Background Chimeric antigen receptor (CAR)-T cells have displayed remarkable efficacy in treating malignant cancers, particularly liquid tumors. CAR-T cells have proven to be a new type of “living” therapeutic by harnessing the patient’s immune system to recognize specific tumor-associated antigens and redirect the engineered T cells to more specifically targeted tumor cells. Considerable research efforts have been invested into developing new CAR structures to increase the scope of targeted cancer types and raise their anti-tumor efficacy. Evaluating the biofunction of CAR-T cells in vitro typically involves a series of labor-intensive co-culture experiments and immunoassays, where reproducibility remains a challenge during the validation of new CAR-T cells due to donor-to-donor variations and other possible factors. In this study, we present a panel of luciferase reporter tumor cell lines that can be utilized to examine the function of CAR-T cells. The panel of selected human tumor cell lines naturally express high levels of clinically relevant CAR-T target antigens on cell surface, such as CD19, CD20 and HER2.

Methods The panel of selected human tumor cell lines naturally express high levels of clinically relevant CAR-T target antigens on cell surface, such as CD19, CD20 and HER2. Antibiotic selection and single cell sorting were performed to isolate stable clones with high luciferase expression via the introduction of a Lenti-LUC2 luciferase reporter into the parental cell lines. The target antigen and luciferase were verified to have expression stability by comparing the low passage and the high passage reporter cells. In addition, these reporter cell lines were characterized and authenticated using cell morphology, growth kinetics, and STR profile. To verify the performance of the target luciferase reporter cell lines, we used the cancer and T cell co-culture experiments. Commercially available CAR-T cells targeting CD19, CD20, and HER2 were employed in this study, with which empty vector-transduced T cells from the same donor were paired as controls. The cytotoxicity of the CAR-T cells against target tumor cells was measured using a luciferase assay, a commercially available potency assay, and a bright field and fluorescence live cell imaging assay.

Results Our results demonstrate that the luciferase reporter system is a simple, robust, and highly sensitive means to measure biological processes in cancer and T cell ex vivo co-cultures.

Conclusions In summary, CAR-T target antigen luciferase reporter cell lines from ATCC provide the well-characterized tools with high reproducibility for studying CAR-T biofunction and validating new CAR-T agents for cancer immunotherapy.