Background While CAR-T cell cancer immunotherapy has been successful in treating hematological malignancies, there have been significant difficulties in adapting these therapies for the treatment of solid tumors. Several factors are responsible for this challenge including increased tumor burden, immunosuppression in the tumor microenvironment, and heterogeneity of tumor associated antigens. Various approaches have been developed to address these challenges, including endowment of CAR T cells with expression of accessory molecules that enhance their function, such as IL-12, to make “armored CARs”. These approaches currently rely on constitutive IL-12 expression, or in case of inducible systems, use of multiple viral vectors, resulting in unregulated delivery or product heterogeneity respectively. Either of these methods can add significant risk of poor activity or toxicity.

Methods To resolve these issues, we developed genetic systems that combine antigen-induced production of an accessory molecule via a synthetic nuclear factor of activated T cells (NFAT) promoter, along with constitutive CAR expression, in a single lentiviral vector referred to as Uni-Vect. We demonstrate the therapeutic application of Uni-Vect in vivo by transient activation-dependent expression of IL-12 in an ovarian cancer model. In a previous clinical trial tumor infiltrating-lymphocytes engineered with NFAT inducible IL-12 expression were tested and clinical activity was observed. However, severe toxicity accompanied by high serum levels of IL-12 and IFN-γ limited further development. We address this challenge by eliminating endogenous TCR-driven activation, which may lead to triggering of undesired NFAT-inducible IL-12 expression. Further, we utilize a genome-editing approach with homology-directed repair as a means of gene integration, to achieve single-step generation of TCR disrupted-inducible IL-12 armored CAR T cells.

Results We compare the Uni-Vect platform to standard methods for armoring CAR-T cells and demonstrate enhanced control over accessory molecule expression in vitro. Further, we demonstrate the functionality of our lentiviral and knock-in products in our in vitro and in vivo models of ovarian cancer. We found that knock-in inducible IL-12 CAR T cells were able to eliminate tumors in all mice while avoiding lethal toxicity due to dysregulated T cell activation.

Conclusions We demonstrate the feasibility of non-viral knock-in of large Uni-Vect constructs that share the benefits of inducible armoring and gene disruption. The modular Uni-Vect platform will set a foundation for potent next generation CAR T cellular products. Our added safety layers and an optimized manufacturing process will support clinical translation for patients with ovarian cancer and beyond.

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REFERENCE