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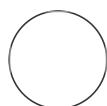
Protocol status: Working
We use this protocol and it's working

In vivo CAR T cell tumor control assay

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Andrea R Daniel: This protocol is adapted from the M. Brown lab at Duke University.



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ABSTRACT

This protocol describes methods for a HCC1954 cell implanted orthotopic tumor model in mice with delivery of T cells expressing HER2 CAR. Tumor volumes are measured to evaluate tumor control by CAR T cell therapy.

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HCC1954 orthotopic tumor model

- 1 Six- to 8-week-old female immunodeficient NOD/SCID gamma (NSG) mice were obtained from Jackson Laboratory and then housed in 12-h light/dark cycles, at an ambient temperature (21 ± 3 °C) with relative humidity ($50 \pm 20\%$) and handled in pathogen-free conditions
- 2 HCC1954s were maintained in DMEM/F12 supplemented with 10% FBS, 100 U ml^{-1} penicillin and $100 \mu\text{g ml}^{-1}$ streptomycin.
- 3 Resuspend cells in 2ml PBS (5×10^7 cells/ml), then diluted 1:1 with Matrigel (2.5×10^7 /ml) thus 2.5×10^6 per 100ul.
- 4 2.5×10^6 HCC1954 cells are implanted orthotopically into the mammary fat pad of NSG mice in 100μL volume of 50:50 (v:v) PBS:Matrigel.
- 5 Upon detection tumor volumes were calculated based on caliper measurements using the formula $\text{volume} = \frac{1}{2} (\text{Length} \times \text{Width}^2)$. Measure tumors every 4-6 days.

CAR T cell delivery

- 6 Human donor T cells were expanded for 9-11 days post-transduction with HER2-CAR-2A-GFP and HER2-CAR-2A-BATF3 constructs before delivery to tumor bearing mice.

- 7 Transduction rates were measured on the day of treatment using flow cytometry and transduction rates exceed 80%.
- 8 T cells were resuspended at 50×10^6 CAR+ cells mL^{-1} in 1X PBS and serially diluted to the appropriate cell concentrations for 200 μL injections of either 10×10^6 , 2×10^6 , 5×10^5 , 2.5×10^5 , or 1×10^5 HER2 CAR+ T cells.
- 9 21 days after tumor implantation randomize mice into groups for CAR T cell injections. Deliver CAR T cells intravenously by tail vein injection.
- 10 Measure the length and width of tumors every 4-6 days using calipers.
- 11 Mice are euthanized before reaching a tumor volume of $2,000 \text{ mm}^3$, the upper threshold defined by the Duke Institutional Animal Care and Use Committee.