Characterization of the synergistic tumor cytotoxicity of agonist DR5 IgM antibody IGM-8444 with chemotherapeutic agents

Poster #6123

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Background

- IGM-8444 is a multivalent IgM agonist that targets tumor necrosis factor (TNF) receptor superfamily member death receptor DR5, which requires multimerization to induce tumor cell apoptosis.
- IGM-8444 exhibits anti-tumor activity in preclinical models with good in vitro and in vivo safety, making it a potentially promising combination partner with standard of care treatment regimens. IGM-8444 is currently being evaluated in a Phase 1 trial (NCT04553692).
- Here we evaluate IGM-8444 single agent and combinatorial cytotoxicity with different classes of chemotherapeutic agents.

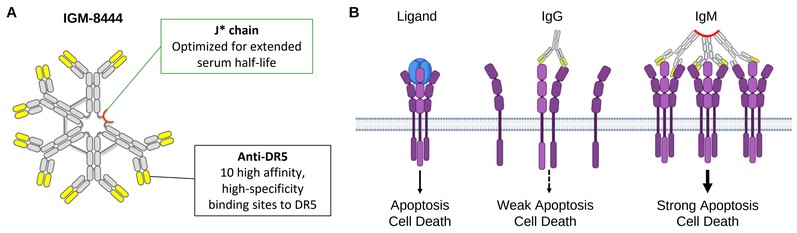

Figure 1. Structure and function of DR5 agonist IGM-8444.
A) IGM-8444 is a monospecific recombinant pentameric IgM antibody with 10 binding sites to DR5. **B)** Schematic illustrating ability of multivalent IgM to efficiently cluster DR5 and induce tumor apoptosis and cell death. Created with BioRender.com.

IGM-8444 Monotherapy Shows a Range of Sensitivity Across Solid Tumor Cell Lines and Xenograft Models

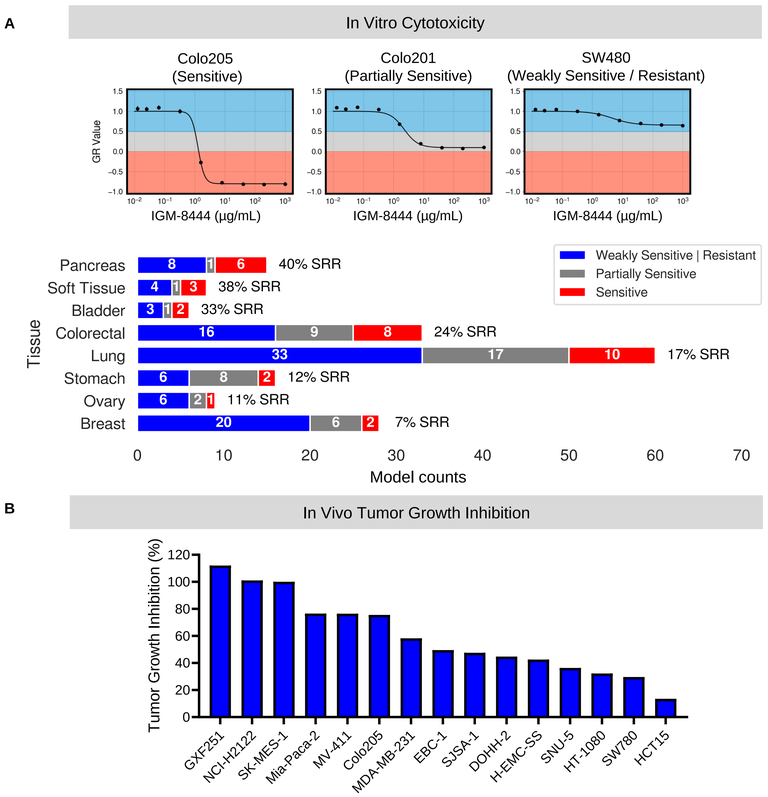

Figure 2. IGM-8444 single agent cytotoxic activity varies across tumor cell lines and xenograft models.
A) IGM-8444 in vitro growth rate (GR)-normalized dose response curves in 3 colorectal cancer cell lines. Sensitive cell lines exhibited a cytotoxic response with maximal activity range of GR<0 (red). Partially sensitive cell lines showed partial cytotoxicity or cytostasis with maximal activity range of 0<GR<0.5 (gray). Weakly sensitive or resistant cell lines had maximal activity of GR>0.5 (blue). 175 solid tumor cell lines from 8 tissue types were treated with IGM-8444 and viability response rates were classified into three tiers, Weakly Sensitive / Resistant (blue), Partially Sensitive (gray) and Sensitive (red). Tissues were sorted based on sensitivity response rate (SRR), or the frequency of cytotoxic response by tissue, and rank ordered. **B)** Tumor cells (or tumor fragments for GXF251 PDX) were implanted s.c. in mice and IGM-8444 was dosed at 5 mg/kg i.v. every other day for a total of 7, 8, or 11 doses. Percent tumor growth inhibition was calculated on the last day that all vehicle-treated animals were on study. GXF251, gastric PDX model. CDX models: NCI-H2122 – non-small cell lung cancer **(**NSCLC), SK-MES-1 – NSCLC, Mia-Paca-2 – pancreatic, MV-411 – acute myeloid leukemia (AML), Colo205 – colorectal carcinoma (CRC), MDA-MB-231 – triple negative breast cancer (TNBC), EBC-1 - NSCLC, SJSA-1 – osteosarcoma, DOHH-2 – non-Hodgkin's lymphoma (NHL), H-EMC-SS – chondrosarcoma, SNU-5 – gastric, HT-1080 – fibrosarcoma, SW780 – bladder, HCT15 – CRC.

IGM-8444 Shows Rapid Intra-Tumoral Pharmacodynamic Activity

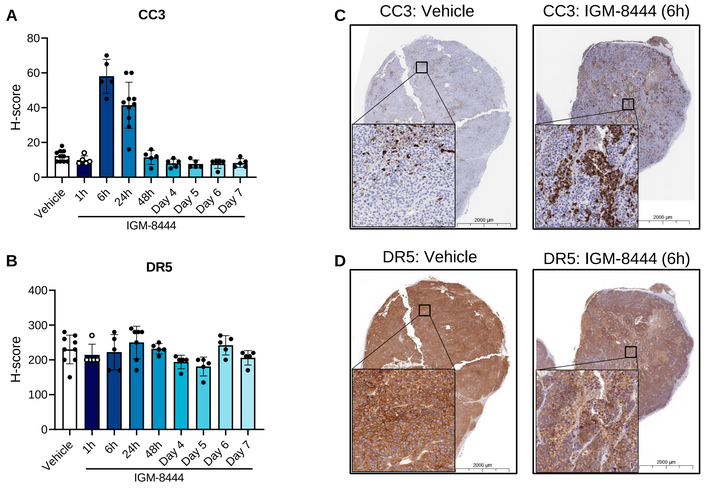

Figure 3. IGM-8444 induces rapid cleavage of caspase 3 at the tumor with a peak 6 hours post-dose.
Colo205 tumor bearing mice were treated with IGM-8444 (5 mg/kg, i.v., single dose) and the tumors were collected at multiple time points post-treatment. The kinetics of cleaved caspase-3 (CC3) expression, as a pharmacodynamic biomarker, and DR5 expression were assessed by immunohistochemistry (IHC) over time. **A)** Cytoplasmic CC3 IHC signal and **B)** plasma membranous DR5 IHC signals on the viable cells were evaluated by H-score. CC3 expression peaked at 6 hours post-dose while DR5 expression was not altered at 6 hours nor at 24 hours post-dose. **C)** Representative images of CC3 expression in a vehicle treated tumor at 24 hours and in an IGM-8444 treated tumor at 6 hours and **D)** DR5 expression in the corresponding tumors, respectively.

IGM-8444 Synergizes with Chemotherapeutic Agents In Vitro

A

		Topoisomerase Inhibitors			Microtubule Inhibitor	Nucleoside Analogs		Alkylating Agents	
Cell line	**Indication**	**Irinotecan**	**Doxorubicin**	**Etoposide**	**Paclitaxel**	**Gemcitabine**	**5-FU**	**Oxaliplatin**	**Carboplatin**
HCT15	CRC	13	10	15	8	9	3	8	6
HT-55	CRC	2	2	5	6	3	0	5	0
NCI-H508	CRC	-1	1	-1	8	-2	1	1	-3
NCI-N87	gastric	8	12	17	13	11	-2	8	5
SNU-5	gastric	2	12	4	3	-1	4	6	2
NUGC-4	gastric	-1	9	-2	7	2	1	1	1
LOU-NH91	NSCLC	12	12	16	10	24	17	2	-1
NCI-H2228	NSCLC	9	16	14	15	9	13	3	2
NCI-H460	NSCLC	5	4	4	5	4	4	3	7
PANC-1	pancreatic	7	7	10	15	14	1	9	4
AsPC-1	pancreatic	5	-1	5	14	15	4	-2	-1
BxPC3	pancreatic	5	2	2	4	9	5	1	1
UM-UC-3	bladder	5	9	8	6	4	-1	4	2
HT-1080	fibrosarcoma	3	2	4	3	5	-6	-1	2

Average Bliss synergy scores: Synergistic | Weakly synergistic | Additive | Weakly antagonistic

B

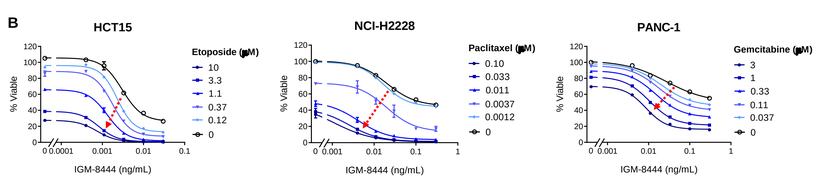

Figure 4. IGM-8444 combination with topoisomerase inhibitors, microtubule inhibitors, and nucleoside analogs shows synergy across multiple cell lines.
14 human solid tumor cell lines were treated with IGM-8444 in combination with 8 chemotherapeutic agents and viability was measured after 72 hours using CellTiter-Glo. **A)** Synergy was calculated using Bliss scores, where an average Bliss score greater than 10 indicates synergy and an average Bliss score between 5 and 10 suggests weak synergy. IGM-8444 combination with irinotecan, doxorubicin, etoposide, paclitaxel, and gemcitabine showed the most synergy across the cell lines screened. Combination with platinum-based alkylating agents trended towards weak synergy or additivity. **B)** Representative cell lines are shown. Dotted red arrow indicates EC50 shift, suggestive of synergy.

IGM-8444 and Chemotherapy Combination Enhances Anti-Tumor Responses In Vivo

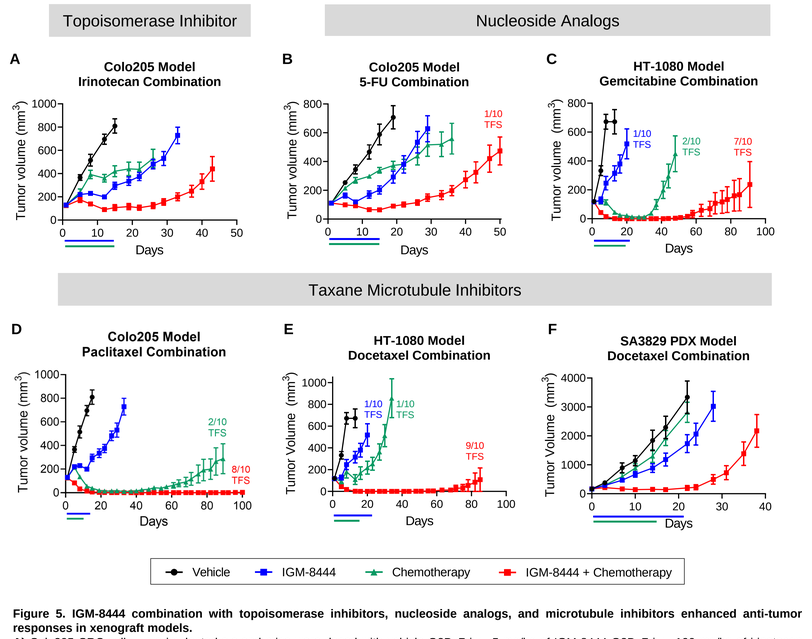

Figure 5. IGM-8444 combination with topoisomerase inhibitors, nucleoside analogs, and microtubule inhibitors enhanced anti-tumor responses in xenograft models.
A) Colo205 CRC cells were implanted s.c and mice were dosed with vehicle Q2Dx7 i.v., 5 mg/kg of IGM-8444 Q2Dx7 i.v., 100 mg/kg of irinotecan QWx3 i.p., or a combination of the IGM-8444 and irinotecan dosing regimens. **B)** Colo205 tumors were implanted and vehicle and IGM-8444 treatment was performed as described in **A)**, and 5-FU was dosed at 100 mg/kg QWx3 i.p. **C)** HT-1080 fibrosarcoma cells were implanted s.c. in mice, followed by treatment with vehicle Q2Dx11 i.v., 5 mg/kg of IGM-8444 Q2Dx11 i.v., 75 mg/kg of gemcitabine Q3Dx7 i.p., or a combination of the IGM-8444 and gemcitabine dosing regimens. **D)** Colo205 tumors were implanted and vehicle and IGM-8444 treatment was performed as described in **A)**, and paclitaxel was dosed at 25 mg/kg Q2Dx5 i.p. **E)** HT-1080 tumors were implanted and vehicle and IGM-8444 treatment was performed as described in **C)**, and docetaxel was dosed at 5 mg/kg QWx3 i.v. **F)** SA3829 Ewing's sarcoma PDX tumor fragments were implanted s.c. and mice were dosed with vehicle Q2Dx11 i.v., 5 mg/kg of IGM-8444 Q2Dx11 i.v., 5 mg/kg of docetaxel QWx3 i.v., or a combination of the IGM-8444 and docetaxel dosing regimens. Horizontal bars in the lower left of each graph indicate the dosing period. Tumor volumes (mean ± SEM) are plotted. Combination of IGM-8444 with these chemotherapeutic agents resulted in enhanced anti-tumor responses compared to either single agent. Several animals in the Colo205 and HT-1080 models that received IGM-8444 in combination with either gemcitabine or a taxane achieved tumor-free survival (TFS) by study end.

Chemotherapeutic Agents Up-Regulate DR5 Expression, a Potential Mechanism for IGM-8444 Synergy

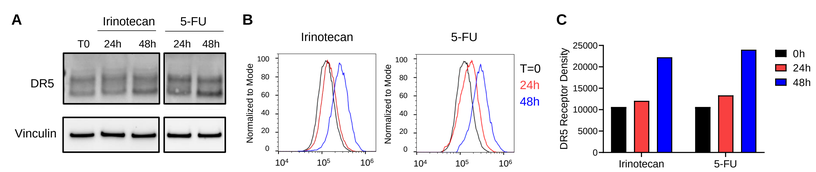

Figure 6. DR5 expression is up-regulated following irinotecan and 5-FU treatment.
Colo205 cells were treated with 3 µM irinotecan or 3 µM 5-FU for 24 or 48 hours. **A)** Cell lysates were run on a reducing gel and analyzed for DR5 or for vinculin loading control by western blot. **B)** Cell surface DR5 expression was assessed by flow cytometry, and **C)** DR5 receptor density was quantitated using PE Quantibrite PE beads. DR5 up-regulation was most pronounced 48 hours post-treatment with chemotherapy.

Summary

- As a monotherapy, IGM-8444 responses range from sensitive to resistant across the tumor cell lines and xenograft models screened
- In a sensitive model, IGM-8444 treatment rapidly induces intra-tumoral cleavage of caspase 3, a pharmacodynamic biomarker
- IGM-8444 was screened in combination with chemotherapeutic agents and topoisomerase inhibitors, microtubule inhibitors, and nucleoside analogs were identified as most synergistic
- Combination of IGM-8444 with these classes of chemotherapeutic agents also enhanced anti-tumor responses in xenograft models, resulting in long-term tumor-free survivors in some cases
- Chemotherapy treatment can up-regulate DR5 expression, providing a potential mechanistic rationale for the enhanced anti-tumor activity observed when these agents are combined with IGM-8444
- The combination of IGM-8444 with FOLFIRI + bevacizumab is currently under evaluation in a Phase 1 study in patients with metastatic colorectal cancer (NCT04553692)