

CLAIMS

1. A method for assessing potency of tumor infiltrating lymphocytes (TILs), comprising:
coculturing TILs and immortalized cells, wherein the immortalized cells comprise a molecule that activates a T cell; and
assessing potency of the TILs.
2. The method of claim 1, wherein the molecule binds to a T cell antigen, optionally, wherein the TILs express the T cell antigen, and further optionally, wherein the T cell antigen is a CD3 antigen.
3. The method of claim 1 or 2, wherein the immortalized cells express the molecule.
4. The method of any one of the preceding claims, wherein the molecule is an antibody or an antibody fragment, optionally wherein:
the antibody fragment is selected from a single-chain variable fragment (scFv), a F(ab')₂ fragment, a Fab fragment, a Fab' fragment, and an Fv fragment;
the antibody or antibody fragment is respectively an OKT3 antibody or OKT3 antibody fragment, optionally, wherein the OKT3 antibody fragment is a membrane-bound OKT3 (mOKT3) scFv, and optionally wherein the mOKT3 scFv is a low-affinity mOKT3 scFv variant; and/or
the molecule binds to CD3 with a dissociation constant (K_D) that is higher than the K_D of mOKT3 scFv, wherein the K_D of mOKT3 scFv is about 5×10^{-10} M.
5. The method of claim 4, wherein the low-affinity mOKT3 scFv variant comprises an amino acid sequence having R55 and Y57 mutations, relative to the amino acid sequence of SEQ ID NO: 2, optionally wherein:
the low-affinity mOKT3 scFv variant comprises an amino acid sequence having R55M and Y57A mutations, relative to the amino acid sequence of SEQ ID NO: 2, optionally wherein the low-affinity mOKT3 scFv variant binds to CD3 with a dissociation constant K_D that is least 250-fold higher than the K_D of mOKT3 scFv; or
the low-affinity mOKT3 scFv variant comprises an amino acid sequence having R55L and Y57T, relative to the amino acid sequence of SEQ ID NO: 2, optionally wherein the low-affinity mOKT3 scFv variant binds to CD3 with a dissociation constant K_D that is least 1000-fold higher than the K_D of OKT3 scFv.

6. The method of any one of claims 1-3, wherein the molecule is selected from bacterial superantigens, optionally *Staphylococcal* enterotoxin B (SEB), phytohaemagglutinin (PHA) and concanavalin A (ConA).
7. The method of any one of the preceding claims, wherein the molecule is a membrane-tethered molecule.
8. The method of any one of the preceding claims, wherein the TILs are engineered TILs (eTILs) optionally wherein the eTILs are edited eTILs, and optionally, wherein the edited eTILs comprise a genomic modification.
9. The method of any one of the preceding claims, wherein the immortalized cells comprise a clonal population of immortalized cells, are immortalized human cells, and/or are engineered cancer cells, optionally the engineered cancer cells are selected from engineered melanoma cells, engineered colorectal cancer cells, engineered bile duct cancer cells, and engineered breast cancer cells.
10. The method of any one of the preceding claims, wherein prior to the coculturing the immortalized cells are plated on an ultra-low attachment surface and/or in a multiwell plate, optionally a 6-well, 24-well, or 96-well plate, optionally wherein the immortalized cells are plated at density of about 5,000 to about 10,000 live cells/culture, optionally wherein the ultra-low attachment surface comprises a hydrophilic, neutrally charged hydrogel coating.
11. The method of claim 10, wherein the immortalized cells are cultured, preferably for about 2 to 6 days, more preferably about 4 days, to produce three-dimensional spheroids.
12. The method of any one of the preceding claims, wherein prior to the coculturing, the TILs were added to a culture of the immortalized cells at an E:T ratio of about 20:1 to 1:1.
13. The method of any one of the preceding claims, wherein the coculturing is for about 4 hours, about 8 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 3 days, about 4 days, or about 5 days, optionally wherein the coculturing is for about 1 day to about 5 days.
14. The method of any one of the preceding claims, wherein the assessing potency comprises:

measuring cytokine release, optionally IFN- γ , IL-2, TNF α and/or IL-6 release, from the immortalized cells, cell death and/or viability of the immortalized cells; and/or measuring growth, cell death, and/or viability of the immortalized cells.
15. The method of claim 14, wherein the measuring comprises performing an assay selected from real-time cell viability assays, ATP cell viability assays, live cell protease

viability assays, tetrazolium reduction cell viability assays, resazurin reduction cell viability assays, dead-cell protease release cytotoxicity assays, lactate dehydrogenase release cytotoxicity assays, and DNA dye cytotoxicity assays.

16. A method for assessing potency of tumor infiltrating lymphocytes (TILs), comprising:
coculturing TILs and a clonal population of engineered cancer cells, wherein the engineered cancer cells express an anti-CD3 antibody or anti-CD3 antibody fragment; and
assessing death and/or viability of the engineered cancer cells.
17. A method for assessing potency of tumor infiltrating lymphocytes (TILs), comprising:
coculturing TILs, immortalized cells, and a bispecific molecule that activates a T cell and binds to the immortalized cells; and
assessing potency of the TILs.
18. A method for assessing potency of polyclonal T cells, comprising:
coculturing polyclonal T cells and immortalized cells, wherein the immortalized cells comprise a molecule that activates a T cell; and
assessing potency of the polyclonal T cells.
19. A method for assessing potency of polyclonal T cells, comprising:
coculturing polyclonal T cells, immortalized cells, and a bispecific molecule that activates a T cell and binds to the immortalized cells; and
assessing potency of the polyclonal T cells.