High valency of IGM-2323, a CD20xCD3 IgM bispecific T cell engager, displaces rituximab binding and induces potent B lymphoma cell killing

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Background

- Rituximab-containing treatment regimens are the standard of care for patients with non-Hodgkin's lymphoma (NHL). However, the majority of patients ultimately experience disease relapse or progression indicating resistance to rituximab (Rtx) therapy.
- IGM-2323 is an engineered high-affinity, high-avidity anti-CD20 pentameric IgM antibody with an anti-CD3 scFv fused to the joining (J) chain. IGM-2323 offers a novel treatment strategy in NHL through two mechanisms: 1) the recruitment of T cells to kill CD20-expressing tumor cells through T cell dependent cellular cytotoxicity (TDCC), and 2) complement-dependent cytotoxicity (CDC).
- We evaluated the activity of IGM-2323 in the presence of rituximab since rituximab can persist in patients after treatment discontinuation, and it can bind to an overlapping epitope on CD20 as IGM-2323. We hypothesized that the high valency of IGM-2323 could displace rituximab, thus enabling potent B cell killing by IGM-2323 even in the presence of high concentrations of rituximab.

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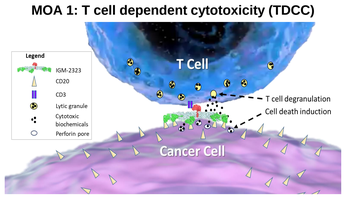

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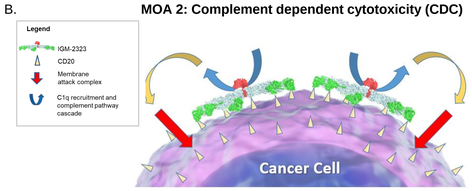


Figure 1. IGM-2323 has two potential mechanisms of action in killing B cell lymphomas: **A)** T cell dependent cytotoxicity (TDCC), and **B)** complement dependent cytotoxicity (CDC).

IGM-2323 and Rtx Bind to an Overlapping Epitope on CD20

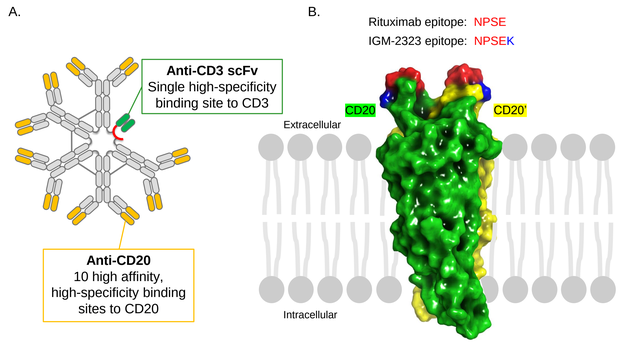
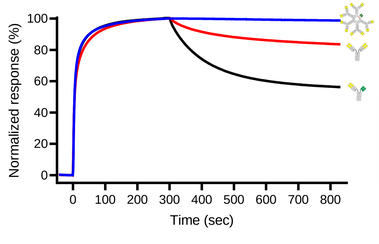


Figure 2. A) Schematic diagram of IGM-2323 with 10 binding domains for CD20 and a single binding domain for CD3 attached to the J-chain. **B)** A model of human CD20 dimer showing that IGM-2323 and Rtx have overlapping epitopes on the extracellular domain. The overlapping residues that bind to IGM-2323 and Rtx are highlighted in red and the unique residue to IGM-2323 in blue.

IGM-2323 Binds with High Affinity to CD20

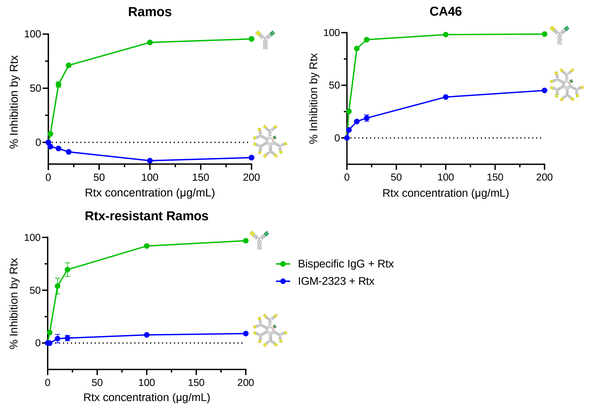


Antibody	$K_D$app (nM)
IGM-2323	0.008
Bivalent anti-CD20 IgG	0.320
Bispecific CD20xCD3 IgG	2.133

Figure 3. The affinity of IGM-2323 and its corresponding bivalent anti-CD20 IgG antibody, and bispecific CD20xCD3 IgG antibody to recombinant human CD20 protein were measured by surface plasmon resonance (SPR). IGM-2323 (blue) bound to human CD20 with an apparent 40- and ~300-fold higher binding affinity (K_D) than the bivalent anti-CD20 IgG (red), and bispecific CD20xCD3 IgG (black), respectively.

IGM-2323 Displaces the Binding of Rtx on B Lymphoma Cells While a Bispecific IgG is Severely Inhibited by Rtx

A.



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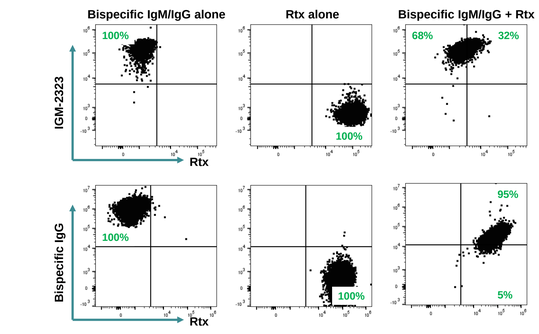


Figure 4. A) Percent inhibition of binding of 200 nM IGM-2323 (blue) or 200 nM bispecific CD20xCD3 IgG (referred to as bispecific IgG) (green) to three human B lymphoma cell lines by Rtx. B cell lines with a range of CD20 expression levels, including wild type Ramos (referred to as Ramos), CD20-low CA46 and a Rtx-resistant Ramos cell variant, were pre-treated with 2 to 200 µg/mL Rtx, and subsequently evaluated for IGM-2323 or bispecific IgG cell binding by flow cytometry. At high concentrations of Rtx, which correspond to reported peak serum concentrations (C_{max}) found in Rtx-treated patients, IGM-2323 displaced the binding of Rtx on human B cell lines. In contrast, binding of a bispecific IgG was inhibited by the C_{max} of Rtx. % inhibition was calculated by comparing the cell binding of IGM-2323 or a bispecific IgG alone +/- Rtx. **B)** Dot plots comparing the binding of 200 nM IGM-2323 or 200 nM bispecific IgG +/- 200 µg/mL Rtx on wild type Ramos cells by flow cytometry. A double positive population shows the binding of the bispecific antibody and Rtx on the same cells.

IGM-2323 CDC of B Lymphoma Cells is Minimally Inhibited by Rtx

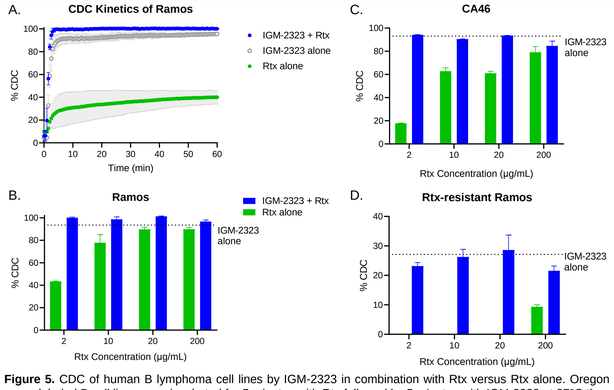


Figure 5. CDC of human B lymphoma cell lines by IGM-2323 in combination with Rtx versus Rtx alone. Oregon green-labeled B cell lines were incubated for 5 minutes with Rtx followed by 5 minutes with IGM-2323 at 37°C then 10% normal human serum (NHS) was added to the cells. **A)** The kinetics of cell death was imaged with a Lionheart automated microscope immediately every 30 seconds for 1 hour using DRAQ7 to quantify dead cells. Percent CDC of 200 µg/mL IGM-2323 in combination with 2 to 200 µg/mL Rtx (blue) compared to 2 to 200 µg/mL Rtx alone (green) was determined for **B)** Ramos, **C)** CA46, and **D)** Rtx-resistant Ramos cells. The horizontal dotted line indicates the % CDC of 200 µg/mL IGM-2323 alone. There was no killing of Rtx-resistant Ramos cells with <200 µg/mL Rtx.

IGM-2323 Maintains Fast Killing Kinetics by CDC in the Presence of Rtx

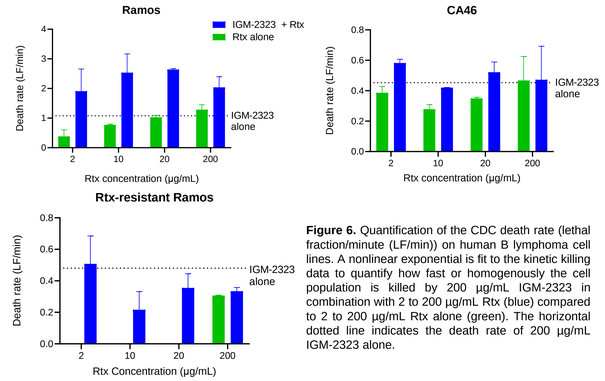


Figure 6. Quantification of the CDC death rate (lethal fraction/minute (LF/min)) on human B lymphoma cell lines. A nonlinear exponential is fit to the kinetic killing data to quantify how fast or homogenously the cell population is killed by 200 µg/mL IGM-2323 in combination with 2 to 200 µg/mL Rtx (blue) compared to 2 to 200 µg/mL Rtx alone (green). The horizontal dotted line indicates the death rate of 200 µg/mL IGM-2323 alone.

No Inhibition of Maximum T Cell Killing by IGM-2323 in the Presence of Rtx Compared to Bispecific IgG

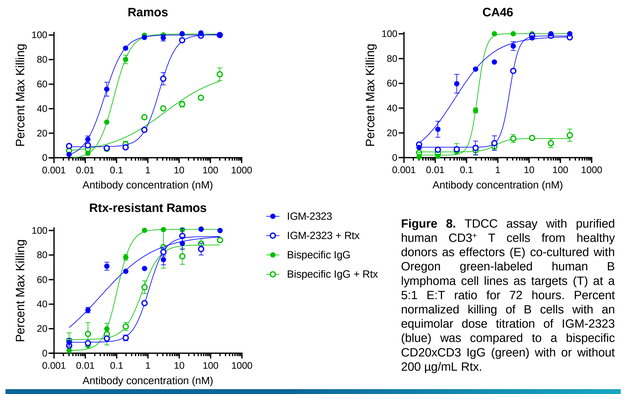
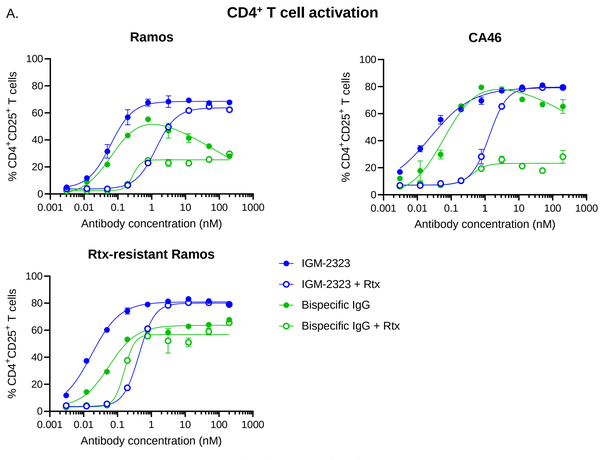


Figure 8. TDCC assay with purified human CD3⁺ T cells from healthy donors as effectors (E) co-cultured with Oregon green-labeled human B lymphoma cell lines as targets (T) at a 5:1 E:T ratio for 72 hours. Percent normalized killing of B cells with an equimolar dose titration of IGM-2323 (blue) was compared to a bispecific CD20xCD3 IgG (green) with or without 200 µg/mL Rtx.

No Inhibition of Maximum T Cell Activation by IGM-2323 in Presence of Rtx Compared to Bispecific IgG

A.



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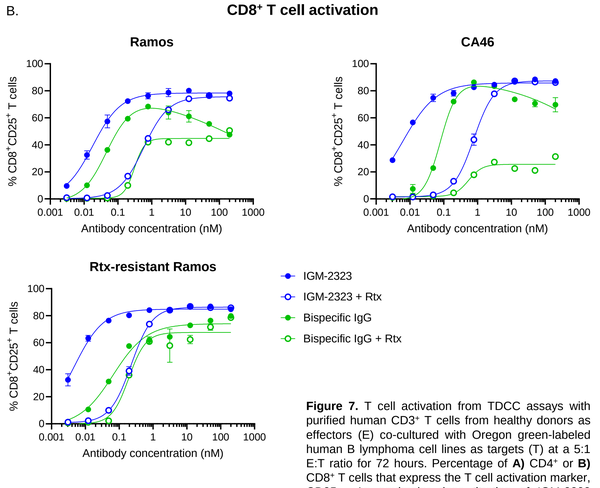
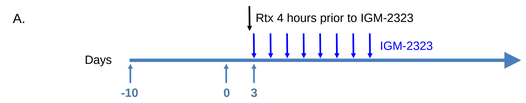


Figure 7. T cell activation from TDCC assays with purified human CD3⁺ T cells from healthy donors as effectors (E) co-cultured with Oregon green-labeled human B lymphoma cell lines as targets (T) at a 5:1 E:T ratio for 72 hours. Percentage of **A)** CD4⁺ or **B)** CD8⁺ T cells that express the T cell activation marker, CD25. An equimolar dose titration of IGM-2323 (blue) was compared to a bispecific CD20xCD3 IgG (green) with or without 200 µg/mL Rtx.

In Vivo Anti-Tumor Activity with IGM-2323 + Rtx is More Efficacious than Single Agents

A.



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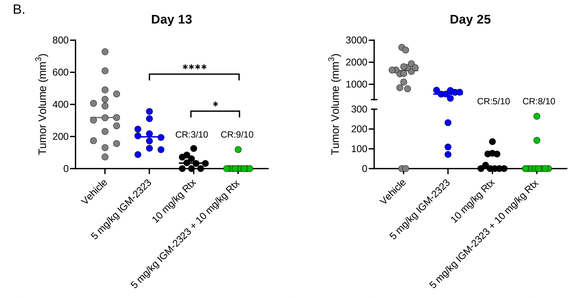

Figure 9. *In vivo* anti-tumor activity of IGM-2323 in the presence of Rtx in a humanized xenograft tumor mouse model. **A)** 10x10⁶ healthy donor PBMCs were engrafted intravenously (i.v.) 10 days prior to subcutaneous (s.c.) inoculation of 3x10⁶ human Raji B lymphoma cells in NSG MHC I/II$^{-/-}$ mice. At 3 days post tumor inoculation, mice (10 to 16 per group) were treated with Vehicle or 5 mg/kg i.v. IGM-2323 q2dx8, or mice were administered a single i.p. dose of wild type Rtx at 10 mg/kg 4 hours prior to 5 mg/kg i.v. IGM-2323 q2dx8. **B)** Tumor volumes (mm³) +/- SEM for each treatment group on days 13 and 25 post tumor inoculation are shown. A Mann-Whitney *U* test was used for statistical analysis, ****p<0.0001, *p<0.02. The combination of IGM-2323 and Rtx has more complete responses compared to each single agent.

Summary

- IGM-2323 bound to human CD20 with an apparent ~300-fold higher binding affinity ($K_D$app) compared to a bispecific CD20xCD3 IgG.
- At high concentrations of Rtx, which correspond to reported peak serum concentrations (C_{max}) found in Rtx-treated patients, IGM-2323 displaced the binding of Rtx on human B lymphoma cell lines.
- The fast killing and potency of IGM-2323 by CDC is maintained in the presence of Rtx.
- In TDCC assays with healthy donor effector T cells, pre-treatment with high concentrations of Rtx only modestly inhibited T cell activation and had no impact to the maximum killing activity (E_{max}) of IGM-2323.
- IGM-2323 in combination with Rtx induces anti-tumor efficacy *in vivo* in a humanized mouse model.
- Our preclinical data indicate that IGM-2323 maintains activity in the presence of Rtx. We plan to explore the combination of IGM-2323 and rituximab in future studies.

Acknowledgments

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