Regional delivery of clinical-grade mesothelin-targeted CAR T cells with cell-intrinsic PD1 blockade: Translation to a phase I trial

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INTRODUCTION

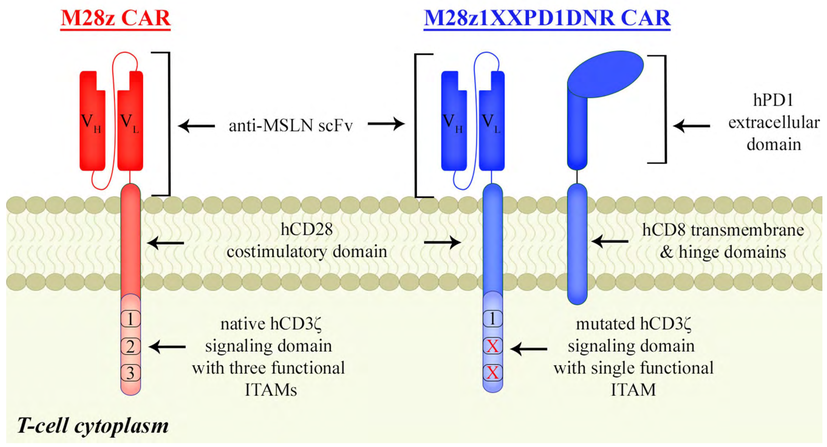
We have previously established the safety and antitumor efficacy of regionally delivered[1], second-generation mesothelin-specific chimeric antigen receptor (CAR) T cells with CD28-costimulatory and CD3ζ signaling domain (M28z) followed by anti-programmed death 1 (PD1) antibody in patients with malignant pleural disease (NCT02414269). To avoid repeated and prolonged administration of anti-PD1 antibody and its off-tumor side effects, we have developed next-generation CAR T cells with modified CD3ζ domain (1XX) bearing loss-of-function mutations within 2 of 3 immunoreceptor tyrosine-based activation motifs (ITAMs)[2], and a PD1 dominant negative receptor (PD1DNR) that provides T-cell intrinsic checkpoint blockade[3] (**Figure 1**).

Herein, we provide evidence of the safety, enhanced antitumor efficacy and superior functional persistence of M28z1XXPD1DNR CAR T cells.

METHODS

CAR and PD1 expression was assessed by flow cytometry. Comparative cytotoxicity upon initial and repeated antigen stimulation was performed by ^{51}Cr-release assay. Antitumor efficacy *in vivo* of a single dose (5×10^4 or 1×10^5 intrapleurally) was investigated in an orthotopic mouse model of pleural mesothelioma by bioluminescence imaging (BLI) and survival analysis. Functional persistence of CAR T cells was assessed in a tumor rechallenge experiment following eradication of pleural tumor. A GLP toxicity study was conducted in male and female mice with body weight, clinical chemistry, hematology and organ pathology assessments post-dose in mice bearing orthotopic mesothelioma xenografts.

Figure 1. M28z1XXPD1DNR: CAR T cells with intrinsic PD1-blockade and modified CD3ζ domain



RESULTS

Figure 2. T cells transduced with M28z1XXPD1DNR overexpress the PD1 extracellular domain

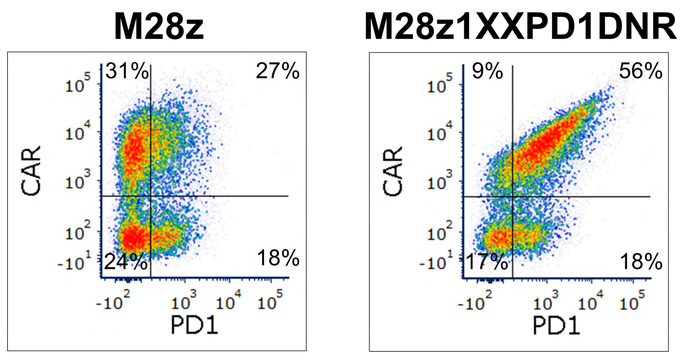


Figure 2. At a similar CAR transduction percentage, T cells transduced with M28z1XXPD1DNR exhibited higher cell-surface expression of the PD1 extracellular domain than M28z CAR T cells due to the expression of PD1DNR.

Figure 3. In a repeated antigen stimulation experiment with mesothelin-expressing target cells, M28z1XXPD1DNR and M28z CAR T cells exhibited similar cytotoxicity upon the 1st and 4th antigen stimulation across multiple effector to target (E:T) ratios. Upon the 7th antigen stimulation, cytotoxicity was substantially reduced for M28z CAR T cells whereas M28z1XXPD1DNR CAR T cells retained cytotoxicity.

Figure 3. M28z1XXPD1DNR CAR T retain cytotoxicity upon repeated antigen stimulation

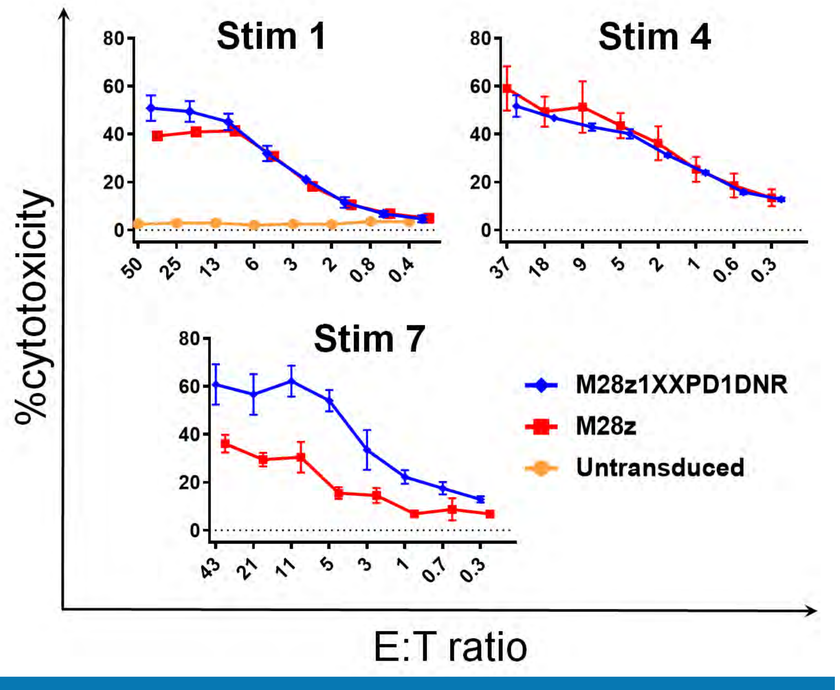


Figure 4. M28z1XXPD1DNR CAR T cells exhibit superior efficacy and functional persistence in mice with mesothelioma compared to M28z

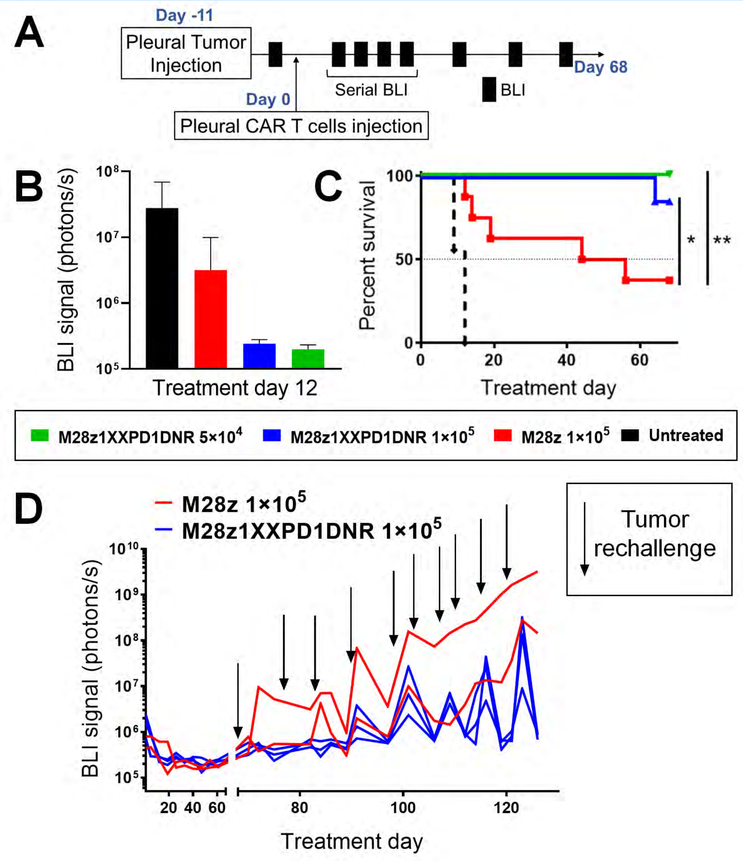


Figure 4. NSG mice bearing pleural mesothelioma were injected with a single intrapleural dose of M28z1XXPD1DNR or M28z CAR T cells (n=7-8) (**A**) and tumor burden was measured by serial BLI (**B**). M28z1XXPD1DNR CAR T cells significantly prolonged survival compared to M28z CAR T cells (**C**). Following eradication of pleural tumor, mice were rechallenged with mesothelin-expressing tumor intraperitoneally. Mice treated with a single intrapleural dose of M28z1XXPD1DNR CAR T cells resisted tumor reestablishment upon 10 tumor rechallenges, indicating that M28z1XXPD1DNR CAR T cells establish systemic immunity and exhibit superior functional persistence (**D**). No toxicities were observed in a GLP toxicity study.

CONCLUSION

The safety, tumor eradication, and functional persistence of M28z1XXPD1DNR CAR T cells supports IND submission and initiation of a phase I clinical trial in patients with advanced mesothelioma and to further extend our investigation to other mesothelin-expressing solid tumors.

REFERENCES

1- Adusumilli PS, Sadelain M, *Sci Transl Med* 2014
2- Feucht J, Sadelain M, *Nat Med* 2019
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