GUIDE

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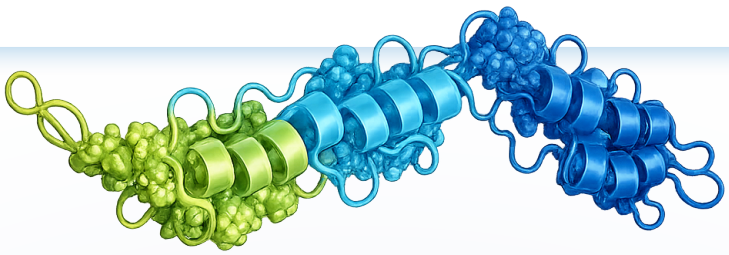


HUMANIZED FCγR MOUSE MODELS FOR THE ASSESSMENT OF THERAPEUTIC ANTIBODIES

Practical considerations for translational research

KEY MESSAGES

- ✓ Fcγ receptors (FcγRs) are **critical determinants of antibody efficacy and safety**, especially for **IgG-based therapeutics**
- ✓ Species differences between murine and human FcγRs **significantly impact antibody binding**, **effector function**, and **PK/PD interpretation**
- ✓ Wild-type mice often provide **misleading results** for Fc-mediated functions (e.g., ADCC, ADCP)
- ✓ Humanized FcγR mouse models **enable translationally relevant evaluation** of: effector function, Fc-engineering strategies, and safety (e.g., cytokine release, immune activation)
- ✓ Model selection directly **impacts data interpretation** and **candidate ranking**
- ✓ Using an inappropriate model can lead to: **false efficacy signals**, **under**- or **over-estimation of toxicity**, and **incorrect Fc-optimization** decisions



INTRODUCTION / BACKGROUND

Therapeutic antibodies rely not only on target binding via Fab regions, but also on Fc-mediated engagement of immune cells.

Fc-FcγR interactions regulate several mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).

Implication: Accurate modelling of FcγR biology is essential for predicting clinical performance.

What are Fcγ receptors?

Fcγ receptors (FcγRs) are cell surface receptors expressed on immune cells that bind the Fc region of IgG antibodies.

Main human FcγRs:

- Activating receptors: FcγRI (CD64), FcγRIIA (CD32A), FcγRIIIA (CD16A) and FcγRIIIB (CD16B)
- Inhibitory receptor: FcγRIIB (CD32B)

In humans, FcγRs are expressed as follows and drive key effector functions:

- NK cells → ADCC (primarily FcγRIIIA)
- Macrophages → phagocytosis (FcγRI, FcγRIIA)
- Dendritic cells → antigen presentation
- B cells → immune regulation (FcγRIIB)

What is their role in the immune response?

FcγRs work as regulators of immune responses.

Engagement of FcγRs leads to:

- Target cell killing (ADCC)
- Phagocytosis (ADCP)
- Cytokine secretion
- Immune complex clearance

Implication for therapeutics: FcγR engagement is often required for efficacy (e.g., oncology antibodies), but can also drive toxicity (e.g. systemic inflammation)

What are the differences between mouse and human FcγR?

Major translational gaps

- Different receptor repertoire (Van Damme et al., 2026): **Humans:** FcγRI, IIA, IIB, IIIA, IIIB vs **Mice:** FcγRI, IIB, III, IV (non-orthologous mapping)
- Different binding affinities: Human IgG subclasses interact differently with mouse FcγRs
- Different expression patterns: Cell-specific expression does not fully overlap, e.g., human NK cells strongly drive ADCC via FcγRIIIA whereas mouse NK cells are less effective, and mouse macrophages rely on FcγRIV (absent in humans), leading to engagement of different effector mechanisms for the same antibody
- Different signaling balance: Activating vs inhibitory receptor ratios differ

Key consequence: Murine FcγR biology does NOT accurately predict human Fc-FcγR interactions, leading to poor translational relevance (Van Damme et al., 2026; Nimmerjahn & Ravetch, 2008; Bruhns, 2012).

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Featuring the genO-hFcγR (scan or click)

Featuring the genO-BRGSF-HIS (scan or click)

HOW TO CHOOSE THE CORRECT MODEL FOR A STUDY

Ask yourself these questions first:

1. Does my antibody engage Fcγ receptors?
2. Is Fc engineering (e.g., glycoengineering, mutations, etc.) part of the strategy?
3. Do I need to assess efficacy, safety, or both?
4. Which cell type needs to be engaged fro a translational assessment?



Comparison of mouse models for testing FcγR engagement

MOUSE MODEL	USE WHEN	AVOID WHEN	ADVANTAGES	KEY LIMITATIONS
Mice expressing murine FcγR (ex: wild-type mice)	Generating preliminary results with cross-reacting antibodies (proof-of-concept experiments)	Screening Fc-dependent antibodies	Simple & Low cost	Poor human FcγR relevance
FcγR KO mice	Mechanism dissection	Searching for translational prediction	Identify Fc contribution	No human relevance
Non-genOway, single-FcγR humanized mice commercially available	General FcγR interaction studies	High-confidence decision making	Assessment of specific receptor activity	· Often non-physiological expression and regulation · Not all receptors are humanized
genOway's humanized genO-hFcγR mice (fully humanized FcγR)	Translational efficacy and safety	Screening murine antibodies that do not engage human Fcγ receptors (surrogates)	Physiological expression of full human FcγR repertoire	
Human immune system (HIS) mice – genO-BRGSF-HIS mice1	Complex immune interactions	Experiments requiring the expression of hFcγR on stromal cells	Human immune cells	High variability and cost

[1]The genO-BRGSF-HIS mouse model develops a human immune system following reconstitution of immunocompromised genO-BRGSF mice with human CD34+ hematopoietic stem cells (HSCs).

Takeaway: Models with physiological expression of the complete human FcγR repertoire provide the highest translational value

PRACTICAL EXAMPLES

What happens if I test an Fc-engineered antibody in WT mice?

Observed effects:

- Reduced or absent Fc-dependent activity

Consequence:

- False negative efficacy
- Misleading ranking of Fc variants

What model should I use to evaluate Fc optimization?

PREFERRED APPROACH

Humanized FcγR Knockin models with:

- Full receptor repertoire
- Physiological regulation

Why:

- Captures binding differences between Fc variants
- Captures functional effects (ADCC/ADCP)
- Captures safety-relevant activation

How to design an ADCC study in mice

Use models expressing human FcγRIIIA (CD16A)

Ensure:

- Presence of functional NK cells
- Correct FcγR expression levels

Select appropriate:

- Tumor model (syngeneic vs xenograft)
- Antibody dose range

Avoid:

- WT mice for human IgG1 ADCC evaluation

What endpoints could be measured?

Efficacy endpoints:

- Tumor growth inhibition
- Target cell depletion
- Survival

Mechanistic endpoints:

- NK cell activation markers
- Phagocytosis assays
- Receptor occupancy

Safety endpoints

- Cytokine profiling
- Immune cell activation status
- Off-target effects

What are the limitations of FcγR mouse models?

Even humanized models:

- May not fully recapitulate human immune complexity due to the differences between murine and human biology, such as the decreased ADCC ability by murine NK cells, and differences between IgG-induced complement responses in humans and mice
- Can differ in cell distribution or activation thresholds

HIS models:

- High variability due to different HSC donors
- Xenogeneic artefacts

Tumor models:

- Lack of fully human tumor microenvironment

Conclusion: FcγR models improve predictivity but must be interpreted within context

FAQ

What is the best FcγR model for antibody studies?

The most appropriate FcγR mouse model is one that expresses the full set of human Fcγ receptors under physiological regulation such as the **genO-hFcγR** model, as this enables accurate recapitulation of Fc-mediated immune engagement and supports translationally relevant assessment of antibody efficacy and safety (Van Damme et al., 2026, Nimmerjahn & Ravetch, 2008; Bruhns, 2012).

When do I need a humanized FcγR mouse model?

A humanized FcγR mouse model is required when evaluating therapeutic antibodies whose mechanism of action depends on Fc-mediated effector functions. This is particularly important in oncology and immunology, where the accurate prediction of ADCC, ADCP and cytokine responses is essential.

How are FcγR models used to evaluate therapeutic antibodies?

Humanized FcγR models are used to assess antibody efficacy, mechanism of action, and safety by enabling *in vivo* engagement of immune effector cells through human Fc receptors, thereby providing insight into ADCC, ADCP, and cytokine-mediated effects. Additionally, PK studies can also be conducted when using mouse models containing both humanized FcγR and humanized FcRn, such as the genO-hFcγR/hFcRn model.

What are the differences between FcγR humanized mouse models?

FcγR humanized mouse models differ in the number of receptors humanized, their expression patterns, and whether they are expressed under endogenous regulatory control, with models recapitulating the full human FcγR repertoire, such as the genO-hFcγR model, generally providing superior translational relevance compared to partial or non-physiological systems.

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Featuring the genO-hFcγR (scan or click)

Featuring the genO-BRGSF-HIS (scan or click)

How are FcγR models used in ADCC studies?

FcγR models allow evaluation of ADCC by supporting the interaction between antibody-opsonized target cells and FcγR-expressing effector cells such as NK cells, enabling quantification of tumor cell killing and immune activation.

How do Fcγ receptors influence antibody safety?

Fcγ receptors influence antibody safety by mediating immune activation and cytokine release, which can contribute to systemic inflammation or adverse events when excessive or non-specific Fc engagement occurs.

How do Fc engineering modifications affect FcγR interactions?

Fc engineering modifications, such as glycoengineering or point mutations, alter the affinity of antibodies for specific Fcγ receptors, enabling tuning of effector functions to enhance or decrease efficacy or reduce toxicity, depending on the context.

How do Fcγ receptors influence antibody efficacy?

Fcγ receptors influence antibody efficacy by determining the strength and quality of effector cell engagement, with activating receptors promoting cytotoxicity and phagocytosis, while inhibitory receptors dampen immune responses, thereby shaping the overall therapeutic outcome (Nimmerjahn & Ravetch, 2008).

How do FcγR polymorphisms impact antibody function?

FcγR polymorphisms can significantly influence antibody binding affinity and downstream effector functions, contributing to variability in patient responses to antibody therapies. Consequently, we developed hFcγR models expressing either the FcγRIIA R131 and FcγRIIIA F158 haplotypes (lower affinity) or the FcγRIIA H131 and FcγRIIIA V158 haplotypes (higher affinity).

Can FcγR models be intercrossed with FcRn or other models?

FcγR models can be intercrossed with FcRn humanized models (genO-hFcγR/hFcRn) or other immune-humanized models (genO-hVISTA/hFcγR, genO-hPD-1/hFcγR, genO-hCTLA-4/hFcγR, genO-hCCR8/hCCL1/hFcγR) expressing the humanized target of the Fab region to simultaneously assess antibody effector function and pharmacokinetics, providing a more comprehensive evaluation of therapeutic antibodies.

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