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ELEVENTH ANNUAL MEETING

# Modeling flotetuzumab-associated CRS in AML using *in vitro* and *in vivo* preclinical models

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## INTRODUCTION

Flotetuzumab (FLZ) is a dual affinity retargeting agent (DART) that is being investigated for the treatment of relapsed and refractory acute myeloid leukemia (AML).

FLZ-mediated anti-leukemia efficacy is often limited by CRS.

## AIM

Developing pre-clinical models is essential to understanding the biology of CRS.

MacroGenics has developed a murine genetic knock-in model that expresses both a murine and human CD3 epsilon exon (hCD3e).

These mice maintain normal signaling through the T-cell receptor and expand and secrete cytokines upon binding to FLZ and human CD123.

**We hypothesize that T-cells harvested from the spleen of immune competent C57/Bl6 human CD3 epsilon knock-in mice will bind FLZ and kill human CD123 expression targets in an *in vitro* killing assay.**

## METHOD

A murine AML cell line, AML1-GFP, was transduced with human CD123 (hCD123) using a lentiviral vector construct and single cell clones expressing high levels of hCD123 were expanded (AML$^{GFP\text{-}hCD123}$).

Spleens from fully immune competent hCD3e$^{HOM}$ C57/Bl6 knock-in mice were harvested for isolation of T-lymphocytes via negative immunomagnetic selection on an AutoMACS machine.

An *in vitro* killing assay was then set up using purified C57/Bl6 hCD123e$^{HOM}$ T-cells and AML$^{GFP\text{-}hCD123}$ cells.

The effector to target ratio was 1:1, with 2.5x10$^5$ cells per well in a 24 well plate. Flow cytometry/FACS was performed at 24 hours.

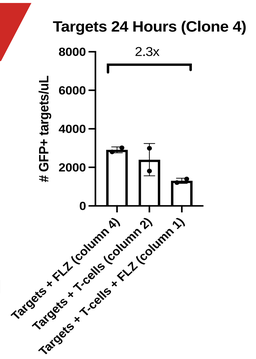
## RESULTS

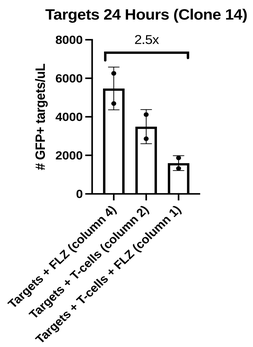
A 2.3-2.5-fold decrease in the number of AML$^{GFP\text{-}hCD123}$ targets at 24 hours was seen in groups treated with T-cells + FLZ compared to controls.

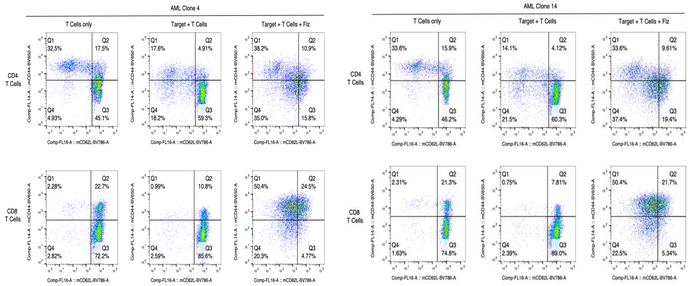
The percentage and MFI expression of activation markers were increased for CD4+ and CD8+ subsets in the treatment group compared to controls.

For CD4+ and CD8+, the addition of FLZ induced phenotypic changes away from naïve (CD62L+/CD44-) and towards central (CD62L+/CD44+) and effector memory (CD62L-/CD44+) subsets.

In the two control groups, naïve T-cells predominated at the 24-hour time point.




**Legend:** The graphs show the number of AML1-GFP targets at 24 hours. The left graph shows clone 4 while the right panel is for clone 14. The x-axis describes three groups, which right to left are targets plus FLZ (column 4), targets and T-cells in co-culture (column 2), and targets, T-cells, and FLZ in co-culture (column 1). Only column 1 contains targets, effector, and drug together. The y-axis shows the number of AML1-GFP labeled targets per microliter in each well. Compared to columns 4 and 2, column 1 shows a decrease in the number of targets at 24 hours. Compared to column 4, the treatment group in column 1 shows a 2.3-fold and 2.5-fold decrease in targets, respectively, indicating AML cell killing.

**Legend:** Flow cytometry/FACS was performed on samples from clones 4 and 14 in co-culture at the 24-hour time point. Each quadrant represents a unique T-cell phenotype. Going clockwise, quadrant 1 represents effector memory T-cells (CD62L-/CD44+), quadrant 2 represents central memory T-cells (CD62L+/CD44+), and quadrant 3 reflects naïve T-cells (CD62L+/CD44-). The three columns of cells from left to right are T-cells alone, targets and T-cells in co-culture, and targets, T-cells, and flotetuzumab (FLZ) in co-culture. Compared to the clone 4 and 14 samples of T-cells alone and targets and T-cells together, the combination of targets, T-cells, and FLZ results in changes in the phenotype of T-cells, away from the naïve markers and towards central memory and effector memory markers. In the former two samples, T-cells are predominantly of the naïve phenotype rather than central or effector memory.

## CONCLUSIONS

The *in vitro* killing assay shows that T-cells from the spleens of immune competent C57/Bl6 hCD123e$^{HOM}$ mice bind to both FLZ and hCD123 on transduced murine leukemia cells.

Co-culture with T-cells + target cells + FLZ showed killing at 24 hours compared to controls.

T-cell activation and proliferation were noted based on cell surface markers.

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