Multimeric Anti-DR5 IgM antibody displays potent cytotoxicity in vitro and promotes tumor regression in vivo

Beatrice Wang, Tasnim Kothambawala, Paul Hinton, Dean Ng, Avneesh Saini, Ramesh Baliga, and Bruce Keyt

IGM Biosciences, Inc., 325 East Middlefield Road, Mountain View, CA 94043

Abstract

Death receptor 5 (DR5) is a member of the TNF receptor superfamily that induces apoptosis upon receptor trimerization. It is expressed on many tumor types and has therefore been an important target for developing antibody based treatments of epithelial, solid tumors. However several agonistic Anti-DR5 IgG antibodies that have demonstrated efficacy in preclinical models have been unsuccessful in clinical trials, likely due to insufficient receptor crosslinking by bivalent IgGs (1). We have developed a multimeric anti-DR5 IgM antibody which has strong avidity for the receptor. The IgM binds Colo205 cells and triggers apoptosis, and is 1000-fold more potent than the respective IgG and 100-fold more potent than crosslinked IgG in vitro. Anti-DR5 IgM displays strong in vitro potency across a panel of tumor cell lines, including ones that are IgG-resistant. Anti-DR5 IgM causes tumor regression and delays tumor growth in various in vivo xenograft models including Colo205, HCT15 and MDA-MB-231. These results support the development of a human anti-DR5 IgM therapeutic with the potential to treat solid tumors.

Multivalent IgM Antibody Structure

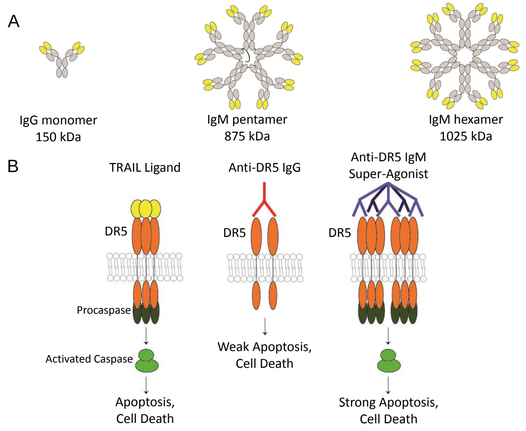


Figure 1. A) Pentameric IgM is composed of 10 heavy chains, 10 light chains, and 1 joining chain (J chain). Hexameric IgM is composed of 12 heavy chains and light chains. **B)** Schematic diagram of DR5-mediated apoptotic signaling when trimerized and thereby activated by endogenous TRAIL ligand, agonistic IgG and IgM. IgM is among a class of multivalent DR5 super-agonists currently under development (2,3).

IgM Assembly

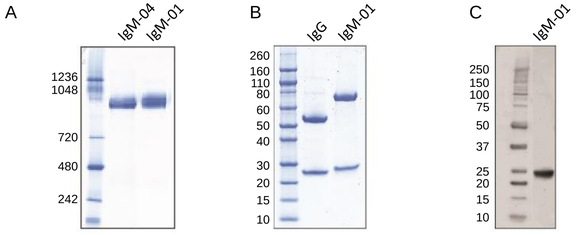


Figure 2. Anti-DR5 IgM-01, IgM-04, and IgG antibodies were run on gels. **A)** Hybrid gel resolves high molecular weight IgMs. **B)** Reduced gel shows heavy and light chains of IgG and IgM. **C)** Anti-J chain western blot confirms presence of J chain in IgM pentamer.

IgM Binds DR5 with Specificity and 10-Fold Greater Avidity than IgG

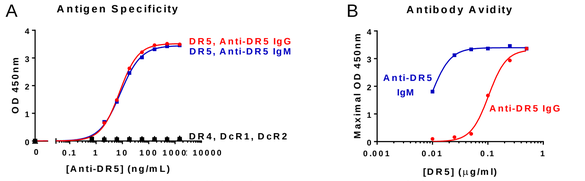
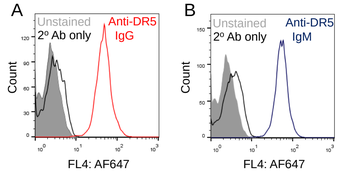


Figure 3. A) DR5, related death receptor DR4, and decoy receptors DcR1 and DcR2 were coated at 2 μg/mL on a plate and antibody binding was measured by ELISA. Anti-DR5 IgM-01 and IgG bind specifically to DR5. **B)** DR5 was coated at decreasing concentrations on a plate. IgM-01 exhibited binding at 10-fold lower antigen density than IgG.

Tumor Cell Binding

Figure 4. A) Colo205 cells were stained with 2 μg/mL anti-DR5 IgG followed by anti-IgG Fc-AF647. Binding was measured by flow cytometry. **B)** Colo205 cells were stained with 1 μg/mL anti-DR5 IgM-01, then anti-IgM-AF647, and measured by flow cytometry.



Anti-DR5 IgM Displays Potent In Vitro Cytotoxicity Across Multiple Tumor Lines

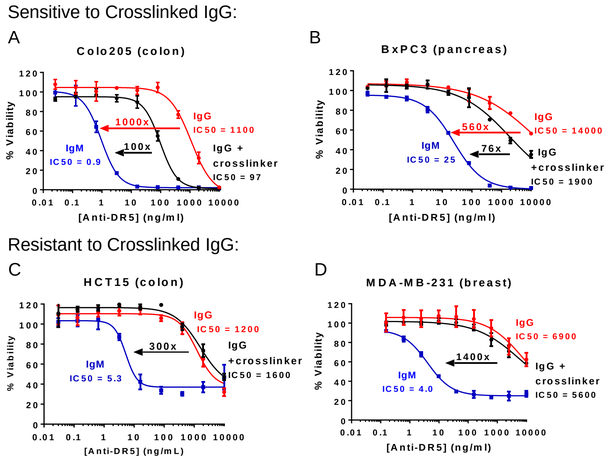


Figure 5. A) Colo205, **B)** BxPC3, **C)** HCT15, and **D)** MDA-MB-231 cells were treated with anti-DR5 IgM-01, anti-DR5 IgG, or anti-DR5 IgG plus a secondary antibody crosslinker for 24 hours, and viability was read using Cell Titer Glo (Promega).

Anti-DR5 IgM Variants Show Improved *In Vitro* Safety

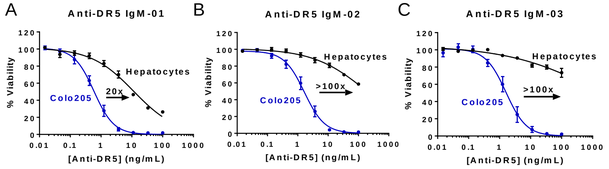


Figure 6. Colo205 was treated as described above. Primary human hepatocytes were plated in collagen coated plates and treated with **A)** anti-DR5 IgM-01, **B)** anti-DR5 IgM-02, or **C)** anti-DR5 IgM-03 for 24 hours, and viability was read using Cell Titer Glo (Promega).

Anti-DR5 IgM Delays Tumor Growth in Xenograft Models

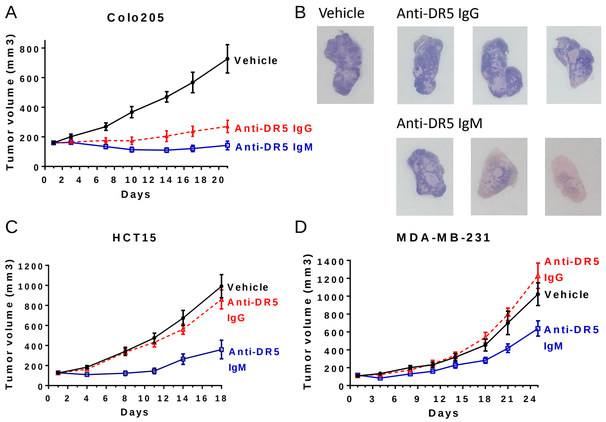


Figure 7. A) Colo205 tumor cells (10^6) were implanted s.c. in 50% Matrigel. Anti-DR5 IgG was administered i.v. with a loading dose of 3 mg/kg, followed by weekly dosing at 2 mg/kg. Anti-DR5 IgM-04 was administered i.v. at 3 mg/kg every other day. **B)** On Day 60, tumors were harvested and stained with H&E and images were taken at 1X. **C)** HCT15 tumor cells (10^7) were implanted s.c, followed by 3 mg/kg i.v. dosing of IgG weekly and IgM-04 every other day. **D)** MDA-MB-231 tumor cells (5 x 10^6) were implanted s.c, followed by 3 mg/kg i.v. dosing of IgG weekly and IgM-04 every other day.

Summary

- Multimeric IgM is a natural platform to agonize DR5 and induce apoptosis
- Anti-DR5 IgM has 10-fold stronger avidity for the receptor than IgG
- Anti-DR5 IgM is 1000-fold more potent than IgG at Colo205 cytotoxicity and >100-fold more potent than crosslinked IgG on many tumor cell lines
- We have generated anti-DR5 IgM variants with improved *in vitro* safety
- Anti-DR5 IgM delays tumor growth in both IgG-sensitive and resistant tumor xenograft models

References

1. Holland P.M. (2014) Death receptor agonist therapies for cancer, which is the right TRAIL? *Cytokine & Growth Factor Rev.* 25: 185.
2. Gieffers C. et al. (2013) APG350 induces superior clustering of TRAIL receptors and shows therapeutic antitumor efficacy independent of cross-linking via FcγR receptors. *Mol Cancer Ther.* 12(12): 2735.
3. Brunker P. et al. (2016) RG7386, a novel tetravalent FAP-DR5 antibody, effectively triggers FAP-dependent, avidity-driven DR5 hyperclustering and tumor cell apoptosis. *Mol Cancer Ther.* 15(5): 946.
4. Email Correspondence: beatrice@igmbio.com, bruce@igmbio.com

Abstract No. 1702 AACR Annual Meeting 2017, April 1-5, Washington D.C.