Enhanced NK and CD8+ T cell proliferation, tumor cytotoxicity and reversal of T cell exhaustion with IGM-7354, an anti-PD-L1 IgM antibody and IL-15 cytokine fusion

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Background

Anti-PD-1/PD-L1 therapies are efficacious in certain cancer indications, but often patients relapse following a primary response. Therefore rational combinations are needed to enhance initial and durable responses of anti-PD-1/PD-L1 therapies. Immunostimulatory cytokine, IL-15 is an attractive combination partner to enhance anti-tumor NK and memory CD8$^+$ T cell expansion and survival.

We are developing 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, designed to deliver IL-15 to PD-L1 expressing tumors to enhance anti-tumor immune responses.

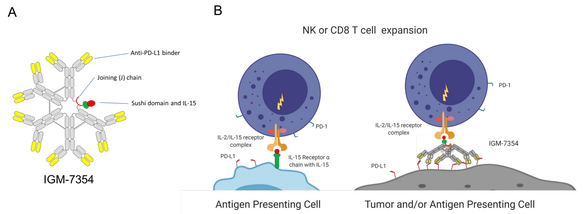

Figure 1. A) Structure of IGM-7354. **B)** Natural presentation of IL-15 versus presentation of IL-15 by IGM-7354 to CD8 or NK cells. The presentation of IL-15 on the PD-L1 IgM can occur on both antigen presenting cells (APCs) and/or PD-L1-expressing tumor cells.

IGM-7354 binds to human and cynomolgus monkey PD-L1 and IL-15 receptor β chain

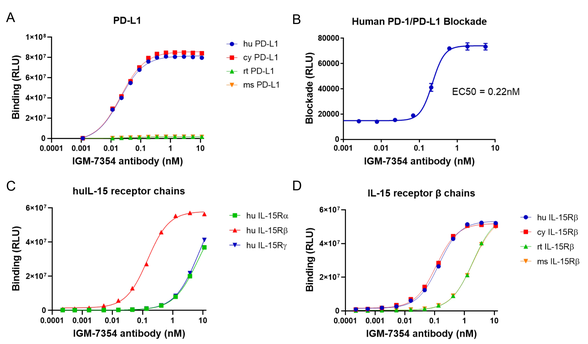

Figure 2. Binding by ELISA to recombinant PD-L1 and IL-15 receptor chains.
A) Species specificity was tested by ELISA with IGM-7354 and recombinant PD-L1 from human (hu), cynomolgus monkey (cy), rat (rt) and mouse (ms). **B)** Blockade of huPD-1/PD-L1 by IGM-7354 with PD-1/PD-L1 Blockade Bioassay. **C)** Binding of IGM-7354 to human IL-15 receptor α, β and γ chains. **D)** 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.

IGM-7354 induces proliferation of NK and CD8+ T cells and phosphorylation of STAT5 *in vitro*

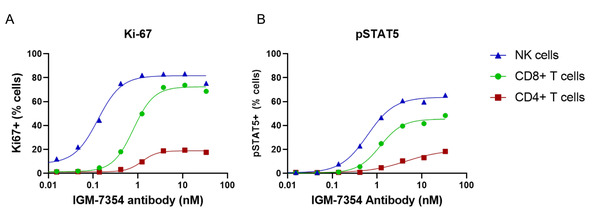

Figure 3. *In vitro* potency of IGM-7354 in PBMC cultures. **A)** Cell proliferation was determined by Ki67 staining of NK, CD8+ and CD4+ T cells after 96hr incubation with IGM-7354. **B)** Phosphorylation of STAT5 was measured by phosphoflow in NK, CD8+ and CD4+ T cells after 4hr incubation with IGM-7354. No Ki67 or pSTAT5 signals were observed with an anti-PD-L1 control IgM lacking the functional IL-15 fusion on the J chain (data not shown).

IGM-7354 reverses T cell exhaustion in an *in vitro* MLR system

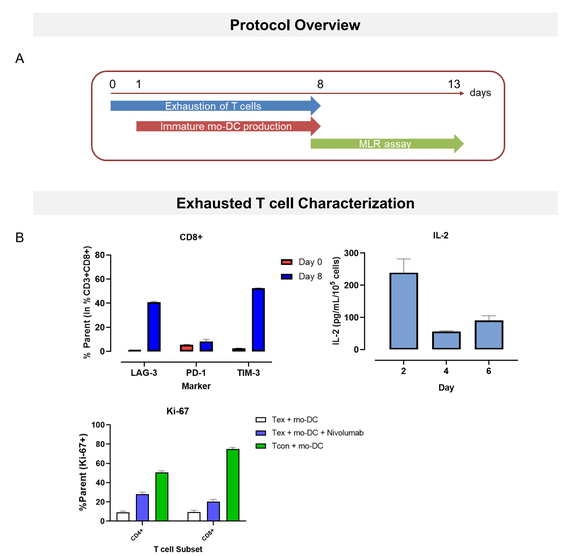

Figure 4. Potency of IGM-7354 in a reversal of T cell exhaustion study.
T cells exhibit an exhausted phenotype after an *in vitro* exhaustion protocol. A) To generate exhausted T cells (T_{ex}), pan-T cells (CD3+) were isolated from PBMC donors and repeatedly stimulated using CD3/CD28 Dynabeads®. At 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. **B)** T_{ex} were phenotyped by flow cytometry to confirm expression levels of PD-1, TIM-3 and LAG-3 by CD8 T cells (on day 0 and day 8). Proliferation was assessed by staining for Ki67. Supernatant collected throughout the process was assessed for IL-2 by ELISA.
IGM-7354 promoted increased secretion of IFNγ, and proliferation (Ki67) by exhausted T cells. 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). **D)** Additionally, the T cell populations were assessed for proliferative responses to the allogeneic stimulus (% Ki67 in CD8 populations). Data are representative of N=6 MLR pairs. Atezolizumab was purchased from Selleckchem.
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).
The T cell exhaustion studies were completed at Antibody Analytics Ltd., BioCity Scotland

IGM-7354 induces the proliferation of NK and CD8+ T cells in cynomolgus monkeys *in vivo*

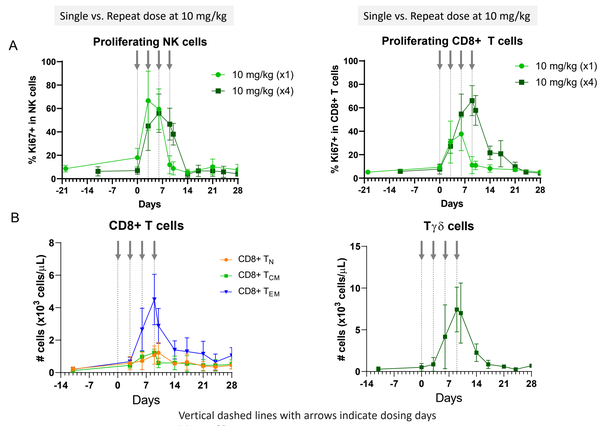

Figure 5. IGM-7354 induces the proliferation of NK and CD8+ T cells in non-human primates *in vivo*.
Cynomolgus monkeys were treated with either one or four doses of IGM-7354 at 10 mg/kg on day 0 or on days 0, 3, 6 and 9 respectively. **A)** Proliferation of NK cells (CD3- CD16+), and CD8+ T cells (CD3+ 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 monkey receiving IGM-7354 at 10mg/kg x 4. Vertical dashed lines with arrows indicate the dosing days (0, 3, 6 and 9).

IGM-7354 enhances PBMC-mediated MDA-MB-231 tumor cytotoxicity *in vitro* and *in vivo*

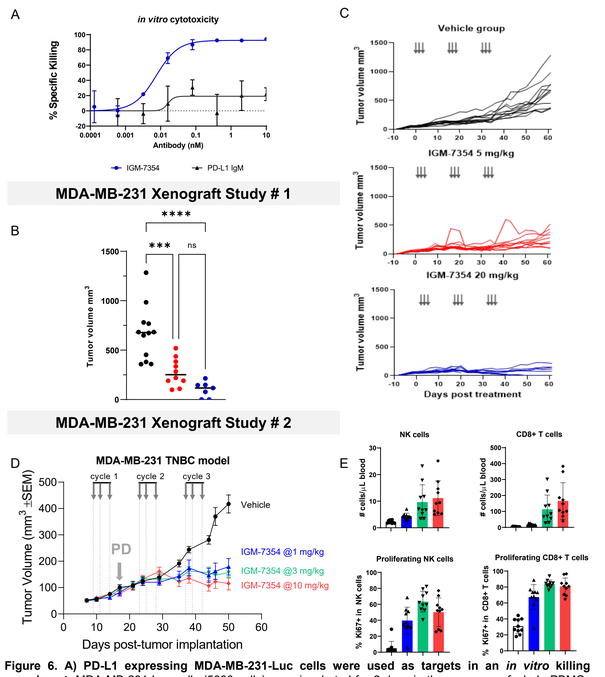

Figure 6. A) PD-L1 expressing MDA-MB-231-Luc cells were used as targets in an *in vitro* killing experiment. MDA-MB-231-Luc cells (5000 cells) were incubated for 6 days in the presence of whole PBMCs at a 3:1 E:T ratio(E=CD8+NK). MDA-MB-231 cell killing was measured by addition of BioGlo (Promega). Shown is a representative experiment of dose dependent killing of the cancer cells. PD-L1 IgM has the same PD-L1 binding units as IGM-7354 but lacks the IL-15 on the joining chain.
MDA-MB-231 were used in an *in vivo* tumor xenograft model (study # 1). MDA-MB-231 tumor cells ($5x10^6$) were implanted s.c. in MHC-/- NSG mice. PBMCs (10^7) were engrafted i.v. 10 days later. IGM-7354 or vehicle was dosed i.p. 2 days later at 5 or 20 mg/kg with the following regimen: Q2d x3, for 3 series of administration (9 doses total) with a week rest between administrations. **B)** Individual tumor size at Day 61 post-dosing. **C)** Individual tumor growth curves. **** p < 0.0001, *** p = 0.0003, ns non-significant (One-way ANOVA, with Tukey's multiple comparison test)
In vivo MDA-MB-231 study # 2 with titration of IGM-7354 dosed at 1, 3 and 10 mg/kg. D) Average tumor growth curves. **E)** Absolute counts of circulating NK and CD8 T cells, and proliferation of NK and CD8 T cells determined by Ki67 staining on whole blood samples at Day17.

IGM-7354 demonstrates additivity and potential synergy in combination with ADCC-mediating antibodies

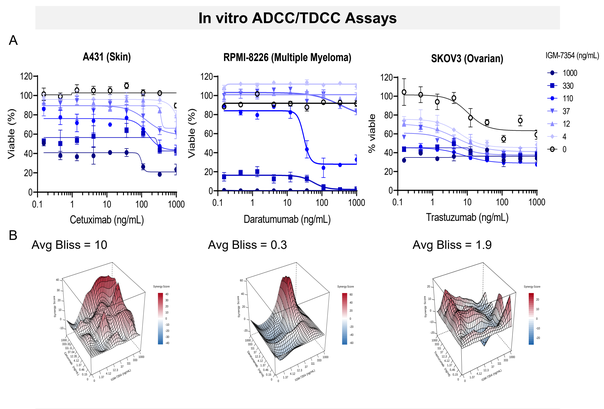

Figure 7. A431, RPMI-8226 and SKOV3 cells were used as targets in an *in vitro* killing experiment with IGM-7354 in combination with Cetuximab, Daratumumab, and Trastuzumab respectively. Luc-labelled cells (5000 cells) were incubated for 5 days in the presence of whole PBMCs at a 3:1 E:T ratio (E=NK) with the ADCC-enabling antibodies added for the last 24 hours. Target cell killing was measured by addition of BioGlo (Promega). **A)** Shown are representative experiments of dose dependent killing of the cancer cells. **B)** Bliss synergy scores were calculated in SynergyFinder and visualized in 3D surface plots. Bliss score > 10 suggests synergy.

RPMI-8226 were used in an *in vivo* tumor xenograft model. RPMI-8226 tumor cells ($1x10^7$) in PBS/Matrigel (1:1) were implanted s.c. in CB17/SCID mice. 14 days later, mice were randomized and IGM-7354 or vehicle was dosed i.p. at 20 mg/kg with the following regimen: Q2d x3, for 3 series of administration (9 doses total) with a week rest between administrations. Daratumumab was dosed i.p. biweekly for 5 weeks. **C)** Average tumor growth curves. **D)** Individual tumor size at Day 28 post-randomization. * p = 0.031, ** p = 0.0091, ns non-significant (One-way ANOVA, with Tukey's multiple comparison test).

Summary

IGM-7354, an anti-PD-L1 –IL-15 fusion IgM antibody:

- Binds equivalently to human and cynomolgus PD-L1 but not rodent.
- Binds equivalently to human and cynomolgus IL-15 receptor β chain through its IL-15 fusion on the J chain. Binding to rodent receptors was 10-15-fold weaker.
- Provides a potent IL-15-dependent proliferation signal to primary human NK and CD8+ T cells *in vitro,* as measured by Ki-67 and STAT5 phosphorylation.
- 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.
- Demonstrates dose-dependent responses in a human MDA-MB-231 xenograft mouse tumor model, with some treated animals having complete tumor regressions.
- Shows additivity and potential synergy in combination with antibodies with ADCC mechanism of action such as Cetuximab, Daratumumab, and Trastuzumab *in vitro* and *in vivo.*
- 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.
- Pharmacodynamic studies in cynomolgus monkeys shows potent proliferation of circulating NK, CD8+ T_{EM} and γδ T cells over the course of the treatment.
- IND filling planned for 2022
- **IGM-7354 may enhance tumor localization of immunostimulatory cytokine IL-15 through the high affinity and high avidity binding to PD-L1 to improve anti-tumor responses and minimize toxicity.**

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