IGM-7354, an immunocytokine with IL-15 fused to an anti-PD-L1 IgM, induces NK and CD8+ T cell-mediated cytotoxicity of PD-L1 positive tumor cells

Poster # 5660

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Background

- Immunostimulatory cytokines are a promising immunotherapy for the treatment of advanced malignancies, but generally have been associated with severe toxicities when administered systemically. The recent development of antibody-cytokine fusion proteins, or immunocytokines, aims to localize cytokine activity to the tumor microenvironment and thus improve their therapeutic index.
- We have developed IGM-7354, a high affinity, high avidity anti-PD-L1 pentameric IgM antibody with an IL-15Rα chain and IL-15 fused to the joining (J) chain. The IGM-7354 immunocytokine was designed to deliver IL-15-mediated stimulation of NK and CD8+ T cells to PD-L1 expressing tumors and antigen-presenting cells, to enhance anti-tumor immune responses. IGM-7354 is currently being evaluated in a first-in-human Phase 1 trial.

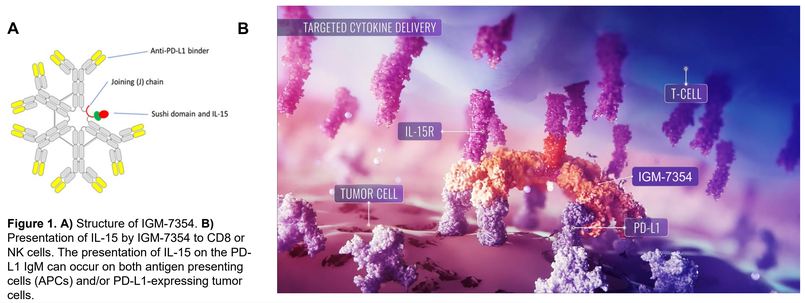

Figure 1. A) Structure of IGM-7354. **B)** Presentation of IL-15 by IGM-7354 to CD8 or NK cells. The presentation of IL-15 on the PD-L1 IgM can occur on both antigen presenting cells (APCs) and/or PD-L1-expressing tumor cells.

IGM-7354 Has a Stronger Avidity for PD-L1 Than for the IL-15 Receptor

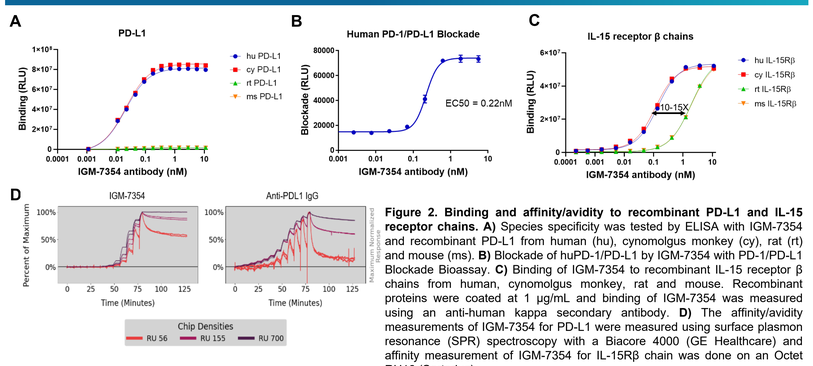

Figure 2. Binding and affinity/avidity to recombinant PD-L1 and IL-15 receptor chains. A) Species specificity was tested by ELISA with IGM-7354 and recombinant PD-L1 from human (hu), cynomolgus monkey (cy), rat (rt) and mouse (ms). **B)** Blockade of huPD-1/PD-L1 by IGM-7354 with PD-1/PD-L1 Blockade Bioassay. **C)** Binding of IGM-7354 to recombinant IL-15 receptor β chains from human, cynomolgus monkey, rat and mouse. Recombinant proteins were coated at 1 μg/mL and binding of IGM-7354 was measured using an anti-human kappa secondary antibody. **D)** The affinity/avidity measurements of IGM-7354 for PD-L1 were measured using surface plasmon resonance (SPR) spectroscopy with a Biacore 4000 (GE Healthcare) and affinity measurement of IGM-7354 for IL-15Rβ chain was done on an Octet RH16 (Sartorius).

	Binding to IL-15Rβ	Binding to PD-L1	
	Kd	Kd	Apparent Avidity
IGM-7354	20.4 nM	4.36 nM	2.69 pM
Anti-PD-L1 IgG	NA	6.78 nM	120.96 pM

IGM-7354 Rescues T cell Exhaustion in an *In Vitro* MLR System

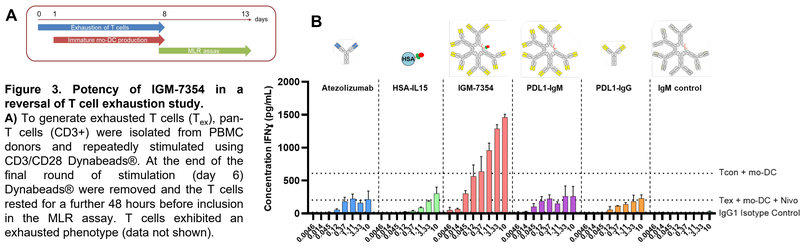

Figure 3. Potency of IGM-7354 in a reversal of T cell exhaustion study.
A) To generate exhausted T cells (T_{ex}), pan-T cells (CD3+) were isolated from PBMC donors and repeatedly stimulated using CD3/CD28 Dynabeads®. At the end of the final round of stimulation (day 6) Dynabeads® were removed and the T cells rested for a further 48 hours before inclusion in the MLR assay. T cells exhibited an exhausted phenotype (data not shown).

IGM-7354 promoted increased secretion of IFNγ by exhausted T cells. B) One-way mixed lymphocyte reactions (MLRs) were used to assess reversal of T_{ex} hypo-responsiveness in the presence of dose titrations of IGM-7354 and control molecules. *In vitro* generated monocytes-derived DCs (mo-DCs) were combined with T_{ex} (1:10 ratio) to generate MLR pairs and cultured for 5 days. **C)** Following completion of the MLR, the supernatants were assessed for levels of IFNγ (by ELISA). Data are representative of N=6 MLR pairs. Atezolizumab was purchased from Sellekchem. **Control MLRs were used to assess reversal of exhaustion:** Control T cells (freshly isolated) + moDCs (Tcon+moDC), T exhausted + moDC + anti-PD-1 IgG (Tex+moDC+Nivo).

IGM-7354 Significantly Enhances CD8 T cell Proliferation Compared to Untargeted IgM-IL-15 In Vitro

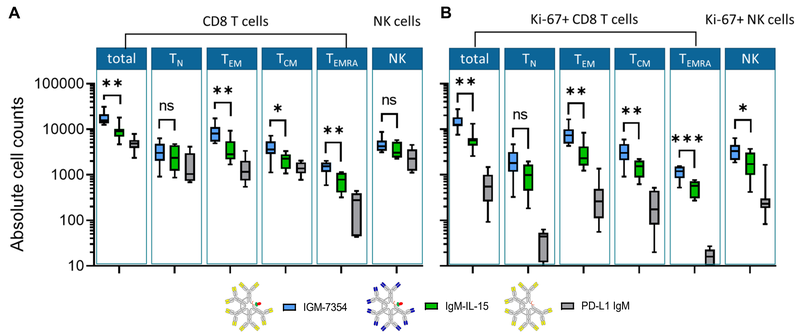

Figure 4. *in vitro* lymphoproliferative activity of IGM-7354. Total and Ki-67+ human NK and CD8 T cells were enumerated by flow cytometry following 7 days in culture with serial dilutions of either IGM-7354, a non-targeted IgM-IL-15 or an anti-PD-L1 IgM (without IL-15). PBMCs were characterized by flow cytometry using a FACSymphony A1 (Becton Dickinson) with CountBright Beads to achieve absolute cell counts. CD8 T cell subpopulation phenotypes were as follows: T naive (T_N) CD3+/CD8+/CCR7+/CD45RA+; T effector memory (T_{EM}) CD3+/CD8+/CCR7-/CD45RA-; T central memory (T_{CM}) CD3+/CD8+/CCR7+/CD45RA- and terminally differentiated T effector memory (T_{EMRA}) CD3+/CD8+/CCR7-/CD45RA+. **A)** Average absolute cell counts for 8 healthy donors at day 7 for CD8 T cells and NK cells. Data shown for 3.3nM antibody concentration for the CD8 T cells, and for 0.37nM antibody concentration for the NK cells. **B)** Average absolute cell counts for 8 healthy donors at day 7 for Ki-67+ CD8 T cells and Ki-67+ NK cells. p values are shown only between the IGM-7354 and IgM-IL-15 data sets. *** p < 0.001, **p < 0.01, *p < 0.05, ns non-significant (One-way ANOVA with Tukey's multiple comparisons test)

IGM-7354 Induces the In Vivo Proliferation of Human NK and CD8+ T cells in Non-Tumor Bearing BRGSF Humanized Mice

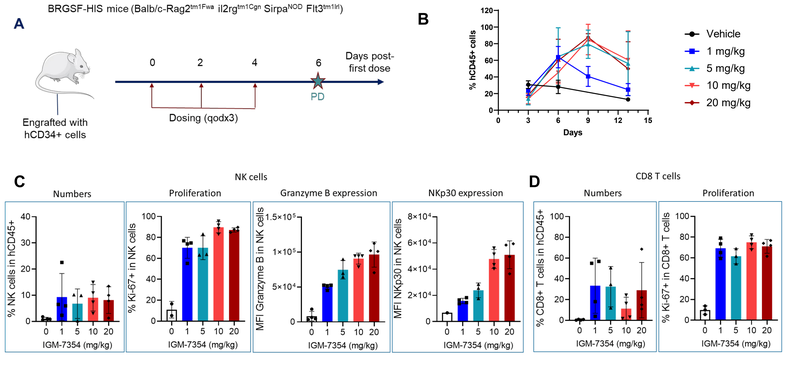

Figure 5. Pharmacodynamics of IGM-7354 in non-tumor bearing BRGSF humanized mice.
A) BRGSF are immunocompromised mice engrafted with human CD34+ from umbilical cord blood. Mice were dosed with IGM-7354 at least 12 weeks post-engraftment. Mice received three doses of IGM-7354 on Day 0 (first day of dosing), Day 2 and Day 4. **B)** Frequency of human CD45+ in blood was analyzed on Days 3, 6, 9 and 13. Pharmacodynamics (PD) of IGM-7354 on NK and CD8+ T cells was evaluated on Day 6. **C)** The NK cell (CD3- CD56+) phenotype was characterized by flow cytometry on whole blood on Day 6, including proliferation with the Ki-67 marker, and activation with the Granzyme B and NKp30 markers. **D)** The proliferation of CD8+ T cells (CD3+ CD8+) in whole blood was analyzed on Day 6.

IGM-7354 Enhances PBMC-Mediated Anti-Tumor Activity In Vitro Against PD-L1-Expressing MDA-MB-231 TNBC

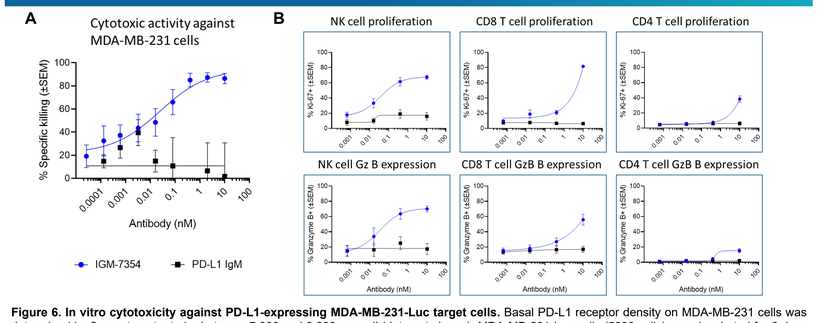

Figure 6. In vitro cytotoxicity against PD-L1-expressing MDA-MB-231-Luc target cells. Basal PD-L1 receptor density on MDA-MB-231 cells was determined by flow cytometry to be between 7,000 and 9,000 per cell (data not shown). MDA-MB-231-Luc cells (5000 cells) were incubated for 6 days in the presence of whole PBMCs at a 3:1 E:T ratio(E=CD8+NK). MDA-MB-231 cell killing was measured by addition of BioGlo (Promega). Shown is **A)** the average cytotoxic activity for 5 healthy donors. **B)** Percentages of Ki-67 and granzyme B (GzB) positive NK and T cells were determined by flow cytometry. Anti-PD-L1 IgM has the same PD-L1 binding units as IGM-7354 but lacks the IL-15 on the joining chain.

IGM-7354 Induces Anti-Tumor Responses in the Humanized MDA-MB-231 Tumor Model

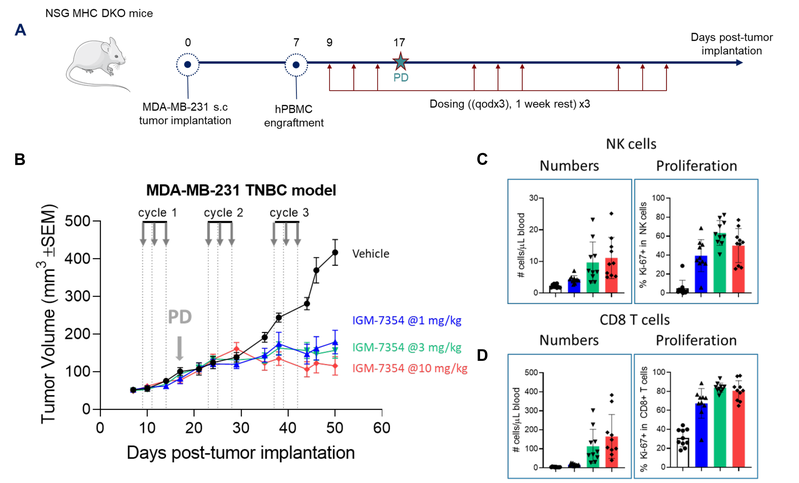

Figure 7. Anti-tumor activity and pharmacodynamics (PD) of IGM-7354 in the MDA-MB-231 humanized tumor model.
A) NSG MHC $I^{-/-}$ MHC $II^{-/-}$ (DKO) mice were implanted s.c with $5x10^6$ MDA-MB-231 tumor cells on Day 0. On Day 7, $10x10^6$ human PBMCs were engrafted i.v and IGM-7354 was dosed two days later for three cycles (each cycle was every other day dosing (qod) for 3 doses, one week rest). **B)** Average of tumor growth (n=10) for the animals dosed with IGM-7354 at 1, 3 or 10 mg/kg or vehicle. **C and D)** NK and CD8+ T cells counts and proliferation were measured on whole blood 3 days post last dose of cycle 1 i.e., Day 17.

IGM-7354 Infusion in Cynomolgus Monkeys Induces the Proliferation of NK, CD8+ and γδ T Cells in In Vivo

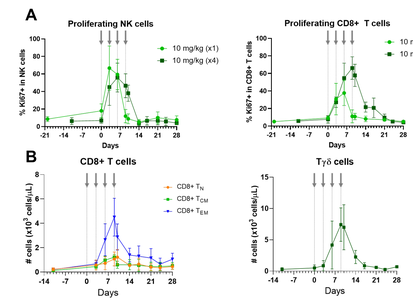

Figure 8. IGM-7354 induces the proliferation of NK and CD8+ T cells in non-human primates *in vivo*.
Cynomolgus monkeys were treated with either one or four doses of IGM-7354 at 10 mg/kg on day 0 or on days 0, 3, 6 and 9 respectively. **A)** Proliferation of NK cells (CD3-CD16+), and CD8+ T cells (CD3+ CD16- CD8+) was determined by Ki67 staining on whole blood samples. **B)** Absolute cell counts of circulating CD8+ Naïve (T_N, CD3+ CD8+ CD95- CD28+), Central Memory (T_{CM}, CD3+ CD8+ CD95+ CD28+), Effector Memory (T_{EM}, CD3+ CD8+ CD95+ CD28-), and γδ T cells in cynomolgus monkeys receiving IGM-7354 at 10mg/kg x 4. Vertical dashed lines with arrows indicate the dosing days (0, 3, 6 and 9).

Summary

IGM-7354, an anti-PD-L1/IL-15 IgM immunocytokine that:

- Binds with high avidity against PD-L1 and lower affinity with the IL-15 receptor through its IL-15-bearing J chain.
- Reverses T cell exhaustion more potently than anti-PD-L1 antibodies or a non-targeted IL-15 fusion molecule alone in an *in vitro* modified MLR model.
- Enhances human NK and CD8 T cells proliferation *in vitro* better than an untargeted IgM-IL-15.
- Increases the proliferation and activation of the cytotoxic NK and CD8+ T cells *in vivo* in non-tumor bearing humanized BRGSF mice.
- Enhances *in vitro* killing of PD-L1 positive MDA-MB-231 cells by human PBMCs compared to anti-PD-L1 IgM lacking the IL-15 fusion. This cytotoxic activity is mediated by NK and CD8 T cells.
- Demonstrates robust anti-tumor activity in the MDA-MB-231 xenograft humanized mouse tumor model.
- Is well tolerated in cynomolgus monkeys at doses up to 10 mg/kg.
- Shows increased proliferation of NK, CD8+ T cells and γδ T cells in cynomolgus monkeys.
- The Phase I clinical trial initiated in January is a first-in-human (FIH), Phase 1, multicenter, open-label study to evaluate the safety, tolerability, and PK of **IGM-7354** in participants with relapsed and/or refractory tumors (NCT05702424). The study design consists of a dose-escalation stage and dose-expansion stage.

IGM-7354 is designed to enhance target delivery of the immunostimulatory cytokine IL-15 through high affinity and high avidity binding to PD-L1 with the potential to improve anti-tumor responses while minimizing toxicity.