Effective IgG1 Fc Silencing: Characterization of Residual Binding and Functional Activity

Fiona Miller, Eric Cruikshank, Sophie Moncrieff, Amy Graham, Dion Gilmour, Kate McDonald, Jessica Walker, Pim Robertson, Rebecca Bonner, Rhiannon Williams, Andrew Baron, Agapitos Patakas, Nichole Cerruti, Lauren Gilfillan, Lewis Campbell and Adele Hannigan

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

Introduction

Monoclonal antibodies (mAbs) are pivotal in mediating effector functions by engaging with Fc gamma receptors on immune cells and initiating the complement cascade via their Fc domains. However, for therapeutic applications where effector function is undesirable, a range of Fc 'silencing' techniques has been developed to mitigate or nullify CDC and ADCC responses. Despite these efforts, residual binding and functional activity are often still detectable. mAbsolve's innovation, the STR mutation, marks a significant advancement in Fc silencing, demonstrating complete cessation of Fc binding and functional activities. Leveraging RoukenBio' advanced platform methodologies, we rigorously assess antibody constructs to ensure effective silencing, setting a new standard in the development of safer therapeutic antibodies.

Anti-CD20 IgG1 variants assessed

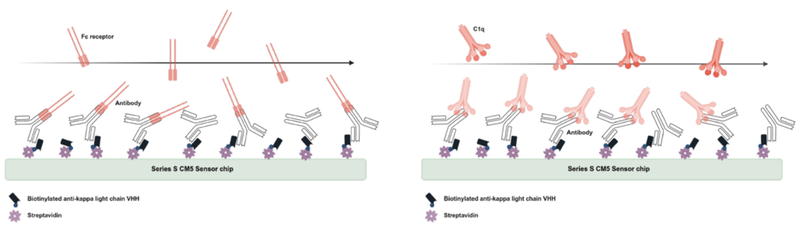
IgG1	IgG2	IgG4
• Wild Type • STR • LALA • LALAPG	• Wild Type • STR	• Wild Type • STR

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 Fc CH2 domain, **fully abrogates Fc binding and effector functions** while preserving other Fc properties.

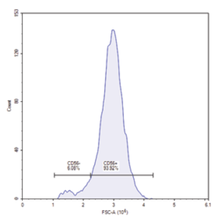
Methods

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

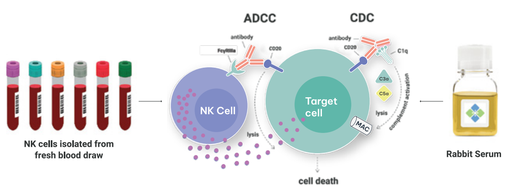
FcγR and C1q Binding



ADCC and CDC



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



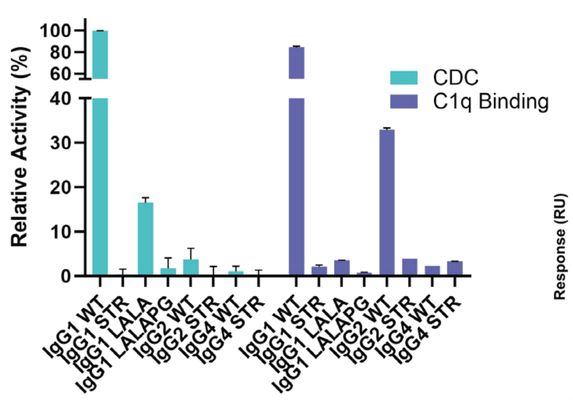
Interested in other ways to characterise Fc binding? **Scan the QR code** to access information on our binding dependency assay

Benefits of RoukenBio's approach

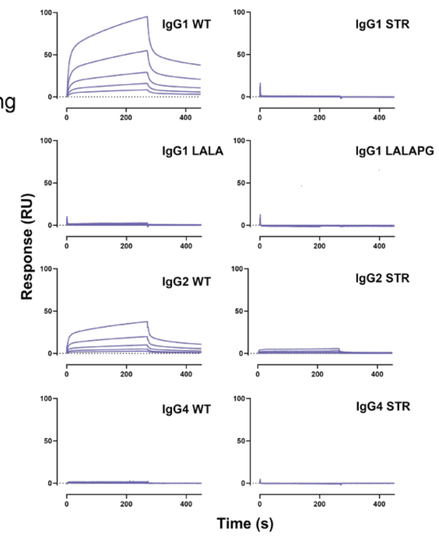
- Platform methods with standard assay designs greatly reduces time spent in optimisation
- Control of donor material and Fc receptor stocks
- Both highly sensitive and highly physiologically relevant methods available
- Target cell line development in-house by our Cell Line Development group
- Low sample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

Results

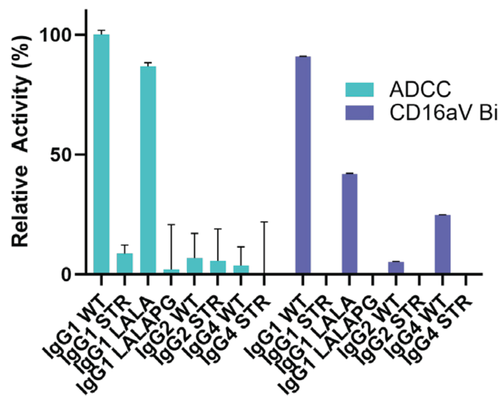
STR mutation achieves inhibition of C1q Binding and CDC across all isotypes



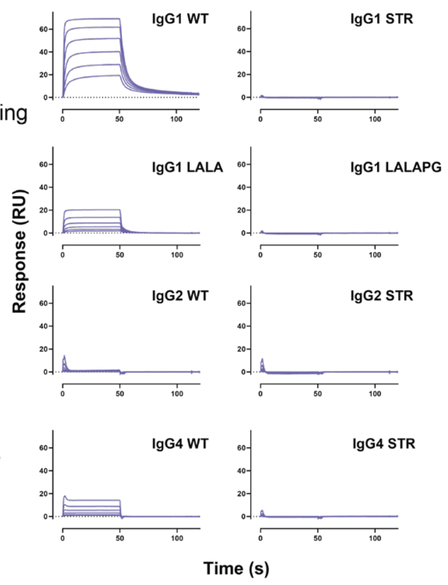
Antibodies with the STR mutation displayed neither significant binding to C1 q nor functional activity in CDC assays across all isotypes. In comparison, IgG2 isotype exhibited 33% of IgG1 WT's C1 q binding, and the LALA mutation showed a mediated <CDC activity of 17% relative to IgG1 WT.



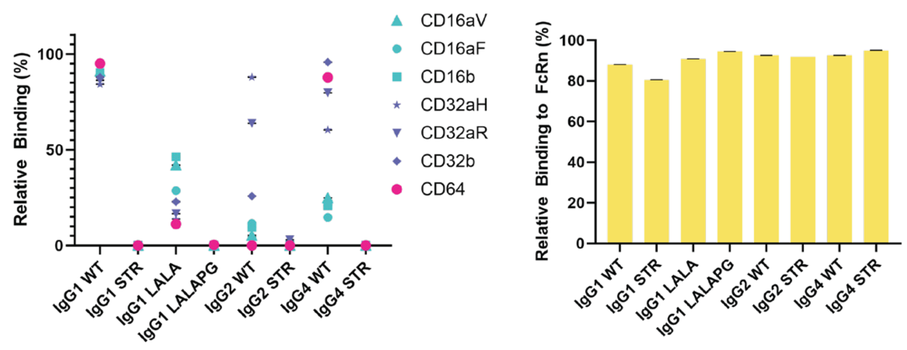
STR mutation nullifies CD16a binding and ADCC functionality across all isotypes



The STR mutation in antibodies led to negligible binding to CD16aV and no detectable functional activity in ADCC assays, as opposed to WT antibodies of all isotypes. By contrast, antibodies with the LALA mutation retained 42% of IgG1 WT's CD16aV binding and mediated 87% of its ADCC efficacy.



STR mutation prevents binding to all FcγR, without impacting binding to FcRn



The STR mutated antibodies demonstrate no binding to all FcγR members. Binding to FcRn is not impacted by the STR mutation, with comparable affinity and normalised binding relative to the IgG1 WT. The LALAPG mutation performed similarly to the STR mutation, while the LALA mutation and IgG2 and IgG4 WT antibodies demonstrated residual binding to the FcγR to varying degrees.

SUMMARY

Our data conclusively shows that neither employing IgG2 and IgG4 isotypes nor the LALA mutation completely abolishes Fc binding and function-evidenced by SPR binding assays and primary cell functional tests. Only the STR mutation achieves complete Fc silencing. These findings highlight the necessity of conducting both binding and primary cell functional assays to fully characterize Fc activity and comprehend the implications of any lingering activity.

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