Assay Name: CAR T cell-mediated cytotoxicity using CFSE and PI

Assay ID: Celigo_01_0009

Description: Measure Chimeric Antigen Receptor (CAR) T cell mediated cytotoxicity by counting total CFSE positive/PI negative live tumor cells

Stains: CFSE (green total cells); PI (red dead cells)

Imaging channels: Bright field, Green, Red

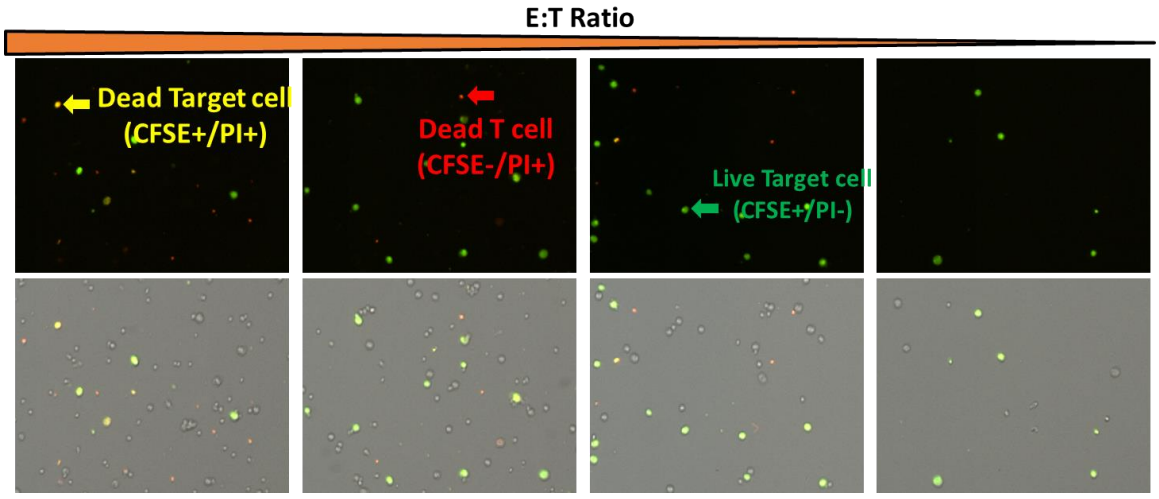
Image analysis algorithm: Celigo software Target 1 + 2 + 3

Methods:

1. Culture and collect Target cells and stain with CFSE
2. Seed the Target cells in the wells of 96-well microplate
3. Add the CAR T cells at different E:T ratios
4. Co-culture the Target cells with cultured CAR T cells for 4 hours and observe the CAR T cell killing
5. Stain the cells in the well with PI to identify the dead cells (Nexcelom, Cat# CS1-0109)
6. Use Celigo and capture images at Target cells over time
7. Use the equation to calculate cytotoxicity
   a. $\%\ Cytotoxicity = \frac{Dead\ Target\ Count}{Dead+Live\ Target\ Count}$

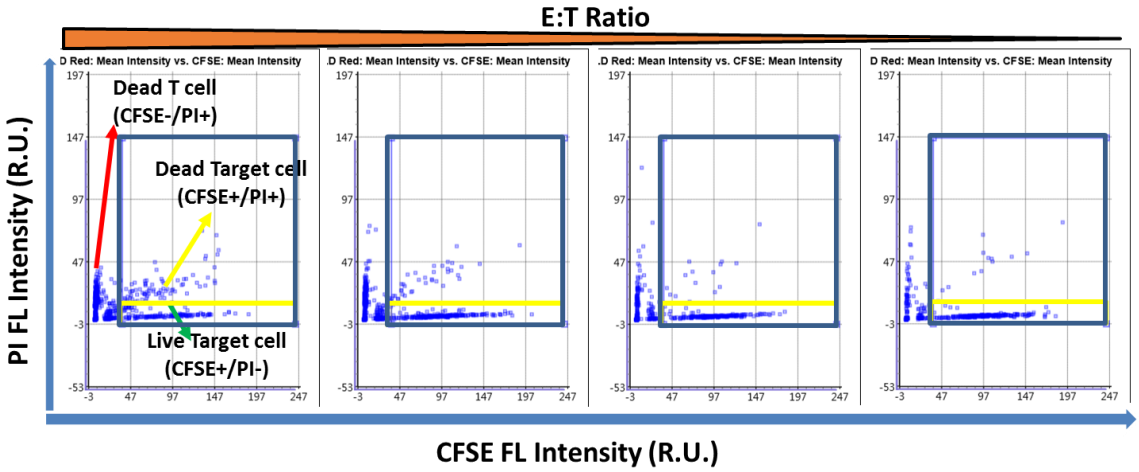
Results:

CAR T cell killing bright field and fluorescent overlay images



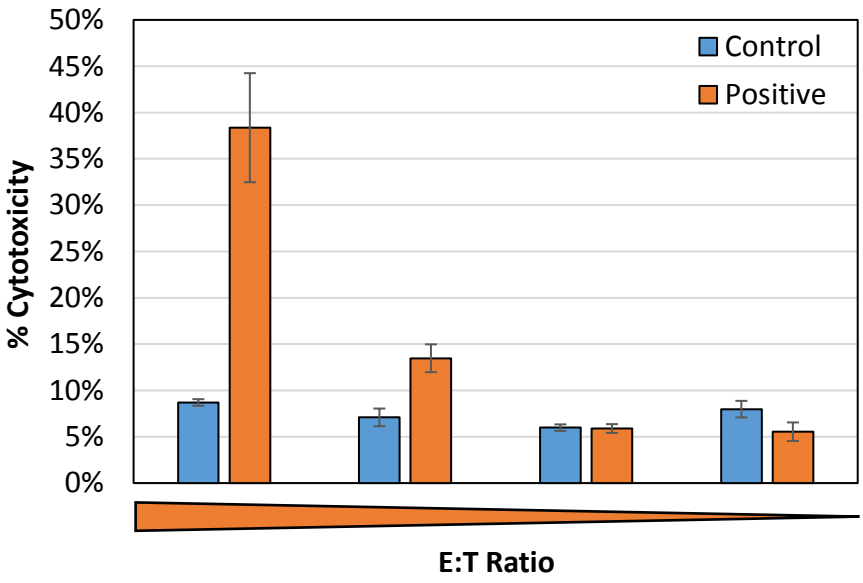
- Visually, more CAR T cells are shown in the bright field overlay at higher E:T ratio

CAR T cell-mediated cytotoxicity gating results



- The number of dead Target cells increased as the E:T ratio increased, which can be visually seen in the scatter plots
- The gating allowed the measurement of number of live and dead Target cells to calculate the final % cytotoxicity

CAR T cell mediated cytotoxicity results



- At the endpoint, a clear cytotoxicity response is shown in respect to the E:T ratios
- The % cytotoxicity is calculated by the equation previously shown
- As the E:T ratio increased, the % cytotoxicity also increased