CD64 CAR-T Therapy Targets Venetoclax-Resistant Monocytic Acute Myeloid Leukemia

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INTRODUCTION

Acute Myeloid Leukemia:

- Annual incidence: 20,800 cases with 11,220 deaths (American Cancer Society, 2024)
- 15% 5-year overall survival

Progress in AML Treatment:

- Treatment with **venetoclax and azacitidine (ven/aza)** induces complete remission in approximately 70% of patients, but the majority relapse (PFS ~18 months)
- Unfortunately, **ven/aza is less effective against AML with a more monocytic (M5) phenotype**
- Relapse after ven/aza is driven by monocytic leukemia stem cells (m-LSCs)
- Monocytic AML cells express CD64
  - ~50% of AML is CD64+; m-LSCs are uniformly CD64+

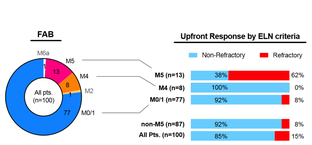

Figure 1: Monocytic AML is more likely to be resistant to venetoclax and azacitidine.

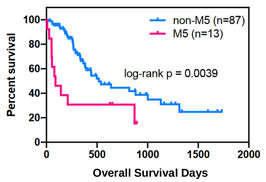

Figure 2: Monocytic AML is associated with inferior overall survival.

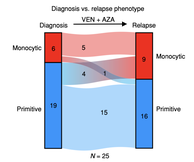

Figure 3: Relapse after ven/aza favors monocytic AML

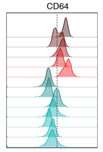

Figure 4: Monocytic AML expresses higher levels of CD64 than AML with primitive phenotype

Pei, et. al., Cancer Discovery, 2020 and 2023

AIM

To optimize CD64 targeted CAR-T cell therapy for the treatment of monocytic AML

CD64 CAR

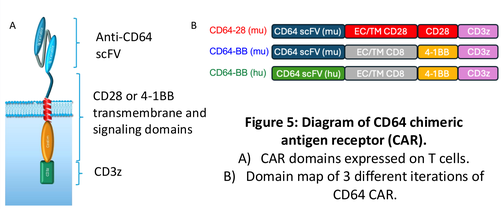

Figure 5: Diagram of CD64 chimeric antigen receptor (CAR).
A) CAR domains expressed on T cells.
B) Domain map of 3 different iterations of CD64 CAR.

RESULTS

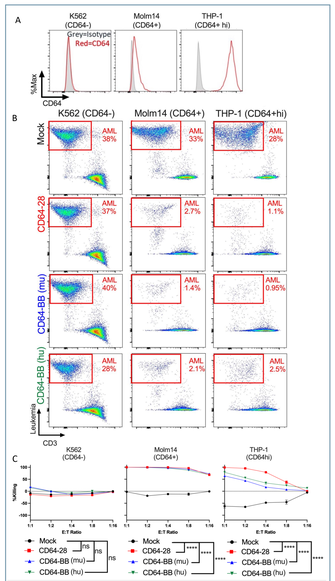

Figure 6: CD64 CAR-T cells exhibit cytotoxicity against CD64+ AML cell lines *in vitro*. A) CD64 expression of AML cell lines. B) CD64+ AML cell lines Molm14 and THP-1 were co-cultured with CD64 CAR T cells at effector to target (E:T ratio) of 1:2 for 18 hours. Remaining percentage of leukemia cells is shown in red. K562 cells serve as a CD64- control. C) Percent killing of AML cells by luciferase assay following co-culture with CAR-T cells at E:T ratios shown.

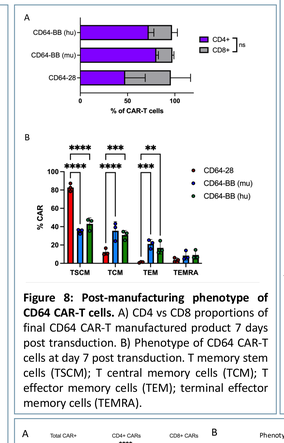

Figure 8: Post-manufacturing phenotype of CD64 CAR-T cells. A) CD4 vs CD8 proportions of final CD64 CAR-T manufactured product 7 days post transduction. B) Phenotype of CD64 CAR-T cells at day 7 post transduction. T memory stem cells (TSCM); T central memory cells (TCM); T effector memory cells (TEM); terminal effector memory cells (TEMRA).

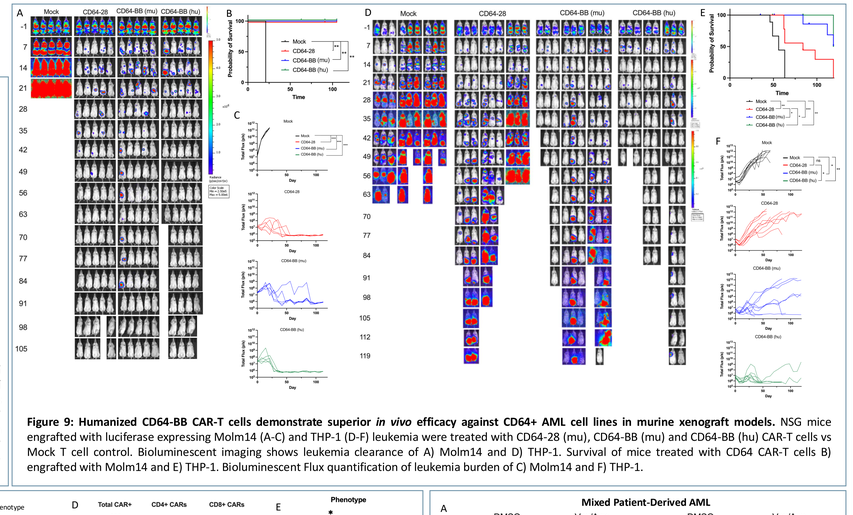

Figure 9: Humanized CD64-BB CAR-T cells demonstrate superior *in vivo* efficacy against CD64+ AML cell lines in murine xenograft models. NSG mice engrafted with luciferase expressing Molm14 (A-C) and THP-1 (D-F) leukemia were treated with CD64-28 (mu), CD64-BB (mu) and CD64-BB (hu) CAR-T cells vs Mock T cell control. Bioluminescent imaging shows leukemia clearance of A) Molm14 and D) THP-1. Survival of mice treated with CD64 CAR-T cells B) engrafted with Molm14 and E) THP-1. Bioluminescent Flux quantification of leukemia burden of C) Molm14 and F) THP-1.

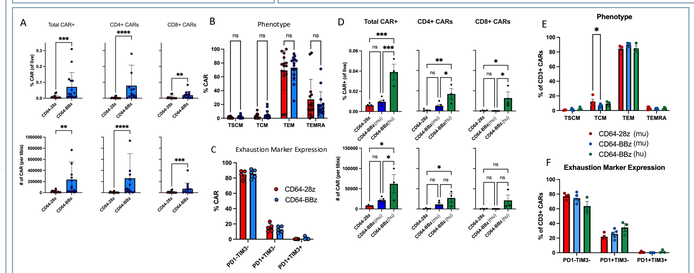

Figure 10: CD64-BB CAR-T cells exhibit enhanced persistence after *in vivo* clearance of AML cell lines compared to CD64-28 CAR-T cells in murine xenograft models. NSG mice were engrafted with luciferase expressing Molm14 and treated with a curative dose of CD64 CAR-T cells. A-C) After leukemia clearance, at day 35, mouse bone marrow was analyzed for A) CAR, CD4, and CD8 expression, B) Phenotype, and C) Exhaustion markers. D-F) NSG mice were treated with curative doses of CD64-28 (mu), CD64-BB (mu) and CD64-BB (hu) CAR-T cells after 100 days, mouse bone marrow was analyzed for D) CAR, CD4, and CD8 expression, E) Phenotype, and F) Exhaustion markers. CD64 CAR-T cells exhibit predominantly T effector memory phenotype and are negative for exhaustion markers PD1 and TIM3 after leukemia clearance.

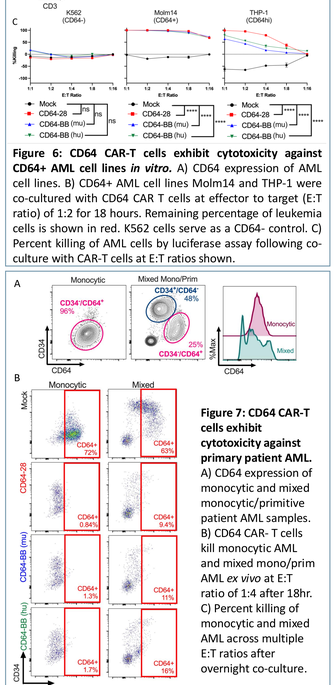

Figure 7: CD64 CAR-T cells exhibit cytotoxicity against primary patient AML. A) CD64 expression of monocytic and mixed monocytic/primitive patient AML samples. B) CD64 CAR- T cells kill monocytic AML and mixed mono/prim AML *ex vivo* at E:T ratio of 1:4 after 18hr. C) Percent killing of monocytic and mixed AML across multiple E:T ratios after overnight co-culture.

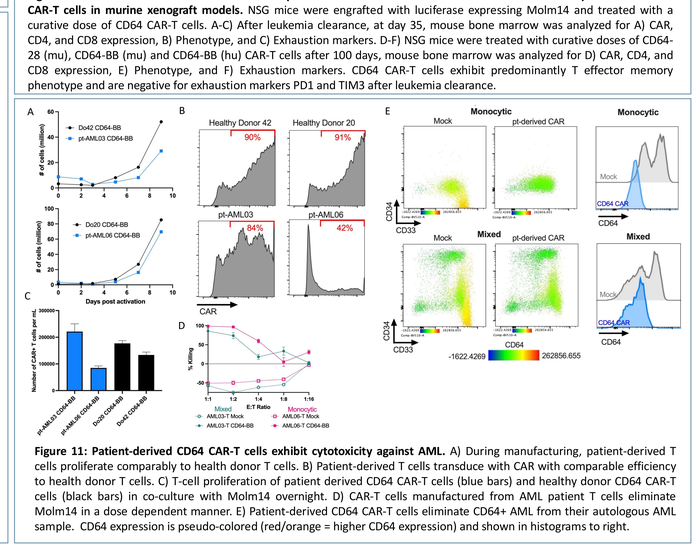

Figure 11: Patient-derived CD64 CAR-T cells exhibit cytotoxicity against AML. A) During manufacturing, patient-derived T cells proliferate comparably to health donor T cells. B) Patient-derived T cells transduce with CAR with comparable efficiency to health donor T cells. C) T-cell proliferation of patient derived CD64 CAR-T cells (blue bars) and healthy donor CD64 CAR-T cells (black bars) in co-culture with Molm14 overnight. D) CAR-T cells manufactured from AML patient T cells eliminate Molm14 in a dose dependent manner. E) Patient-derived CD64 CAR-T cells eliminate CD64+ AML from their autologous AML sample. CD64 expression is pseudo-colored (red/orange = higher CD64 expression) and shown in histograms to right.

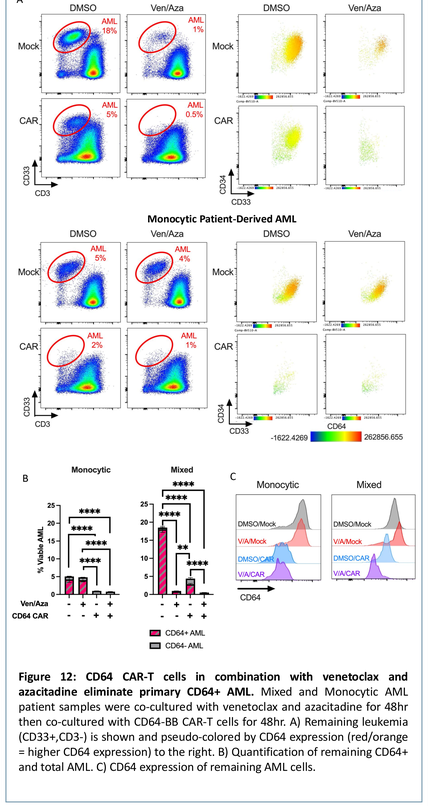

Figure 12: CD64 CAR-T cells in combination with venetoclax and azacitadine eliminate primary CD64+ AML. Mixed and Monocytic AML patient samples were co-cultured with venetoclax and azacitadine for 48hr then co-cultured with CD64-BB CAR-T cells for 48hr. A) Remaining leukemia (CD33+,CD3-) is shown and pseudo-colored by CD64 expression (red/orange = higher CD64 expression) to the right. B) Quantification of remaining CD64+ and total AML. C) CD64 expression of remaining AML cells.

CONCLUSIONS

- CD64 CAR-T cells are effective against CD64+ AML
  - CD64-BBz with humanized binder demonstrates more potent *in vivo* AML clearance
- CD64-BBz CAR-T cells exhibit ~10-fold greater persistence relative to CD64-28z
- Patient-derived CD64 CAR-T cells are functional against autologous CD64+ AML
- Treatment with Ven/Aza specifically reduces the CD34+/CD64- (primitive) AML subpopulation, whereas our CAR-T cells eliminate the monocytic CD64+ subpopulation
- Combination of Ven/Aza with CD64-BB CAR-T cell therapy may be a promising novel therapeutic strategy for monocytic AML

FUTURE DIRECTIONS

- Assess CD64 CAR-T cell safety and preliminary efficacy in a **phase I/II clinical trial** in patients with relapsed/refractory AML
- Assess persistence and exhaustion of CD64 CAR-T cells in patients
- Explore factors apart from CAR:antigen engagement that drive CD64 CAR-T cell efficacy
- Explore the potential on target/off tumor and off target toxicities of CD64 CAR-T therapy
- Examine production of cytokines which may increase AML proliferation and survival

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