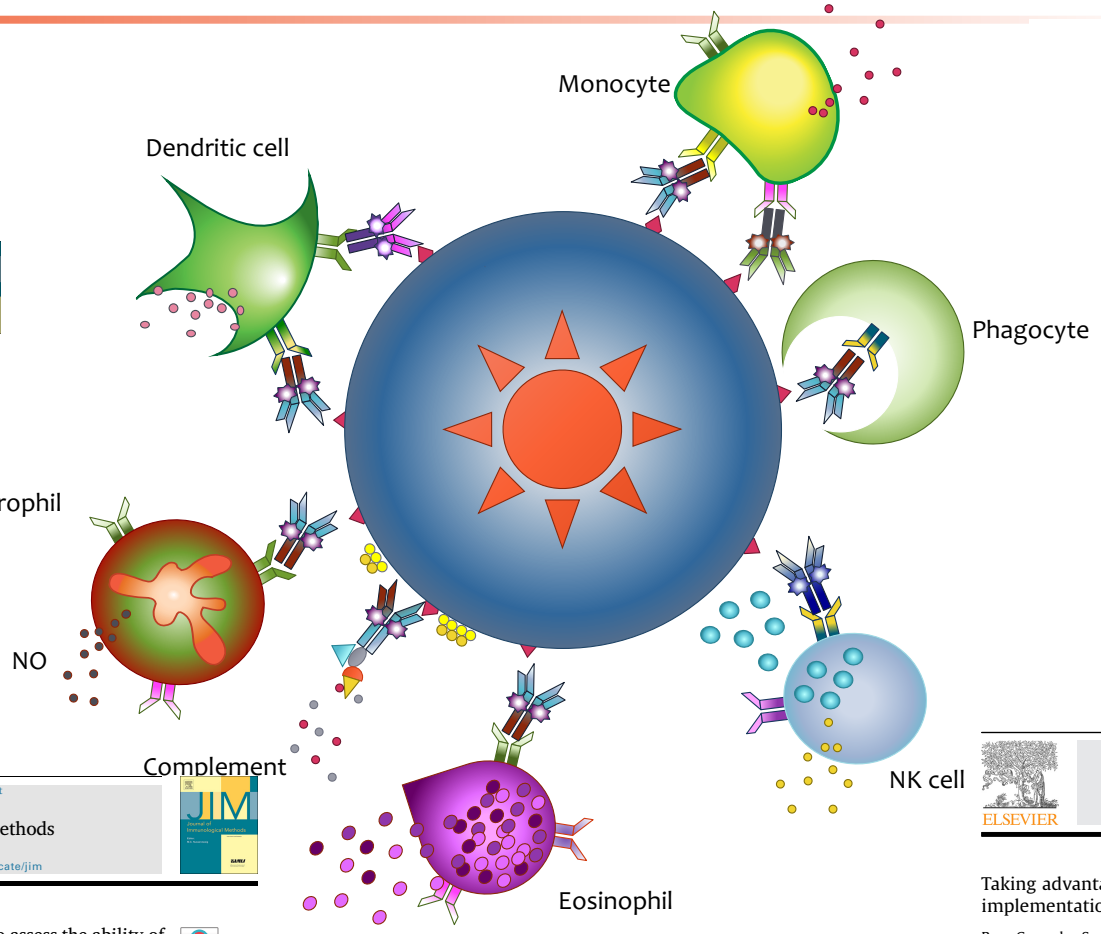
Robust assays exist to evaluate many antibody Fc effector functions



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A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis

Christina B. Karsten[a,1], Nickita Mehta[a,1], Sally A. Shin[a], Thomas J. Diefenbach[a], Matthew D. Slein[a], Wiktor Karpinski[a], Edward B. Irvine[a,b], Thomas Broge[a], Todd J. Suscovich[a], Galit Alter[a,*]

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Research paper

A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation☆

Stephanie Fischinger[a,b], Jonathan K. Fallon[a], Ashlin R. Michell[a], Thomas Broge[a], Todd J. Suscovich[a], Hendrik Streeck[b], Galit Alter[a,*]

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Research paper

A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples

Margaret E. Ackerman[a,*], Brian Moldt[b,1], Richard T. Wyatt[b], Anne-Sophie Dugast[a], Elizabeth McAndrew[a], Stephen Tsoukas[a], Stephanie Jost[a], Christoph T. Berger[a], Gaia Sciaranghella[a], Qingquan Liu[a], Darrell J. Irvine[a,c], Dennis R. Burton[a,b], Galit Alter[a]

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[b] Department of Immunology and Microbial Science and IAVI Neutralizing Antibody Consortium, The Scripps Research Institute, La Jolla, CA, United States
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Biotechnology Reports
journal homepage: www.elsevier.com/locate/btre

Taking advantage of a high-throughput flow cytometer for the implementation of an ADCC assay for regulatory compliance

Rosa Camacho-Sandoval[a,1], Alexis Jiménez-Uribe[a,1], Alejandra V. Tenorio-Calvo[a], Carlos A. López-Morales[a], Leslie Muñoz-García[a], Alejandra Montes-Luna[a], Héctor Leonardo García-Xolalpa[d], Marco Velasco-Velázquez[b], Lenin Pavón[c], Sonia Mayra Pérez-Tapia[a,*], Emilio Medina-Rivero[a,*]

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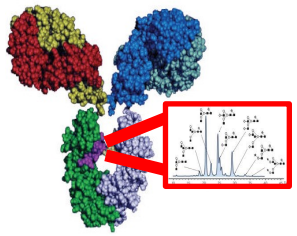
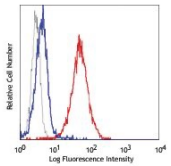
SeromYx has commercialized this in an industrialized platform



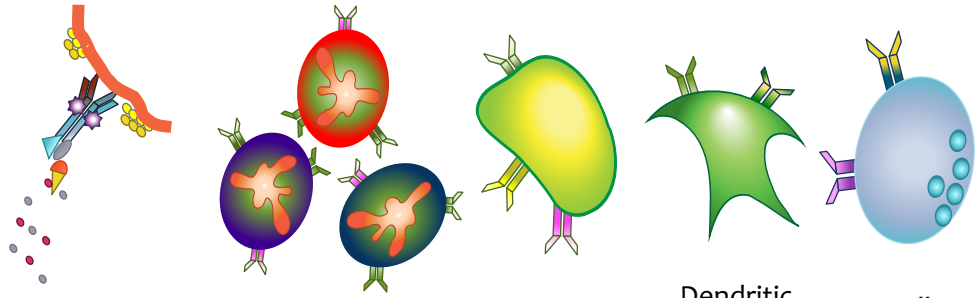
Glycan Analysis



Fc-receptor Binding

Biophysical assays

Antibody Fc receptor binding, Antibody glycosylation



Complement | Granulocytes | Monocyte | Dendritic cells | NK cell

10 functional assays

ADCP, ADCC, CDC, ADNKA, ADEP, ADBP, ADCD, ADNP, ADDCP, ADMB

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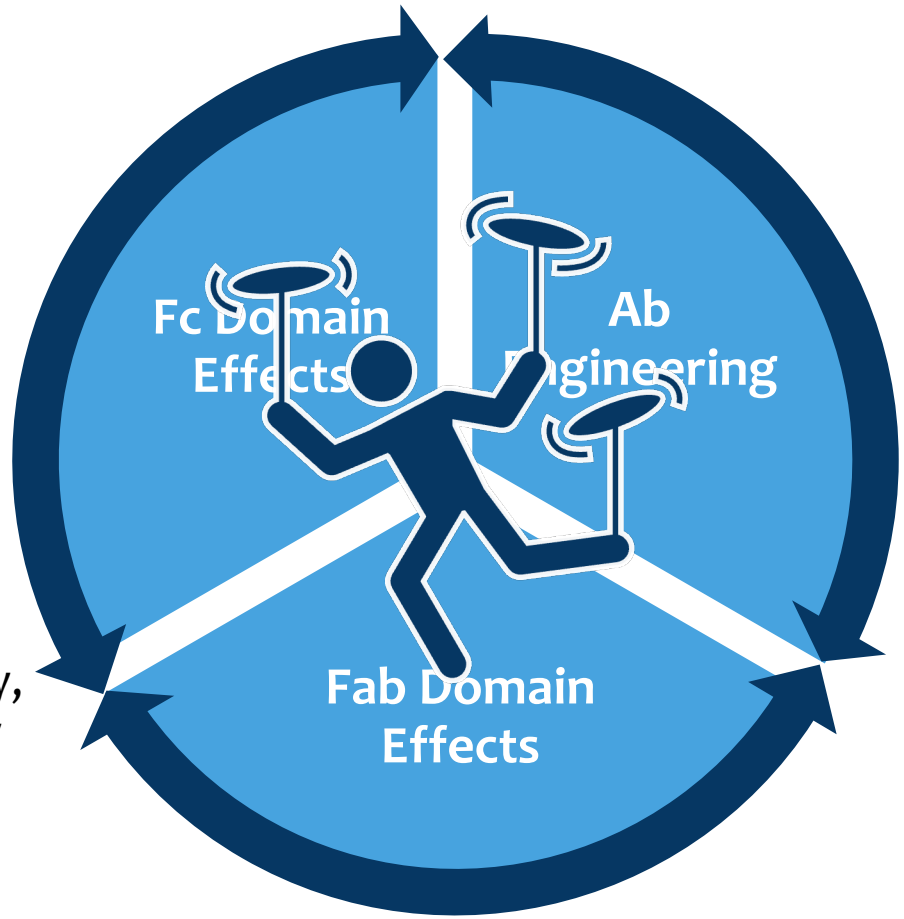
Understanding, optimizing and predicting function is a delicate balancing act

Fc Domain Effects

- Isotype/Subclass
- Glycosylation

Fab Domain Effects

- Affinity
- Valency
- Epitope specificity, binding geometry
- Fab/Fc allostery



Ab Engineering

- Affinity maturation
- Targeting multiple epitopes
- Modulating antibody valency

- Driving IgG self-assembly
- Tuning effector function by target location
- Modifying complement binding
- Modifying FcγR binding
- Extending half-life

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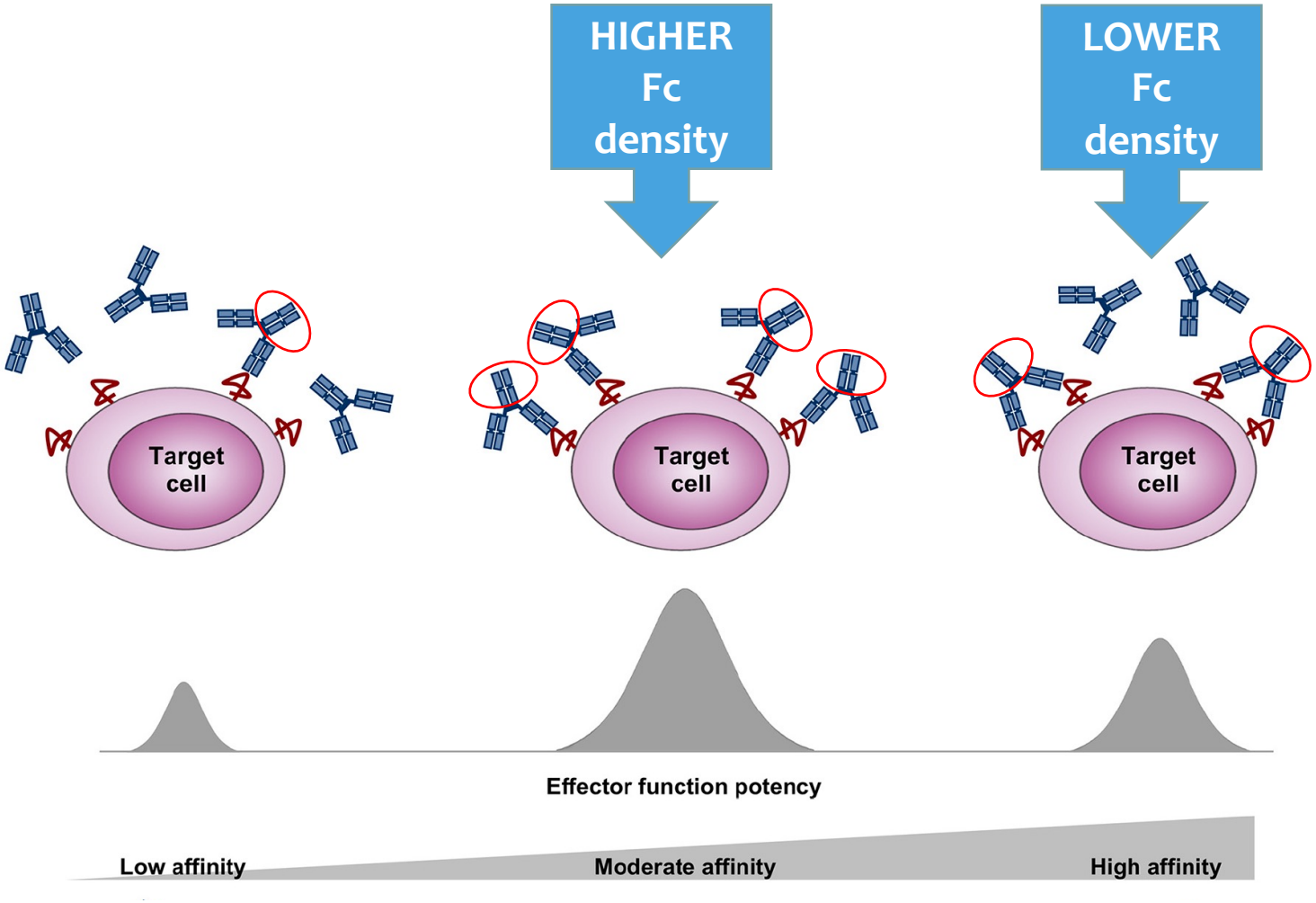
Higher antigen binding affinity does not always correlate with increased Fc effector function



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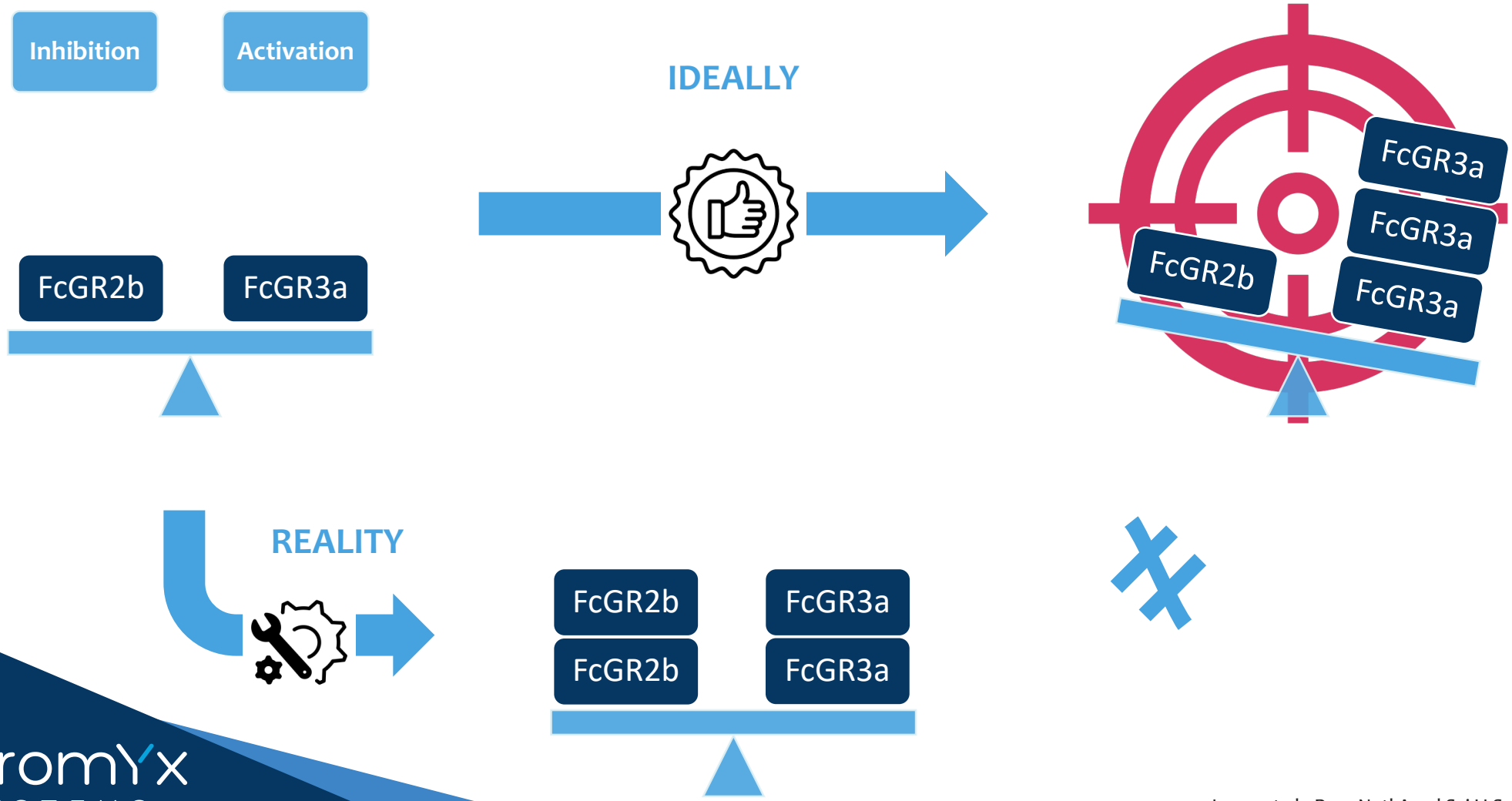
Mazor et al. PLoS One. 2016;11(6):e0157788.
Wang et al. MAbs. 2020;12:1690959.

Modifying the Fc to target specific FcγRs can inadvertently impact its binding to other FcγRs and product efficacy



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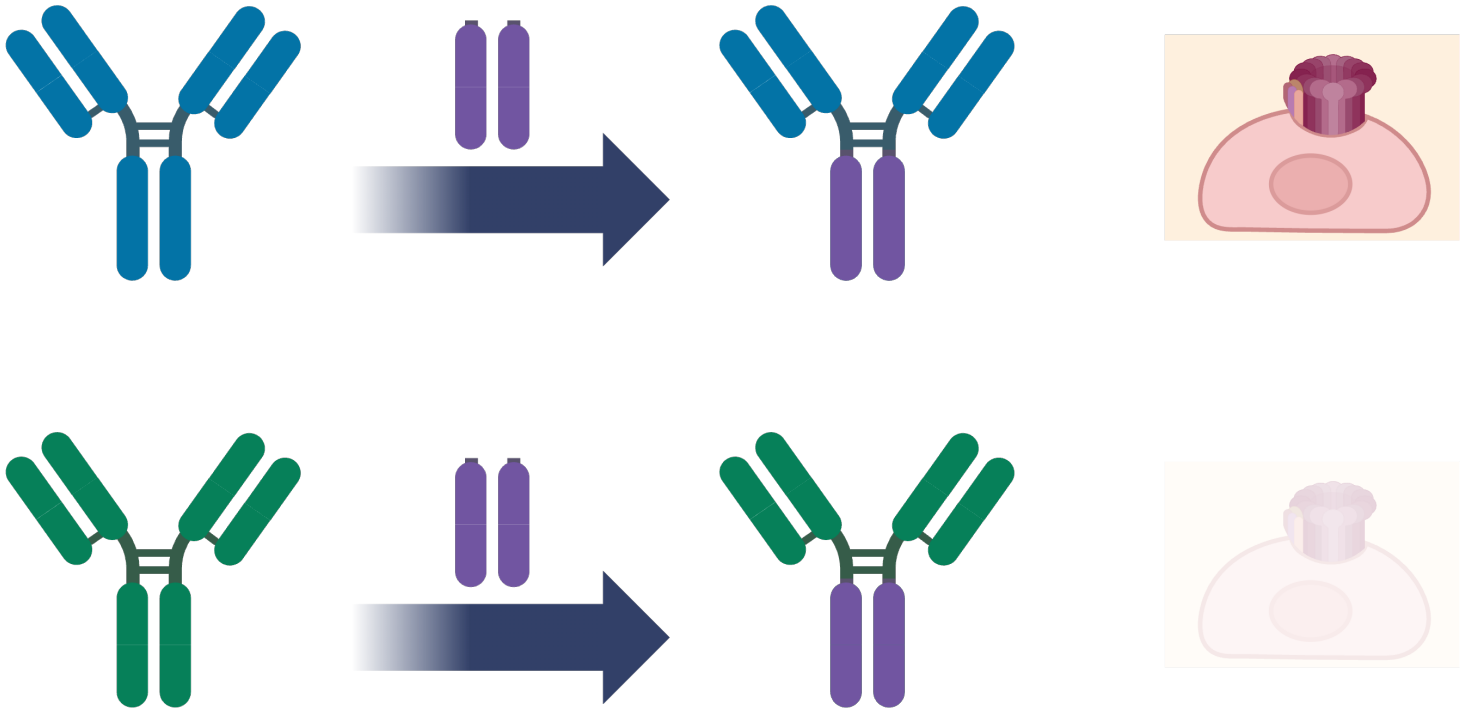
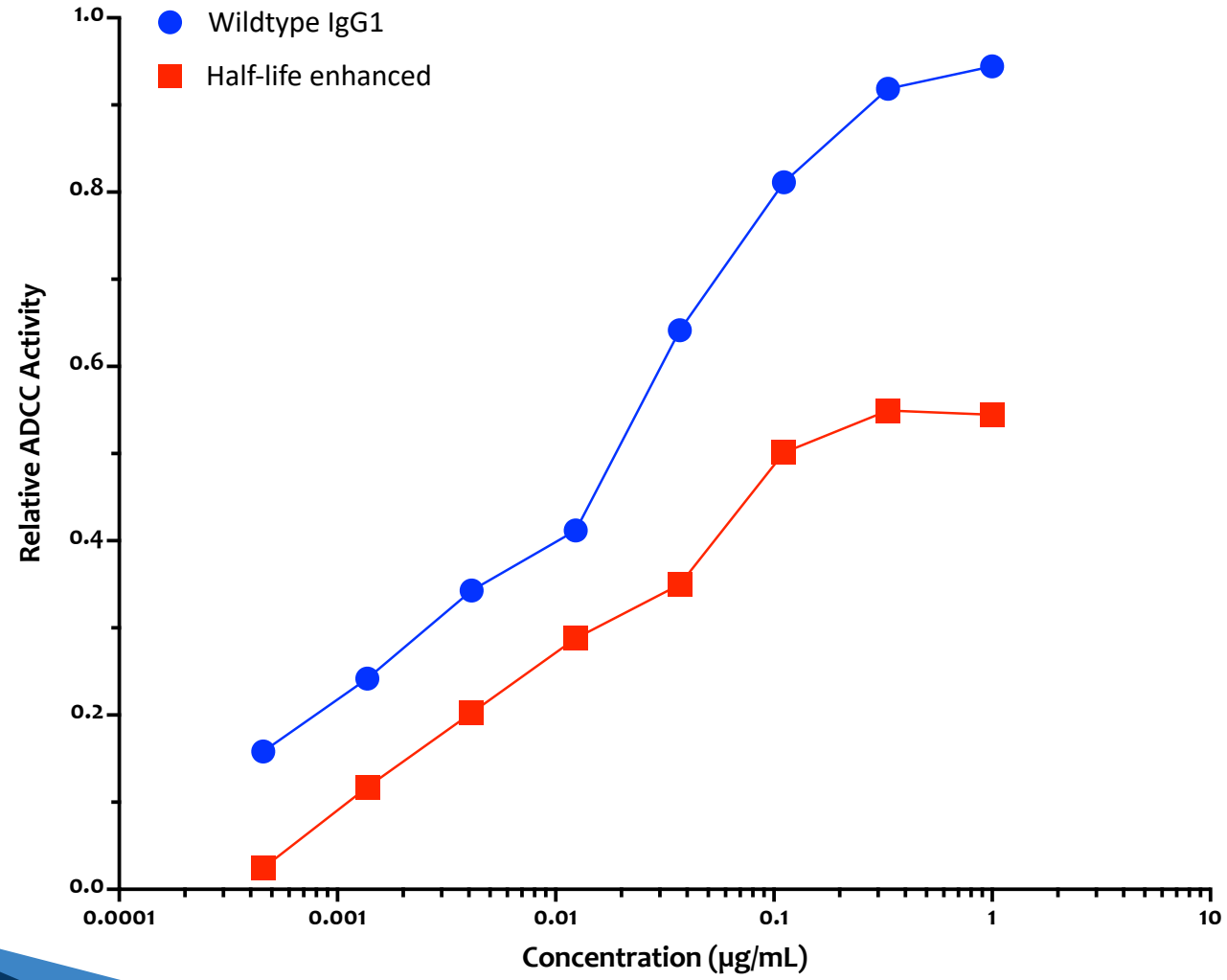
Lazar et al., Proc Natl Acad Sci U S A. 2006;103:4005-10.

Transferability of Fc modifications to other antibodies is difficult to predict



Moore et al., MAbs. 2010;2:181-9.

Impact of half life extension is unpredictable



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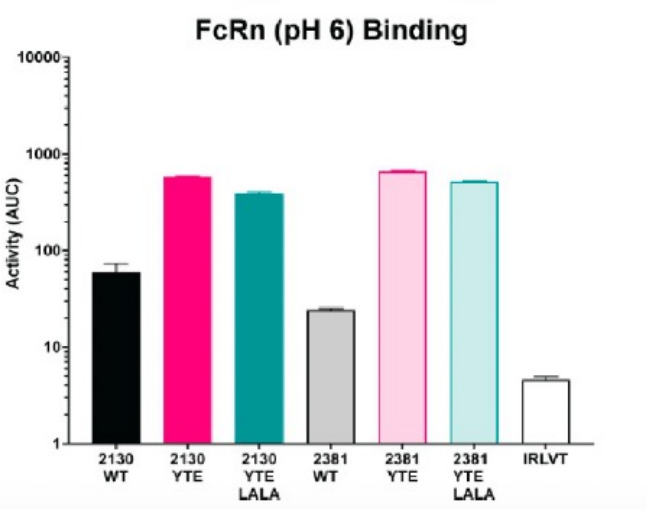
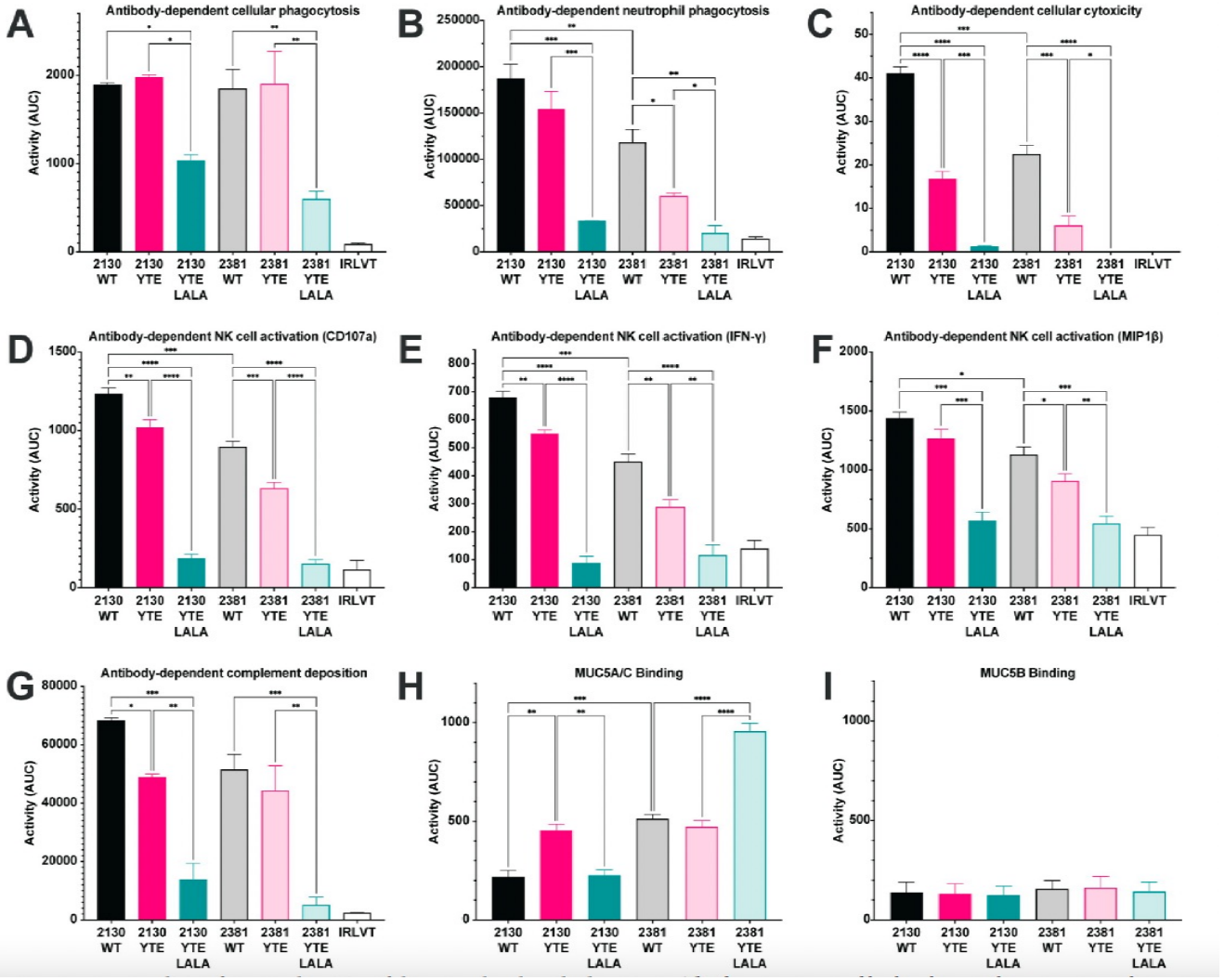
SARS-CoV-2 Spike-targeting mAbs: Half-life extending modifications reduce ADCC, NK cell activation, ADNP, and ADCD, while showing no effect on ADCP

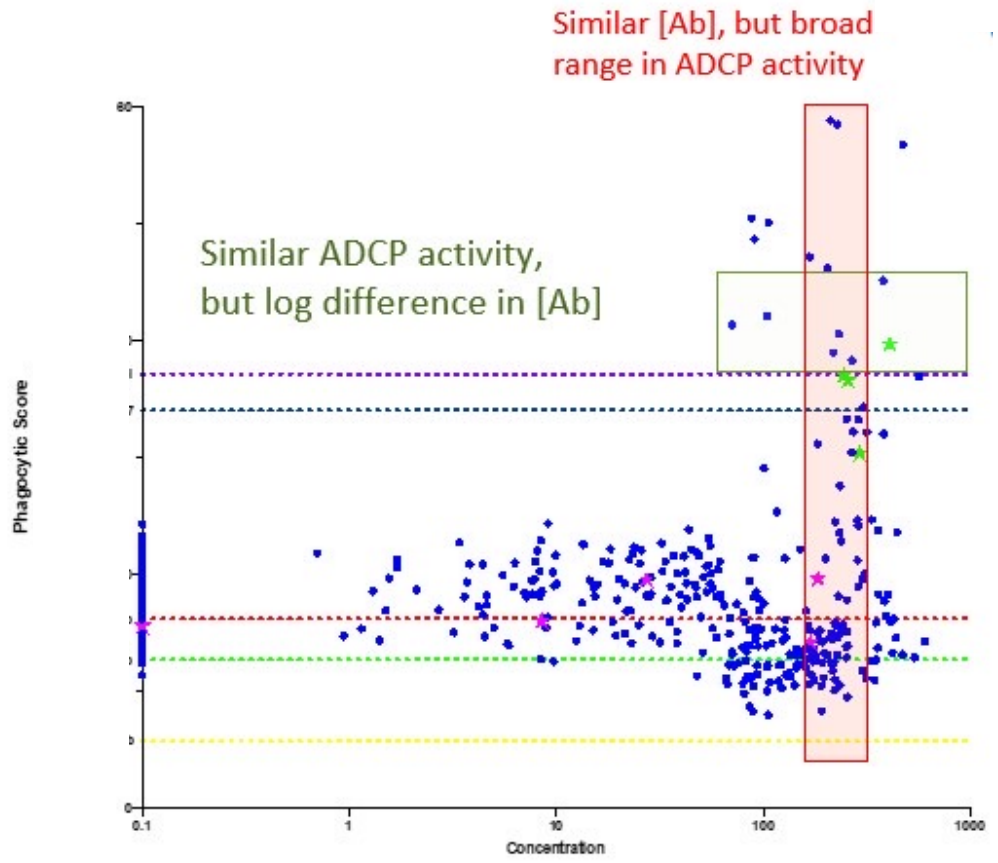




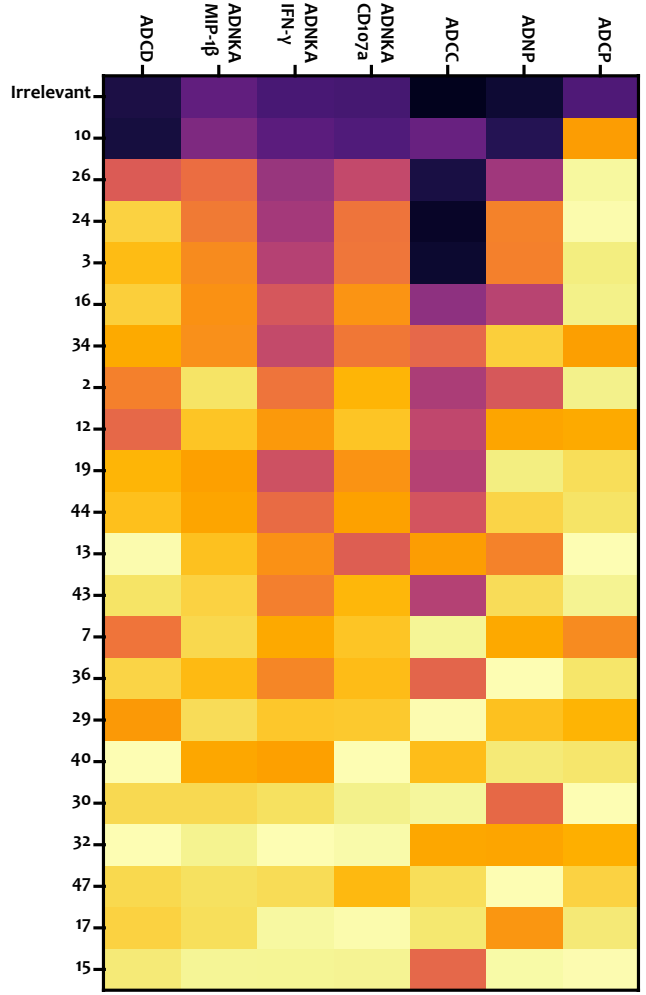
House et al., PLoS One. 2022;17:e0267796.

We measure outcome: Variations in function with identical Fc driven by epitope specificity and/or affinity

Phagocytic activity of 598 mAbs on the same Fc but targeting different epitopes



Functions of a panel of mAbs in clinical development/in use



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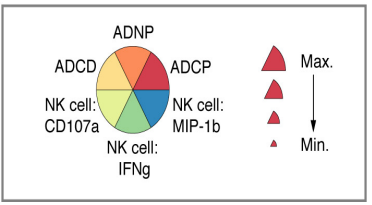
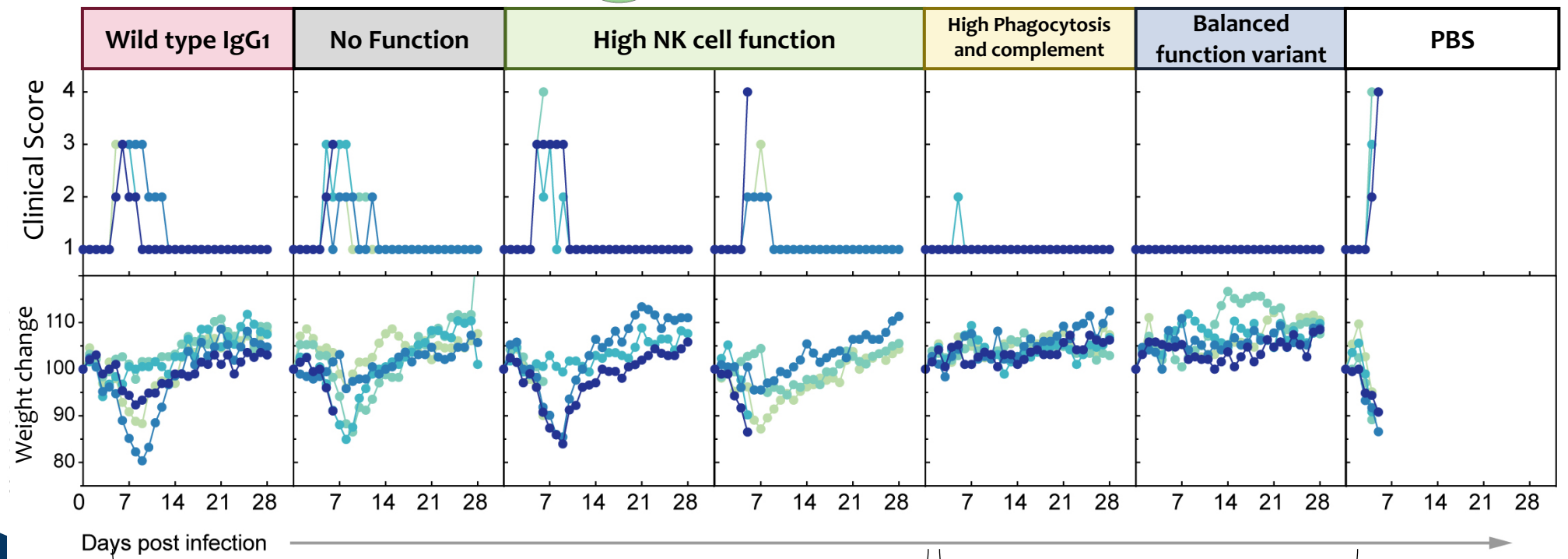
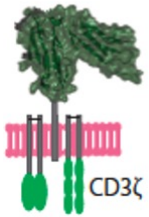
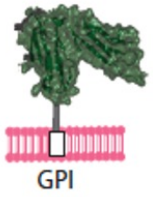
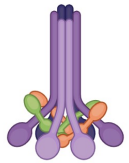
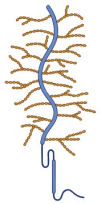
Optimizing treatment efficacy: Assessing effector function outcomes in a library of mAbs with a single Fab on variable Fc regions





Gunn et al, Immunity. 2021;54:815-828.e5.

Breadth of SeromYx Systems assay offerings

FcγRI (CD64)	FcγRIIa (CD32a)	FcγRIIb (CD32b)	FcγRIIIa (CD16a)	FcγRIIIb (CD16b)	C1q	Mucin
						
Binding Assay	Monocytes ADCP Basophils ADBP Eosinophils ADEP DCs ADDCP Binding Assay	Binding Assay	NK Cells ADCC Binding Assay	Binding Assay	Complement CDC ADCD Binding Assay	Mucin ADMB

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Adapted from Delidakis et al., Annu Rev Biomed Eng. 2022;24:249-274.

Critical attributes integrated into the assays

- **Robustness:** extensive development and optimization of each assay component
- **GCLP quality:**
  - **Precision**
  - **Linearity**
  - **Specificity**
  - **Sensitivity**
- **Adaptability**: variety of antigens and sample matrices –Never found an antigen we can't work with, but antigen quality is critical.
- **High-throughput:** 1000s of samples in a single experimental run

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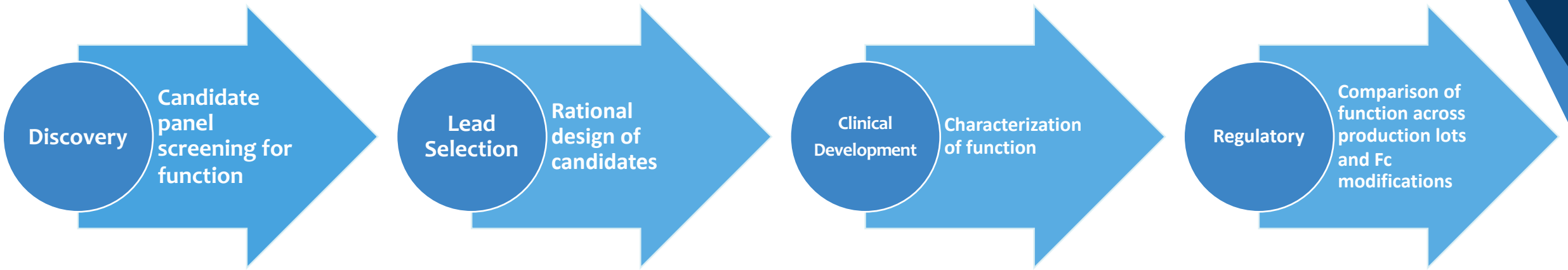
Antigen-specific characterization: Added value throughout the mAb discovery and development process



- Finding the right mAb with the desired mix of function.

- Fc specificity
- Improved effector function
- Safety and efficacy

- IND enabling

- BLA enabling

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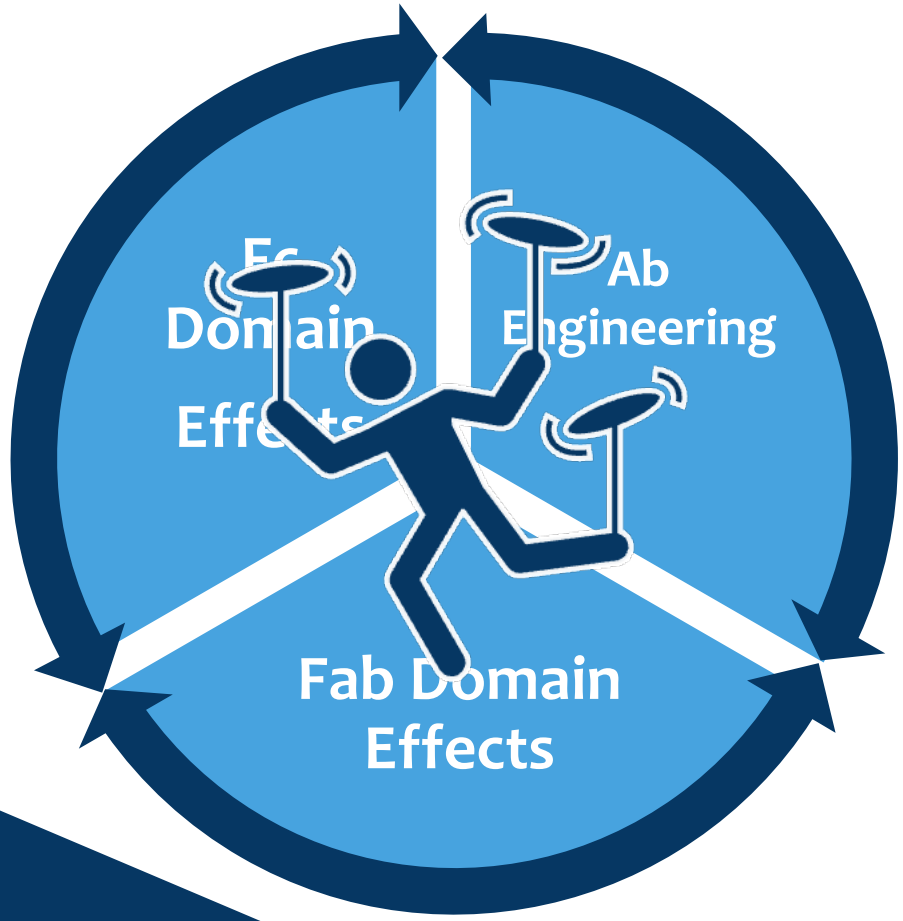
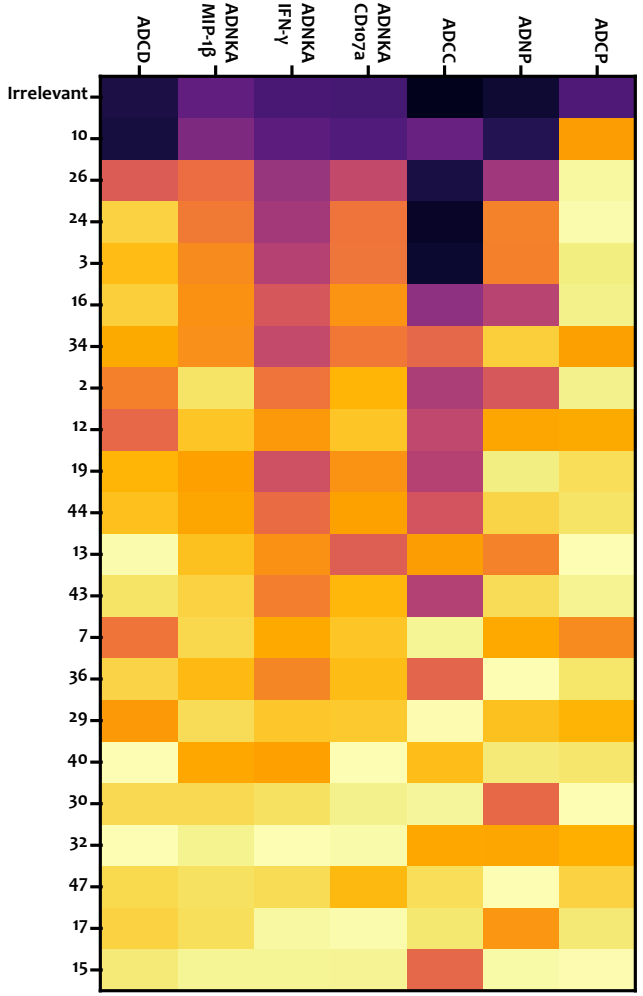
Managing the risk imposed by biological complexity can be achieved through the assessment of outcomes





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Thank you!

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