CD16-IL15-CLEC12A Trispecific Killer Engager (TriKE) Drives NK Cell Expansion, Activation, and Antigen Specific Killing of Cancer Stem Cells in Acute Myeloid Leukemia

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BACKGROUND

- A majority of all deaths from hematopoietic malignancies are caused by acute myeloid leukemia (AML) which has has a poor five-year survival rate of 26% highlighting the need for new therapies.
- The most common antigen used to target AML cells is CD33 however there are many limitations of developing therapies against CD33.
  1. Not all cancer cells express CD33. This is especially significant in patients with refractory AML

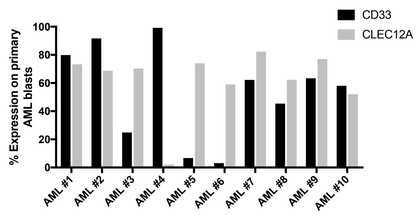

Figure 1: Percentage of CD33 and CLEC12A surface expression measured by flow cytometry analysis of primary AML samples from 10 patients

  2. All cells of the myeloid lineage and some cells of the lymphoid lineage like activated NK cells and T cells express CD33 leading to off-target toxicity
  3. Cancer stem cells, which are thought to facilitate relapse do not express CD33
- To address these limitations, we aim to target a novel antigen called C-type Lectin-like molecule 1 (CLL-1) or CLEC12A.
  1. CLEC12A is highly expressed on AML cells. About 70% of CD33 negative cells express CLEC12A
  2. The expression of CLEC12A is restricted to a subset of myeloid cells limiting off-target toxicity
  3. CLEC12A is present on leukemic stem cells but not hematopoietic stem cells
- To target cancer cells using Natural Killer (NK) cells, we developed a tri-specific killer engager (TriKE) molecule containing an anti-CD16 heavy chain antibody that activates NK cells, an IL-15 molecule that drives NK cell priming, expansion and survival, and an anti-CLEC12A single chain variable fragment (scFv) that engages cancer targets.

METHODS

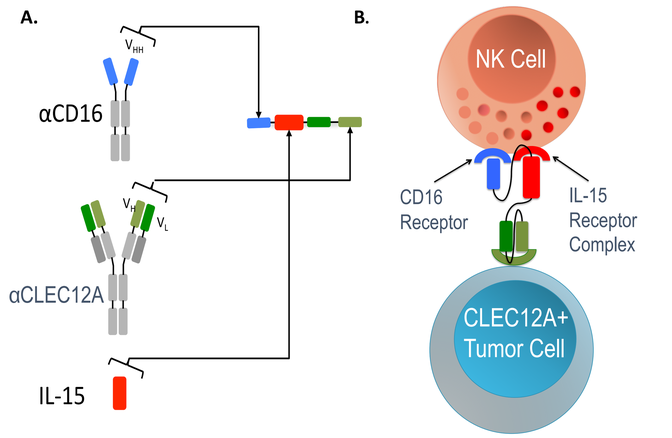

Figure 2: Schematic of the CD16-IL15-CLEC12A TriKE and mechanism of action: (A) The TriKE contains an anti-CD16 heavy-chain antibody constructed by our group by incorporating the CDRs of a llama anti-CD16 V_{HH} into a humanized V_{HH} backbone. This is linked to a wild type IL-15 molecule which is linked to the scFv from an anti-CLEC12A antibody. The TriKE is produced in a mammalian system with Expi-293 cells and contains a His tag which is used to purify the molecule. (B) The TriKE creates an immunological synapse between a CLEC12A+ tumor cell and NK cell promoting release of cytotoxic granules and secretion of cytokines that kills the target cell.

RESULTS

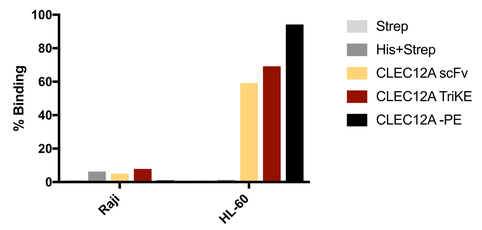

Figure 3: The CD16-IL15-CLEC12A TriKE binds specifically to targets that express CLEC12A: CLEC12A+ HL-60 and CLEC12A- Raji targets were incubated with the CLEC12A TriKE or scFv at equimolar concentrations. Binding was assessed by an anti-His antibody that binds to the His tag on the TriKE or scFv. A secondary Streptavidin antibody was used that was detected by flow cytometry.

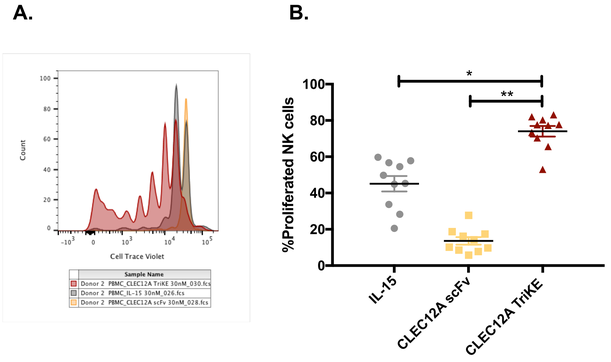

Figure 4: The CD16-IL15-CLEC12A TriKE promotes proliferation of NK Cells: (A) PBMCs were labeled with Cell Trace and incubated with equimolar concentrations of IL-15, CLEC12A scFv or CLEC12A TriKE for 7 days. The NK cell population was assessed by evaluating dilution of Cell Trace dye in the CD56+CD3- population using flow cytometry (B) Percentage of proliferated NK cells was calculated using FlowJo Analyzer. Statistics reflect significant differences between the groups as calculated with a One Way ANOVA, * $P < 0.05$, ** $P < 0.005$, N = 10.

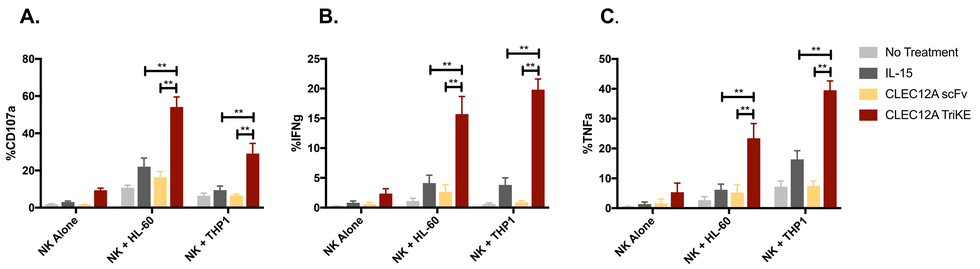

Figure 5: The CD16-IL15-CLEC12A TriKE induces strong degranulation and cytokine production against AML target cells: PBMCs were incubated with CLEC12A+ HL-60 and THP1 cells at a 2:1 effector to target ratio in the presence of IL-15, CLEC12A scFv or CLEC12A TriKE at equimolar concentrations. (A) Surface CD107a, to evaluate degranulation, (B) intracellular IFNg, (C) TNFa to evaluate inflammatory cytokine production, were assessed on $CD56^+CD3^-$ NK cells by flow cytometry. Statistics reflect significant differences between the groups as calculated with a One Way ANOVA, ** $P < 0.005$, N = 6.

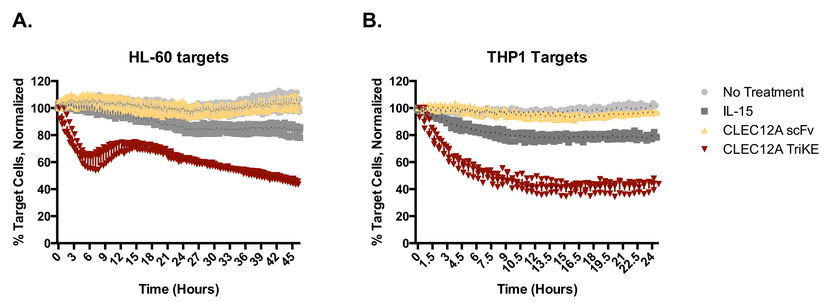

Figure 6: The CD16-IL15-CLEC12A TriKE induces killing of AML targets: Enriched NK cells were incubated with CLEC12A+ (A) HL-60 and (B) THP1 targets at a 2:1 effector to target ratio in the presence of IL-15, CLEC12A scFv or CLEC12A TriKE at equimolar concentrations. The target cells were labeled with a Cell Trace Far Red dye and a Caspase 3/7 green apoptosis assay reagent (Essen Biosciences). Killing was assessed using an Incucyte Zoom machine and analyzed by normalizing cell numbers to initial number of target cells.

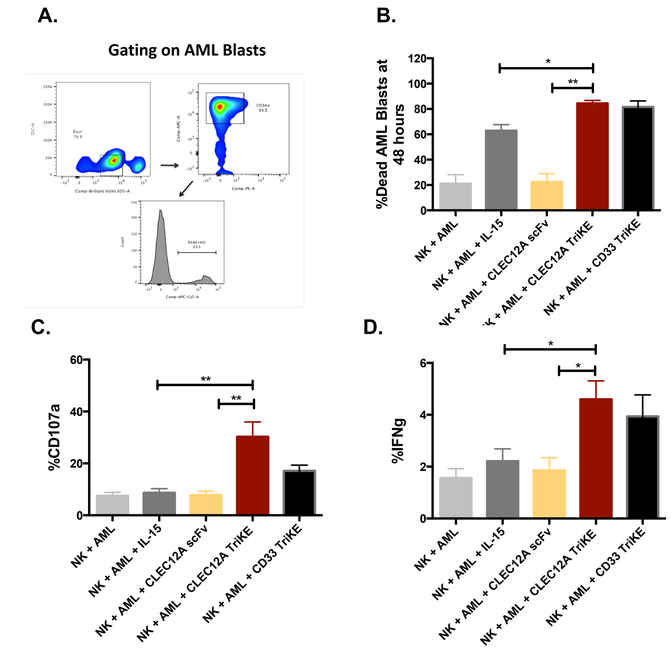

Figure 7: The CD16-IL15-CLEC12A TriKE induces killing of primary AML targets *in vitro*. Enriched NK cells were incubated with primary AML blasts at a 2:1 effector to target ratio in the presence of IL-15, CLEC12A scFv, CLEC12A TriKE or CD33 TriKE at equimolar concentrations. (A) Gating scheme to identify AML blasts using FlowJo Analyzer. (B) Percentage of killing of AML blasts as assessed by the live dead marker after gating on blasts cells after 48 hours (C) Surface CD107a expression to evaluate degranulation (D) intracellular IFNg to evaluate inflammatory cytokine production, were assessed on $CD56^+CD3^-$ NK cells by flow cytometry after 4 hours. Statistics reflect significant differences between the groups as calculated with a One Way ANOVA, *<0.05 ** $P < 0.005$, N = 10.

CONCLUSIONS AND FUTURE DIRECTIONS

The CD16-IL-15-CLEC12A TriKE:

1. Binds specifically to target cells expressing CLEC12A
2. Promotes proliferation of NK cells
3. Enhances function of NK cells
4. Promotes killing of AML cell lines in Incucyte zoom assays
5. Induces killing of primary AML Blasts

Future Experiments

- Assess *in vivo* functionality using a patient derived xenograft mouse model developed by our collaborator Dr. Craig Eckfeldt
- Assess *in vivo* functionality in a xenograft mouse model using HL-60 cells
- Test efficacy of dual targeting of CLEC12A and CD33

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