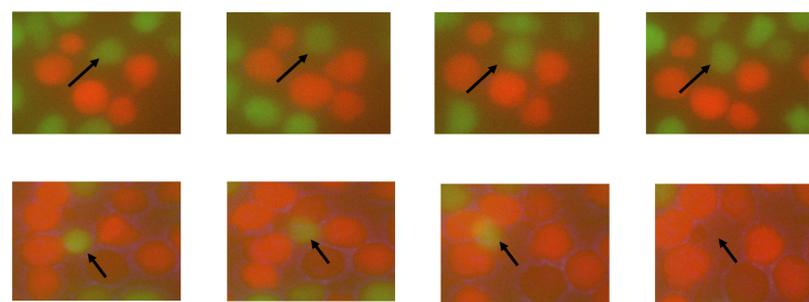


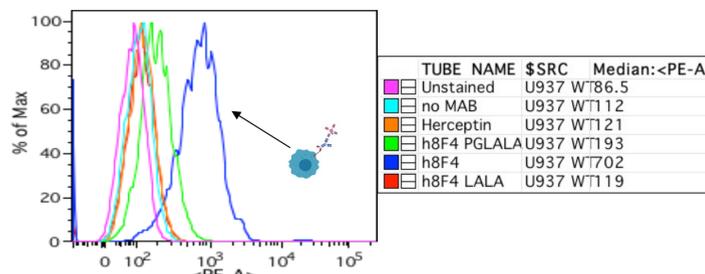
## Background

- Acute Myeloid Leukemia (AML) is a clonal disorder of the haemopoietic progenitor cells<sup>1</sup>.
- 8F4, a T cell receptor (TCR)-like monoclonal antibody (mAb)<sup>3</sup>, has been developed to potentially treat patients and has demonstrated a high activity against AML cells, *in vivo*.
- 8F4 binds with high affinity to a conformational epitope of PR1/HLA-A2 via its FAB region<sup>2</sup>.
- Previous experiments have demonstrated that 8F4 also binds to Fc Receptors (FcR) on AML cells, as presented in Figure 2.
- Binding of AMLs through 8F4 via the Fab region and FcR is hypothesized to be the cause of their mutual destruction, as is demonstrated in Figure 1.
- To further validate the role of FcγRI (CD64) and FcγRII (CD32) in the mechanism of action of 8F4, double knockout cells were developed through CRISPR to test AML cells' susceptibility to 8F4.
- To exclude the possibility of errors in CRISPR that could affect susceptibility to 8F4, both receptors were placed back into the double knockout AML cells through cloning and transduction.



**Figure 1. Binding of AML cells through 8F4.** U937-A2 (green) and U937 WT (red) at 20 minute intervals. U937- WT cells were incubated without 8F4 (top) and with 8F4 (bottom).

## Background and Rationale



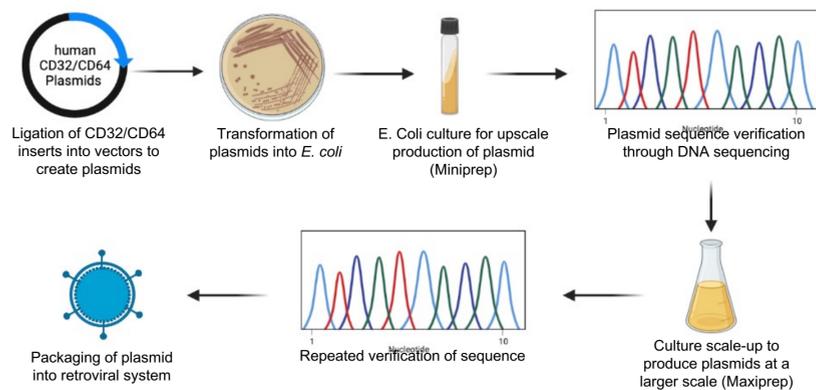
**Figure 2. 8F4 binding via Fc region.** After incubating A2 negative cells with 8F4, the PR1/HLA-A2 tetramer was added. U937 showed that 8F4 binds successfully to both the cell line via Fc region and the tetramer.

## Hypothesis

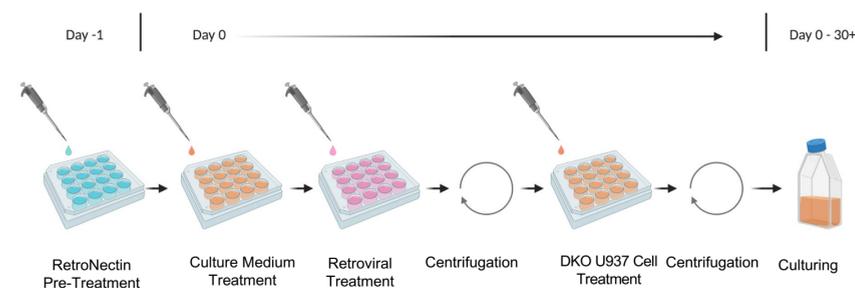
Double knockout acute myeloid leukemia cells will regain susceptibility to 8F4 after addition of FcγRI and FcγRII, validating FcRs' role in the mechanism of action of 8F4.

## Methods

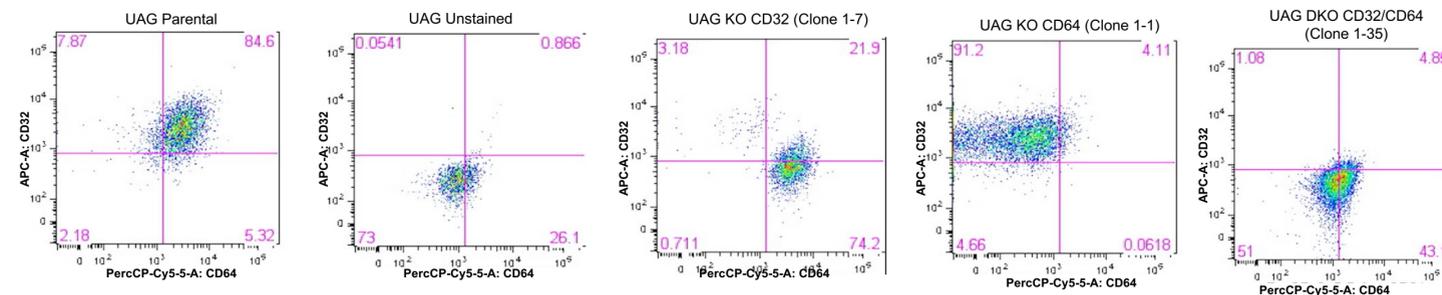
Development and construction of human CD32/CD64 constructs.



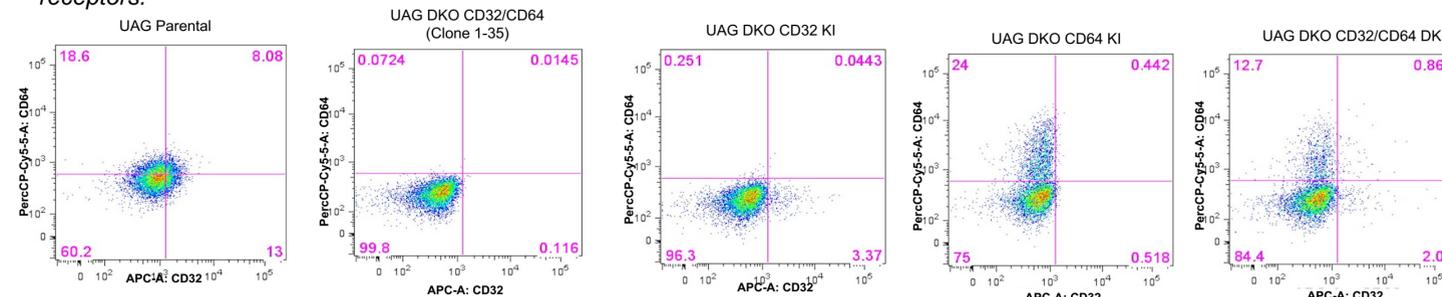
## Retroviral Transduction and Cell Culture



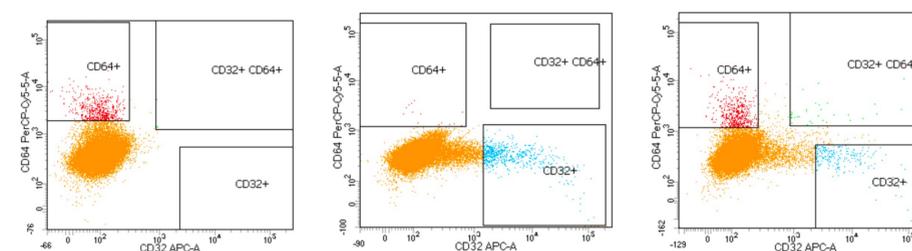
## Results



**Figure 3. Staining of DKO U937 Cells.** Staining with anti-CD64 and anti-CD32 was done to test expression of corresponding receptors.



**Figure 4. Staining on Transduction Day Two.** 24% of CD64-transfected cells were CD64 positive, 3.4% of CD32-transfected cells were CD32 positive and 0.867% of CD32/CD64-transfected cells were CD32/CD64 positive.



**Figure 5. Cell Sorting on Transduction Day Three.** CD64+, CD32+ and CD32/CD64+ were selected for further cell expansion.

## Conclusions

- Successful transductions produced DKO U937 cells that expressed CD32, CD64 and CD32/CD64.
- Ongoing *in vivo* experiments (not shown) have preliminarily demonstrated that DKO U937 cells exhibit a **decreased susceptibility** to 8F4.
- Further experiments must be done *in vivo* to test transduced DKO U937 cells' susceptibility to 8F4 in comparison to DKO U937 cells.
- These experiments could disregard errors in CRISPR/Cas 9 and could **further validate** FcγRI or FcγRII's role in the mechanism of action of 8F4.

## References

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