Standard cell-based assays for cytokine release and cellular proliferation of murine CAR T cells.

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ABSTRACT

Two standard cell-based assay to assess the function of murine CAR T cells, which we regularly performed at the end of the process of generating those CAR T cells (see "Retroviral transduction of primary murine CD8 T cells").

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

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Procedure for the preparation of adherent tumor cells (targe...

1 Aspirate media from T75 flasks; wash with 10ml PBS to remove complete DMEM media (cDMEM).
Add 5mL of Cell Dissociation Media (0.5mM EDTA in PBS); incubate at 37C for 10-20 mins.

Pipet vigorously to dissociate the cells, transfer cells to a 15 mL tube, and wash the flask with 5mL mouse T cell media (mTCM) to combine in the tube; centrifuge at 400rcf for 4mins.

Resuspend cells in 1mL mTCM; count cells by hemocytometer or automated cell counter.

Note

mTCM recipe:
RPMI 1640 (+HEPES)
10% heat-inactivated FBS
1 mM HEPES
100 U/mL penicillin/streptomycin
1 mM sodium pyruvate
50 µM b-mercaptoethanol
50 U/mL human IL-2 (Peprotech)
Filter through 0.2um before use.

cDMEM recipe:
DMEM (gibco)
10% heat-inactivated FBS
100 U/mL penicillin/streptomycin
2mM L-Glutamine
25mM HEPES
Filter through 0.2um before use.

2 In new 15mL tubes, prepare 1.5×10^6 cells / 6mL mTCM in 15mL tubes; irradiate the cell lines with 100Gy (10,000 rad) in a Cesium irradiator.

3 For the ELISA plate: pipet 100µL (2.5×10^4) of the target cells to 96-well plate wells. For no-target controls, use 100µL mTCM. For positive control, use 100µL of 2X PMA/Iono stimulation cocktail (eBioscience).

4 Create a mirror-image of the 96-well plate for the "CellTrace" plate.

Preparation of the ELISA-plate effector cells

5 Harvest the transduced mouse T cells (see protocol titled "Retroviral transduction of primary murine CD8 T cells" for details) and transfer them to a 15mL falcon tube. Centrifuge cells at 400rcf for 6mins; resuspend in 1mL mTCM; count; prepare 3×10^6 T cells / 6mL mTCM in a new tube.

6 Aliquot 100 µL (5×10^4 cells) to the respective wells in 96-well “ELISA” plates; mark the time; place at 37C for 24 hr. Save 4mL (2×10^6 T cells) for CellTrace labeling (see below).

7 After 24 hours, carefully harvest 100 uL from each well without disturbing the pellets. The supernatant can then be frozen at -20C until the samples are required to perform the ELISA.

Preparation of the CellTrace-plate effector cells

8 Meanwhile, with the hood lights turned off, prepare a 1:1000 CellTrace solution in PBS (consult the manufacturer's protcol for detailed instructions). Prepare enough volume to label 1e6 cells / mL.

Add 10mL PBS to each 4mL T cell suspension; centrifuge at 400rcf for 6mins; resuspend cells are 1e6 / mL CellTrace solution.

9 Incubate in the 37ºC incubator for 20 mins. Then quench with five times the staining volume and incubate in the 37ºC incubator for an additional 5 mins. Centrifuge at 400rcf for 6mins; resuspend in 4mL mTCM.

10 Distribute 100µL aliquots (5×10^4 cells) to the labeled wells of “CellTrace” plate. Wrap plate in foil; mark the time; place in the incubator for 48 hr.

11 At 48 hrs, gently mix the effector cells before transfering the samples to a new U-bottom 96 well plate. Immunophenotype the samples with the appropriate markers (e.g. CD8, transduction marker, congenic marker, etc..). Attention should be given to the spectral overlap between the respective CellTrace reagent used and the markers used in staining.