CD123 Directed IgM T-cell Engager, IGM-2537, Demonstrates Potent in vitro and in vivo Activity with Minimal Cytokine Release

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Introduction

- Despite recent progress, the outlook for patients with acute myeloid leukemia (AML) remains poor. Novel therapies are needed for effective treatment of AML, especially in the relapsed/refractory setting.
- CD123, the IL-3 receptor alpha chain (IL-3Rα), is highly expressed on both leukemic blasts and leukemic stem cells and thus is an attractive therapeutic target in AML.
- CD123-based immunotherapies including bispecific antibodies or CAR-T cells have shown clinical promise, but cytokine release syndrome is a major safety concern with dose limiting toxicities.
- IGM-2537, a novel IgM antibody-based CD123xCD3 T cell engager (TCE), has 10 binding sites for CD123, and a single binding site for CD3ε, providing high affinity, high avidity binding to CD123$^+$ malignant cells with co-engagement of CD3$^+$ T cells leading to T cell induced cytotoxicity with low or minimal cytokine release in preclinical study.

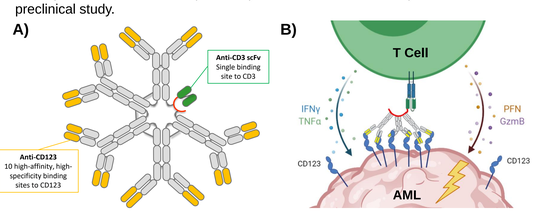

Figure 1. A) Structure of IGM-2537. **B)** Schematic mode of action (MoA) of IGM-2537 in T-cell dependent cellular cytoxicity (TDCC).

IGM-2537 activates T cells and induces cytotoxicity of the MV4-11 AML cell line

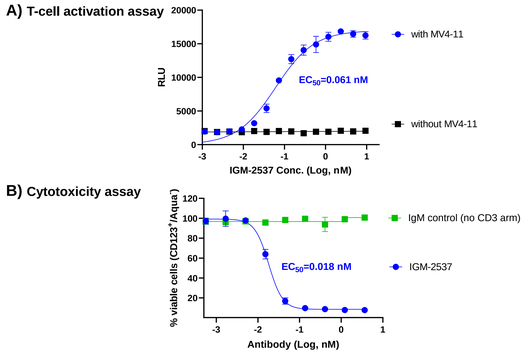

Figure 4. A) Jurkat/NFAT reporter cells (Promega) were either alone or co-mixed with AML cell line MV4-11, then incubated with various concentrations of IGM-2537. After incubation overnight, Bio-Glo reagent was added to plate followed by measurement of NFAT activation luminescence signal using a luminescence plate reader. **B)** IGM-2537-mediated, redirected T-cell cytotoxicity of tumor cells was evaluated in an in vitro TDCC assay using human PBMCs as effector cells and MV4-11 as target cells at an E/T ratio of 10:1. After 72 hr incubation, a flow-based viability assay was used to assess CD123$^+$Aqua$^-$ viable cells. The percentages of viable cells following IGM-2537 treatment were calculated by normalization of MV4-11 viable cells from no IGM-2537 treatment condition.

IGM-2537 inhibits tumor growth in humanized xenograft MV4-11 AML model

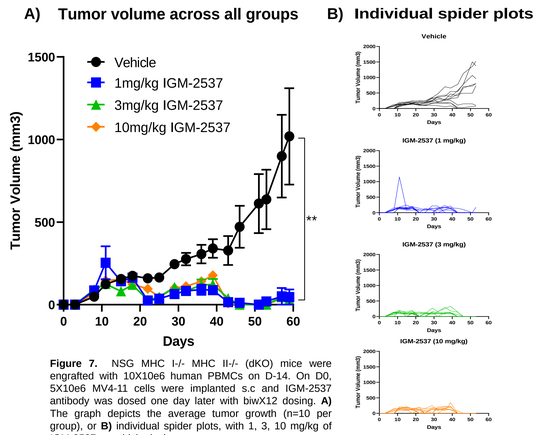

Figure 7. NSG MHC I-/- MHC II-/- (dKO) mice were engrafted with 10X10e6 human PBMCs on D-14. On D0, 5X10e6 MV4-11 cells were implanted s.c and IGM-2537 antibody was dosed one day later with biwX12 dosing. **A)** The graph depicts the average tumor growth (n=10 per group), or **B)** individual spider plots, with 1, 3, 10 mg/kg of IGM-2537 or vehicle dosing.

Single dose of benchmark CD123xCD3 IgM induces minimal/no cytokine release in cyno monkeys

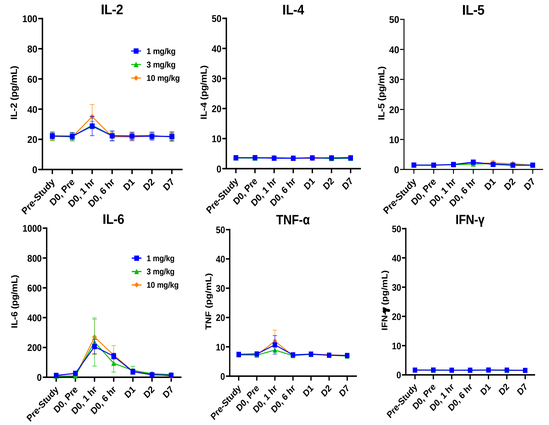

Figure 10. Cynomolgus monkeys were treated with a single dose of benchmark CD123(7G3-derived)xCD3 IgM on Day 0. Serum samples were collected at different time points as shown. Serum cytokines, including IL-2, IL-4, IL-5, IL-6, TNF-α and IFN-γ were measured with the non-human primate Th1/Th2 cytometric bead array (CBA) assay.

IGM-2537 has high affinity, specificity and binds to unique regions of CD123

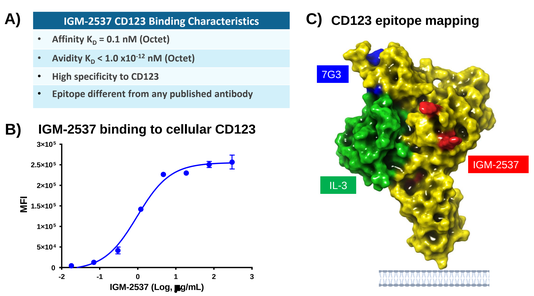

Figure 2. A) Affinity, avidity, specificity and epitope mapping of IGM-2537. **B)** IGM-2537 binding to cellular CD123 on HEK293 cells transfected with CD123 (shown). IGM-2537 was specific for CD123 binding as demonstrated by a Retrogenix screen of 5828 membrane proteins or cell surface-tethered secreted proteins (not shown). **C)** CD123 ECD structure showing key residues of the IGM-2537 epitope as determined by Ala scanning in the D2 region (red). The IGM-2537 epitope does not overlap with 7G3 (blue) or IL-3 (green).

IGM-2537 has significantly lower cytokine release in vitro than a CD123xCD3 IgG benchmark molecule

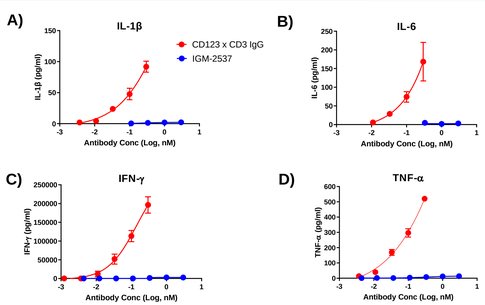

Figure 5. IGM-2537-mediated T cell cytokine release was evaluated and compared with a CD123xCD3 IgG benchmark using an in vitro TDCC assay in which human PBMCs were used as effector cells and MV4-11 as AML target cells at an E/T ratio of 10:1. The supernatants from the TDCC assay were collected after 72 hrs and evaluated by a cytokine multiplex MSD assay to determine the cytokine levels as indicated in **A), B), C)** and **D)**.

Single dose of benchmark CD123xCD3 IgM depletes CD123$^+$ basophils in cyno monkeys

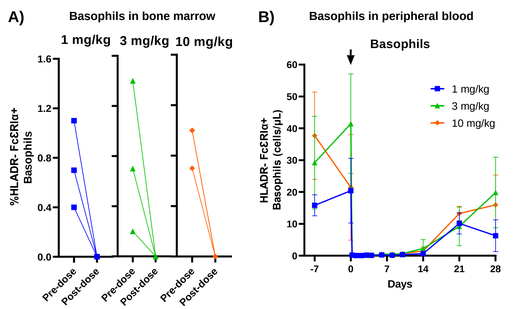

Figure 8. A) Frequency of cynomolgus monkey basophils (HLADR$^-$FcεRIα$^+$) in bone marrow were analyzed pre-dose and 2 days post-dose IV infusion with 1, 3 and 10 mg/kg of the benchmark CD123(7G3-derived)xCD3 IgM. **B)** Absolute cell counts of basophils (HLADR$^-$FcεRIα$^+$) in peripheral blood were measured pre- and post-IgM infusion. Black arrow and vertical dashed line indicate dosing at Day 0.

Tolerability, cytokine level and PD effect comparison of CD123xCD3 IgM with reported data for IgG TCE

	Maximum tolerated dose	Cytokine release level	Depletion of CD123$^+$ basophils
Benchmark CD123xCD3 IgM	10,000 μg/kg (highest dose tested)	Minimal/no cytokine release, not dose-dependent **IL-6 < 300 pg/mL TNF-α < 20 pg/mL**	Complete depletion in PB and BM (single dose)
CD123xCD3 IgG	10-100 μg/kg*	Significant cytokine release,* dose-dependent **IL-6 > 10,000 pg/mL TNF-α > 5,000 pg/mL**	Complete depletion in PB and BM (single dose)*

* based on reported data (Chu, ASH, 2014)

IGM-2537 binds to CD123 and CD3 antigens

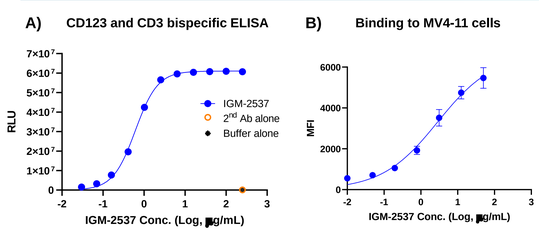

Figure 3. **A)** A bispecific ELISA assay was conducted using CD123 antigen coated-plates followed by incubation with various concentrations of IGM-2537, then followed by addition of CD3ε-His tag. The binding signal was measured after addition of anti-Penta-His HRP antibody. **B)** IGM-2537 binding to AML cell line MV4-11 was analyzed by flow cytometry after incubation of MV4-11 with various concentrations of IGM-2537, followed by adding an anti-IgM detection antibody.

IGM-2537 eliminates AML leukemia cells but spares normal hematopoietic progenitor cells

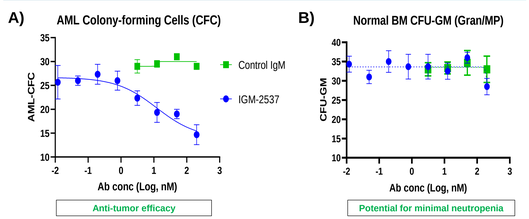

Figure 6 The effects of IGM-2537 on AML clonogenic progenitors (CFC) and normal BM CFU-GM progenitor cells were assessed in a semi-solid methylcellulose assay following liquid culture of BM samples (3 days) with optimal conditioned medium and various concentrations of IGM-2537 as shown. After incubation at 37°C, 5% CO_2 for a total of 14-16 days, and the resultant colonies were assessed & scored microscopically. Shown in **A)** is one representative result from 7 separate AML donor assays; shown in **B)** is one representative result from 3 independent normal BM donor assays.

Single dose of benchmark CD123xCD3 IgM transiently depletes CD123$^+$ pDCs in cyno monkeys

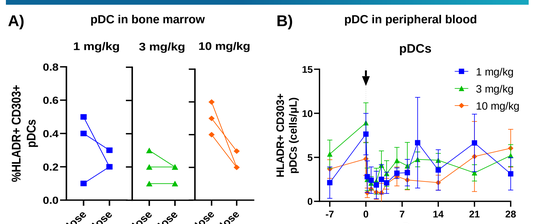

Figure 9. A) The frequency of pDCs (HLADR$^+$CD303$^+$) in cynomolgus monkey bone marrow was analyzed pre-dose and 2 days post-dose IV infusion of 1, 3 and 10 mg/kg of the benchmark CD123(7G3-derived)xCD3 IgM. **B)** Absolute cell counts of pDCs (HLADR$^+$CD303$^+$) in peripheral blood pre- and post-IgM infusion. Black arrow and vertical dashed line indicate dosing at Day 0.

Summary and Conclusions

IGM-2537

- Binds to human CD123 with high affinity, avidity, and specificity
- Co-engages with both CD123 and CD3ε, leading to T cell activation and T cell-redirected killing of AML cells
- Did not induce significant cytokine release in vitro (IFN-γ, TNF-α, IL-1β and IL-6, etc.)
- Demonstrated excellent in vivo anti-tumor efficacy with tumor growth inhibition observed at doses as low as 1 mg/kg, consistent with its potent in vitro TDCC activity
- Eliminated AML leukemic colony forming cells but spared normal BM progenitor cells.
- A benchmark, cynomolgus monkey cross reactive CD123xCD3 IgM demonstrated excellent tolerability with little to no cytokine production though depleted CD123$^+$ basophils & pDCs

Utilizing the IgM framework in IGM-2537 to target CD123 for T-cell mediated cytotoxicity may potentially provide a superior therapeutic window in the treatment of AML

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