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Construction and Evaluation of Interleukin 3 (IL3)-Zetakine–Redirected Cytolytic T Cells for Treatment of CD123-Expressing Acute Myeloid Leukemia

Rebecca A. Moeller,[1] Sadik Kassim,[1] Julian Scherer[1]

[1]*Vor Biopharma Inc., Cambridge, MA, USA*

INTRODUCTION

- Acute myeloid leukemia (AML) is an aggressive bone marrow malignancy characterized by the presence of leukemic blasts in peripheral blood.
- Poor AML prognoses[1] are largely attributable to high rates of disease relapse, primarily due to cluster of differentiation 123 (CD123)+ leukemic stem cells (LSCs).[2,3]
- CD123, the alpha chain of the IL3 cytokine receptor,[4] has been identified as a favorable therapeutic AML target, overexpressed in LSCs and blasts.[5,6]

OBJECTIVE

- 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 (TMD) and intracellular costimulatory and CD3ζ signaling domains.
- The constructs were transduced into Jurkat cells with lentiviral vectors (LVVs). T-cell activation via CD69 expression was assessed via flow cytometry of sorted IL3-zetakine+ Jurkat cells after coculture 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

Fig. 1. CD123 Highly Expressed in AML LSCs and Correlates With Reduced Patient Survival

- LSCs highly express CD123 and have the capacity for self-renewal, which can lead to disease relapse. (**A**)
- High expression levels of CD123 in patients with AML correlate with significantly reduced survival. (**B**)[8]

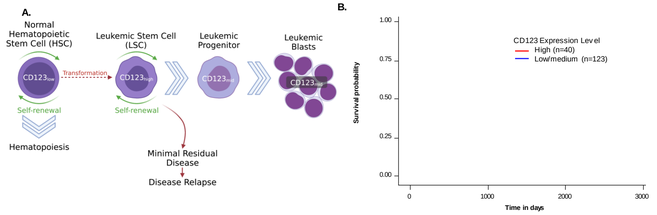
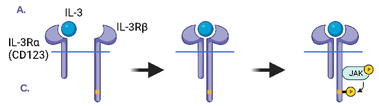
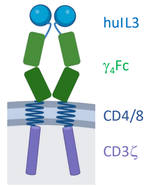


Fig. 2. CD123:IL3 Binding and IL3-Zetakine Constructs

- CD123 (IL3Rα) binds IL3 ligand. IL3Rβ (CSF2RB) joins the complex to initiate janus kinase/signal transducer and activator of transcription pathway signaling. (**A**)



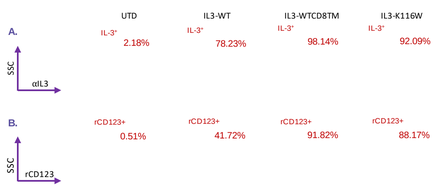


Construct	Cytokine	Hinge	TMD	Stimulatory Domain
IL3-WT	huIL3(WT)	IgG4 [SmP]	CD4	CD3zeta
IL3-WTCD8TM	huIL3(WT)	IgG4 [SmP]	CD8	CD3zeta
IL3-K116W	huIL3(K116W)	IgG4 [SmP]	CD4	CD3zeta

- The dimerized structure of IL3-zetakine constructs, consisting of extracellular human IL3 (huIL3) tethered to an Fc gamma 4 (g4Fc) hinge region, CD4 or CD8 TMD, and a CD3ζ intracellular signaling domain. (**B**)
- Table detailing domains of the IL3-zetakine constructs. (**C**)

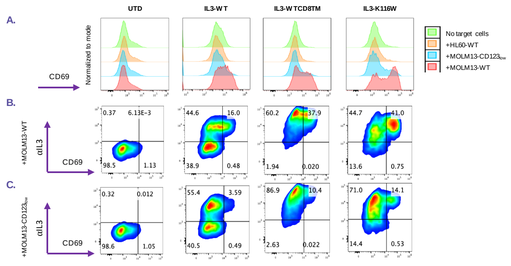
Fig. 3. IL3-Zetakine Expression Detectable in Transduced TIB153 Jurkat Cells With Anti-IL3 Antibody and rCD123 Protein

- Wild-type TIB153 Jurkat cells were transduced with LVV for each of the indicated zetakine constructs or left untransduced (UTD), then subjected to flow cytometry 7 days after transduction.
- To detect IL3-zetakine expression, cells were stained with anti-IL3 antibody (αIL3) (**A**) or biotinylated recombinant CD123 protein (rCD123, **B**) bound to fluorescence-tagged streptavidin.



- Both staining methods indicate IL3-zetakine expression in ≤98% of all TIB153 Jurkat cells.

Fig. 4. TIB153 Jurkat Cells Transduced With IL3-Zetakine Constructs Exhibit CD123-Specific Activation



- Mean fluorescence intensity (MFI) of CD69 expression: detection via flow cytometry on UTD or IL3-zetakine–transduced TIB153 Jurkat cells cocultured with or without CD123-expressing target cells for 24 hours at an effector to target cell ratio of 1:1. (**A**)
- CD69 expression of transduced TIB153 Jurkat cells as a function of zetakine expression by anti-IL3 (αIL3) staining, cocultured with *MOLM13*^{WT} ($CD123_{high}$; **B**) or *MOLM13*^{MUT} ($CD123_{low}$) target cells. (**C**)

N=3 for all samples. ***p<0.001, one-tailed student's T-test.

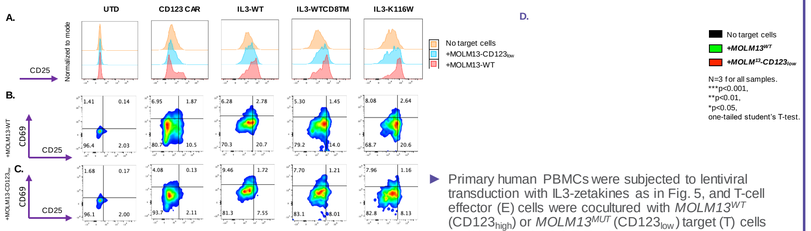
- Transduced TIB153 Jurkat cell CD69 expression in coculture with *MOLM13*^{WT} ($CD123_{high}$), *MOLM13* ($CD123_{low}$), or *HL60*^{WT} ($CD123_{null}$) target cells. (**D**)

Fig. 5. IL3-Zetakine Expression Detectable in Primary T Cells Using Anti-IL3 Antibody

- Expression levels varied across transduced conditions in a range from 6%–16% of CD3+ T cells.

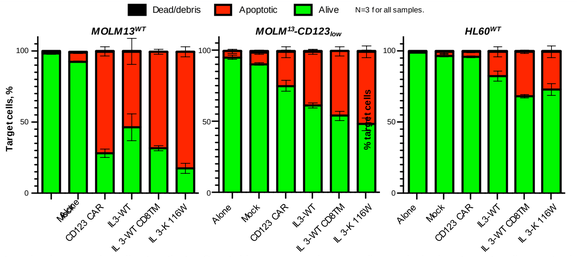
- Primary human peripheral blood mononuclear cells (PBMCs) were activated by Transact (CD3/CD28 containing activation matrix) and either left UTD or transduced with lentivirus for each indicated zetakine construct and subjected to flow cytometry 7 days after transduction.
- Flow analysis pregated on CD3+ T cells and IL3-zetakine expression was detected by anti-IL3 antibody (αIL3).

Fig. 6. Primary T Cells Transduced With IL3-Zetakine Constructs and CD123 CAR Exhibit CD123-Specific Activation



- Primary human PBMCs were subjected to lentiviral transduction with IL3-zetakines as in Fig. 5, and T-cell effector (E) cells were cocultured with *MOLM13*^{WT} ($CD123_{high}$) or *MOLM13*^{MUT} ($CD123_{low}$) target (T) cells for 24 hours at a 1:1 E:T ratio
- MFI of CD25 detected via flow cytometry on CD3+ T cells. (**A**)
- IL3-zetakine–induced activation, indicated by CD25 (X axis) and CD69 (Y axis) expression of transduced CD3+ T cells cocultured with *MOLM13*^{WT} ($CD123_{high}$, **B**) or *MOLM13*^{MUT} ($CD123_{low}$) target cells. (**C**)
- Transduced CD3+ T-cell expression of CD25 in coculture with *MOLM13*^{WT} ($CD123_{high}$) or *MOLM13*^{MUT} ($CD123_{low}$) target cells. (**D**)

Fig. 7. Primary T Cells Transduced With IL3-Zetakine Construct Exhibit CD123-Specific Cytotoxicity



- Primary human PBMCs were subjected to lentiviral transduction with IL3-zetakines as in Fig. 5 and a positive control CD123 CAR construct.
- Transduced and UTD E cells were cocultured with MOLM13 T cells for 24 hours at a 1:1 E:T ratio. Target cell health was assessed by flow cytometry: alive cells, viability and annexin V-negative; apoptotic cells, viability negative and annexin V-positive; dead cells/debris, viability and annexin V-positive.
- The bar graph indicates that IL3-zetakine–expressing T cells are inducing target cell apoptosis to a similar or greater extent as the CD123 CAR–expressing cells, especially in the CD123low-expressing MOLM13MUT and HL60 target cells.

CONCLUSION

- This work establishes IL3-zetakines as a viable alternative to traditional CD123-targeted, antibody-based 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 specifically kill CD123-expressing AML cells, further illustrating the potential of this novel class of therapeutics.

References

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Acknowledgments

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