# Allogeneic CAR T cells with deoxycytidine kinase knockdown demonstrate resistance to fludarabine

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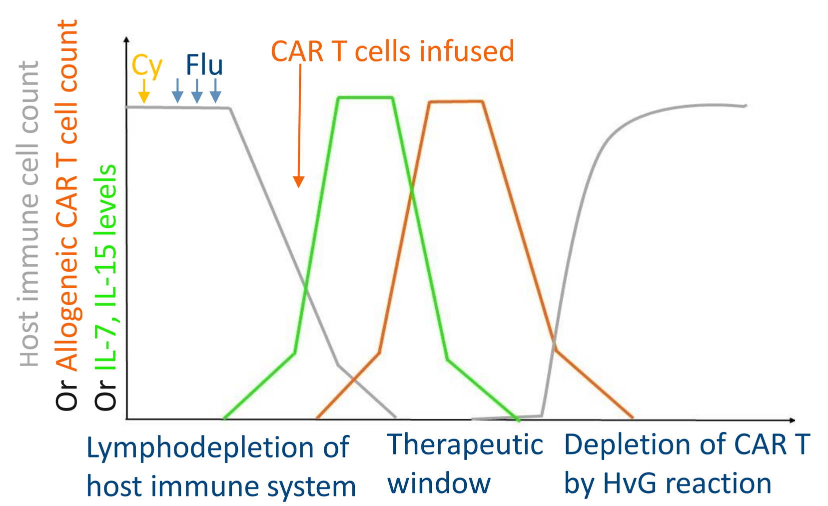
## INTRODUCTION

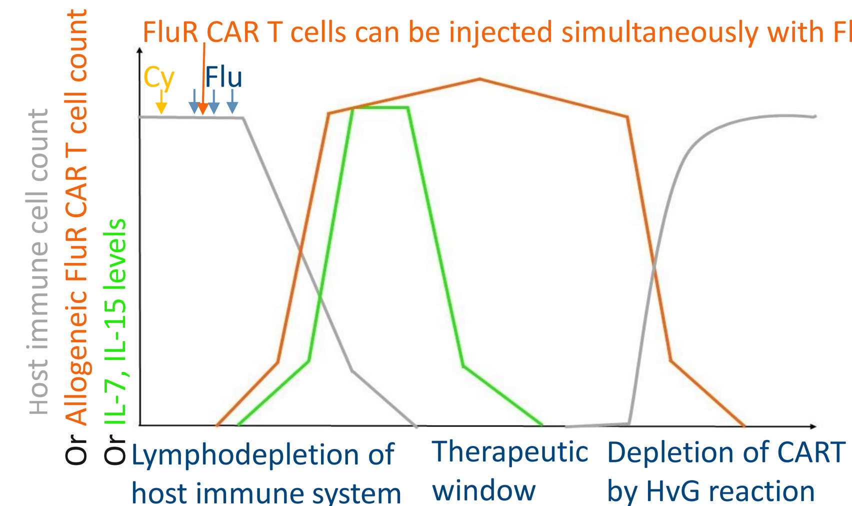
Clinical outcomes in CAR T therapy correlate with engraftment, expansion and persistence of CAR T cells. In order to facilitate engraftment and expansion, a lymphodepleting regimen consisting of cyclophosphamide and fludarabine precedes CAR T infusion. This creates niches for infused CAR T cells and stimulates beneficial homeostatic cytokine production. As these compounds are also toxic to CAR T cells, administering the proper doses of both the conditioning drugs and the cell therapies with appropriate timing can be a challenge. Here, we describe a way to protect CAR T cells from fludarabine toxicity by knocking down a gene called deoxycytidine kinase (dCK), which converts fludarabine from the prodrug form to an active compound resulting in Fludarabine resistant allogeneic CAR T (FluR CAR T).

**Fig 1. Schematic representation of allogeneic CAR T (left) vs FluR CAR T (right) adoptive transfer and potential clinical use**

Rapid rise and fall of IL-7 and IL-15 shortly after lymphodepletion. Narrow therapeutic window for CAR T to proliferate before they are rejected by HvG




Resistance to fludarabine should allow for simultaneous administration of allogeneic FluR CART suppressing CAR T rejection, providing better cytokine support and improving engraftment response

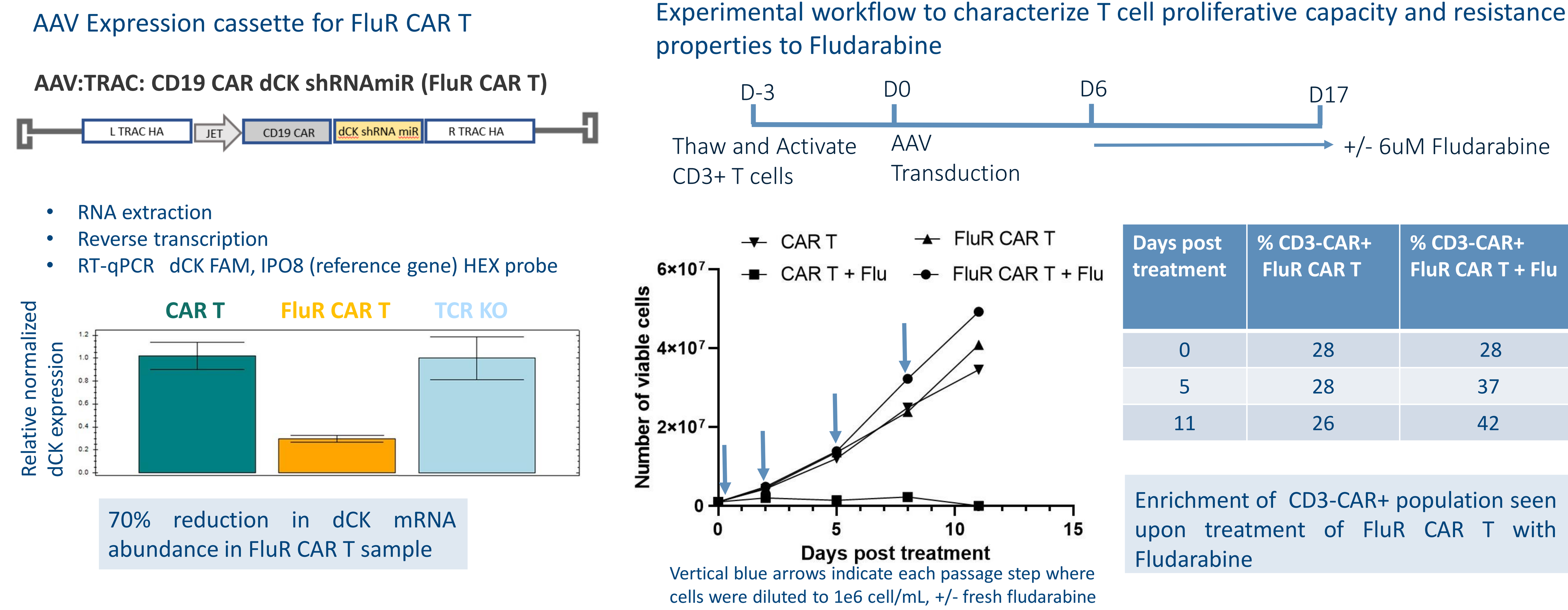



## METHODS

Knockdown of dCK was accomplished using an RNAi sequence featuring a dCK-specific shRNA sequence embedded into a micro-RNA backbone. The resulting RNAi sequence demonstrated the potency of shRNA and the stability of a microRNA. Using Precision BioSciences' ARCUS gene editing technology and AAV-mediated targeted transgene insertion strategy, we disrupted the endogenous T cell receptor and inserted a transgene encoding a CD19-specific CAR and a dCK-specific RNAi sequence. Cells produced in this manner were exposed to CD19+ target cells in vitro and in immune-deficient mice and CAR T proliferation and target killing were monitored in the presence and absence of fludarabine.

## RESULTS

**Fig 2: CAR T cells expressing dCK shRNAmiR reduce dCK mRNA abundance, conferring resistance and the ability to proliferate in the presence fludarabine and also works as a selection system helping in CAR enrichment**

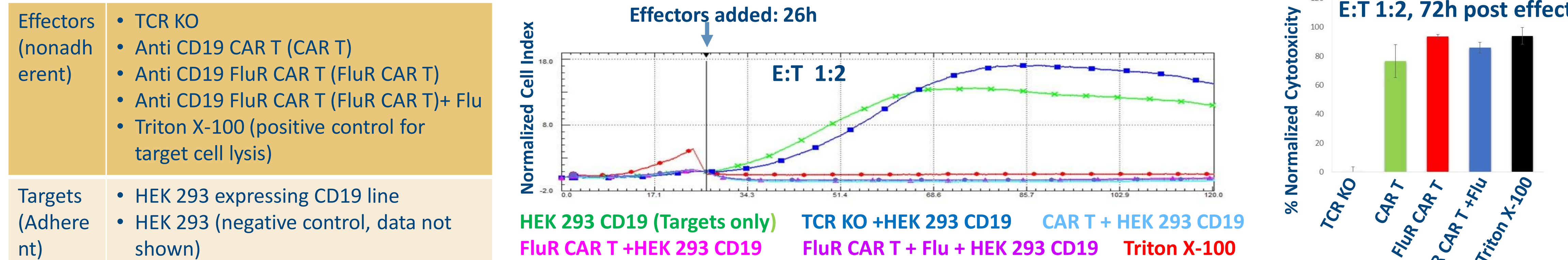



| Days post treatment | % CD3-CAR+ FluR CAR T | % CD3-CAR+ FluR CAR T + Flu |
|---|---|---|
| 0 | 28 | 28 |
| 5 | 28 | 37 |
| 11 | 26 | 42 |

Enrichment of CD3-CAR+ population seen upon treatment of FluR CAR T with Fludarabine

**Fig 3: Anti-CD19 CAR T cells with dCK knockdown (FluR CAR T's) in the presence of fludarabine display efficient antitumor response to CD19 expressing tumor cells In vitro**

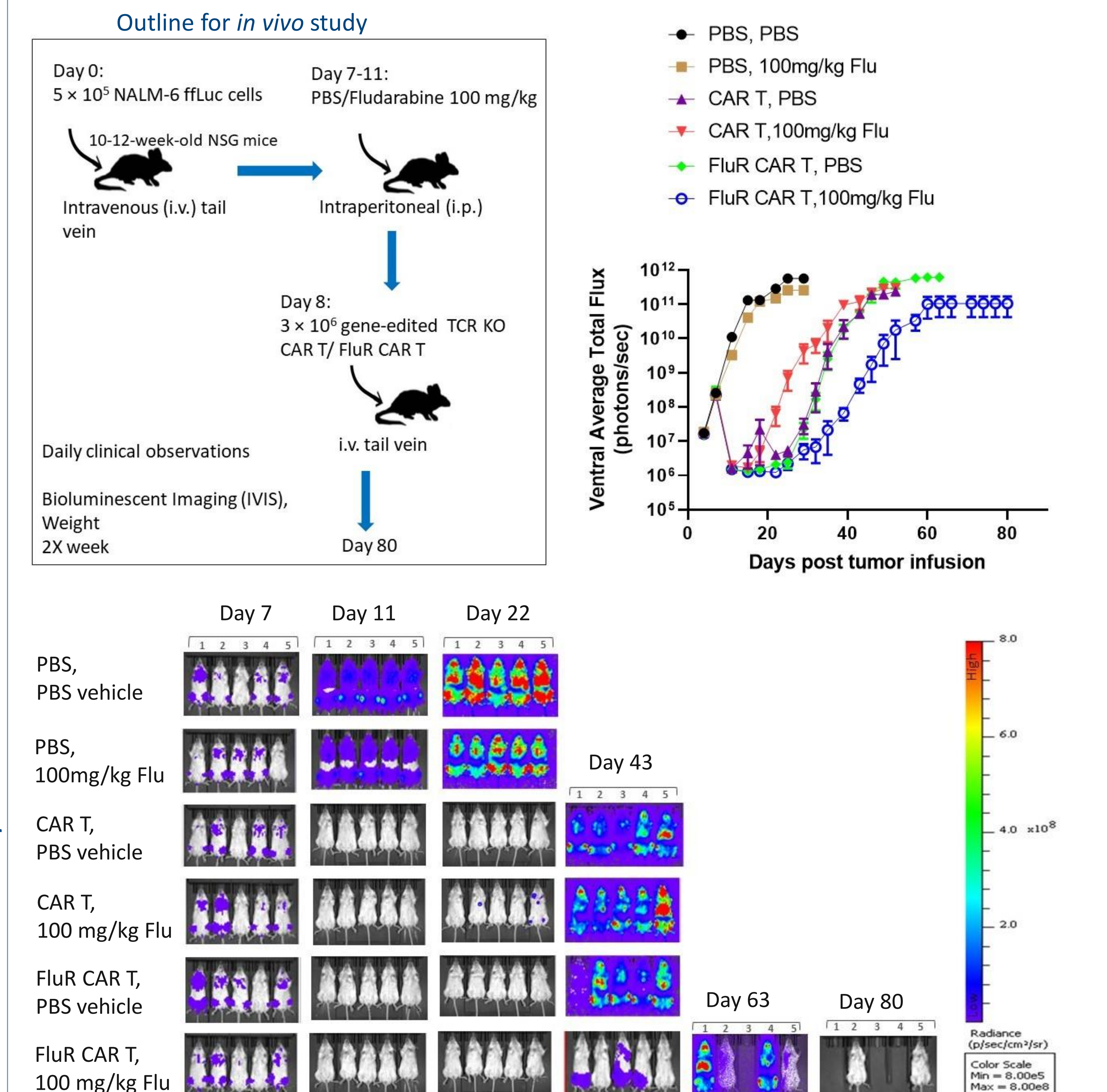
3 days post transduction CAR T and FluR CAR T cells were treated +/- 6uM fludarabine for 8 days. Cells were then depleted of CD3+ cells. Enriched CD3⁻ cells were cultured for 3 additional days in the presence of IL-15 + IL-21 and then tested in xCELLigence real-time cell analysis (RTCA) assay. Real-time cell analysis (RTCA) is a technique based on impedance and microsensor electrodes and is used as a label-free, real-time system to detect the killing of target cells by effector cells. Step1 : Adherent target cells (i.e. tumor cells) are first seeded in the wells of an electronic microtiter plate. Adhesion of target cells to the gold microelectrodes impedes the flow of electric current between electrodes. Impedance is measured as a unitless parameter called Cell Index. Step 2: Nonadherent effector added, which themselves do not cause change in impedance. Step 3: If the effector cells attack the target cancer cells, the destruction of the tumor cells is reflected by a decrease in Cell Index over time.



| | |
|---|---|
| Effectors (nonadherent) | • TCR KO<br>• Anti CD19 CAR T (CAR T)<br>• Anti CD19 FluR CAR T (FluR CAR T)<br>• Anti CD19 FluR CAR T (FluR CAR T)+ Flu<br>• Triton X-100 (positive control for target cell lysis) |
| Targets (Adherent) | • HEK 293 expressing CD19 line<br>• HEK 293 (negative control, data not shown) |



**Fig 4: Anti CD19 CAR T cells with dCK knockdown (FluR CAR T's) in the presence of fludarabine show enhanced tumor clearance and survival compared to mice treated with Anti CD19 CAR T's alone or Anti CD19 CAR T cells plus fludarabine in a Murine Model of Acute Lymphoblastic Leukemia (ALL)**




## CONCLUSIONS

- CAR T cells expressing a dCK-specific RNAi feature resulted in 70% reduction in dCK mRNA abundance, and conferred resistance to fludarabine *in vitro* and *in vivo*.
- These data suggest that the drug resistance feature may enable allogeneic CAR-T cells to be simultaneously administered with fludarabine, suppressing rejection of CAR T and improving CAR T engraftment and expansion. This synergy between conditioning and CAR T therapy may improve clinical outcomes by enhancing effector persistence and tumor clearing.

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