Preclinical Characterization of NGM936, a Novel Bispecific T Cell Engager Targeting ILT3 for the Treatment of Acute Myeloid Leukemia With Monocytic Differentiation

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Background

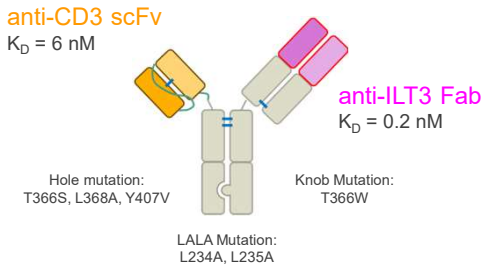
Acute myeloid leukemia (AML) with monocytic differentiation Acute myeloid leukemia (AML) with monocytic differentiation [M4 and M5 AML per the French-American-British (FAB) classification] accounts for 25–30% of all AML cases and has a particularly poor prognosis, with a <15% five-year overall survival rate and an increased risk of bone marrow and extramedullary relapse after stem cell transplant compared with other AML subtypes. The use of T cell engagers in AML has been limited by the lack of suitable target antigens with high specificity for AML cells and minimal expression on healthy bone marrow progenitors. Immunoglobulin-like transcript 3 (ILT3, also known as LILRB4) is a highly specific marker of AML with monocytic differentiation and is not expressed on non-monocytic blood cells or hematopoietic progenitor cells. This highly restricted expression profile makes ILT3 a promising target for monocytic AML, with the potential to enable effective elimination of AML cells with less toxicity than current therapies. Here, we report the pre-clinical characterization of NGM936, an ILT3-based T cell engager for the treatment of monocytic AML and other ILT3+ hematologic malignancies.

NGM936 is a monovalent scFv-Fab bispecific on an effectorless human IgG1 backbone (LALA) with a knob-in-hole Fc design for heterodimeric assembly. NGM936 demonstrates sub-nM affinity towards ILT3 of human origin (K_D = 0.2 nM at 25°C by surface plasmon resonance) and is not cross-reactive towards other LILRA/B family members.

A panel of more than 30 ILT3 x CD3 engagers in various formats was generated and screened in assays to measure both T cell-dependent cytotoxicity and cytokine release. NGM936 was identified for its ability to potently induce T cell-dependent cytotoxicity (TDCC) against ILT3+ AML cells while inducing minimal cytokine release. In both whole blood cytokine release assays and in TDCC assays in which cytokine secretion was measured after target engagement, induction of TNF-α, IL-6, IFN-γ, and IL-2 by NGM936 was considerably lower than that induced by a vibecotamab biosimilar (CD123 x CD3). NGM936 induced potent cytotoxicity when both expanded and naïve T cells were used as effectors. Importantly, NGM936 efficiently ablated tumor cells with a range of ILT3 expression, from ~1500 copies/cell to ~40,000 copies/cell. In addition, NGM936 failed to induce T cell-dependent cytotoxicity against CD34+ hematopoietic stem cells or non-monocytic immune cells, consistent with the lack of ILT3 expression on these cell types. In *ex vivo* cultures of primary M5 AML bone marrow, NGM936 induced a dose-dependent depletion of AML cells and a concordant increase in T cell proliferation and activation. Finally, NGM936 induced a dose-dependent depletion of circulating tumor cells in a xenograft model in which irradiated, immunodeficient NSG mice were engrafted with human PBMCs and human ILT3+ AML cells.

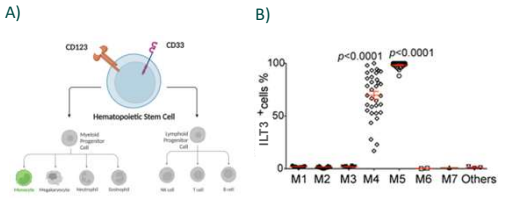
NGM936 thus represents a promising new treatment strategy for monocytic AML, with the potential to eliminate monocytic leukemia cells while minimizing the myelotoxicity associated with ablation of healthy bone marrow.

NGM936



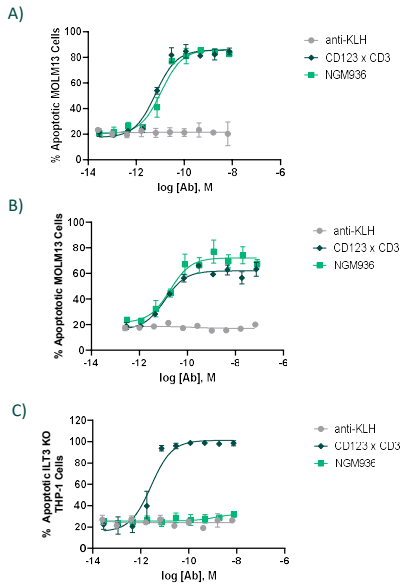
Results

Figure 1. The Expression Pattern of ILT3 Makes it an Ideal Target for AML with Monocytic Differentiation



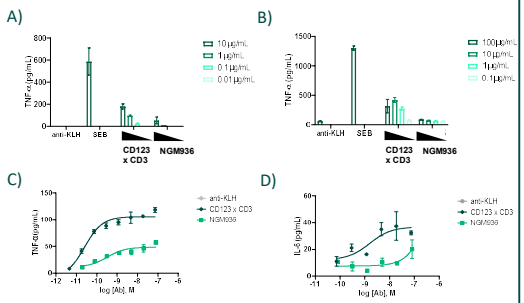
A) Common targets of T cell engagers for AML, such as CD123 and CD33, are expressed on hematopoietic stem cells and are broadly expressed across mature myeloid-lineage cells. In contrast, the expression of ILT3 is restricted to monocytic cells. B) ILT3 is specifically expressed on AML with monocytic differentiation [M4 and M5 AML according to the French-American-British (FAB) classification] and is particularly highly and uniformly expressed in M5 AML. Figure from Deng M., *Nature*, 2018 (PMID 30333625).

Figure 2. NGM936 Potently Induces T Cell-Dependent Cytotoxicity Against ILT3+ AML Cells



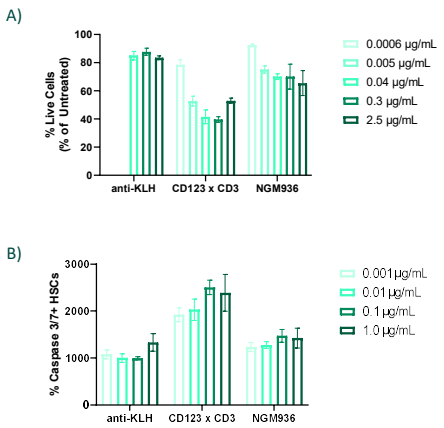
A) T cell-dependent cytotoxicity induced by NGM936 against MOLM13 cells (a human M5 AML cell line), using expanded T cells as the effector cells. B) T cell-dependent cytotoxicity induced by NGM936 against MOLM13 cells, using naïve human PBMCs as the effector cells. C) T cell-dependent cytotoxicity induced by NGM936 against an ILT3 knockout human AML cell line (THP-1). A vibecotamab biosimilar (CD123 x CD3) was included as a comparator.

Figure 3. NGM936 Induces Low Cytokine Release in Multiple Assay Formats



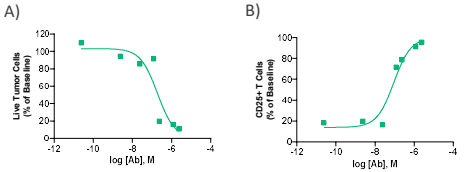
A-B) TNF-α secretion induced by NGM936 was measured in whole blood cytokine secretion assays, with the antibodies added in soluble (A) or plate-coated (B) format. SEB was included as a reference molecule. C-D) TNF-α (C) and IL-6 (D) release were measured in cytotoxicity assays using naïve human PBMCs as the effectors. In both assay formats, NGM936 induced significantly less cytokine release than the vibecotamab biosimilar (CD123 x CD3), despite having similar potency in cytotoxicity assays.

Figure 4. NGM936 Does Not Induce Apoptosis in Healthy Bone Marrow Progenitor Cells



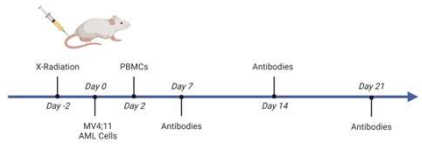
A) CD34+ hematopoietic stem cells (StemCell Technologies) were combined with allogeneic T cells and a dose titration of NGM936 or a vibecotamab biosimilar, and the percentage of live HSCs remaining after 24 hrs was measured by flow cytometry. B) Apoptosis induction in CD34+ HSCs was measured by flow cytometry for cleaved caspase 3/7.

Figure 5. NGM936 Induces T Cell Activation and Tumor Cell Killing in Primary M5 AML Bone Marrow Cultures



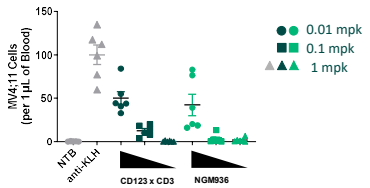
The activity of NGM936 was evaluated in the Native Tumor Microenvironment platform (Vivia Biotech), in which whole bone marrow samples from patients with M5 AML were cultured with a dose titration of NGM936, and T cell activation and tumor cell depletion were evaluated by flow cytometry. Results were normalized to the baseline values for each donor. NGM936 induced dose-dependent tumor cell depletion (A) and T cell activation (B) in primary M5 AML bone marrow samples. Data from a representative donor are shown (out of 4 donors evaluated).

Figure 6. Human AML Xenograft Model



NSG mice (female, 6 weeks old) were irradiated (1 Gy) 48 hrs prior to administration of AML cells. NucLight Red-labeled MV4;11 cells (8 x 10^6 cells/mouse) were administered by tail vein injection. Two days later, mice were injected with human PBMCs (1 x 10^7 cells/mouse) by tail vein injection. Mice were administered antibodies i.v. once per week (0.01, 0.1, or 1 mpk) beginning 7 days after PBMC administration. Blood was drawn weekly, and the circulating tumor burden was quantified by flow cytometry using Precision Count beads (BioLegend).

Figure 7. NGM936 Reduced the Circulating Tumor Burden in a Human AML Xenograft Model



Quantification of the circulating tumor burden in mice engrafted with human PBMCs and a human monocytic AML cell line after 2 weeks of treatment with NGM936 or a vibecotamab biosimilar.

Acknowledgements

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