High Avidity Anti-CD20 IgM Antibody for Enhanced Complement-Dependent Cell Killing of Low CD20 Expressing Tumor Cells

Ramesh Baliga, Keyu Li, Paul Hinton, Tim Luger, Marigold Boe, Dean Ng and Bruce Keyt
IGM Biosciences, Inc., 320 Logue Avenue, Mountain View, CA 94043

Abstract

IgM antibodies are the first line of defense generated by our immune system in response to foreign antigens. Typically, IgMs have low affinity individual interaction with the target antigen, but due to the multivalent penta- or hexameric scaffold, the IgM isotype exhibits very high avidity to specific cell surface antigens. Few IgMs have been developed as therapeutics because of challenges in gaining high level expression and difficulties with manufacturing. IGM Biosciences has overcome many of these challenges and prepared an IgM designed to bind the B-cell antigen CD20 with high avidity. This IgM antibody, IGM-2320, is able to bind CD20 antigen greater than 1000x better than the corresponding IgG version rituximab (1) in an ELISA format. Using a pair of CD20 expressing tumor cell lines, we demonstrate functional effects such as complement dependent cytotoxicity (CDC) at more than 100x higher potency than corresponding IgG's. *Ex vivo* assays with human and cynomolgus complement-preserved whole blood indicate that the IgM format enables rapid CDC based killing of target cells within 4 hrs, with EC_{50} values greater than 20x better than the corresponding IgG. Further studies in antibody engineering as well as *in vivo* animal models are in process. These initial preclinical data provide promise for the therapeutic potential of this polyvalent antibody format for treatment of resistant and/or refractory B-cell diseases, such as chronic lymphocytic leukemia (CLL) or Non-Hodgkins' lymphoma (NHL).

IgM Antibodies

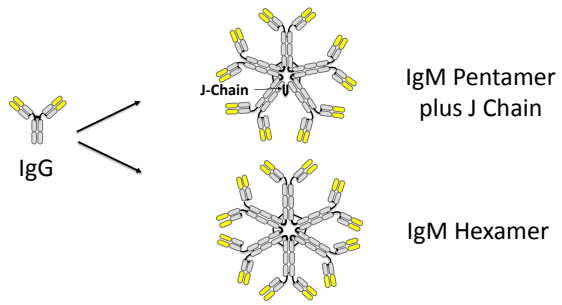

Figure 1: Schematic diagrams of IgG vs IgM with or without the joining or J-chain. Compared to the two antigen binding units of an IgG, IgM pentamers (2) have 10 binding units and IgM hexamers have 12 binding units. This allows high avidity binding of antigens and hence access to antigens expressed at low density.

Gel Based Characterization of αCD20 IgM

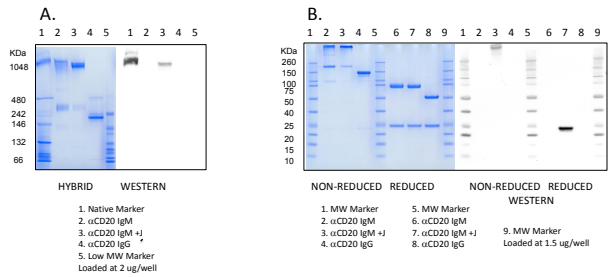

Figure 2: A. Hybrid gels were used to resolve the large MW (approx.1MDa) IgM pentamer and hexamers from component monomers/IgG. Western blots with antibody to J-chain was used to determine if the IgM multimer contains the J-chain. B. Proper assembly was verified using non-reducing and reducing-gels with corresponding western blots for detecting J-chain.

Antibody Binding to Cell Surface CD20 Antigen

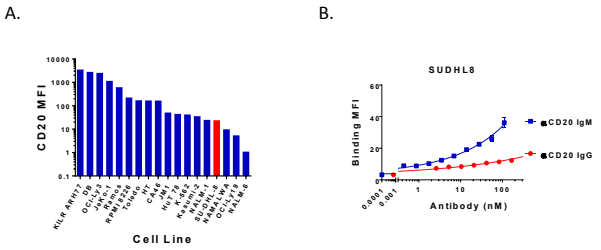

Figure 3: A. A panel of 18 tumor cell lines was chosen for differential expression of CD20 antigen. Antigen expression was measured using a commercial anti-CD20 antibody using flow cytometry. CD20 MFI ranged from 3250 (KILR ARH77) to 1 (NALM-6). B. The avidity advantage of IgM format was demonstrated using SUDHL-8 (CD20 MFI=22) and directly labeled αCD20 IgG and IgM.

Antibody Binding vs CD20 Antigen Density

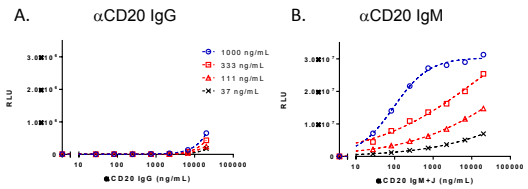

Figure 4: A. Binding of αCD20 IgG to low levels of Trx-His6-CD20 antigen (Acros Biosystems). Chemiluminescent ELISA was able to detect some binding at the highest concentration of antigen coating on plate. B. By contrast, αCD20 IgM+J was able to show greater than 1000X better binding at the same low antigen concentrations.

CDC Activity vs CD20 Antigen Density

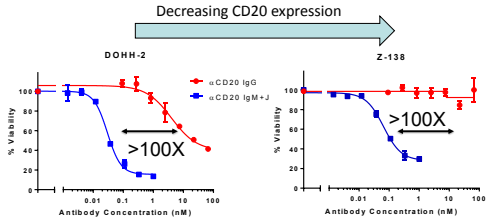

Figure 5: Complement dependent cytotoxicity (CDC) of αCD20 IgM and IgG format showed >100X better cell killing of both DOHH-2 (high CD20 expressing) and Z-138 (low CD20 expressing) by IgM format antibody, demonstrating the clear advantage of using a high-avidity multimeric format vs the traditional IgG format or IgG's designed for improved CDC (3).

Tumor Cell Killing in Whole Blood

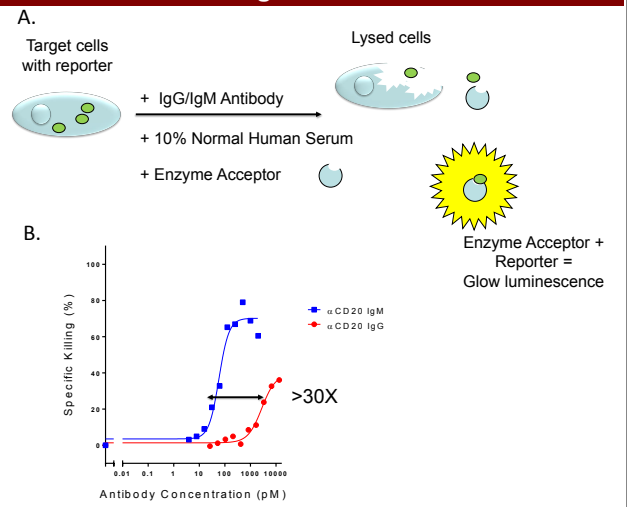

Figure 6: A. KILR assay for cell lysis (DiscoverX). ARH77 cell line carrying the reporter, when lysed by action of an antibody, releases the reporter allowing complementation and glow luminescence from turnover of substrate in detection reagent. **B.** αCD20 IgG and αCD20 IgM based killing of high level CD20 expressing tumor cell line ARH77. Rapid killing was observed with 15% whole, complement preserved human blood even as early as 4 hrs after co-incubation. The IgM format achieved >30X better killing than IgG in this ex-vivo experiment.

Summary

- We have engineered V_H and V_L regions of IgM's to bind the CD20 antigen on B-cells and lymphoma cell lines
- Engineered IgM's were characterized using hybrid, non-reduced and reduced PAGE gels to demonstrate purity and assembly
- CD20 binding IgM bound antigen >1000X better than the IgG antibody particularly at low antigen density
- Functional activity on tumor cell lines showed CDC >100X better than rituximab IgG on low CD20 expressing cell lines
- CD20 binding IgM showed 30X better cell killing activity than IgG in a whole blood in presence of complement

References

1. Du, J. et al. (2007) Structural basis of recognition of CD20 by therapeutic antibody Rituximab *JBC 282:15073-80.*
2. Perkins SJ et al. (1991) Solution structure of human and mouse IgM *J. Mol. Biol.*221:1345-1366.
3. Teeling JL et al. (2006) The biological activity of human CD20 antibodies is linked to unique epitopes on CD20 *J. Immunol.* 177:362-371.
4. Email Correspondence: ramesh@igmbio.com, bruce@igmbio.com