A Bispecific IgM Antibody Format for Enhanced T cell-Dependent Killing with Minimal Cytokine Release

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Abstract

We have developed a novel T cell engaging bispecific antibody platform that utilizes the high avidity of IgM antibodies, but further combines this with the high affinity and specificity of IgG antibodies. By grafting the affinity matured binding domains of IgG antibodies onto the multimeric framework of an IgM antibody and fusing a CD3 binding single chain Fv domain to the J-chain, the resultant engineered IgM T cell bispecific antibody demonstrates strong binding to specific targets while limiting over-stimulation of engaged T cells. One of our bispecific IgM antibodies, IGM-2323, binds CD20 antigen with more than 1000-fold increased avidity and mediates complement dependent cytotoxicity (CDC) of CD20-expressing cells with greater than 100-fold higher potency when compared to the corresponding IgG bispecific. IGM-2323 also shows highly potent T cell dependent cytotoxicity, even on cells with very low cell surface expression of CD20 and on rituximab-resistant variants of Ramos cells. Significantly, IGM-2323 exhibits vastly reduced cytokine release in vitro and in vivo, with at least equivalent T cell dependent killing of CD20-expressing target cells, providing a potentially safer and more effective bispecific format than IgG-based T cell bispecifics. These data indicate that IgM-based bispecifics can induce T cell engagement and very potent cytotoxicity that can be dissociated from the cytokine release unlike what has been observed with other IgG-based bispecific antibodies. In vivo efficacy studies in humanized NSG mice indicated that doses as low as 3 µg/mouse cause complete B cell elimination. Similarly, bispecific IgM completely depletes circulating B cells in cynomolgus monkeys at doses of 300 µg per kg. Furthermore, in these primate studies, durable depletion of B cells in spleen and lymphoid tissues was noted without any observable adverse effects despite repeated doses at the highest dose tested, 25 mg/kg. Low levels of transiently increased circulating IL-6 and no increased levels of TNF-alpha and IFN-gamma were observed in these primate studies. These preclinical data demonstrate the potential for broad application of this novel modular IgM-based bispecific antibody format. A Phase I dose-escalation study of IGM-2323 is currently on-going in patients with relapsed/refractory Non-Hodgkin's Lymphoma.

Bispecific IgM Format

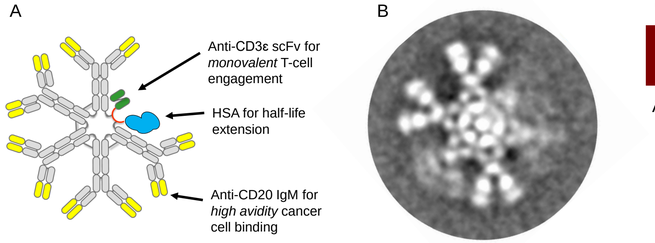


Figure 1.A. Schematic diagram of a bispecific IgM. For IGM-2323, the variable regions of IgM were designed to bind CD20 and the scFv fused to J-chain binds CD3.

Figure 1.B. Cryo-electron micrograph of bispecific IgM showing the asymmetric structure of the pentameric IgM with the J-chain fusion.

High Avidity Binding of Antigen by Anti-CD20 IgM

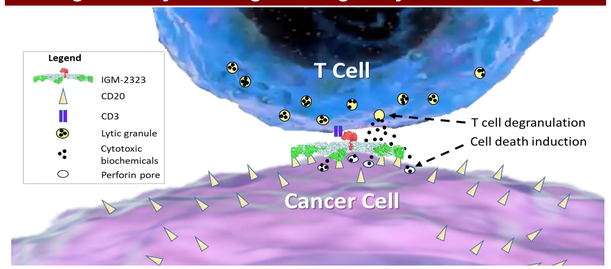


Figure 2. Schematic diagram of T-cell engagement by B-cell bound IGM-2323. We believe that the bispecific IgM format allows simultaneous engagement of multiple antigens on the target cell surface and engages a single CD3 on T-cells for each IgM, resulting in a more "physiological" immune synapse compared to BiTEs or bispecific IgG format.

Superior Binding of Bispecific IgM, Potent Killing

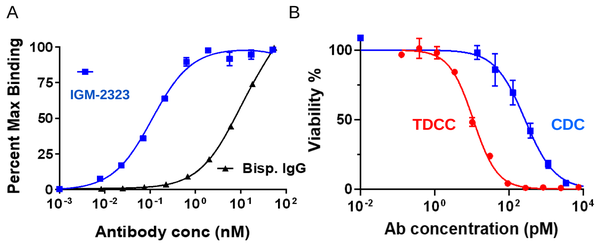


Figure 3.A. IGM-2323 or IgG with identical CD20 binding variable domains to CD20 ECD coated on a plate at 10 µg/mL. Highly avid IgM allows >1000x more potent binding than IgG.

Figure 3.B. TDCC is more potent than CDC in vitro. T cell dependent cellular cytotoxicity (TDCC) of Ramos cells using CD8+ T-cells at effector:target ratio of 5:1 for 48 hrs exhibits more potent killing (EC50=8.6 pM), whereas complement-dependent cytotoxicity of IGM-2323 on Ramos cells indicates an EC50 of 250 pM

IGM-2323 Kills Rituximab-Resistant Ramos Cells and Standard Ramos Cells at Low E:T Ratios

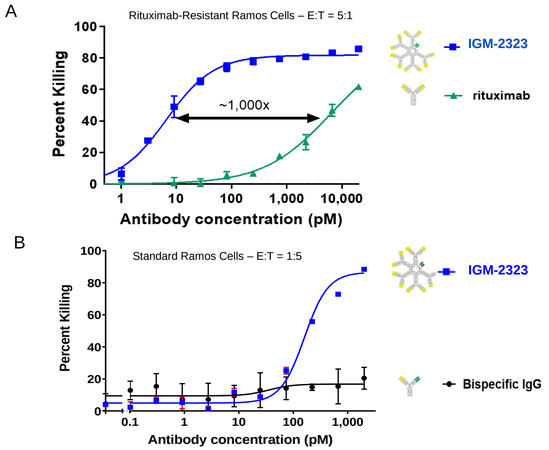


Figure 4.A. Comparison of cell killing activity of rituximab resistant cells in human PBMCs with 10% serum. A rituximab resistant clone of Ramos cells was created by serial outgrowth of CDC survivors over six cycles. Bispecific IgM IGM-2323 shows ~1000x better killing of this rituximab resistant cell line compared to rituximab with EC50 of 6.6 pM vs > 6 nM.

Figure 4.B. Bispecific IgM IGM-2323 exhibits more potent TDCC against standard Ramos cells at low effector to target ratios than the bispecific IgG. T cell dependent cellular cytotoxicity (TDCC) of Ramos cells using CD8+ T-cells at an effector:target ratio of 1:5 for 48 hrs – the IgM-based bispecific exhibits potent killing, whereas the IgG-based bispecific does not.

Bispecific IgM Shows Low Cytokine Release In Vitro

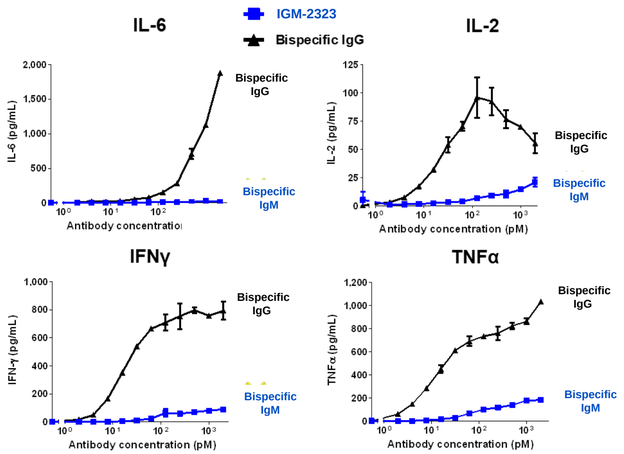


Figure 5. In vitro comparison of four cytokines elevated in "cytokine release syndrome" observed with bispecific IgG's. IGM-2323 or a bispecific IgG with the same CD20 and CD3 binding units were incubated with human PBMCs from mixed donors for 48 hours and the supernatant assayed for cytokines using flow cytometry. Levels of cytokine release observed for IGM-2323 are orders of magnitude lower than that for the corresponding bispecific IgG indicating potential for greater therapeutic window using bispecific IgM T cell engagers.

IGM-2323,-2324 Show Efficacy In Cynomolgus Monkeys

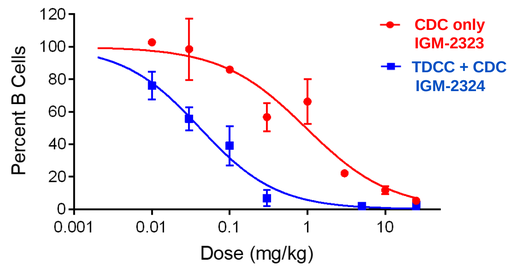
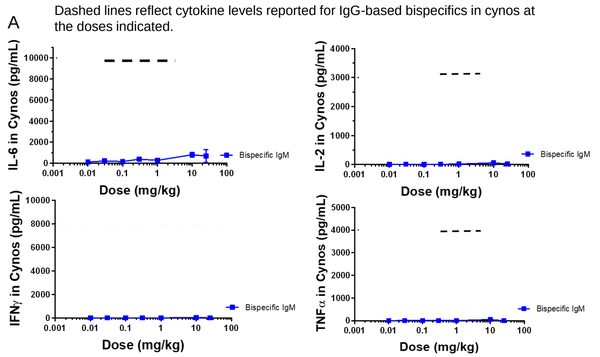


Figure 6. Depletion of peripheral B-cells upon treatment of Cynomolgus monkeys with a single dose of either IGM-2323 (not bind to cynomolgus monkey CD3) or IGM-2324 (does bind to cynomolgus monkey CD3). The dose response curves indicate the bispecific IgM IGM-2324 with dual mechanism of B-cell killing (both CDC and TDCC), has an EC50 of 0.04 mg/kg vs IGM-2323 which only has CDC based B-cell killing and shows an approximate 25x higher EC50 of 0.94 mg/kg for B-cell depletion at 24 hours post administration.

IGM-2324 Shows Minimal Elevation of Cytokines in Cynomolgus Monkey Studies



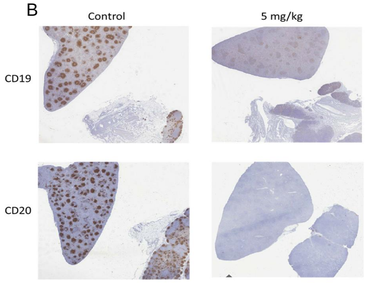


Figure 7.A. Cytokine levels were measured in a GLP toxicology study conducted in Cynomolgus monkeys with IGM-2324. Minimal elevation of cytokines was observed at the maximal tested dose of 25 mg/kg in comparison to reported data (dotted lines) for competing bispecific IgG's at ≤ 3 mg/kg.

Figure 7.B. Immunohistochemistry to detect CD19 and CD20 positive B cells in non-human primate spleen and mesenteric lymph nodes from animals administered with IGM-2324.

Summary

- We have developed a novel bispecific IgM platform that allows highly avid engagement of tumor antigens with monovalent engagement of CD3 on T cells.
- Bispecific IGM-2323 exhibits 100x greater binding to CD20 antigen on a tumor cell line compared to a corresponding bispecific IgG
- Highly potent CDC and TDCC based killing of tumor cell lines is demonstrated in vitro, together with potent killing of rituximab resistant cells, even at low E:T ratios
- Importantly, TDCC activity of IGM-2323 does not result in excessive cytokine release in vitro, commonly associated with CRS in clinical trials with other bispecific mAbs or CART therapy
- IGM-2323 has potent activity in vivo in NSG mice (data not shown) as well as in cynomolgus monkeys, with durable B cell depletion from spleen and lymph nodes
- Very low cytokine release, durable depletion of B-cells in cyno monkeys and potent killing of rituximab resistant cells support clinical development of IGM-2323 as a bispecific IgM for the treat of Non-Hodgkin's lymphoma patients
- Phase I clinical trial in progress. See ClinicalTrials.gov ID: NCT 04082936

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