Engineering CAR-T Cells for Inducible and Localized Secretion of Multi-Antigen-Specific T Cell Engagers in Acute Myeloid Leukemia

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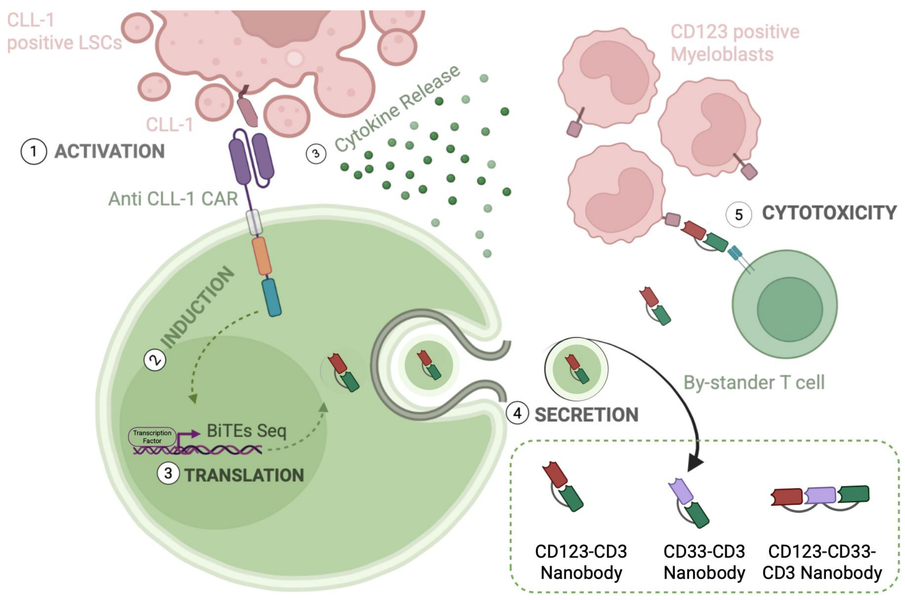
INTRODUCTION

Relapse in acute myeloid leukaemia (AML) is driven by a chemo-resistant, self-renewing population of **leukaemic stem cells (LSCs**). For AML, CAR-T therapy has not matched its success in B-cell malignancies, hampered by **antigenic heterogeneity** across LSCs and blasts and by **on-target/off-tumor toxicity**, since candidate antigens such as CD33 and CD123 are also expressed on normal haematopoietic progenitors. CLL-1 is expressed on most LSCs but is largely absent from normal HSCs, making it an attractive, HSC-sparing CAR target — yet its heterogeneous expression across bulk blasts means no single antigen can eliminate both LSCs and the broader myeloblast population.

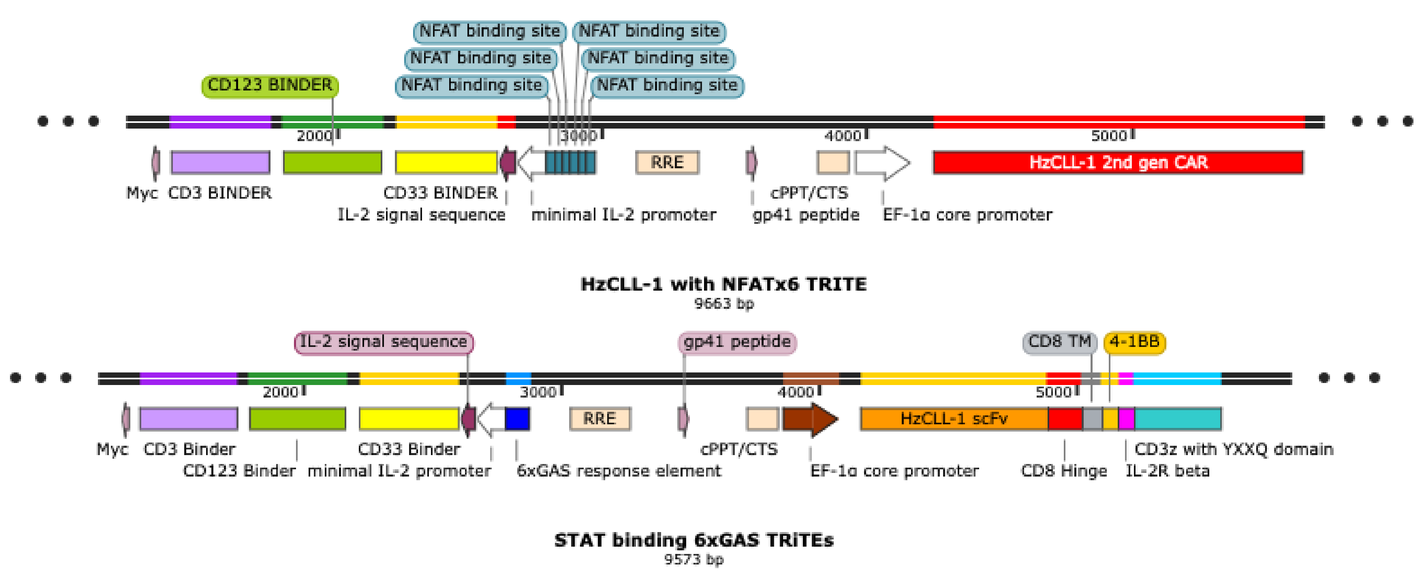
APPROACH

We engineered CLL-1-targeting CAR-T cells that, upon **antigen-driven activation, secrete T-cell engagers** via an NFAT/STAT-responsive expression cassette. Secretory anti-CD33 and anti-CD123 engagers — each paired with an anti-CD3 nanobody — were designed alongside a tri-specific anti-CD33/CD123/CD3 construct, to identify which format best recruits bystander T cells against CLL-1^{low} LSCs or Myeloblasts. Because engagers have a **short half-life** and are **secreted locally within the CLL-1-positive marrow** niche rather than administered systemically, T-cell engagement with **CD33+/CD123+ cells should stay confined** to that niche, **limiting robust toxicity to LSCs and myeloblasts** and **sparing normal myeloid progenitor**s and other healthy tissues.



Graphical Representation of approach to overcome antigen heterogeneity and toxicity towards healthy hematopoietic and myeloid cells.

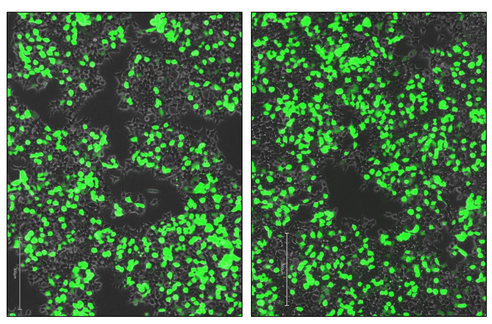
DESIGN



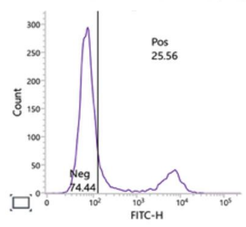
Graphical Representation of gene cassette designs using NFAT and STAT binding domains.

RESULTS

Transient Transfection: GFP expression after 24 and 48h.



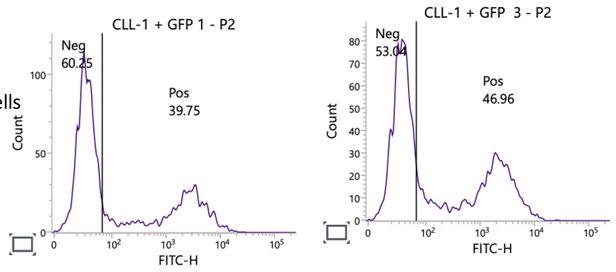
TU/mL = no. of cells transduced x fraction of expression x DF / Volume of virus used (mL)



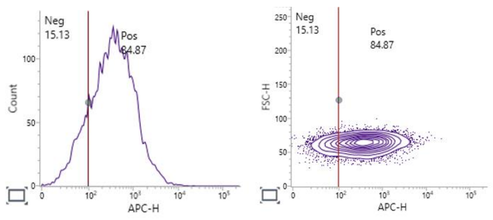
TU/mL = 1.2 x 10^5 x 0.255 / 0.001

TU/mL = 3 x 10^7

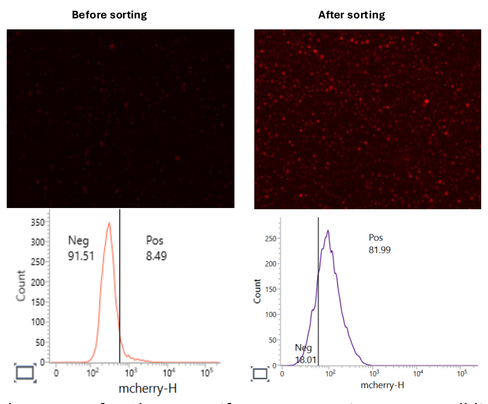
Expression in Primary T cells at MOI=1 and MOI=3



Testing the expression of designed CARs on HEK293T, engineering LVV, calculating titre and testing their transduction and expression on primary T cells



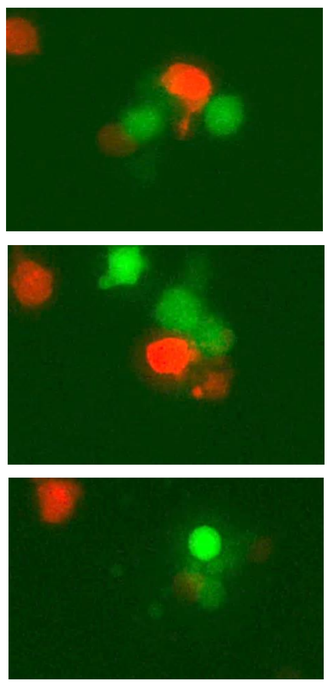
CAR Expression (Without GFP using APC conjugated G4S Linker Ab) – **84.87%**



Development of mCherry Luciferase expressing THP-1 cell line

The mCherry Luciferase expressing THP-1 cells were co-cultured with CLL-1 CAR-T+GFP cells at E:T ratio of 1. The BLI signal was captured 24 hours after set up of culture. The CAR-T cells were rechallenged by THP-1 every 72 hours. The CAR-T efficacy and persistence were tested for 20 days following the above protocol.

The microscopy images are stills from a movie capturing the cytotoxic ability of **CLL-1 CAR-Ts** against **THP-1 cells**



CONCLUSION

Once validated, this inducible platform would let a single CAR-T product eliminate both the LSC reservoir responsible for relapse and the heterogeneous bulk blast population responsible for disease progression — while limiting the systemic toxicity that has constrained multi-antigen approaches in AML.

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