Assessing Absence of Effector Function: Binding and Primary Cell-Based Assays for Biosimilar Development

Andrew Baron*, Alison Kerr*, Rebecca Bonner, Eric Cruikshank, Lauren Gilfillan, Dion Gilmour, Amy Graham, Kate McDonald, Fiona Miller, Sophie Moncrieff, Pim Robertson, Jessica Walker, Rhiannon Williams

RoukenBio, Rouken Discovery Centre, Braidhurst Industrial Estate, Motherwell, ML1 3ST, UK

Corresponding authors: abaron@rouken.bio & akerr@rouken.bio

Introduction

Demonstrating similarity to the reference product is essential to ensure comparable safety and efficacy for a biosimilar. For some formats, such as IgG2, IgG4 and engineered IgG1 variants, this may include confirming the absence of Fc-mediated binding and function. Regulators such as the CDSCO, FDA and EMA require robust evidence that all pertinent biological activities, whether present or absent, are matched to the originator, as differences may impact clinical outcomes. However, assumptions about complete Fc silencing can be misleading, for example, LALA mutations often considered 'silent' may retain receptor interactions and residual activity. Advanced binding and functional assays are therefore critical to characterise Fc properties with high sensitivity and physiological relevance. This approach supports biosimilar developers in meeting regulatory expectations and achieving a totality of evidence framework for comparability.

IgG variant panel assessed for effector function

IgG1
- Wild Type
- STR
- LALA
- LALAPG

IgG2
- Wild Type
- STR

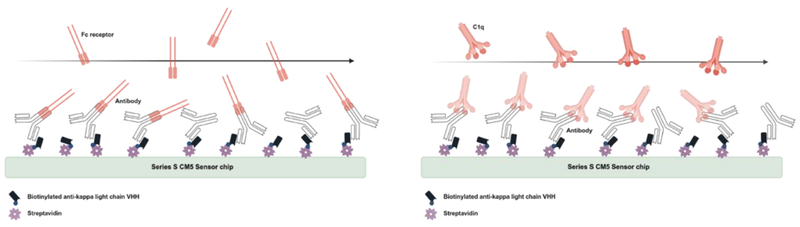
IgG4
- Wild Type
- STR

The **IgG1** isotype exhibits **strong FcγRIIIa and C1 q affinity,** enabling ADCC and CDC activity. **IgG2** and **IgG4** isotypes show **reduced effector function** due to lower affinities, serving as safer alternatives **with retained measurable activity.** The **LALA mutation,** commonly used for silencing, **decreases Fc interactions and functions.** The **LALAPG** mutation **further reduces** these residual activities. Conversely, the **STR mutation,** involving three alterations in the Fe CH2 domain, **fully abrogates Fe binding and effector functions** while preserving other Fc properties.

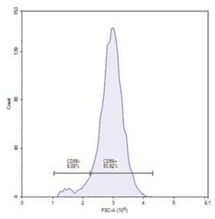
Methods

The antibodies were subject to testing in binding and functional assays, using RoukenBio's platform methodologies and standard assay designs.

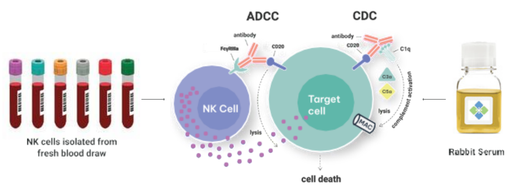
FcγR and C1q Binding



ADCC and CDC



Flow cytometry staining of the effector preparation demonstrated >90% purity of the CD56+ cell population

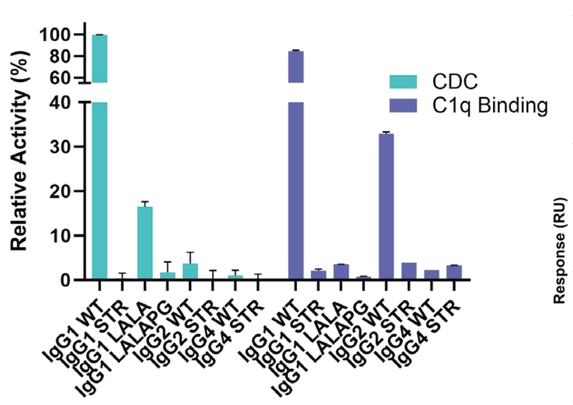


Benefits of RoukenBio's approach

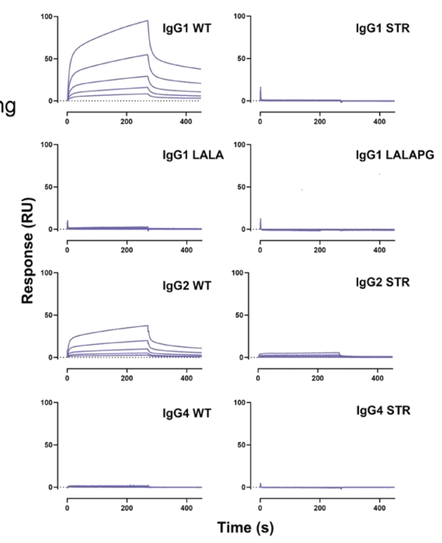
- Platform methods with standard assay designs greatly reduces time spent in optimisation
- Control of donor material and Fc receptor stocks
- Both highly sensitive and highly physiologically relevant methods available
- Custom target cell line development in-house by our highly experienced Cell Engineering team
- Low sample volume requirements

Results

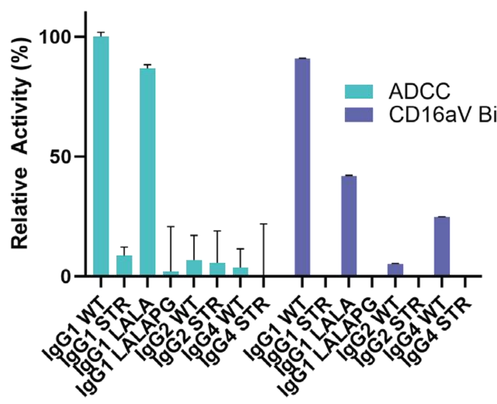
C1q Binding and CDC shows minimal activity across the IgG variant panel



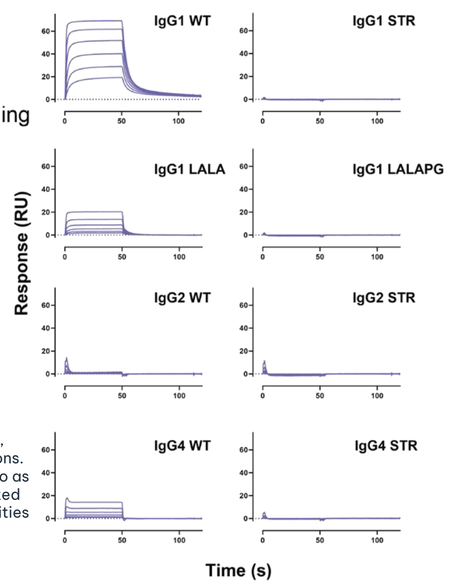
IgG1 wild type showed high C1q binding and CDC activity. In contrast, the variant panel demonstrated minimal effector function overall, with only modest residual activity observed for IgG1 LALA. Data highlights the importance of orthogonal testing and confirmation of residual function.



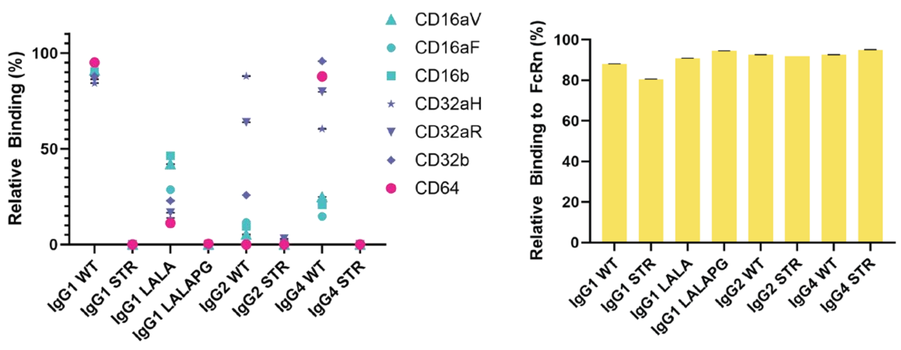
CD16a (FcγRIIa) binding and ADCC is not always as 'silent' as expected



IgG1 wild-type showed strong CD16a binding and ADCC activity, while most of the variant panel significantly reduce these functions. By contrast, the IgG1 LALA mutation which is typically referred to as 'silenced' retained 42% of IgG1 WT's CD16aV binding and mediated 87% of its ADCC activity. The clinical significance of these activities should be carefully considered.



Distinct FcγR binding profiles highlight considerations for Biosimilars



All IgG variants exhibited unique binding patterns across the Fcγ receptors. While IgG1 wild-type showed strong interactions, variants such as LALA, IgG2, and IgG4 retained binding to certain receptors, including CD64 and CD32, which are implicated in ADCP, whereas CD16 is more relevant to ADCC. Fc receptor engagement is not uniformly abolished by variant selection. For biosimilar development, the specific FcγR binding characteristics of the chosen IgG format is critical, influences effector functions and ultimately, clinical outcomes.

SUMMARY

Demonstrating Fc functional similarity is critical for biosimilar antibodies to ensure comparable safety and efficacy to the reference product. Our data show that IgG subclasses and engineered variants exhibit distinct Fcγ receptor binding profiles and effector functions. While IgG1 wild-type engages multiple receptors strongly, variants such as LALA, IgG2, and IgG4 retain selective interactions with receptors linked to ADCP and ADCC, highlighting that Fc silencing is not absolute. Complement activation (C1q binding and CDC) and ADCC activity are similarly reduced but not uniformly abolished across variants. These findings emphasise the importance of comprehensive Fc characterisation, covering binding and functional assays to understand residual activity and its clinical relevance.

Scan the QR code and take the digital version of this poster home in your pocket.