Interactions of Fcγ receptors with an IgG4 format, anti-FcRn monoclonal antibody, rozanolixizumab

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* At the time of the study

Objective

Testing the binding and functional consequences of FcγR engagement by humanized anti-FcRn mAbs, in particular the IgG4 mAb rozanolixizumab

Background

- FcRn expressed on phagocytic leukocytes as well as on non-hematopoietic cells, prolongs the circulating half-life of IgG by facilitating its recycling from endosomes (**Figure 1**). This process can preserve the half-life of pathogenic autoantibodies and therefore targeting FcRn is of interest as a therapeutic approach in autoimmune diseases that are driven by IgG autoantibodies, such as myasthenia gravis
- Rozanolixizumab is a humanized anti-FcRn mAb of the IgG4P sub-class, the 'P' denoting a Ser to Pro mutation to prevent so-called Fab arm exchange. Rozanolixizumab is currently in clinical development for the treatment of IgG autoantibody-driven diseases, and has demonstrated clinical efficacy in patients with generalized myasthenia gravis[1]
- IgG4 is a common format for therapeutic mAbs due to its intrinsic lower affinity for FcγRs (which are present on immune cells) and because it does not engage C1q, and therefore inadvertently activate the complement pathway
- The current *in vitro* study explored the binding and functional consequences of direct FcγR engagement by rozanolixizumab and another anti-FcRn agent, MST-HN-Fc (*aka* efgartigimod) which is a mutated IgG1 Fc fragment with a higher affinity for FcRn over wild-type Fc

Design/methods

- Binding of fluorophore-labeled (Alexa-647) anti-FcRn agents and relevant controls was investigated by flow cytometry using HEK293 cells stably expressing the high affinity FcγR, FcγRI. IgG competition for binding was tested by titrating an IVIg preparation
- Surface expression of FcγRs following incubation with anti-FcRn agents was investigated by high-content imaging using human monocyte-derived macrophages (derived from adherent monocytes cultured for 5 days in M-CSF). After stimulation with interferon-γ, macrophages were incubated over time with test agents and then stained for FcγRI and FcγRII expression. Western blotting experiments were also conducted to measure intracellular levels of FcγRs
- Cytokine release following incubation with anti-FcRn antibodies was measured from human whole blood, HUVECs and macrophage cultures. After incubation with rozanolixizumab offered either in solution, plate immobilized, crosslinked with an $F(ab')_2$ fragment anti-human Fc or bead-immobilized, the release of a range of pro-inflammatory cytokines (IFNγ, IL-12p70, IL-1β, IL-6, IL-8, IL-10 and TNFα) was assessed by the MesoScale Discovery method

Results

- As predicted, rozanolixizumab was able to bind to FcγRI in cell-based assays (**Figure 2**)
  - The binding was completely inhibited by exogenous IgG, including at concentrations considerably lower than one would observe in patients treated with rozanolixizumab
  - Other Fc-containing agents, such as MST-HN-Fc and isotype control reagents, demonstrated similar properties
- Antibody bipolar bridging (ABB) is the capacity through which a mAb concomitantly binds its membrane antigen through its Fab domain and a membrane Fc receptor via its Fc region
  - Rozanolixizumab was able to mediate ABB, leading to a down-regulation of FcγRI (but not FcγRII) from the cell surface on macrophages (**Figure 3**).
  - The down-regulation in FcγRI was dependent on both the Fab and the Fc regions, since a Fab version of rozanolixizumab and the MST-HN-Fc did not mediate a reduction
  - Western blotting showed a reduction in FcγRI immunoreactivity with rozanolixizumab, but not following incubation with rozanolixizumab Fab, consistent with internalization and degradation of FcγRI induced by ABB
  - ABB mediated by rozanolixizumab was completely inhibited by low concentrations of exogenous IgG
- Studies incorporating HUVEC and whole blood exposed to soluble and cross-linked rozanolixizumab showed that there was no release of the pro-inflammatory cytokines IL-6, and TNFα (**Figure 4**)
  - The anti-CD52 mAb, Campath was shown to induce cytokine production both in soluble and cross-linked formats
  - There was also no cytokine release using an IgG1 format of rozanolixizumab
  - The same conclusions were drawn from experiments conducted under other conditions and where other cytokines were measured

Figure 1 FcRn and the mechanism of action of rozanolixizumab

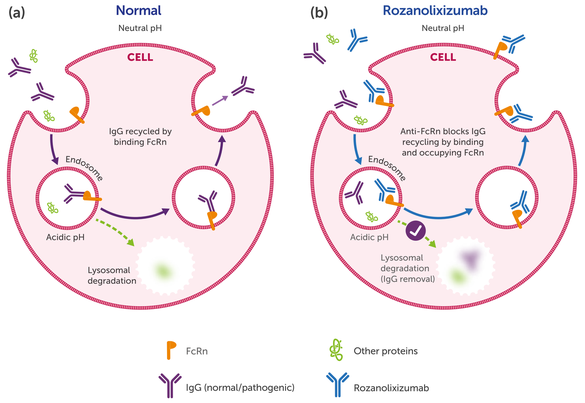
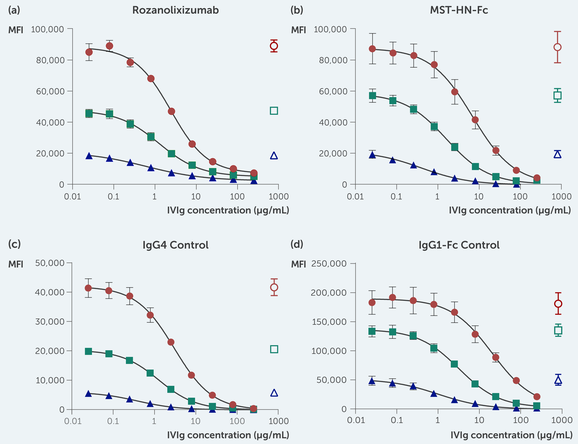
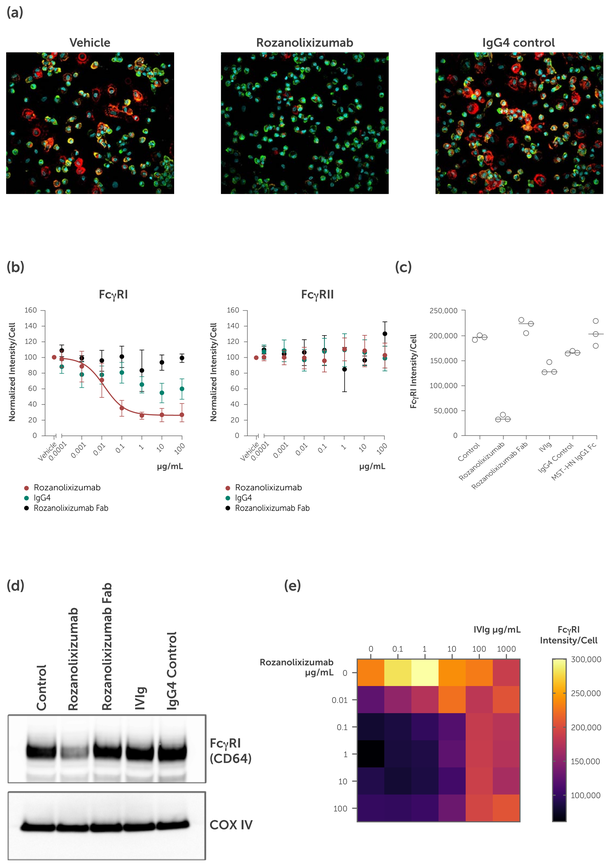


Figure 2 Competitive inhibition of the binding of various Fc-containing agents to FcγRI-expressing cells by IVIg



Competitive binding of Alexa-647-labeled rozanolixizumab (A), MST-HN-Fc (B), and their respective IgG4 IgG (C) and IgG1 Fc (D) isotype controls with IVIg. Three concentrations of each test agent were tested at 25 nM ●, 5 nM ■, and 1 nM ▲ and inhibition of binding was compared in the absence of IVIg at 25 nM ○, 5 nM □ and 1 nM △. The data show median fluorescence intensity (MFI) and are the mean of n=3 experiments ± SD.

Figure 3 Rozanolixizumab specifically reduces the surface labeling of FcγRI on human macrophages, but only in the absence of exogenous human IgG



(A) Surface expression of FcγRI (red; labeled with APC-conjugated anti-CD64) and FcγRII (green; labeled with FITC-conjugated anti-CD32) on interferon (IFN)γ stimulated (2.0 ng/mL, overnight) monocyte-derived macrophages (differentiated with M-CSF [50 ng/mL] for 5 days), and treated with vehicle (PBS), rozanolixizumab or IgG4 isotype control (1.0 µg/mL), for 4 hours. Cells were fixed and labeled with Hoechst (cyan) before image acquisition by confocal microscopy. Images are maximum intensity projections from consecutive confocal z-stacks of representative fields of view. (B) Quantification of FcγRI and FcγRII for macrophages as described in (A) treated with rozanolixizumab, IgG4 control, or rozanolixizumab Fab for 4 hours. Points represent mean and error bars = SEM of normalized data from three independent experiments. (C) FcγRI expression on macrophages as described in (A) treated with rozanolixizumab (10 µg/mL), rozanolixizumab Fab (10 µg/mL), IVIg (100 µg/mL), ET904 (IgG4 isotype control; 10µg/mL) or MST-HN-Fc mutant (10 µg/mL). Data show mean and individual data points from triplicate cultures. (D) SDS-PAGE/Western Blotting analysis of FcγRI in macrophages following culture with rozanolixizumab. (E) Macrophages were cultured with the indicated concentrations of IVIg and rozanolixizumab for 72 hours followed by quantification of FcγRI surface expression. The heat map shows the staining intensity of FcγRI.

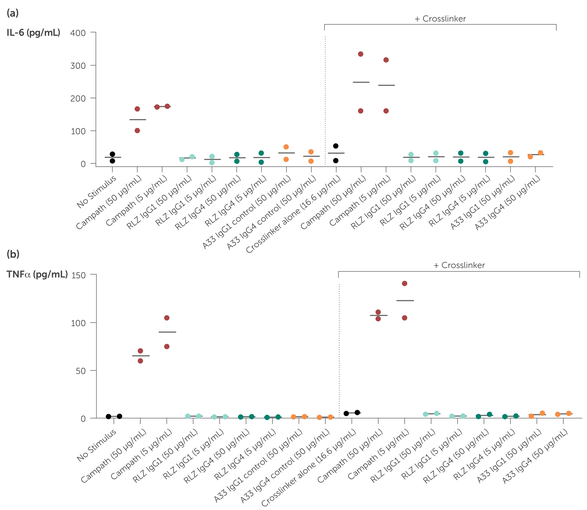
Summary and conclusions

Although rozanolixizumab, like other IgG4 antibodies, can bind to FcγRs and mediate ABB (a functionally relevant response) *in vitro*, these events are completely inhibited by low levels of exogenous IgG (far lower than would be expected even in a patient treated with rozanolixizumab)

Moreover, there was no evidence for cytokine production by immune cells exposed to rozanolixizumab under a broad range of conditions

Overall, the data challenge the relevance and interpretation of *in vitro* FcγR binding assays performed in the absence of competing IgG and support the use of IgG4 as a suitable format for therapeutic mAbs

Figure 4 Rozanolixizumab does not induce the release of IL-6 (a) or TNFα (b) from whole blood/HUVEC cultures



Human whole blood was added to confluent HUVEC cultures in the presence of soluble or cross-linked antibodies (using a cross-linking anti-human Fc antibody at 1/3 of test antibody concentration) (● rozanolixizumab [RLZ] in IgG1 format; ● rozanolixizumab [RLZ] in IgG4 format; ● Campath; or ● isotype control mAbs) for 24 hours at 37°C before supernatants were harvested for subsequent cytokine quantification. Each dot is the mean of 2 determinations from one of two separate donors.

Disclosures of interest: All the studies reported here were funded by UCB Pharma. All authors except for NB & OQ were employees of UCB Pharma when all studies were carried out: OQ was an employee of Celentyx Ltd when some of the experiments were conducted and NB was an employee at Celentyx Ltd throughout the study; GM is now an employee of AstraZeneca UK; and GC is now an employee of Dark Blue Therapeutics.

Abbreviations: ABB, Antibody bipolar bridging; FcRn, neonatal Fc Receptor; FcγR, Fc-gamma receptor; HUVECs, human umbilical vein endothelial cells; Ig, immunoglobulin; IVIg, intravenous immunoglobulin; mAbs, monoclonal antibody.

References: Bril *et al. Lancet Neurol.* 2023, 22: 383

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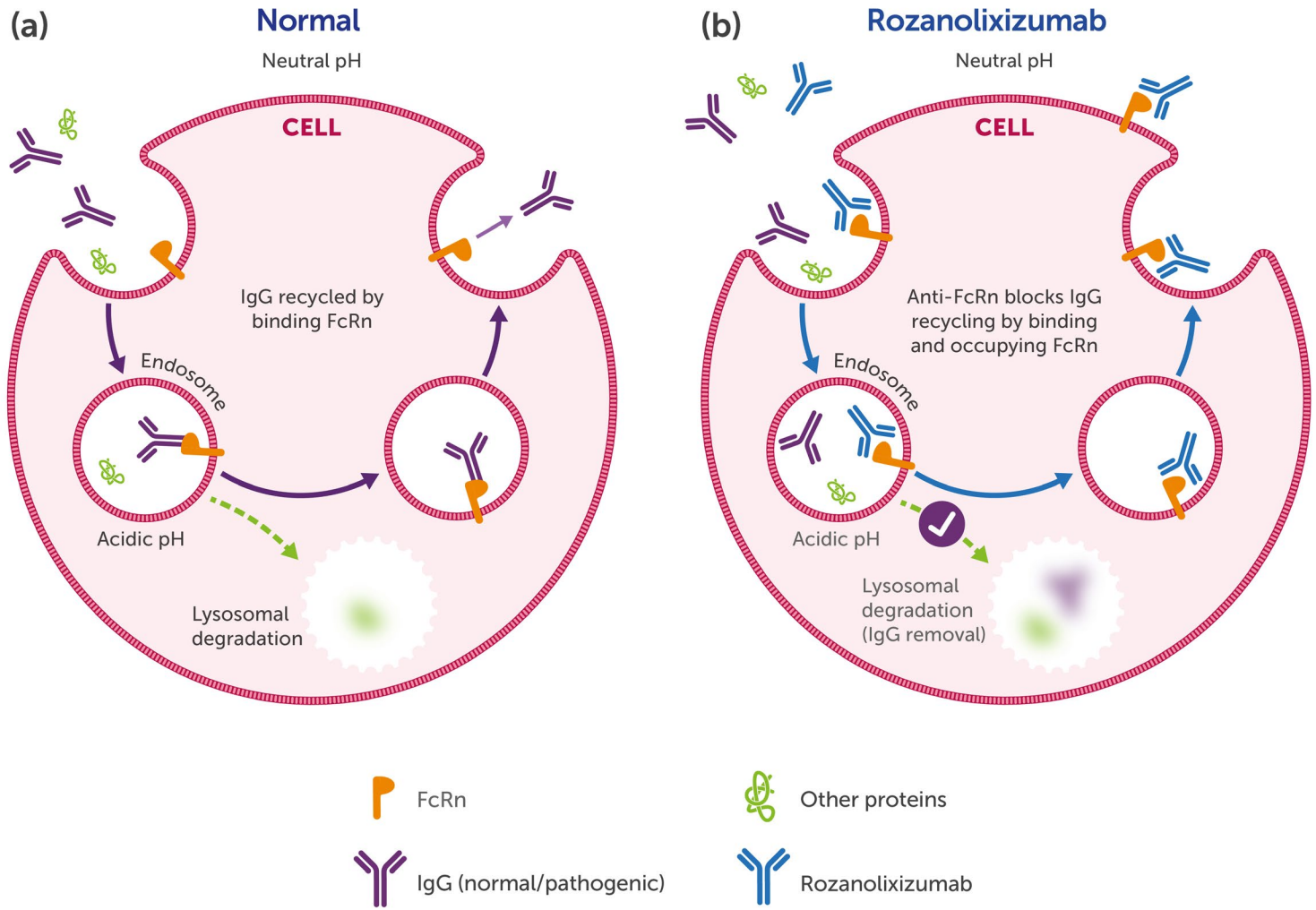

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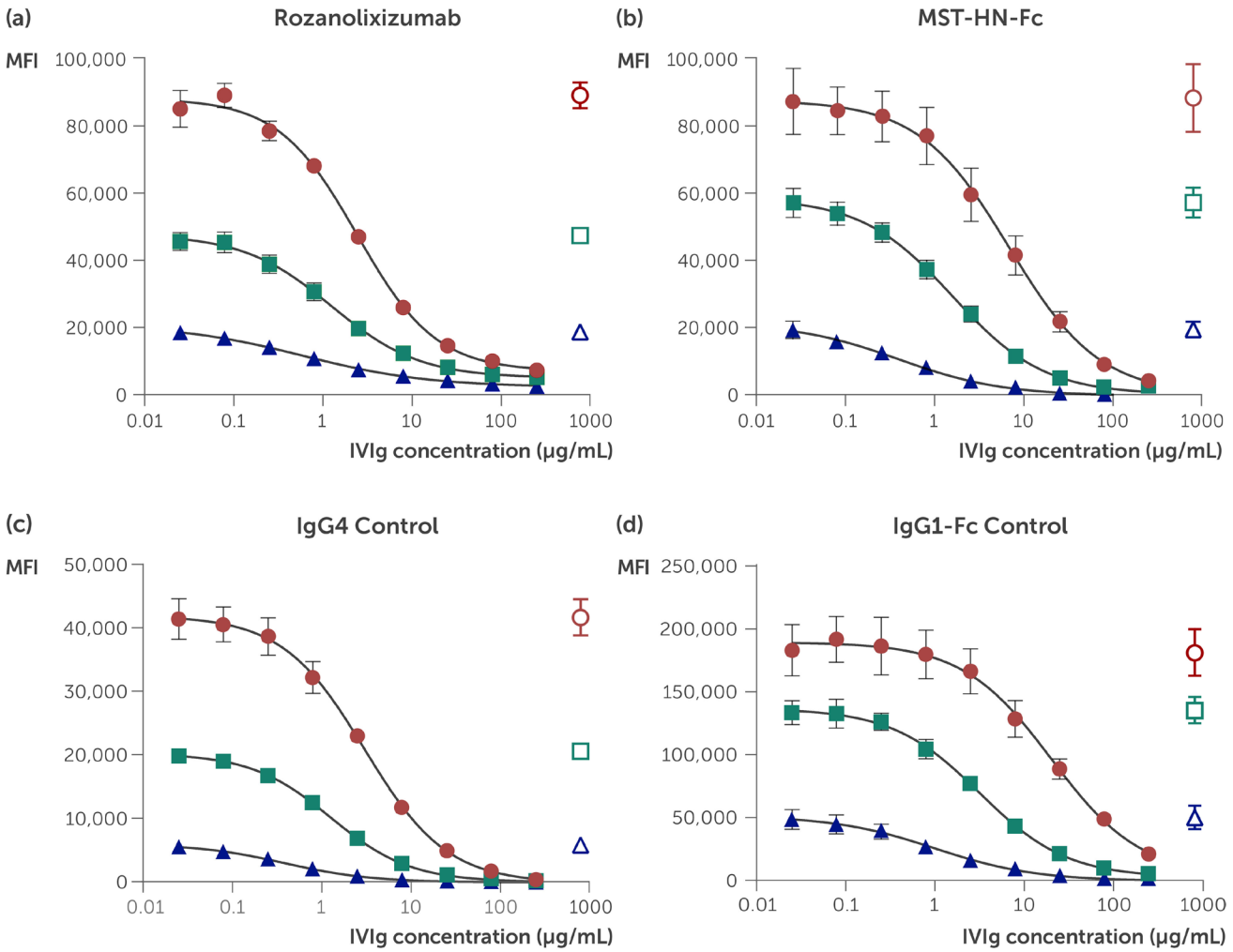

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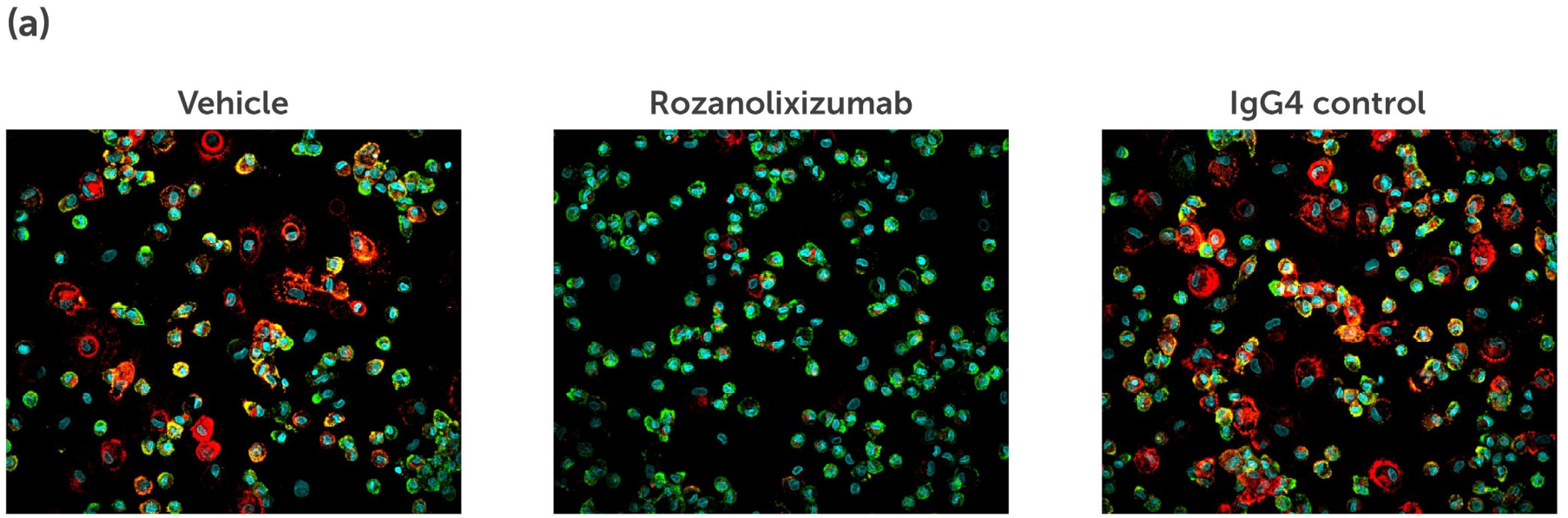

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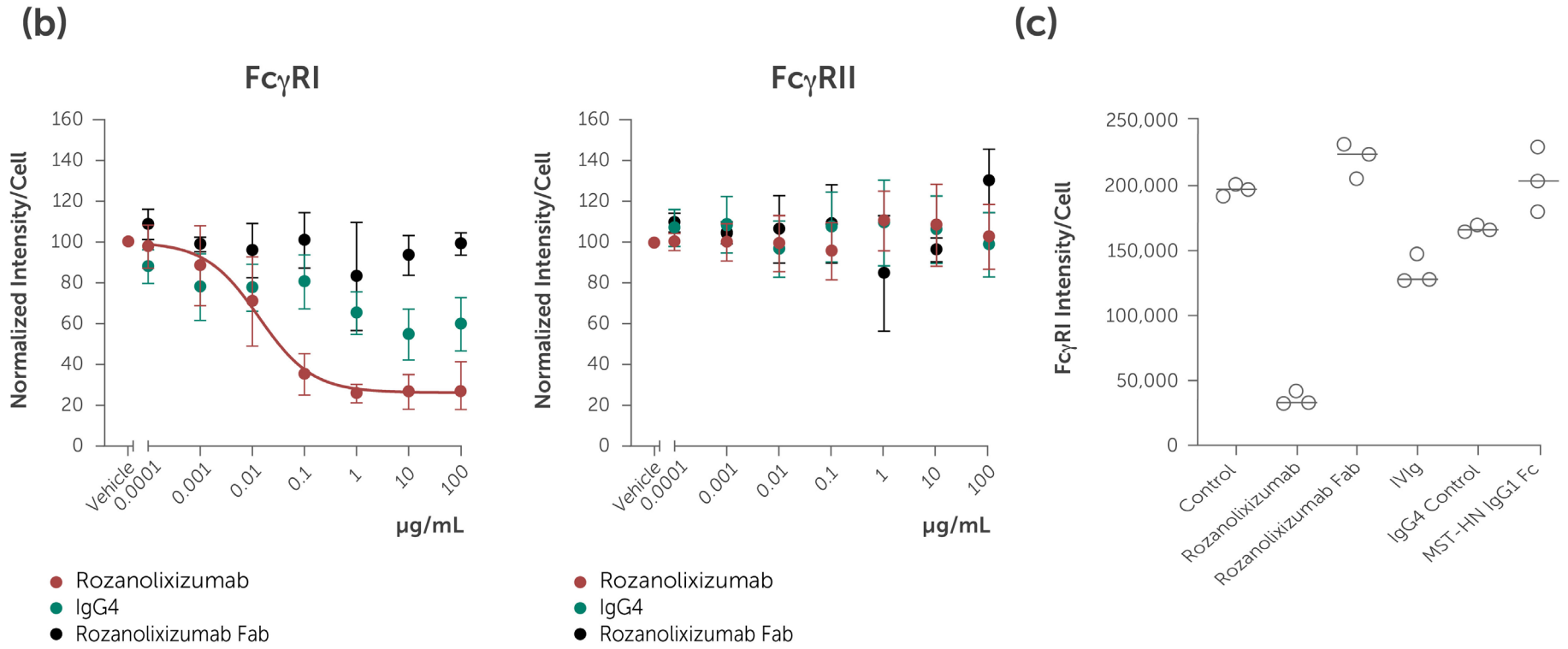

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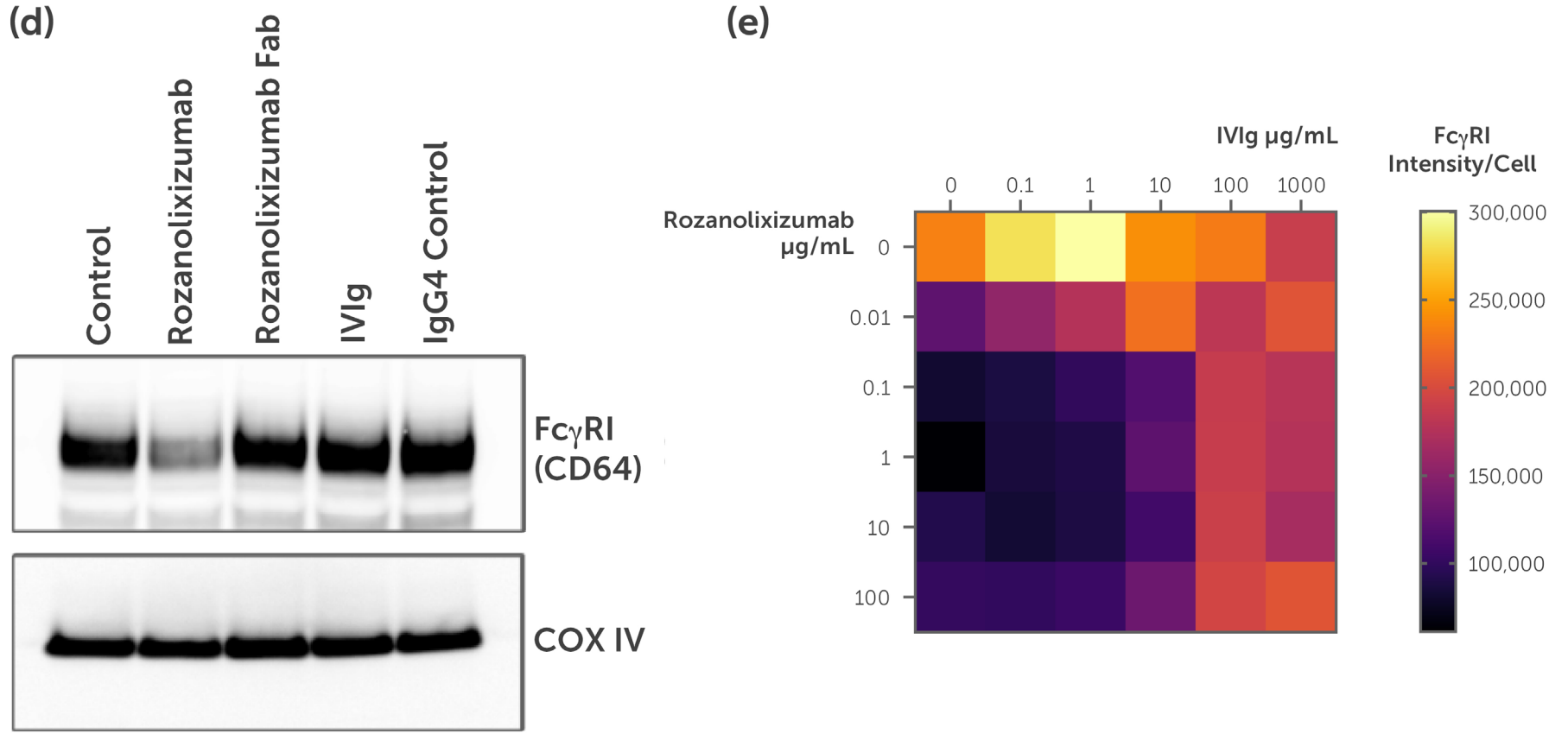

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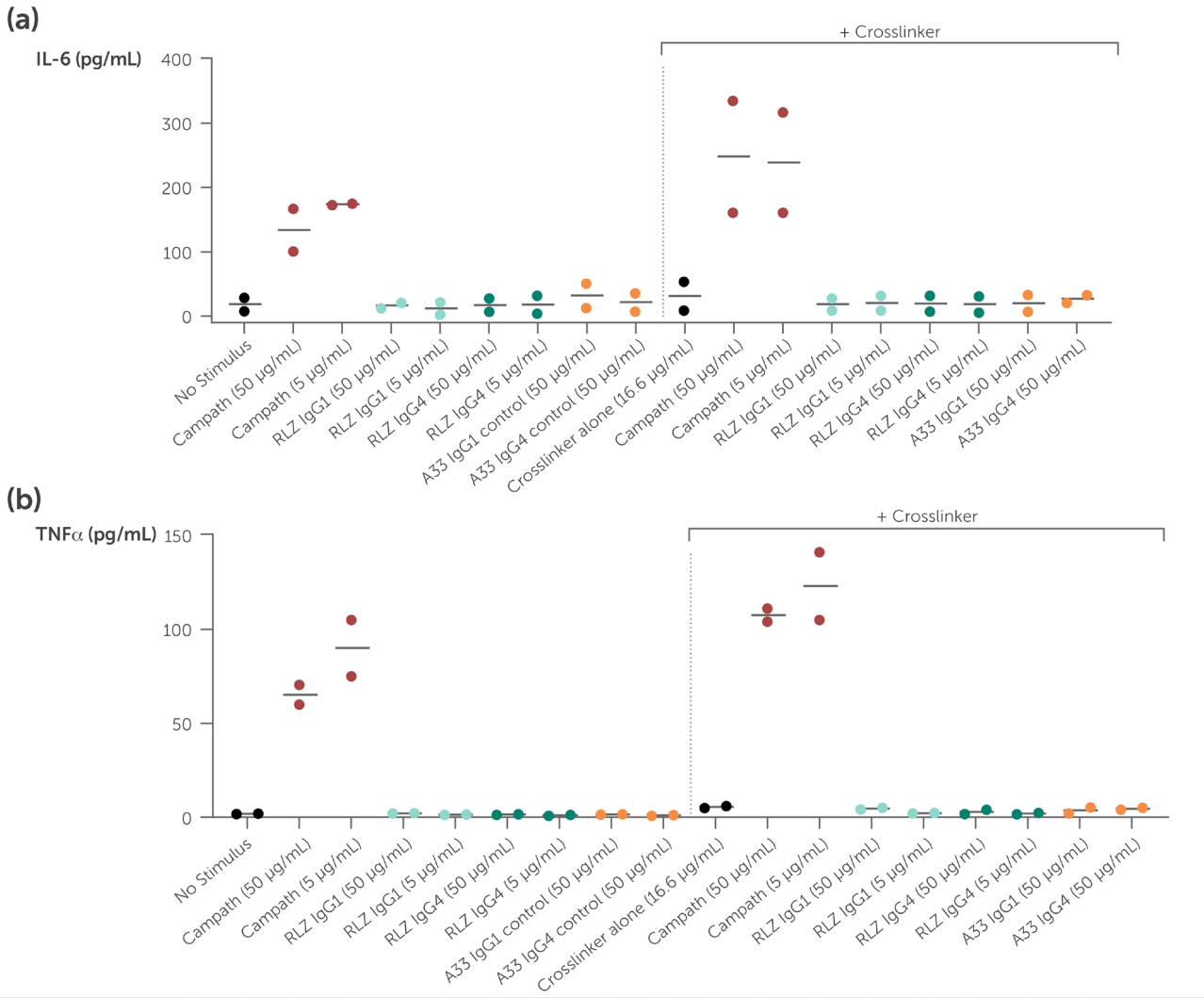

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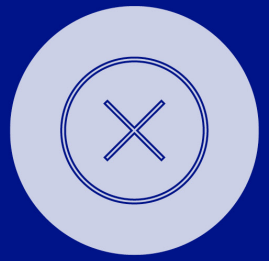

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