Poster # 2933

Novel CD123xCD3 Bispecific IgM Antibody, IGM-2537, Potently Induces T-cell Mediated Cytotoxicity of Acute Myeloid Leukemia Cells 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 studies.

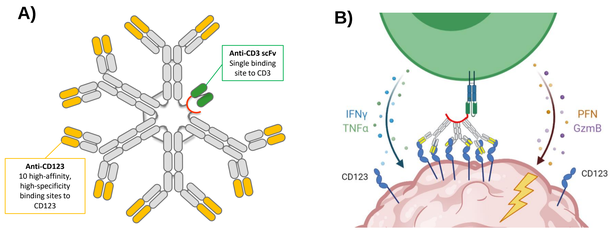


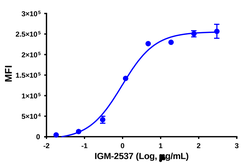
Figure 1. A) Structure of IGM-2537. **B)** Schematic mode of action (MoA) of IGM-2537 in T-cell dependent cellular cytotoxicity (TDCC).

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

A)

IGM-2537 CD123 Binding Characteristics
Affinity K_D = 0.36 nM (Octet)
Avidity K_D < 0.001 nM (Octet)
High specificity to CD123
Differentiated epitope

B) IGM-2537 binding to cellular CD123



C) CD123 epitope mapping

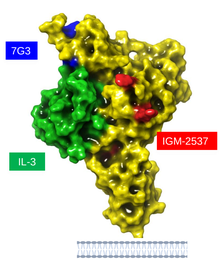
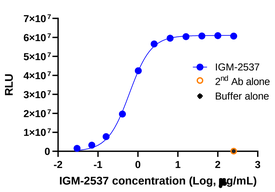


Figure 2. A) Affinity, avidity, specificity and epitope mapping of IGM-2537. **B)** IGM-2537 binding to cellular CD123 on HEK293 cells transfected with CD123 (shown). IGM-2537 was specific for CD123 binding as demonstrated by a Retrogenix screen of 5828 membrane proteins or cell surface-tethered secreted 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 binds to CD123 and CD3 antigens

A) CD123 and CD3 bispecific ELISA



B) Binding to MV-4-11 cells & CD3 T cells

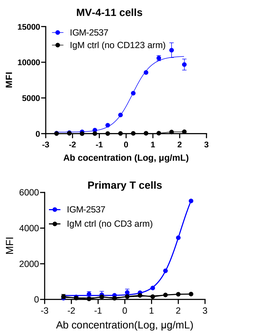
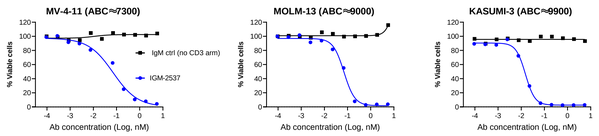


Figure 3. A) A bispecific ELISA assay was conducted using CD123 antigen coated-plates followed by incubation with various concentrations of IGM-2537, then followed by addition of CD3ε-His tag. The binding signal was measured after addition of anti-Penta-His HRP antibody. **B)** IGM-2537 binding to AML cell line MV-4-11 & primary T cells were analyzed by flow cytometry after incubation of cells with various concentrations of IGM-2537, followed by adding an anti-IgM detection antibody.

IGM-2537 induces cytotoxicity of AML cell lines accompanied by T cell activation & proliferation

A) In vitro cytotoxicity



B) In vitro T cell activation & proliferation

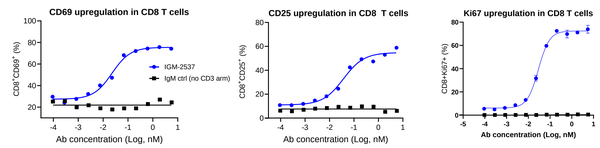


Figure 4. A) IGM-2537-mediated, redirected T-cell cytotoxicity of tumor cells was evaluated in an in vitro TDCC assay using human PBMCs as effector cells and MV-4-11, MOLM-13 and KASUMI-3 as target cells at an E/T ratio of 10:1. After 72 hr incubation, a luminescent-based viability assay was used to assess viable cells. The percentages of viable cells following IGM-2537 treatment were calculated by normalization of viable cells from no IGM-2537 treatment condition. **B)** T cell activation and proliferation were also profiled by staining PBMCs with CD4/CD8/CD25/CD69/Ki67 Ab cocktails, following 72-hr incubation of AML target cell lines with human PBMCs at an E/T ratio of 10:1. Shown are representative T cell activation and proliferation profiles in CD8 T-cell subset in co-culture containing MV-4-11 cells. Similar T cell activation and proliferation profiles were also observed in CD4 T-cell subset (data not shown).

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

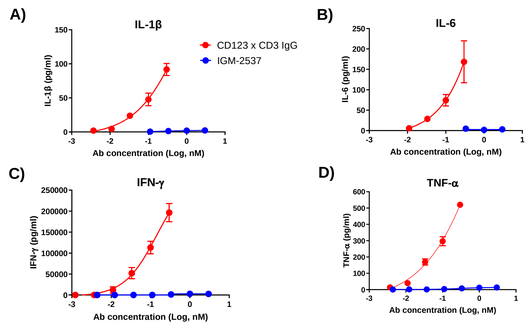
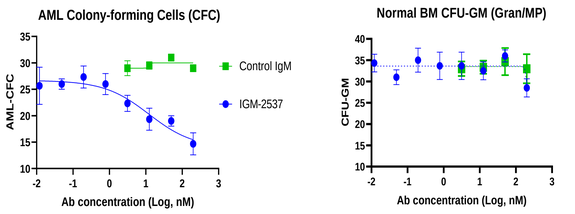


Figure 5. IGM-2537-mediated T cell cytokine release was evaluated and compared with a CD123xCD3 IgG benchmark using an in vitro TDCC assay in which human PBMCs were used as effector cells and MV-4-11 as AML target cells at an E/T ratio of 10:1. The supernatants from the TDCC assay were collected after 72 hrs and evaluated by a cytokine multiplex MSD assay to determine the cytokine levels as indicated in **A), B), C)** and **D)**.

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



Anti-tumor activity ex vivo

Potential for minimal neutropenia

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.

IGM-2537 inhibits tumor growth in humanized xenograft MV-4-11 AML model

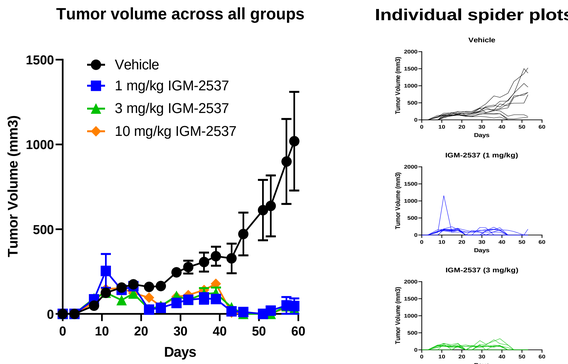


Figure 7. NSG MHC I-/- MHC II-/- (dKO) mice were engrafted with 10X10e6 human PBMCs on D-14. On D0, 5X10e6 MV-4-11 cells were implanted s.c and IGM-2537 antibody was dosed one day later with biwX12 dosing. **A)** The graph depicts the average tumor growth (n=10 per group), or **B)** individual spider plots, with 1, 3, 10 mg/kg of IGM-2537 or vehicle dosing.

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

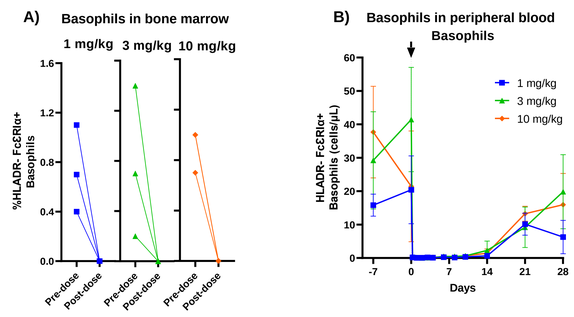


Figure 8. A) Frequency of cynomolgus monkey basophils (HLADR$^-$FcεRIα$^+$) in bone marrow were analyzed pre-dose and 2 days post-dose IV infusion with 1, 3 and 10 mg/kg of the benchmark CD123 (7G3-derived)xCD3 IgM. **B)** Absolute cell counts of basophils (HLADR$^-$FcεRIα$^+$) in peripheral blood were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

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

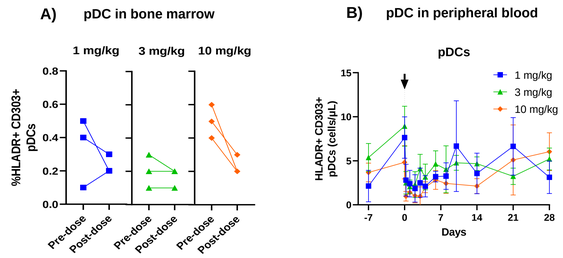


Figure 9. A) The frequency of plasmacytoid dendric cells (pDCs, HLADR$^+$CD303$^+$) in cynomolgus monkey bone marrow was analyzed pre-dose and 2 days post-dose IV infusion of 1, 3 and 10 mg/kg of the benchmark CD123(7G3-derived)xCD3 IgM. **B)** Absolute cell counts of pDCs (HLADR$^+$CD303$^+$) in peripheral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

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

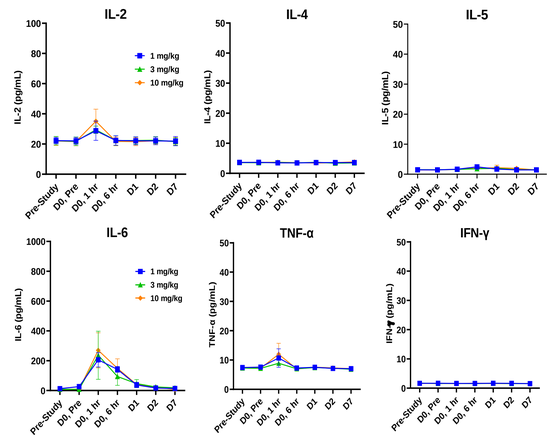


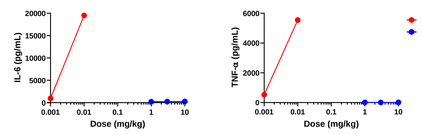
Figure 10. Cynomolgus monkeys were treated with a single dose of benchmark CD123(7G3-derived)xCD3 IgM on Day 0. Serum samples were collected at different time points as shown. Serum cytokines, including IL-2, IL-4, IL-5, IL-6, TNF-α and IFN-γ were measured with the non-human primate Th1/Th2 cytometric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

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

	Maximum tolerated dose in cyno monkeys	Depletion of CD123$^+$ basophils in cyno monkeys
Benchmark CD123xCD3 IgM	10,000 µg/kg (highest dose tested)	Complete depletion in PB and BM (single dose)
CD123xCD3 IgG*	10-100 µg/kg	Complete depletion in PB and BM (single dose)

*Chu, ASH, 2014

Cytokine induction level in monkey study



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 activity 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