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
Antibodies mediate an array of functions beyond binding
Robust assays exist to evaluate many antibody Fc effector functions

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

1. Introduction

Immune correlates of protection

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

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

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

A breakthrough application of flow cytometry in flow phenotyping of clinical samples.
SeromYx has commercialized this in an industrialized platform

Biophysical assays

Antibody Fc receptor binding, Antibody glycosylation

10 functional assays

ADCP, ADCC, CDC, ADNKA, ADEP, ADBP, ADCD, ADNP, ADDCP, ADMB
Understanding, optimizing and predicting function is a delicate balancing act.

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

**Fc Domain Effects**
- Isotype/Subclass
- Glycosylation

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

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

Transferability of Fc modifications to other antibodies is difficult to predict

Impact of half life extension is unpredictable
SARS-CoV-2 Spike-targeting mAbs: Half-life extending modifications reduce ADCC, NK cell activation, ADNP, and ADCD, while showing no effect on ADCP.

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

Similarity between WT, non-functional and high NK cell functional mAbs

mAbs with balanced functional activity display efficacy

Breadth of SeromYx Systems assay offerings

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

**Discovery**
- Candidate panel screening for function

**Lead Selection**
- Rational design of candidates
- Fc specificity
- Improved effector function
- Safety and efficacy

**Clinical Development**
- Characterization of function
- IND enabling

**Regulatory**
- Comparison of function across production lots and Fc modifications
- BLA enabling

- Finding the right mAb with the desired mix of function.
Managing the risk imposed by biological complexity can be achieved through the assessment of outcomes.
Thank you!

Lenny Moise, PhD
lenny.moise@seromyx.com
+1.401.743.0310