Mechanistic evaluation of anti-DR5 IgM antibody IGM-8444 with potent tumor cytotoxicity, without *in vitro* hepatotoxicity

Short title (characters including spaces): 34 (max allowed 35)

Mechanism of anti-DR5 IgM IGM-8444

Beatrice T. Wang, Tasnim Kothambawala, Kevin C. Hart, Xingjie Chen, Melanie Desbois, Susan E. Calhoun, Poonam Yakkundi, Rodnie A. Rosete, Yuan Cao, Katie Cha, Thomas J. Matthew, Ling Wang, Paul R. Hinton, Maya K. Leabman, Genevive Hernandez, Maya F. Kotturi, Eric W. Humke, Angus M. Sinclair, Bruce A. Keyt

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

Death receptor 5 (DR5) is a member of the tumor necrosis factor (TNF) receptor superfamily that activates the extrinsic apoptotic pathway when bound and multimerized by its ligand, TNF-related apoptosis inducing ligand (TRAIL). DR5 is broadly expressed on solid and hematologic cancers and has been targeted with both recombinant TRAIL and agonistic antibodies in the clinic. However, these therapeutics have been unsuccessful due to lack of efficacy or due to hepatotoxicity. We have developed IGM-8444, an engineered pentameric IgM with 10 binding sites specific for DR5, which is designed to multimerize DR5 to selectively and potently induce tumor cell apoptosis while sparing hepatocytes. Here, we describe the rationale behind the selection of IGM-8444 as our clinical candidate.

A panel of agonistic DR5 antibodies were evaluated for DR5 binding affinity, epitope, and *in vitro* potency versus hepatotoxicity. Antibodies formatted as an IgM showed enhanced potency when compared to an IgG with the same binding domain. IGM-8444 binds an epitope on DR5 within cysteine-rich domain 1 (CRD1) that competes with TRAIL binding. While the binding affinities of the panel of anti-DR5 antibodies were comparable, IGM-8444 was selected from a subset of anti-DR5 IgM antibodies capable of potently killing tumor cells without exhibiting cytotoxicity of primary human hepatocytes *in vitro*. Further mechanistic studies examined the kinetics of apoptotic induction by IGM-8444 and other DR5 agonists. Interestingly, we noted that DR5 agonists with the fastest kinetics of tumor cell apoptotic induction also displayed the most hepatotoxicity *in vitro*. In spite of the kinetic differences, IGM-8444 has similar maximal cytotoxicity *in vitro* and comparable anti-tumor efficacy in xenograft mouse tumor models when compared with an IgM antibody targeting a different DR5 epitope. In cynomolgus monkeys, IGM-8444 showed no evidence of hepatotoxicity or other adverse events when dosed repeatedly up to 30 mg/kg, the highest dose tested. These preclinical properties of IGM-8444 provide an opportunity for enhanced tumor cytotoxicity without additional hepatotoxicity when combined with standard of care agents. Indeed, we have demonstrated enhanced anti-tumor efficacy by combining IGM-8444 with chemotherapies such as 5-FU and irinotecan in colorectal cancer models, as well as combining with Bcl-2 inhibitor ABT-199 in hematological malignancy models.

In summary, we have evaluated the mechanism by which IGM-8444 agonizes DR5, which potently kills tumor cells without accompanying hepatotoxicity. IGM-8444 is currently being evaluated in a Phase 1 study as a single agent and in combination with chemotherapy-based regimens in patients with solid cancers and NHL (NCT04553692).