Agonistic Death Receptor 5 (DR5) IgM antibody IGM-8444 induces tumor cell apoptosis *in vitro* and *in vivo* and has a favorable *in vitro* safety profile

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Background

- Tumor necrosis factor receptor (TNFR) superfamily member death receptor DR5 requires multimerization to induce apoptosis and tumor cytotoxicity (Pan et al., 2019, Cell *176*, 1477)
- While agonistic IgG antibodies targeting DR5 have demonstrated evidence of preclinical efficacy, limited clinical efficacy was observed likely due to insufficient receptor multimerization in the tumor microenvironment
- Some multivalent DR5 agonists have shown signs of clinical efficacy but also liver toxicity
- We have developed IGM-8444, a novel multivalent anti-DR5 IgM antibody that effectively clusters DR5 to induce tumor cytotoxicity *in vitro* and *in vivo*

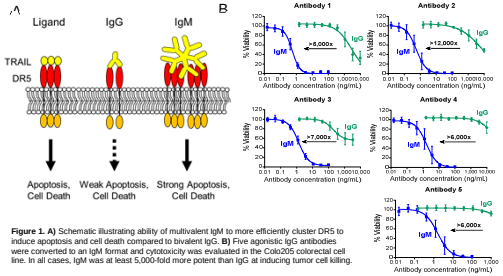

Figure 1. A) Schematic illustrating ability of multivalent IgM to more efficiently cluster DR5 to induce apoptosis and cell death compared to bivalent IgG. **B)** Five agonistic IgG antibodies were converted to an IgM format and cytotoxicity was evaluated in the Colo205 colorectal cell line. In all cases, IgM was at least 5,000-fold more potent than IgG at inducing tumor cell killing.

IGM-8444 Binds and Induces Apoptosis in Tumor Cells

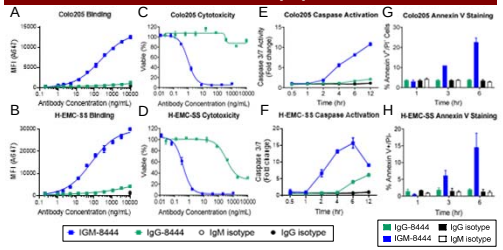

Figure 2. IGM-8444 binding and cytotoxicity was evaluated in the Colo205 colorectal and H-EMC-SS chondrosarcoma cell lines. Cells were incubated with IGM-8444, IgG-8444 (an IgG with the same binding domain), or isotype controls. **A)** and **B)** Binding was measured by flow cytometry using an anti-human kappa antibody. **C)** and **D)** Cells were treated with IGM-8444 or IgG-8444 for 24 hours and viability was measured using CellTiter-Glo. **E)** and **F)** Cells were treated with 1 µg/mL of each antibody and caspase activation was measured using the Caspase 3/7-Glo reporter assay. **G)** and **H)** Cells were treated with 1 µg/mL of each antibody and apoptosis was determined as a percentage of Annexin V positive PI negative cells by flow cytometry.

IGM-8444 Does Not Induce Killing of Primary Human Hepatocytes *In Vitro*

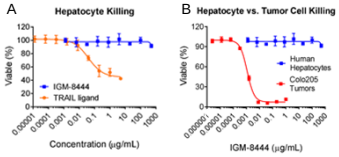

Figure 3. A) Primary pooled human hepatocytes were treated with IGM-8444 or recombinant human TRAIL ligand, and viability after 24 hours was measured using CellTiter-Glo. Similar results were achieved in individual human hepatocyte donors with varying states of liver disease. **B)** Comparison of IGM-8444 cytotoxicity of Colo205 tumor cells versus pooled primary human hepatocytes in a 24-hour assay.

IGM-8444 Induces Cytotoxicity Across Multiple Tumor Cell Line Indications

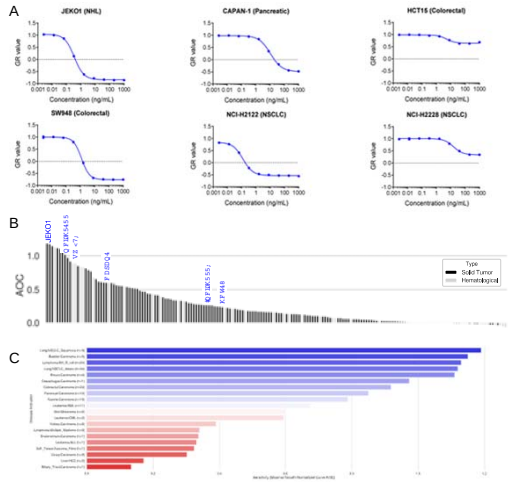

Figure 4. A) Examples of various IGM-8444 cytotoxicity responses. Tumor cell lines (n=190) were treated with IGM-8444 and viability was measured after 96 hours using CellTiter-Glo. Viability data was normalized for growth rate using GR Calculator and resulting GR values were plotted versus concentration. **B)** Distribution of area-over-the-curve (AOC) for the growth rate normalized dose response curve of 157 solid and 53 hematological cancer cell lines. **C)** Indications are sorted and scaled by the distribution of maximal AOC in order of sensitive (blue) to insensitive (red).

Combination of IGM-8444 with Chemotherapy Results in Enhanced Tumor Cytotoxicity *In Vitro*

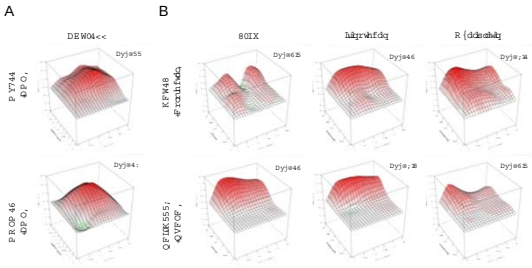

Figure 5. Synergistic response of combination chemotherapy and IGM-8444 in solid tumor and AML cell lines. **A)** AML cell lines MOLM13 and MV411 experienced synergy on average across the combination dose spectrum (Bliss>5). **B)** HCT15 and NCI-H2228 showed high average synergistic cytotoxicity (Bliss>5, red). Normal human hepatocytes experienced less toxicity in synergy than solid tumor cell lines (data not shown).

IGM-8444 is Efficacious as a Monotherapy in CDX and PDX Mouse Tumor Models

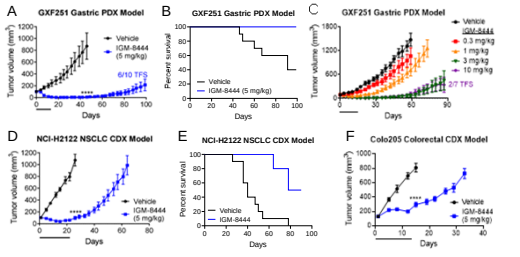

Figure 6. A and **B)** GXF251 is a gastric PDX model that expresses moderate levels of DR5 by IHC. GXF251 tumor fragments were implanted s.c. and IGM-8444 was dosed at 5 mg/kg i.v. q2dx7, or **C)** at 0.3, 1, 3, or 10 mg/kg i.v. q2dx7. IGM-8444 induced tumor regressions and some animals in the 5 and 10 mg/kg treatment groups were tumor-free by study end. **D** and **E)** NCI-H2122 cells were implanted s.c. and IGM-8444 was dosed at 5 mg/kg i.v. q2dx11.Tumor regressions were observed during the dosing period. **F)** Colo205 cells were implanted s.c. and IGM-8444 was dosed at 5 mg/kg i.v. q2dx7. Tumor stasis was observed during the dosing period. Mann-Whitney U tests were used to compare tumor volume in treated versus control groups on the last day that all vehicle animals were on study. ****p<0.0001. Bar below x-axis indicates dosing period. TFS, tumor-free survivor.

Combination of IGM-8444 with Chemotherapy and ABT-199 Enhances *In Vivo* Efficacy

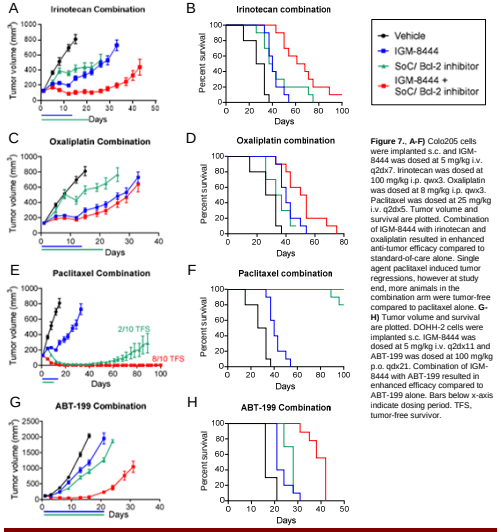

Figure 7. A-F) Colo205 cells were implanted s.c. and IGM-8444 was dosed at 5 mg/kg i.v. q2dx7. Irinotecan was dosed at 100 mg/kg i.p. qwx3. Oxaliplatin was dosed at 8 mg/kg i.p. qwx3. Paclitaxel was dosed at 25 mg/kg i.v. q2dx5. Tumor volume and survival are plotted. Combination of IGM-8444 with irinotecan and oxaliplatin resulted in enhanced anti-tumor efficacy compared to standard-of-care alone. Single agent paclitaxel induced tumor regressions, however at study end, more animals in the combination arm were tumor-free compared to paclitaxel alone. **G-H)** Tumor volume and survival are plotted. DOHH-2 cells were implanted s.c. IGM-8444 was dosed at 5 mg/kg i.v. q2dx11 and ABT-199 was dosed at 100 mg/kg p.o. qdx21. Combination of IGM-8444 with ABT-199 resulted in enhanced efficacy compared to ABT-199 alone. Bars below x-axis indicate dosing period. TFS, tumor-free survivor.

Summary

- Anti-DR5 antibodies showed > 5000-fold increased *in vitro* potency as IgMs compared to IgGs
- When compared to an IgG, IGM-8444 induces more rapid and profound apoptosis
- IGM-8444 is highly potent across 38 cell lines and PDXs evaluated from 21 tumor types but does not kill primary human hepatocytes *in vitro* at concentrations well above those required to kill tumor cell lines
- As a monotherapy, IGM-8444 induces anti-tumor efficacy in CDX and PDX models *in vivo*, including durable and dose dependent tumor regressions in a gastric PDX
- Combinations of IGM-8444 with standard of care chemotherapy and targeted agents enhance tumor cytotoxicity both *in vitro* and *in vivo*
- These data support the development of IGM-8444 to treat solid and hematologic cancers and an IND is projected to be filed in 2020

Abstract No. 518, 2020 AACR Virtual Annual Meeting, June 22-24