Luciferase reporter cancer cell lines facilitate CAR-T development

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Abstract

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. 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. 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. 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.

Background

Chimeric Antigen Receptor T (CAR-T) Cells

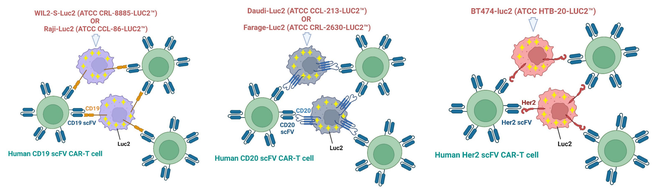
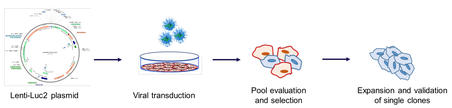


Figure 1: CAR-T Target Luciferase Reporter Cells. Schematic showing CAR-T target cells with expression of CD19-positive WIL2-S-Luc2 and Raji-Luc2, CD20-positive Daudi-Luc2 and Farage-Luc2, and HER2-positive BT-474-Luc2 being surrounded and attacked by CD19-, CD20-, and HER2-targeting CAR-T cells, respectively. Created with BioRender.com.

Table 1. ATCC luciferase-expressing cell lines

Luciferase Cell Line	ATCC® No.	Tissue/Disease	Target
WIL2-S-Luc2	CRL-8885-LUC2™	B Lymphoblastoid Cell	CD19
Raji-Luc2	CCL-86-LUC2™	Burkitt’s Lymphoma	CD19
Daudi-Luc2	CCL-213-LUC2™	Burkitt’s Lymphoma	CD20
Farage-Luc2	CRL-2630-LUC2™	Lymphoma	CD20
BT-474-Luc2	HTB-20-LUC2™	Breast ductal carcinoma	HER2

Generation of Luciferase-expressing Cell Lines



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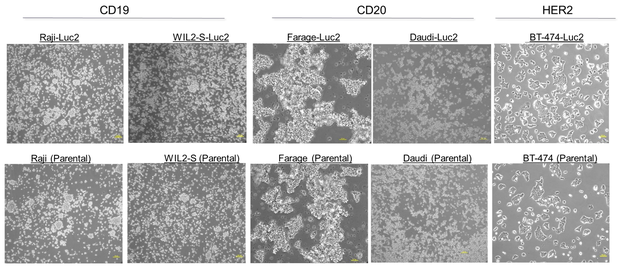
- Kiesgen S, et al. Comparative analysis of assays to measure CAR T cell-mediated cytotoxicity. Nat Protoc 16: 1331-1342, 2021. PubMed: 33589826.
- Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J 11: 69, 2021. PubMed: 33824268

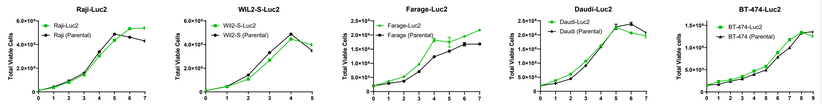
Results

Characterization of CAR-T target cell lines

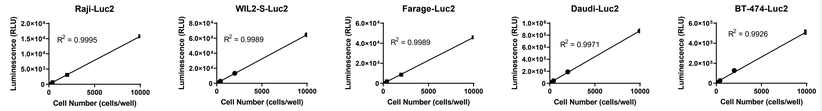
A. Growth Curves of CAR-T Luciferase reporter cell lines are similar to the parental cell line



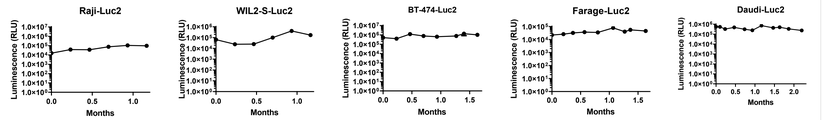
B. Growth Curves of CAR-T Luciferase reporter cell lines are similar to the parental cell line



C. Luciferase activity has a linear correlation between bioluminescence intensity and cell number



D. Luciferase expression is stable for over 30 population doublings



E. Antigen expression is stable for over 30 population doublings

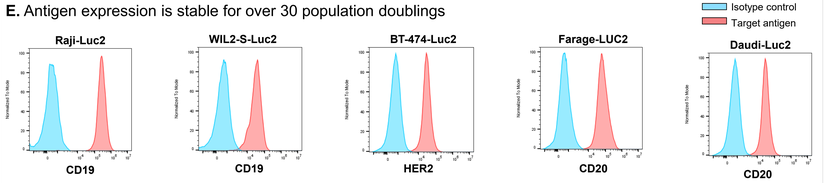
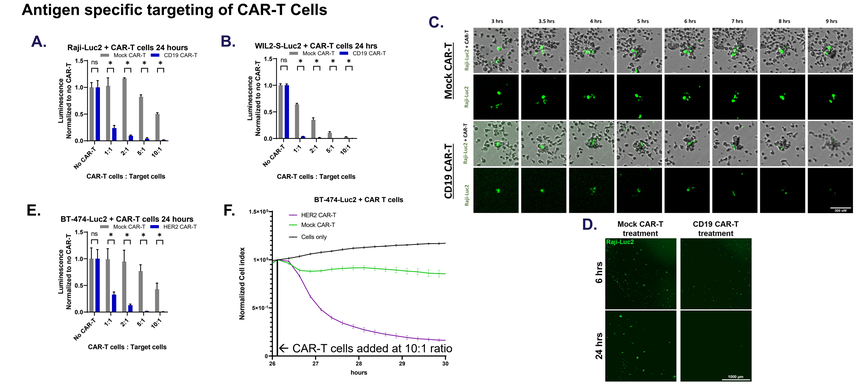


Figure 2. Characterization of CAR-T target cell lines. (A) Cell morphology of the luciferase expressing cell lines was observed under microscopy and images were captured via digital camera (scale bar = 100 mm). (B) 2 x 10^5 cells/mL were seeded into T25 flasks and automated cell counting was used to generate growth curves. (C) Luciferase assay was performed by using Bright-Glo™ (Promega®) Luciferase Assay System and a luminescence plate reader. Data showed a linear correlation between bioluminescence intensity and cell number. (D) To verify the stability of luciferase expression, the cells were maintained in culture for 30 population doublings. The luciferase expression was monitored every week by using the luciferase assay. (E) Flow cytometry analysis was performed to assess the CAR-T target antigen expression levels CD19, CD20, and Her2 (pink) on the tumor cell lines compared to isotype controls (blue).

Antigen specific targeting of CAR-T Cells



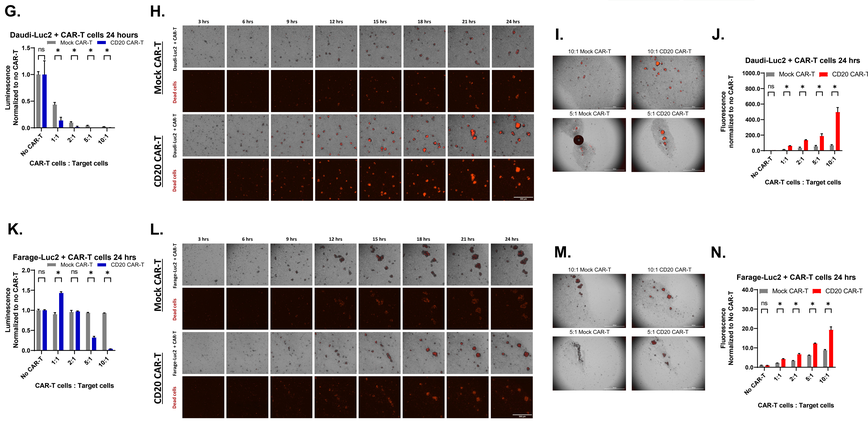


Figure 3. CAR-T cytotoxicity assays on CAR-T Luciferase reporter cell lines. Raji-Luc2 (A), WIL2-S-Luc2 (B), BT-474-Luc2 (E), Daudi-Luc2 (G), or Farage-Luc2 (K) (5 x 10^3 cells) were targeted by CAR-T cells at ratios of 1:1, 2:1, 5:1, and 10:1 and cell killing was measured by luciferase assay. (* = significant difference and ns = not significant using unpaired t test, with a single pooled variance) (A, B, E, G, K). Raji-Luc2 cells were stained with Vybrant™ (Thermo Fisher) DiO dye and real-time fluorescent imaging was measured every 30 minutes for 24 hours during the co-culture of Raji-LUC2 cells with CAR-T cells. After 24 hours of co-culture CD19 CAR-T cells showed a decrease in fluorescent cells as compared to 6 hours; in a co-culture with Mock CAR-T cells numerous Raji-LUC2 cells were present (C). (D) Two stained Raji-Luc2 cells (Green) from the co-culture experiment were tracked for 6 hours and became surrounded by CAR-T cells resulting in a decrease of fluorescence when treated with CD19 CAR-T, as compared to co-cultures with Mock-CAR-T cells. HER2 CAR-T cells were used to target 2 x 10^4 HER2 positive BT-474-Luc2 at a 10:1 ratio and cell killing was measured using the xCELLigence system (F). Daudi-Luc2 (H) or Farage-Luc2 (L) cells (5 x 10^3) were co-cultured with CD20 CAR-T cells or Mock CAR-T cells in the presence of Incucyte Cytotox red dye in the medium and real-time fluorescent imaging was measured every hour for 24 hours, resulting in an increase of fluorescence intensity when co-cultured with CD20 CAR-T as compared to co-cultures with Mock-CAR-T cells. After 24 hours of co-culture with CD20 CAR-T cells, Daudi-Luc2 (I) or Farage-Luc2 (M) showed an increase in the number dead (red) fluorescent cells as compared co-culture with Mock CAR-T cells. The clustered red fluorescence was quantified and compared (J, N).

Conclusion

The functionally validated CAR-T target luciferase reporter tumor cells exhibit naturally high expression of the target antigens CD19, CD20, and HER2 and provide a robust, straightforward method for testing CAR-T activity.

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