140 ALLOGENEIC CAR T CELLS WITH DEOXYCYTIDINE KINASE KNOCKDOWN DEMONSTRATE RESISTANCE TO FLUDARABINE

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Background 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.

Methods To protect CAR T cells from fludarabine toxicity, we have knocked down deoxycytidine kinase (dCK), which converts fludarabine from the prodrug form to an active compound. This 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 micro-RNA. 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 We observed that the inclusion of the RNAi feature resulted in 70% reduction in dCK mRNA abundance, and conferred resistance to fludarabine in vitro. Moreover, treatment of tumor-bearing mice with fludarabine and dCK knockdown CAR T cells resulted in enhanced tumor clearance and survival compared to mice receiving CAR T cells alone or fludarabine plus dCK replete CAR T cells.

Conclusions CAR T cells expressing a dCK-specific RNAi gene exhibited resistance to fludarabine in vitro and in vivo. This 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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Ethics Approval The animal study conducted was approved by the Institutional Animal Care and Use Committee (IACUC) of Mispro Biotech.

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