CONSTRUCTION AND EVALUATION OF INTERLEUKIN 3 (IL3)-ZETAKINE REDIRECTED CYTOLYTIC T CELLS FOR THE TREATMENT OF CD123 EXPRESSING ACUTE MYELOID LEUKEMIA

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Background Acute Myeloid Leukemia (AML) is an aggressive bone marrow malignancy, characterized by the presence of leukemic blasts in the peripheral blood of patients. Poor AML prognoses¹ are largely attributable to high rates of disease relapse, of which CD123+ leukemic stem cells (LSCs) are the primary cause.²⁻³ CD123, the alpha-chain of the IL3 cytokine receptor,⁶ has been identified as a favorable therapeutic AML target, overexpressed in both LSCs and blasts.⁴⁻⁵ We sought to direct T cells to CD123+ AML cells via cell surface tethered IL3 (termed "IL3-zetakine").⁷ The use of a zetakine instead of a chimeric antigen receptor (CAR) construct enables structure-guided site-directed mutagenesis to increase binding affinity and alter target cell signaling without detrimental T cell hyperactivation.

Methods Zetakine constructs were designed using IL3 sequences bound to a transmembrane domain and intracellular costimulatory and CD3z signaling domains. The constructs were transduced into Jurkat cells with lentiviral vectors (LVV). T cell activation via CD69 expression was assessed via flow cytometry of sorted IL3 zetakine-positive Jurkat cells after co-culture with MOLM13 AML cells. Lead constructs were selected based on initial transduction percentage and activation response. In vitro functionality of each IL3 zetakine was tested with LVV transduced primary T cells by flow cytometry.

Results Zetakine constructs yielded a wide range of transduction percentages in Jurkat cells (0 – 98%) prior to sorting. In co-cultures with CD123+ MOLM13 AML cells, Jurkat cells expressing wildtype IL3 constructs lacking a costimulatory domain induced the highest level of CD69 expression (18.7% CD69+ T cells) in an antigen-specific manner (5.3-fold increase of CD69+ T cells over those cultured with MOLM13 CD123KO cells). The K110E mutant IL3 was reported to exhibit a 40-fold increased affinity over wildtype,⁸ but it showed no detectable zetakine function. However, additional mutant IL3 zetakines increased Jurkat cell activation up to 5.8-fold. Antigen-specific increases in CD69, as well as CD25, surface expression were also observed with zetakine-transduced primary T cells co-cultured with MOLM13 cells, in addition to target cell killing comparable to antibody-based CD123CAR T-cells.

Conclusions This work establishes IL3 zetakines as a viable alternative to traditional CD123-targeted CAR constructs. Structure-guided IL3 zetakine mutants with altered affinity and activation profiles will further our understanding of CD123-specific cytotoxicity modulation without inducing acute T cell hyperactivation and exhaustion. These results indicate the ability of IL3 zetakine-expressing T cells to kill CD123-expressing AML cells and illustrate the potential of this novel class of therapeutics.

REFERENCES

1. Gansel C, et al. Very poor long-term survival in past and more recent studies for relapsed AML patients: the ECOG-ACRIN experience. *American journal of hematology* 2018;10.1002/ajh.25162.
2. Shlush LI, et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. *Nature* 2017;547(7661):104–108.
3. Hanekamp D, Cloos J, Schuurhuis GJ. Leukemic stem cells: identification and clinical application. *International Journal of Hematology* 2017;105(5):549–557.

4. Bras AE, et al. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. *Cytometry part B: Clinical Cytometry* 2019;96(2):134–142.
5. Sugita M, Guzman ML. CD123 as a therapeutic target against malignant stem cells. *Hematology/Oncology clinics of North America* 2020;34(3):553–564.
6. Mingyue S, et al. CD123: a novel biomarker for diagnosis and treatment of leukemia. *Cardiovascular & Hematological Disorders-Drug Targets* 2019;19(3):195–204.
7. Kahlon KS, et al. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res* 2004;64(24):9160–6.
8. Bagley CJ, et al. A discontinuous eight-amino acid epitope in human interleukin-3 binds the alpha-chain of its receptor. *J Biol Chem* 1996;271(50):31922–8.

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