Antibody-Mediated Effector Function: Learning and Challenges from Early Discovery to Development

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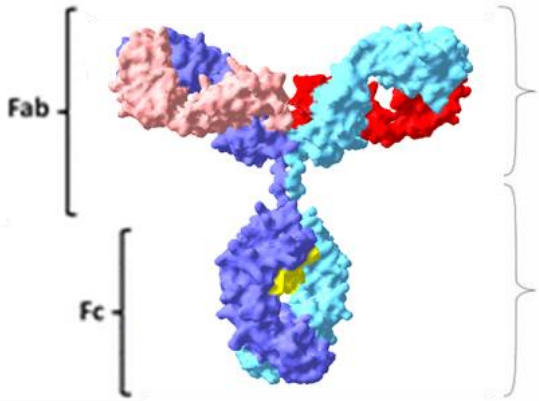
Agenda

- Overview of Antibody (Ab)-Mediated Effector Function
- Ab-Effector Function Testing During Life Cycle Development of Drug
- Transition Challenges from Discovery to Development
  - Mouse to Human transition
  - Choice of Appropriate Assay Format When Multiple Receptors Are Part of MOA
  - Finding Appropriate Target Cell Lines
    - Representative of Physiological Target Expression
    - Stability Over Assay Runs
- Summary

Bimodal Nature of Antibodies: Implications for Safety and Efficacy



Protein of interest

Receptor extracellular domain
Cytokine
Enzyme

Fc region

FcγR binding → ADCC
C1q binding → CDC
FcRn binding → Half-life

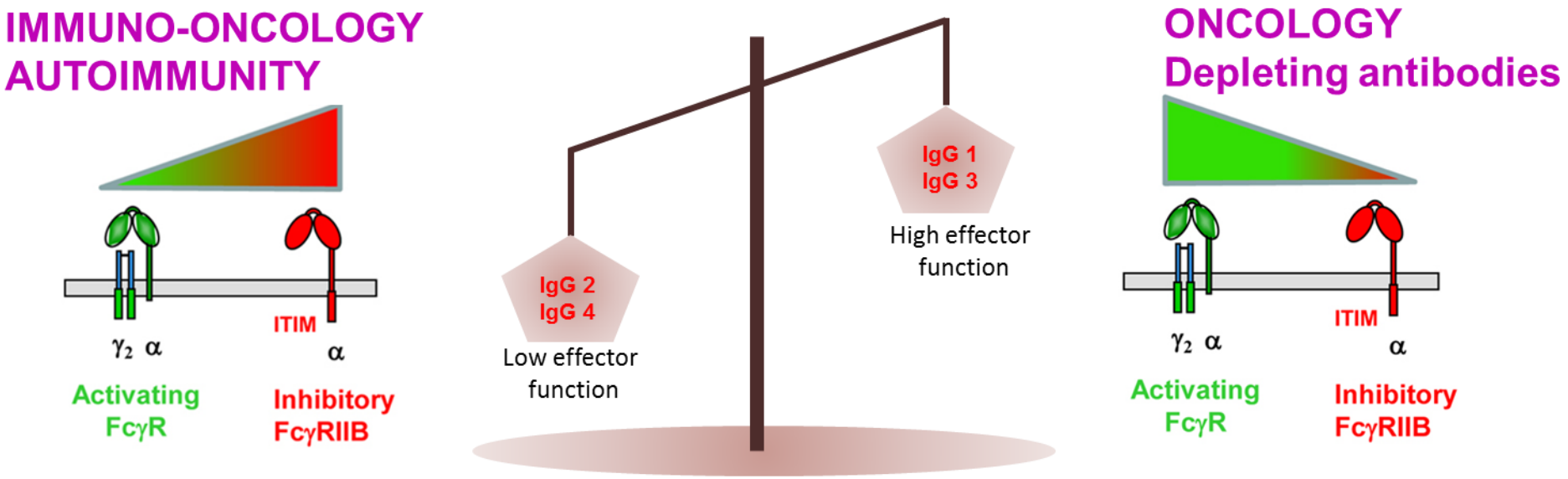
Effector function potential → Linked to safety profile & therapeutic efficacy

Table 1 Key features of the four IgG isotypes

	IgG1	IgG2	IgG3	IgG4
FcRn[e]	+	+	+	+
Effector functions				
C1[e]	++	–	+++	–
FcgRI[e]	+++	–	+++	++
FcgRII[e]	+	±	+	?
FcgRIIIa/b[e]	+	–	+	±

Salfeld et al, Nature Biotech (2007)

Is Effector Function Always Non-Desirable?



- Beneficial where target cell depletion is **NOT** desired- Ex. autoimmunity targets, immuno-oncology targets
- Fc-engineering of antibody to ablate effector function

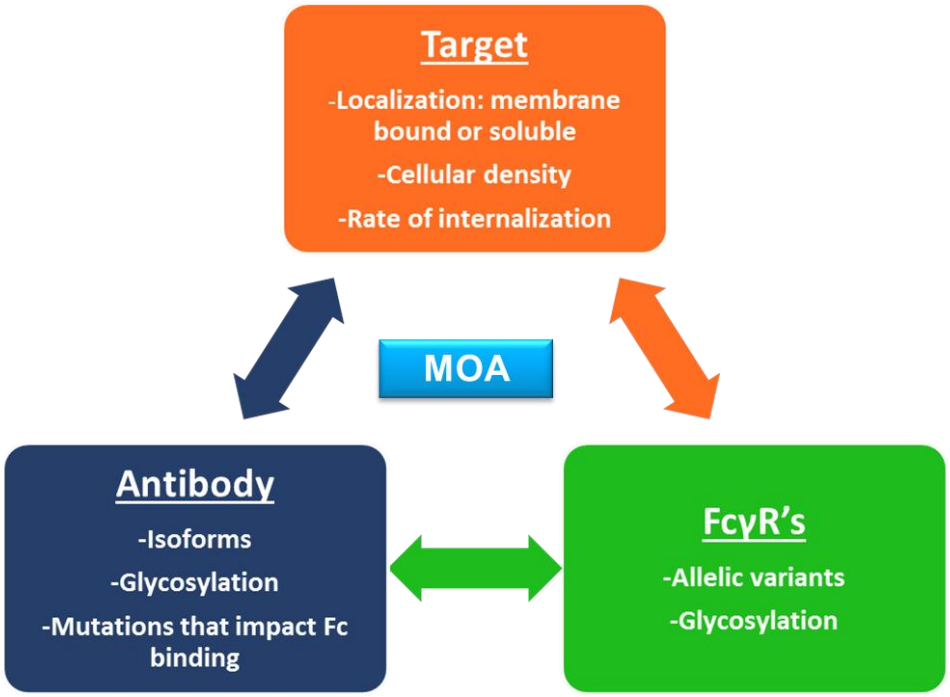
- Beneficial where target cell depletion is desired MOA
- Fc-engineering of antibody to enhance effector function

Effector Function Potential: Interplay with MOA

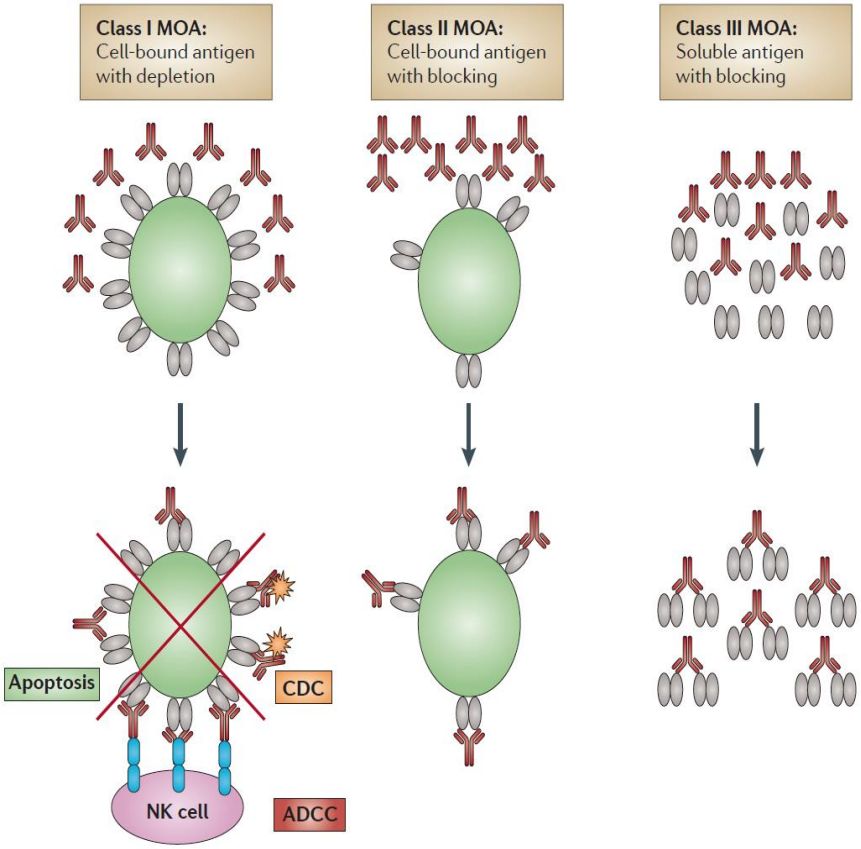
OPINION

Advances in the assessment and control of the effector functions of therapeutic antibodies

Xu-Rong Jiang, An Song, Svetlana Bergelson, Thomas Arroll, Bhavin Parekh, Kimberly May, Shan Chung, Robert Strouse, Anthony Mire-Sluis and Mark Schenerman



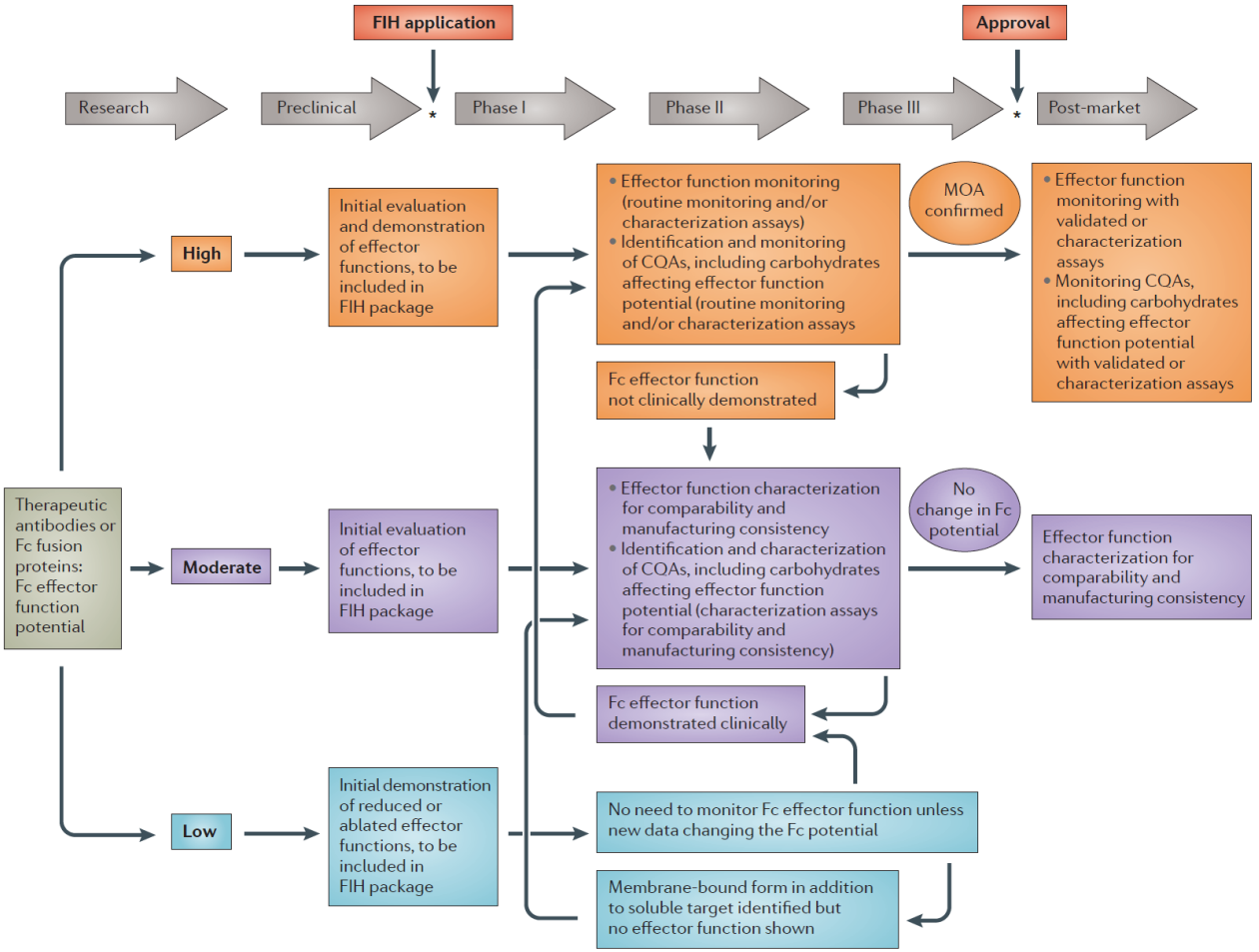
Nature Reviews Drug Discovery, 2011 (10), 101



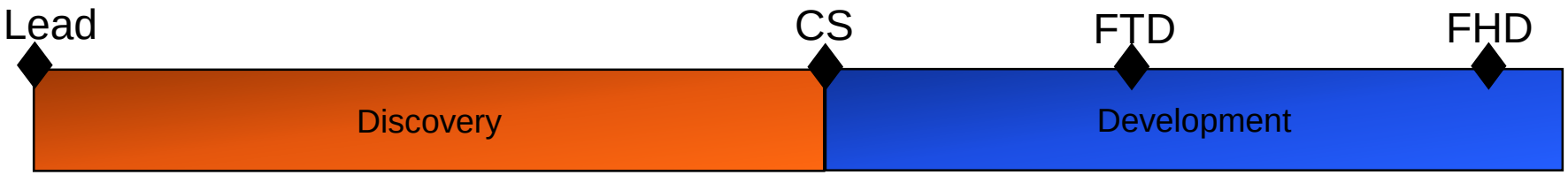
Effector Function Testing: Continuous Monitoring

Effector Function Status is impacted by isotype, Fc engineering or Glyco-engineering

Jian et al; Nature Reviews Drug Discovery, 2011 (10), 101



Transitioning Effector Function Cellular Assays from Discovery to Development



Discovery

- **Purpose: Establish the presence c effector f establish**
- **Assay Fo could eitl or cell lin or overex**
- ***In vivo* st link of eff MOA**
- **Species: Hum**

Development

- **Purpose: An assay MOA that monitor**

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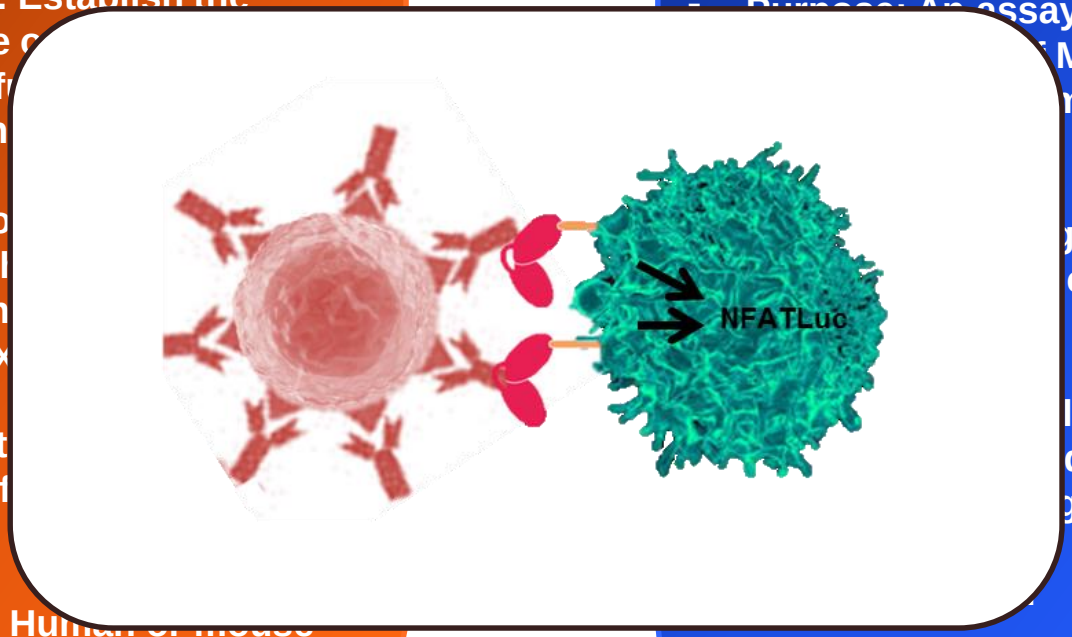
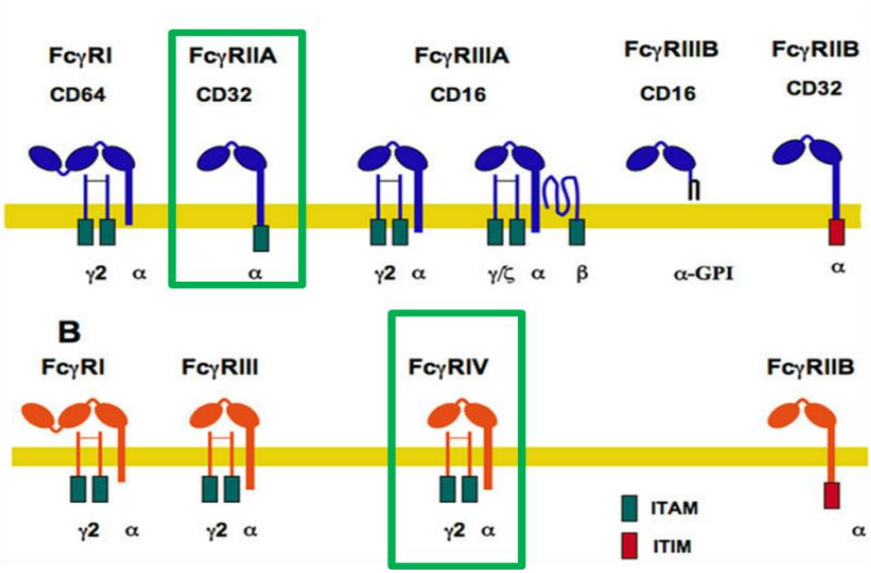


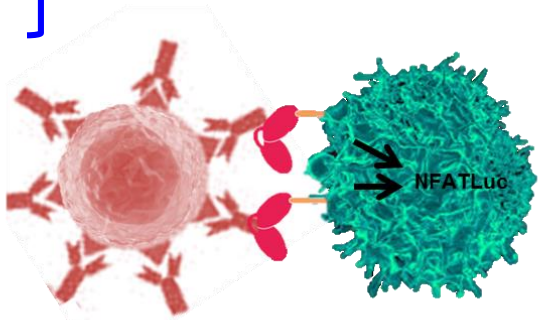
Case Study #1: Transitioning Effector Function Data from Mouse to Human

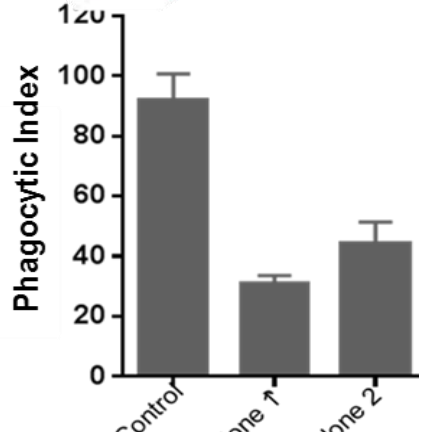
- Understanding of putative MOA of therapeutic antibody within the context of a disease comes from *in vivo* mouse studies
  - Mouse and human FcγR repertoire is very different
  - Mouse IgG isotypes different than humans
  - Human and mouse FcγR (s) exhibit differences in IgG binding pattern's
  - Human and mouse FcγR (s) differ in lineage expression profiles
  - Reporter cell-based assays use cell lines expressing individual FcγR's

These differences add to the complexity of crossing over experimental data between the two species

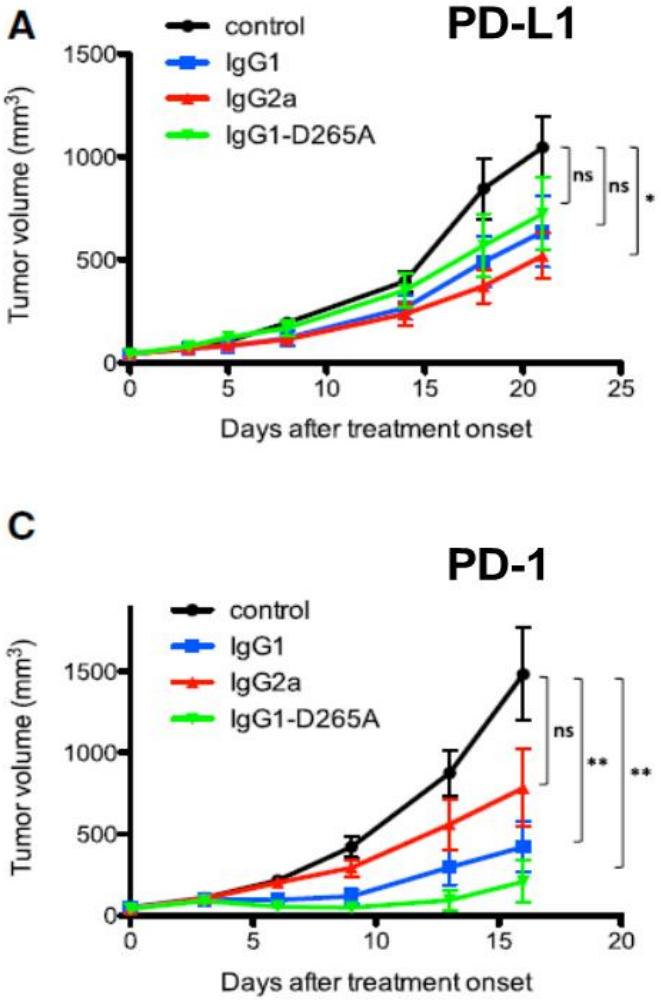


Advances in Bioscience and Biotechnology, Vol.4 No.4A(2013)





Transitioning *in vivo* Results to Design Cell-based Effector Function Assays



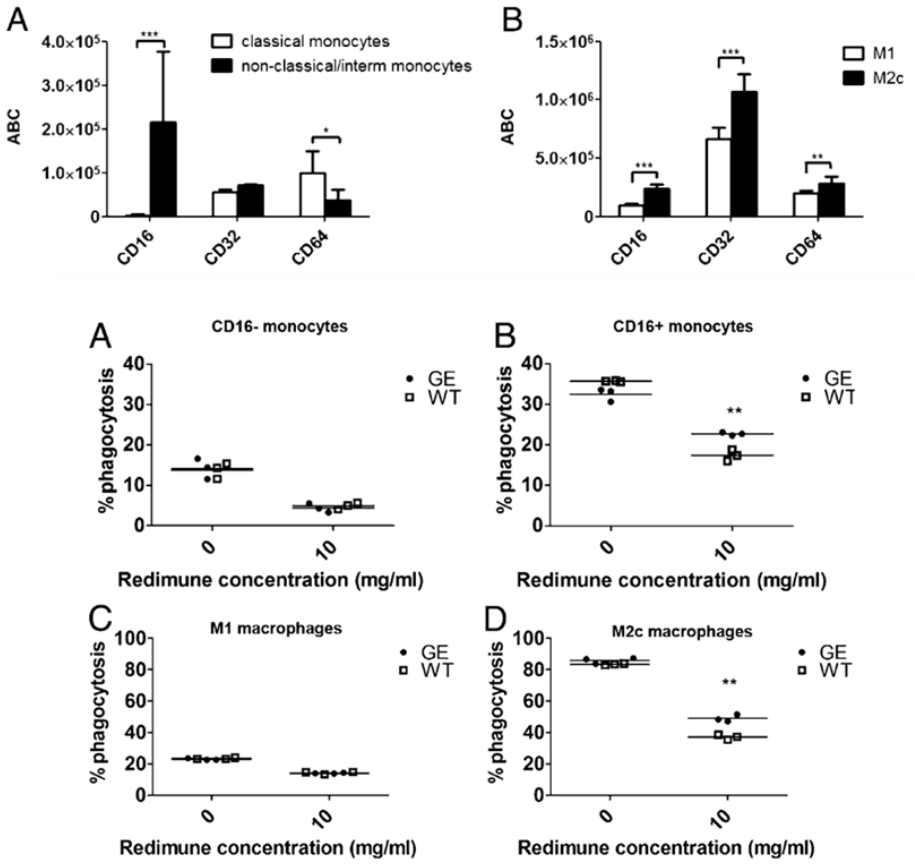
Dahan R et al, Cancer Cell, 2015, 285

- Effector function independent trans-co engagement of agonist CD40 Ab with FcγRIIb is MOA.
- PD-L1 blocking antibody requires trans engagement with activating receptor for optimal *in vivo* efficacy.
- On the contrary interaction with FcγR's reduces efficacy of PD-1 blocking antibody in *in vivo* mouse tumor models.
- Therefore, choice of backbone is dictated by a better understanding of MOA of antibody and a thorough understanding of biology (QoD)

Using mice surrogate antibody data to understand relevant human FcγR-engagement and its link to MOA is a challenge

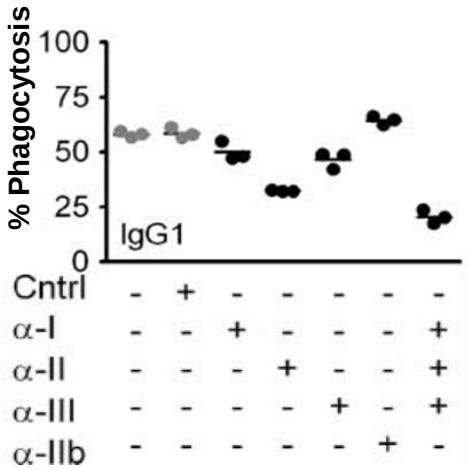
Case Study #2: Choosing a Reporter Assay When Multiple FcγR's Are Involved in MOA

Evidence for FcγRIIIa

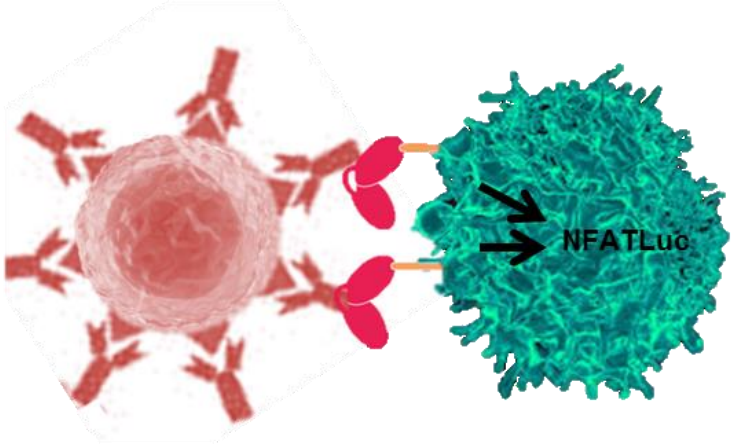


Herter S, et al, *J Immunol* 2014; 192:2252-2260

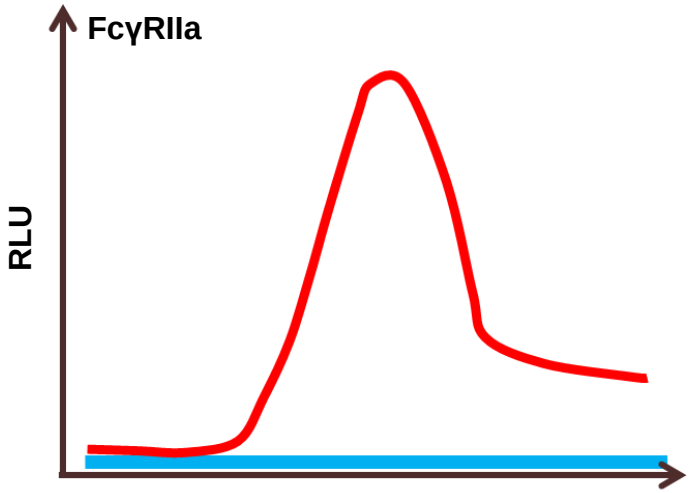
Evidence for FcγRIIa

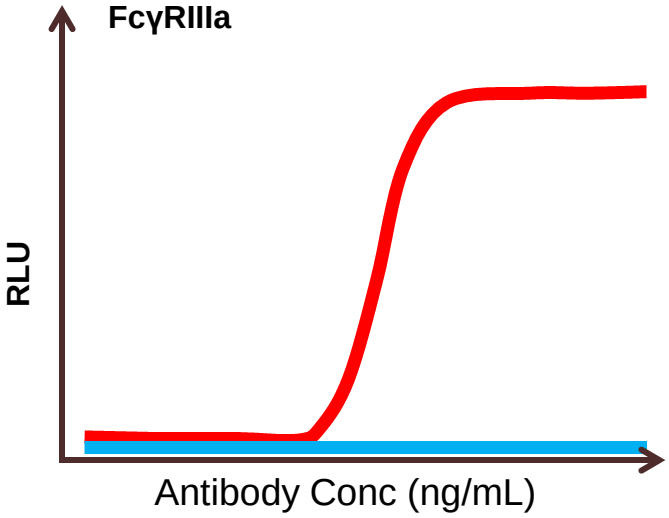


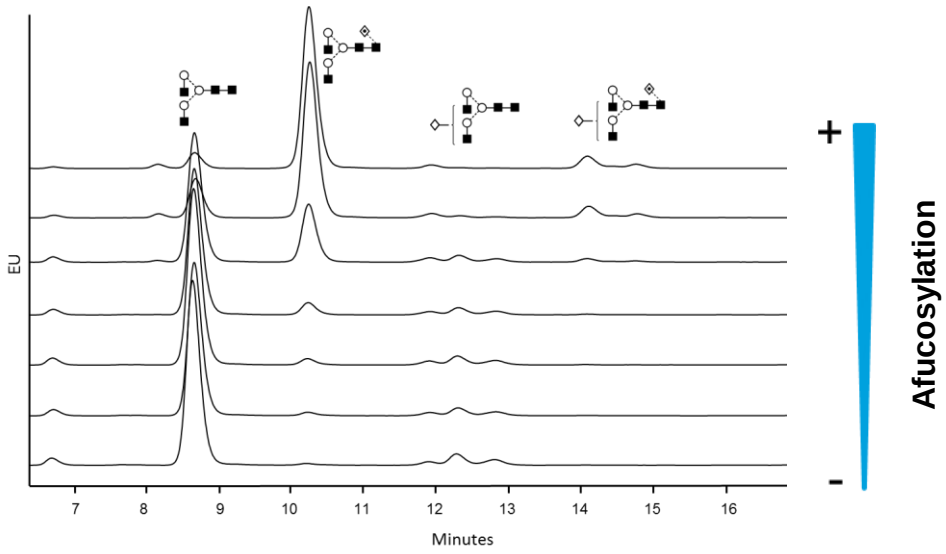
Richard et al, Mol Cancer Ther 2008;7(8). August 2008)

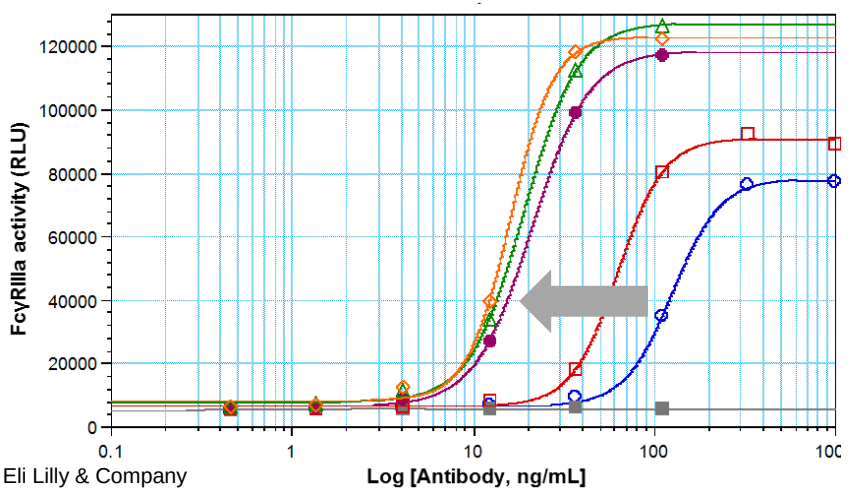


Should QC Assay be Selected Based on Its Ability to Monitor CQA?

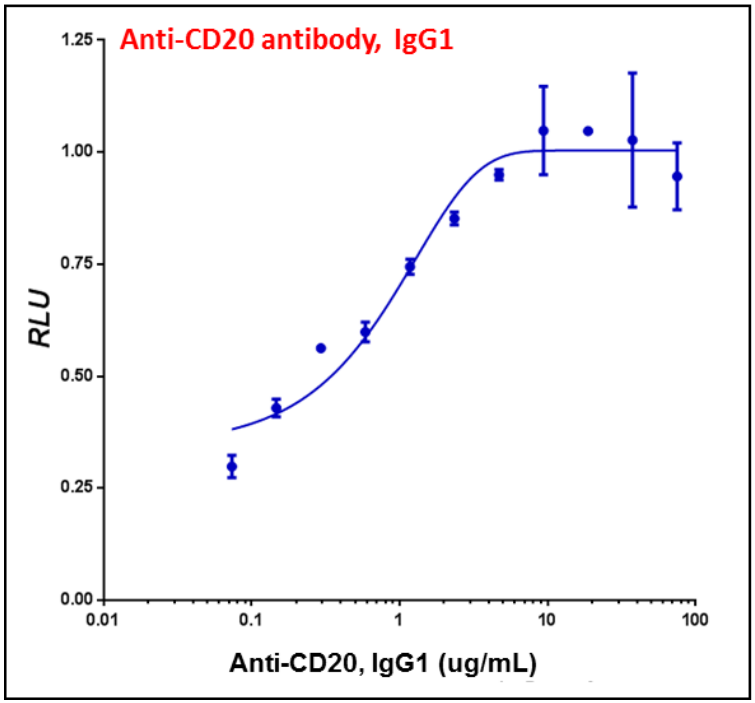


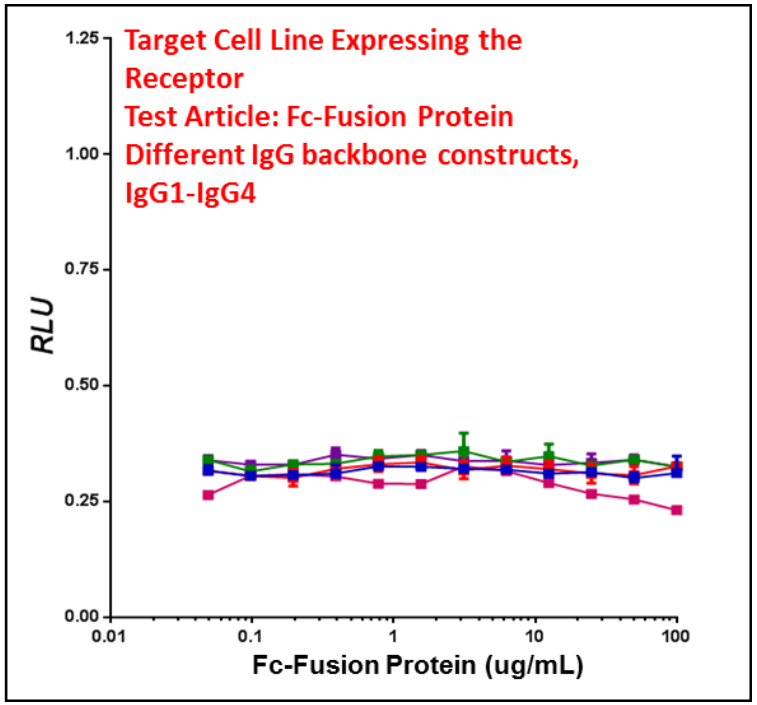


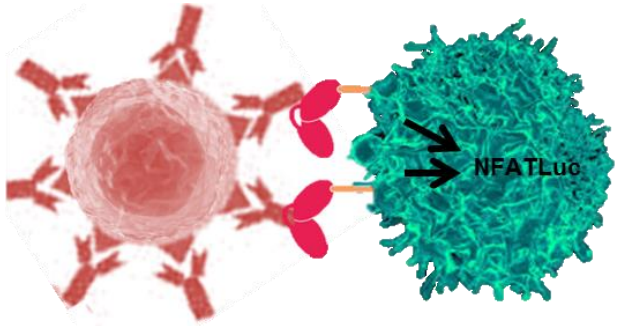




Case Study # 3: Choosing an Appropriate Target Cell Line with Endogenous Receptor Expression

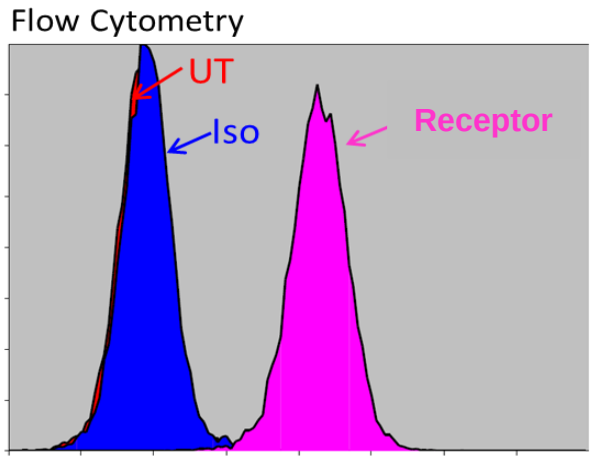


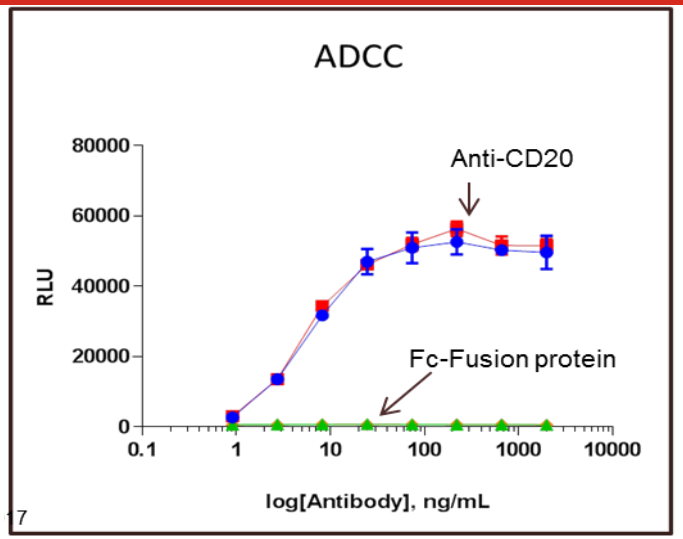


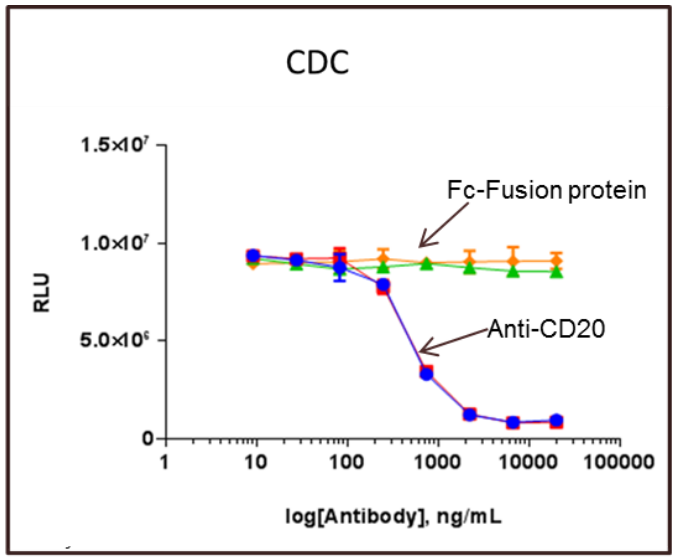


- None of the IgG-fusion constructs gave a response in FcγRIIIa activity assays.
- Is it the Cell Line issue? Expression issue?

Selecting a Target Cell Line with a Second Positive Marker for Effector Function



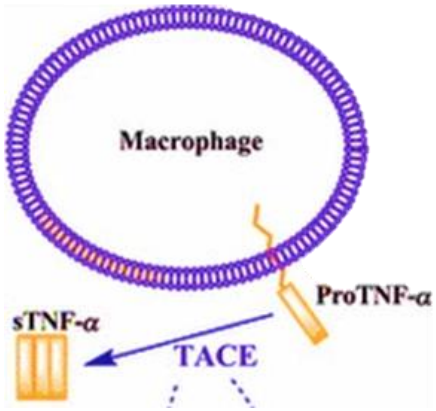




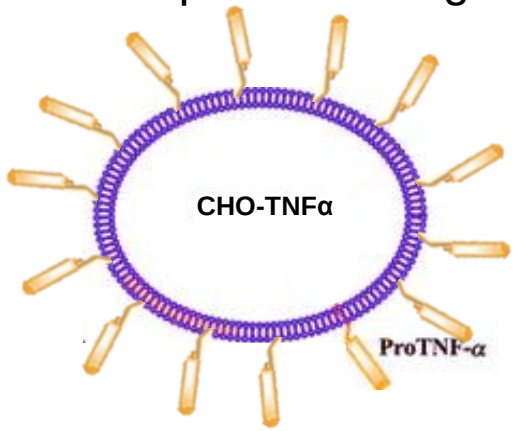
- The Biological function of the receptor was confirmed via Signaling experiments on the new Target Cell Line.
- If IgG1-Fc functionality does not respond in the FcγRIIIa activity assays do we need to confirm this with multiple target cell lines?
- However, it is not always easy to find cell lines with physiologically relevant target expression

Providing an Appropriate Context When Choosing An Overexpression Target Cell Line

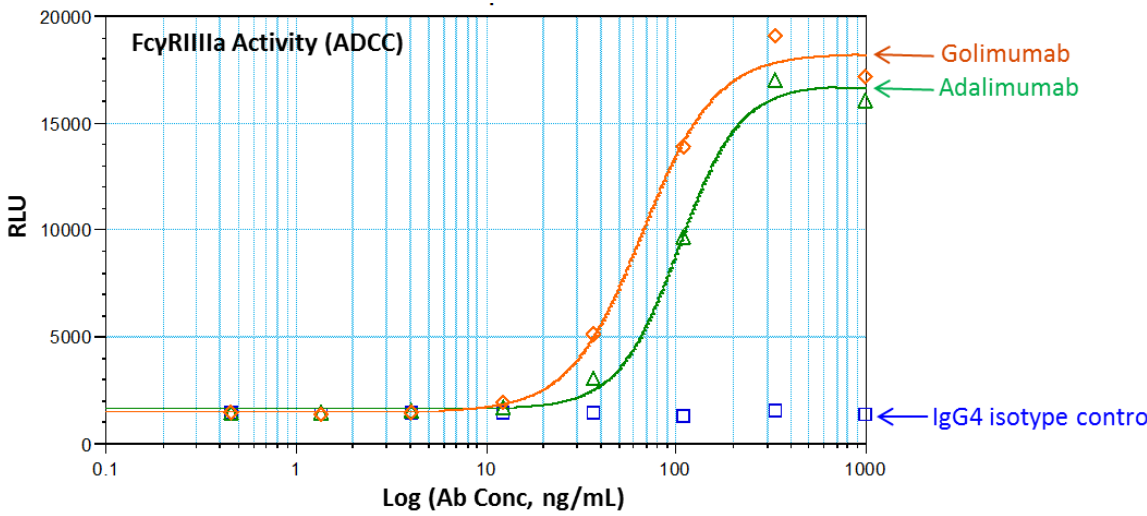
Physiological Expression: Transient



Overexpressed Antigen



- Data with overexpression systems tend to be exaggerated and may not be representative of true physiological scenario's.
- It is important to put the data obtained within appropriate context.
- Clinical data from and knowledge from previous efforts may be helpful in this regard.



Summary

- Effector function is a critical component of life cycle management of therapeutic Mab. It is reflective of
  - Therapeutic Efficacy
  - Safety/risk
  - Lot to Lot variation (CQA)
- The goals of discovery work on ab-mediated effector function testing is very different than development work
- Transition Challenges include
  - Mouse to human transition
  - Multiple receptors involved in MOA
  - Finding suitable target cell lines with physiologically relevant cell lines
- It is important to integrate the knowledge of FcγR-MOA into an appropriate cell-based assay as early as possible and use previously available clinical data to drive testing some of the molecules

Acknowledgments

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