CART T CELL *IN VITRO* SAFETY TESTING STRATEGY

Investigative Toxicology Laboratory

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NIH NATIONAL CANCER INSTITUTE
DCTD Division of Cancer Treatment & Diagnosis

Frederick National Laboratory for Cancer Research

Why Assess Binding of CAR-T Cells to Normal Human Tissues/Cells?

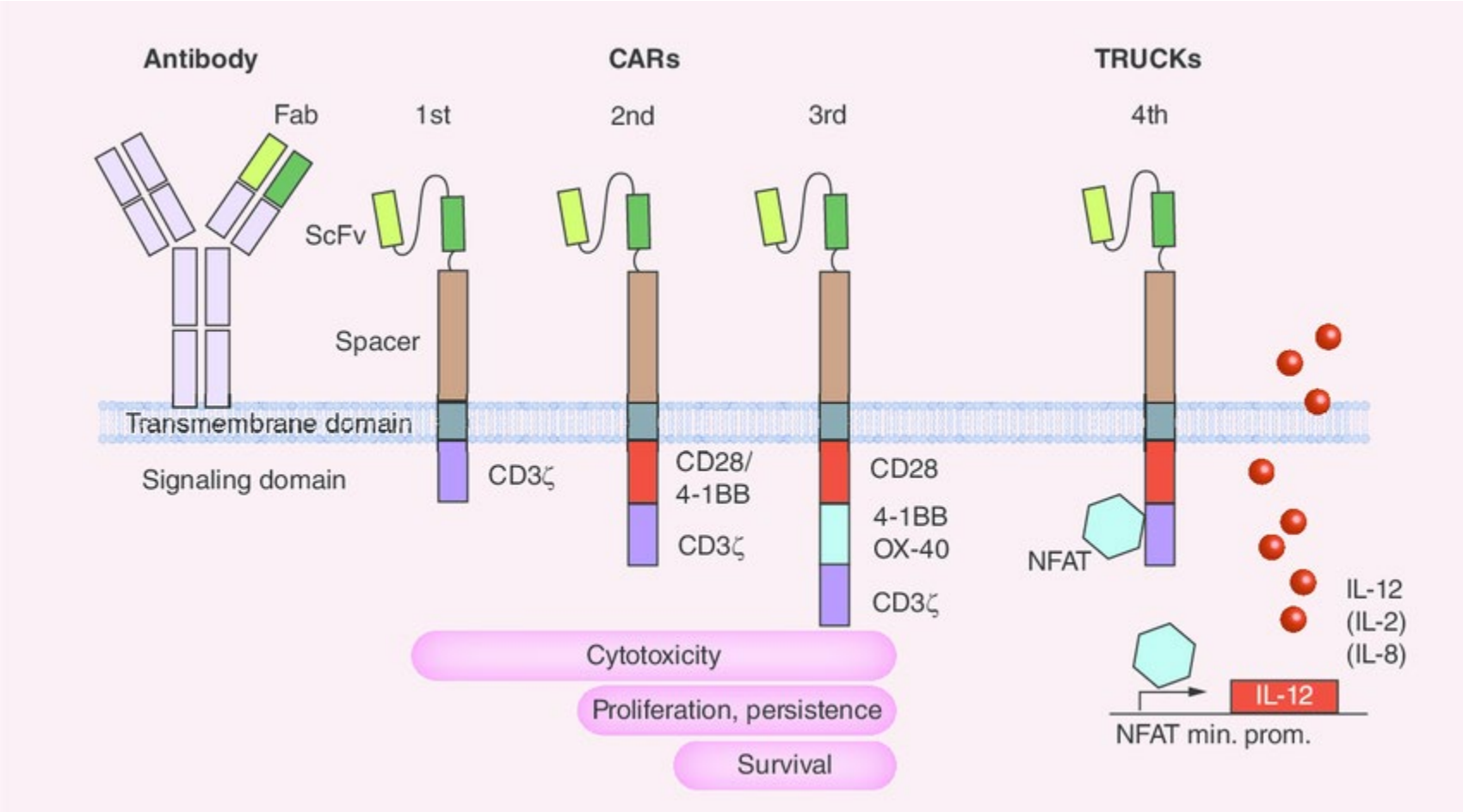
From 1997 FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use:

Non-target tissue binding may have serious consequences, particularly when pharmacologically active antibodies or cytotoxic immunoconjugates are used.

Accordingly, cross-reactivity studies with human tissues should always be conducted prior to phase 1 to search for cross-reactions or non-target tissue binding.

Preclinical evaluation of the antigen recognition domain should include assessment of the specificity and affinity for the target antigen to evaluate the potential for on-target/off-tumor and off-target toxicities. Undesired targeting of healthy/normal tissue that express the intended target antigen (on-target/off-tumor), as well as unintended targeting of other antigens expressed on healthy/normal tissue is a safety concern.

Why Assess Binding of CAR-T Cells to Normal Human Tissues/Cells?



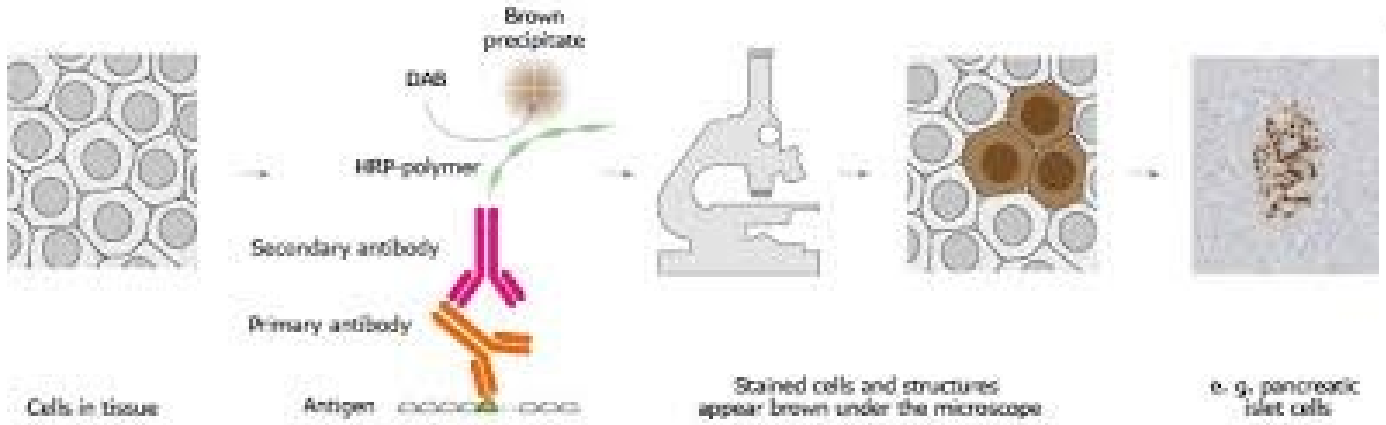
Kalaitsidou M et al., Immunotherapy 2015. 7(5): 487-497

Potential Adverse Consequences of CAR-T Cell Binding to Human Tissues/Cells

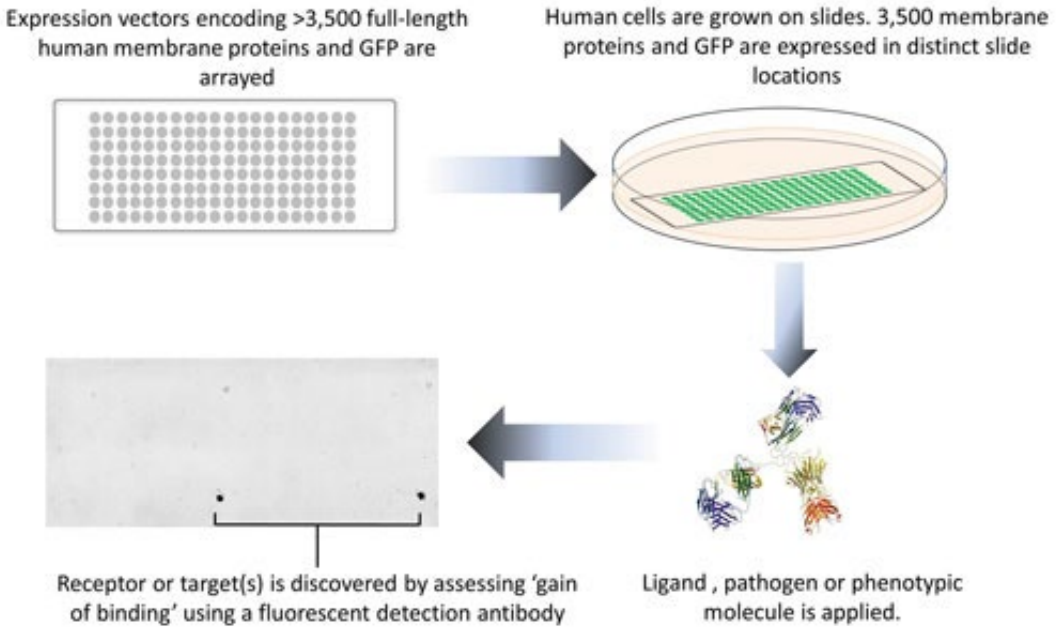
- On target/on tumor – cytokine release syndrome, tumor lysis syndrome
- On target/off tumor – destruction of healthy B-cells (CD19), cardiovascular toxicity (HER2)
- Off target/off tumor – cardiotoxicity due to cross-reactivity with titin

Binding Assessment Methods

Immunolabeling



Array-based Screen

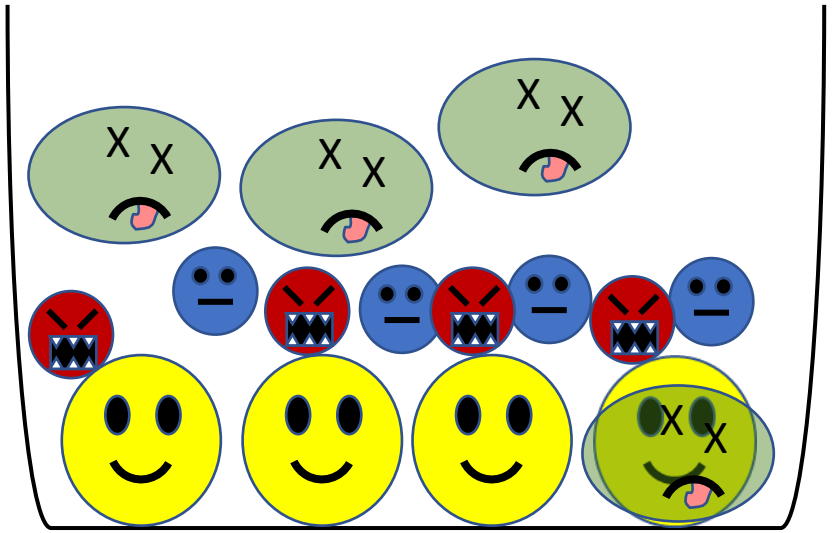


- Protein chip screen
- In situ hybridization
- Flow cytometry

From: Soden, J and Freeth, J (2013) Removign the Receptor Deconvolution Bottleneck: Power tool aims to uncover novel disease-relevant drug targets. Genetic Engineering and Biotechnology News. 33 (14)

Human Induced Pluripotent Stem Cell Derived Cells Used as a Model of Normal Cells in a Co-culture System

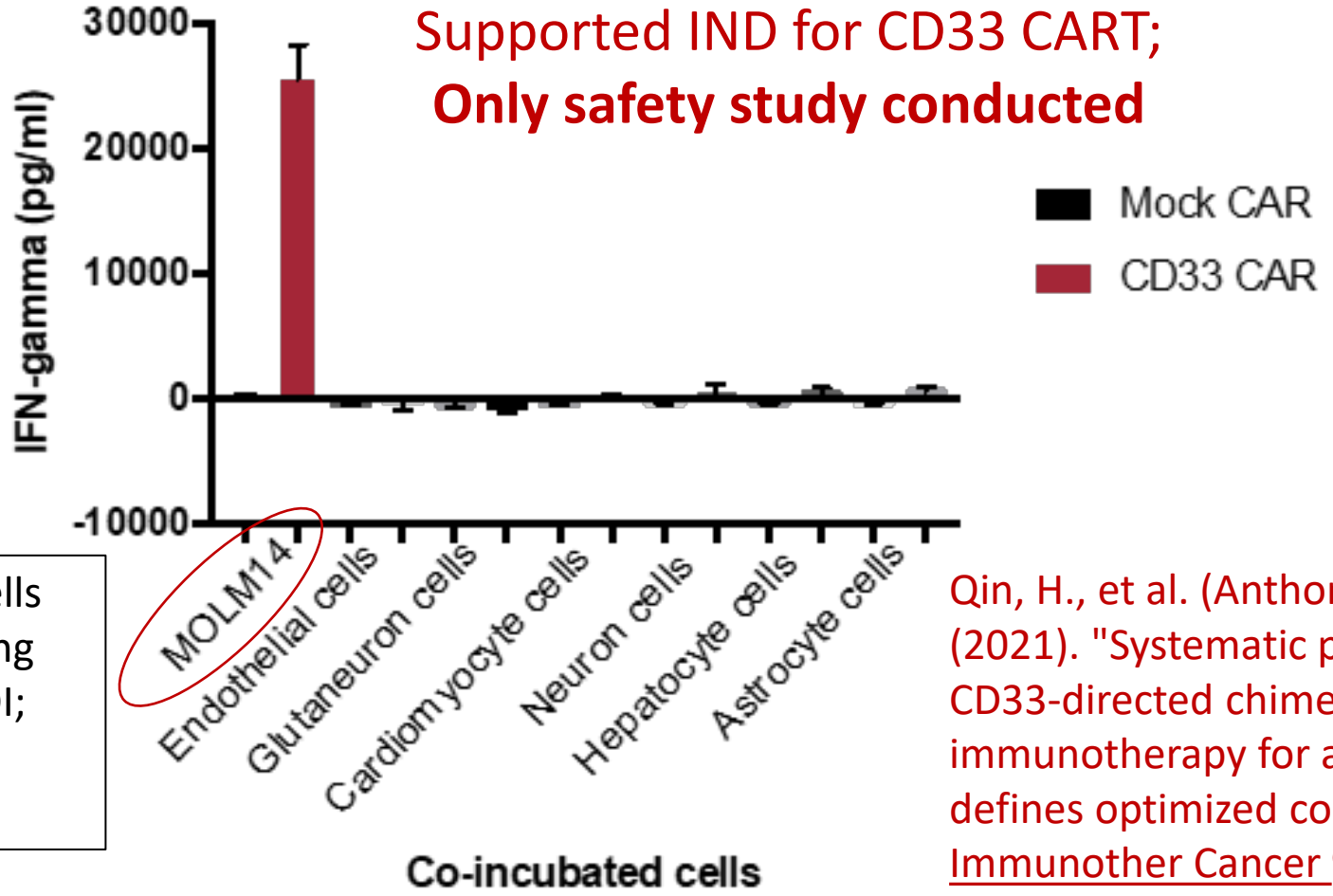
Cell types to be tested are intended to be broadly representative of target cells for potential toxicity after on- or off-target binding



Reactivity of CD33 CAR-T Cells Co-cultured with CD33+ Tumor Cell Line and a Panel of Human Induced Pluripotent Stem Cell Derived Models of Normal Cells

Supported IND for CD33 CART;

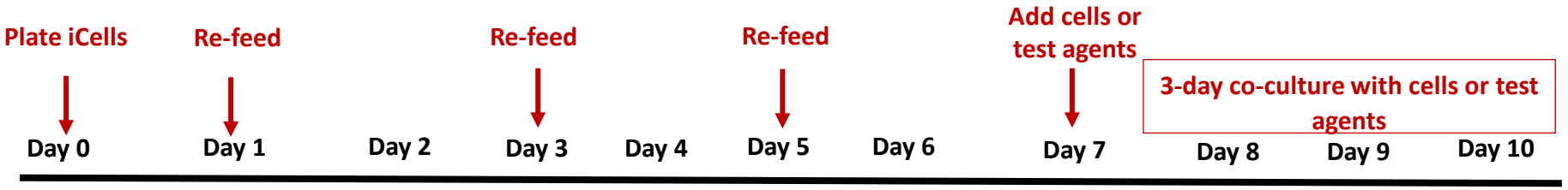
Only safety study conducted



Effector and target cells cultured 1:1 ratio using hiPSC-iCells from FCDI; Studies conducted in Terry Fry's lab at NCI

Qin, H., et al. (Anthony Welch, Terry Fry) (2021). "Systematic preclinical evaluation of CD33-directed chimeric antigen receptor T cell immunotherapy for acute myeloid leukemia defines optimized construct design." J Immunother Cancer **9**(9).

Investigative Toxicology Laboratory CAR-T Cell Binding Assessment Workflow



96-well
MEA plate

Hamilton Microlab
STAR

Continuous monitoring

96-well
regular plate

96-well
MEA plate

MAESTRO Z

MAESTRO PRO

LDH release - Supernatant
ATP and/or Caspase 3/7 - Lysate

Tecan M1000 Plate
Reader

Cytokine panel - Supernatant

BioPlex 200 multiplex
platform

Human Induced Pluripotent Stem Cell Derived Cells Used as a Model of Normal Cells

Cell types to be tested are intended to be broadly representative of target cells for potential toxicity after on- or off-target binding

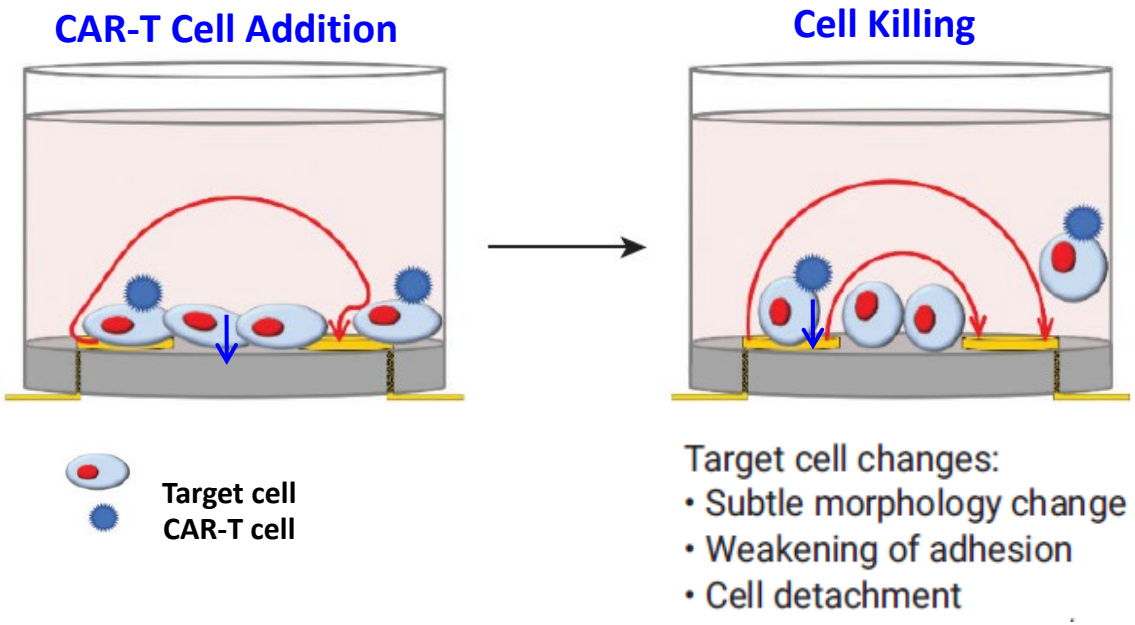
hiPSC Cells Planning to Use	**Other hiPSCs of Interest**
iCell Cardiomyocytes	iCell Motor Neurons
iCell Endothelial Cells	iCell Retinal Pigmented Epithelial Cells
iCell GlutaNeurons	iCell Microglia
iCell GABANeurons	iCell Mesenchymal Stem Cells
iCell Astrocytes	iCell Hematopoietic Progenitor Cells
iCell Hepatocytes	hiPSC Derived Intestinal Epithelial Cells (Takara)
iCell Peripheral Neurons	Others as available?

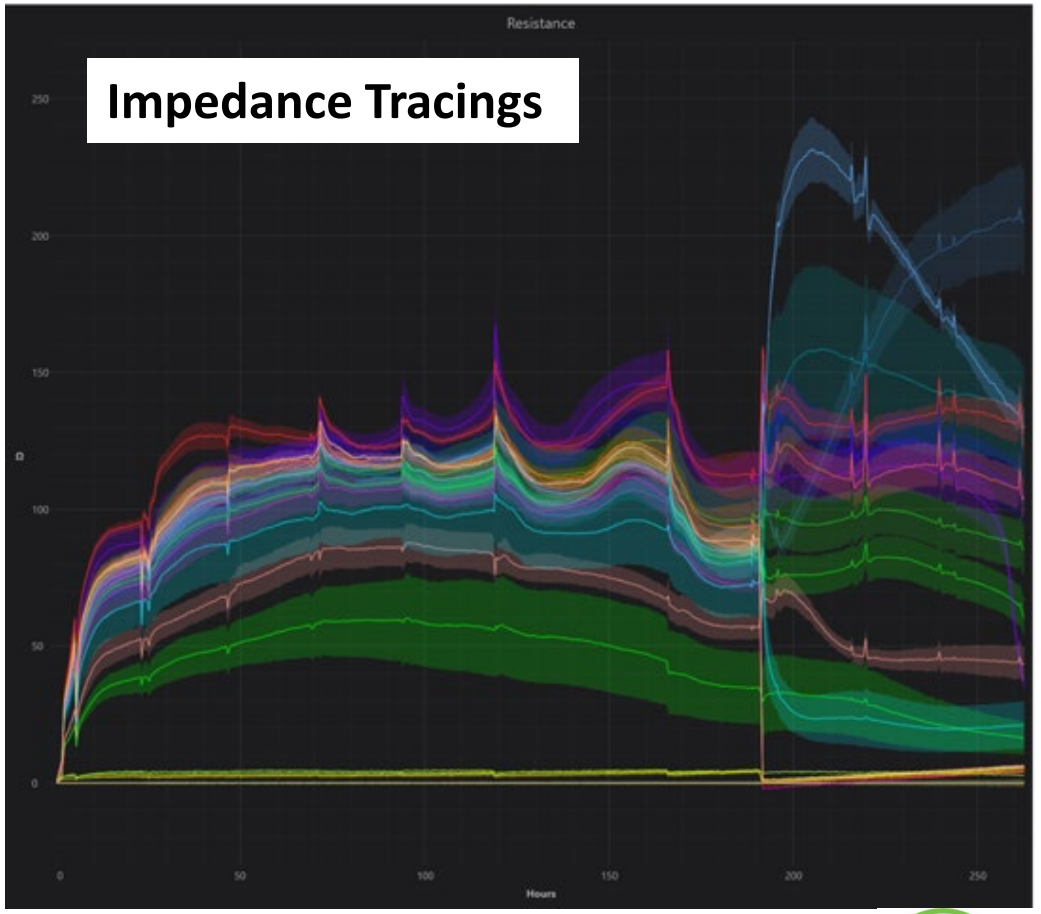
Readouts of CAR-T Cell Co-culture Binding Assessment

Impedance:

Surrogate for cytotoxicity (specifically for target cells; requires NO modification of target cells)

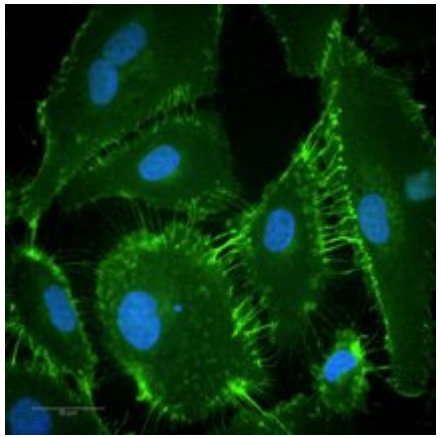
Technology



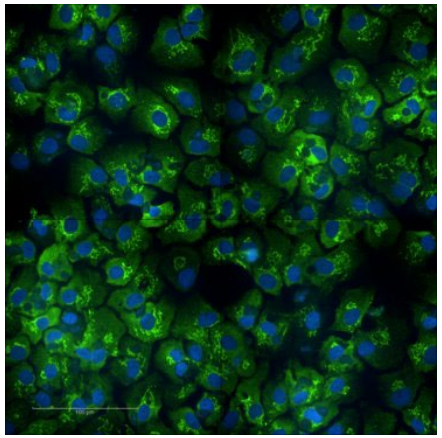


Readouts of CAR-T Cell Co-culture Binding Assessment

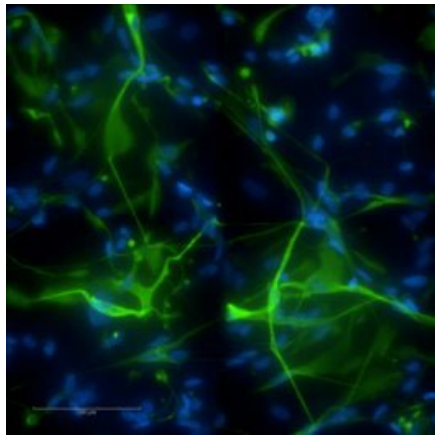
Endothelial cells
CD31



Hepatocytes
Albumin

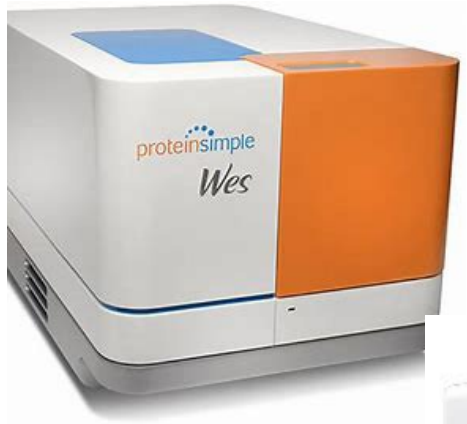


Astrocytes
GFAP

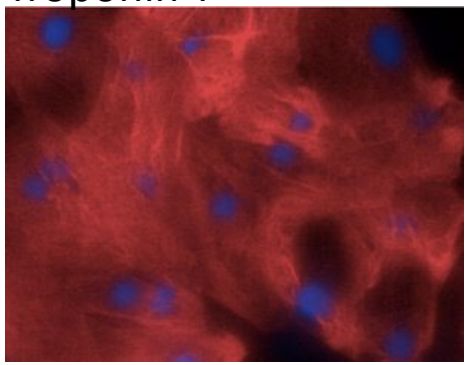


Target expression:

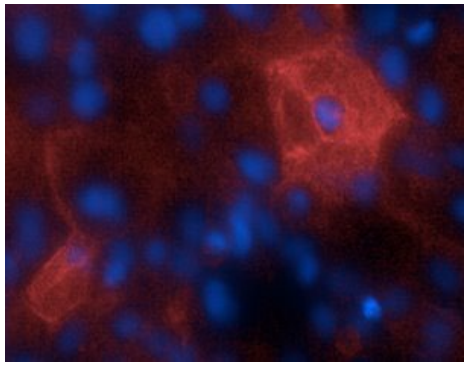
Western blots
Flow cytometry
Immunohistochemistry/imaging



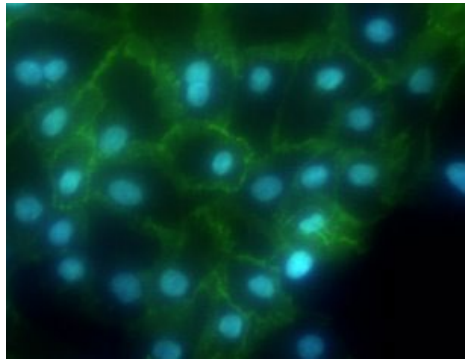
Cardiomyocytes
Troponin T

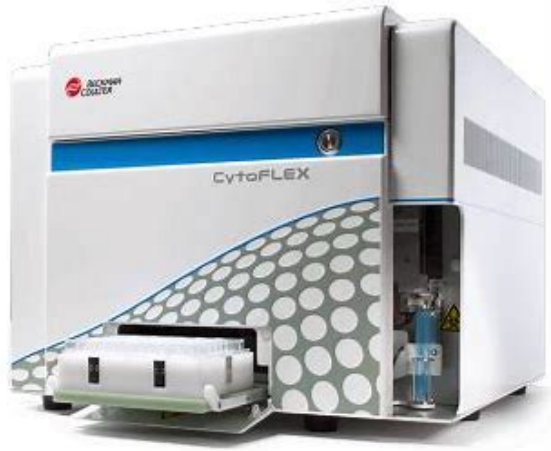


Cardiomyocytes
Sarcomeric α-actinin



Cardiomyocytes
N-cadherin

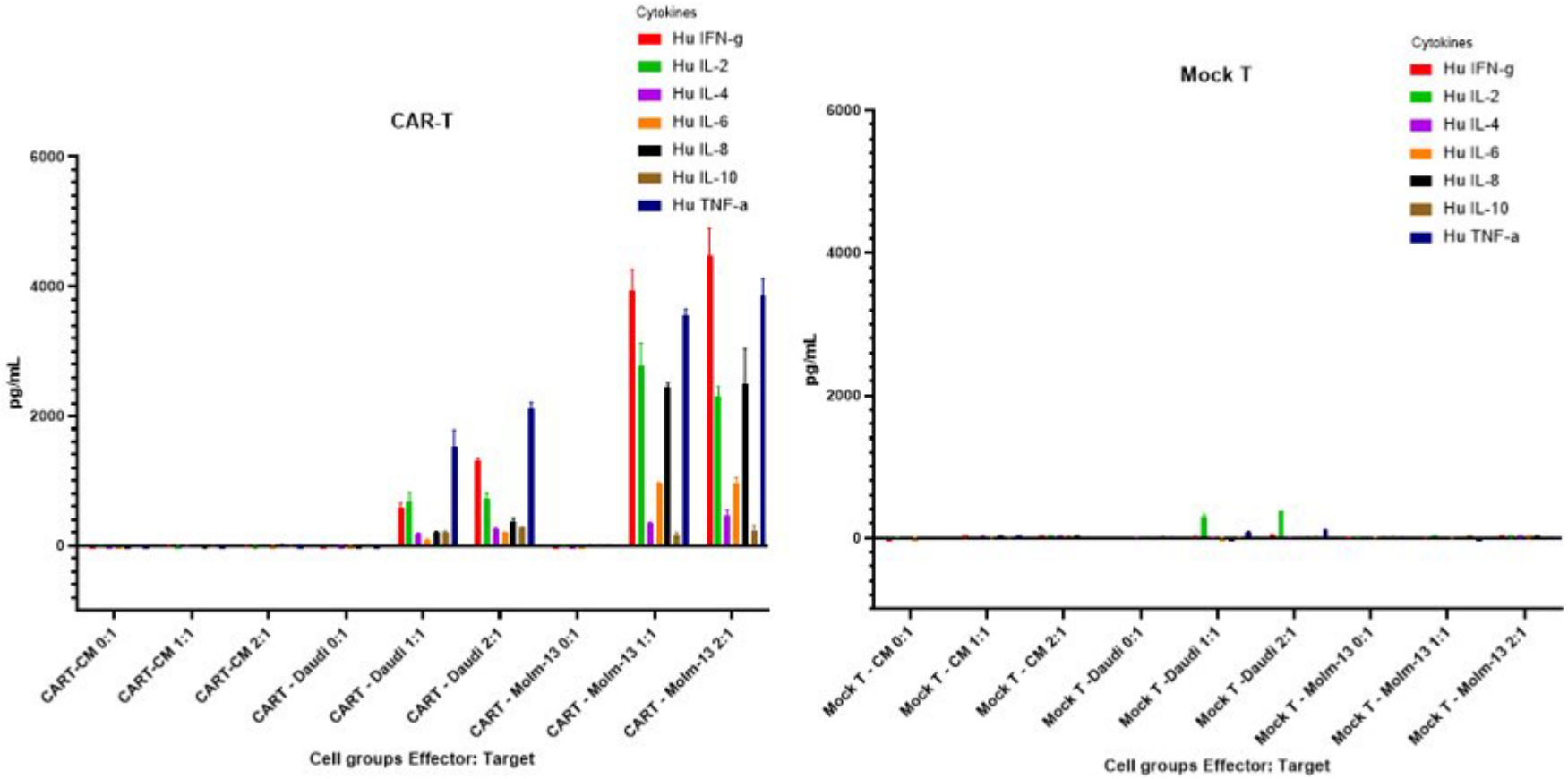




Readouts of CAR-T Cell Co-culture Binding Assessment

Biochemical endpoints:

Cytokine release

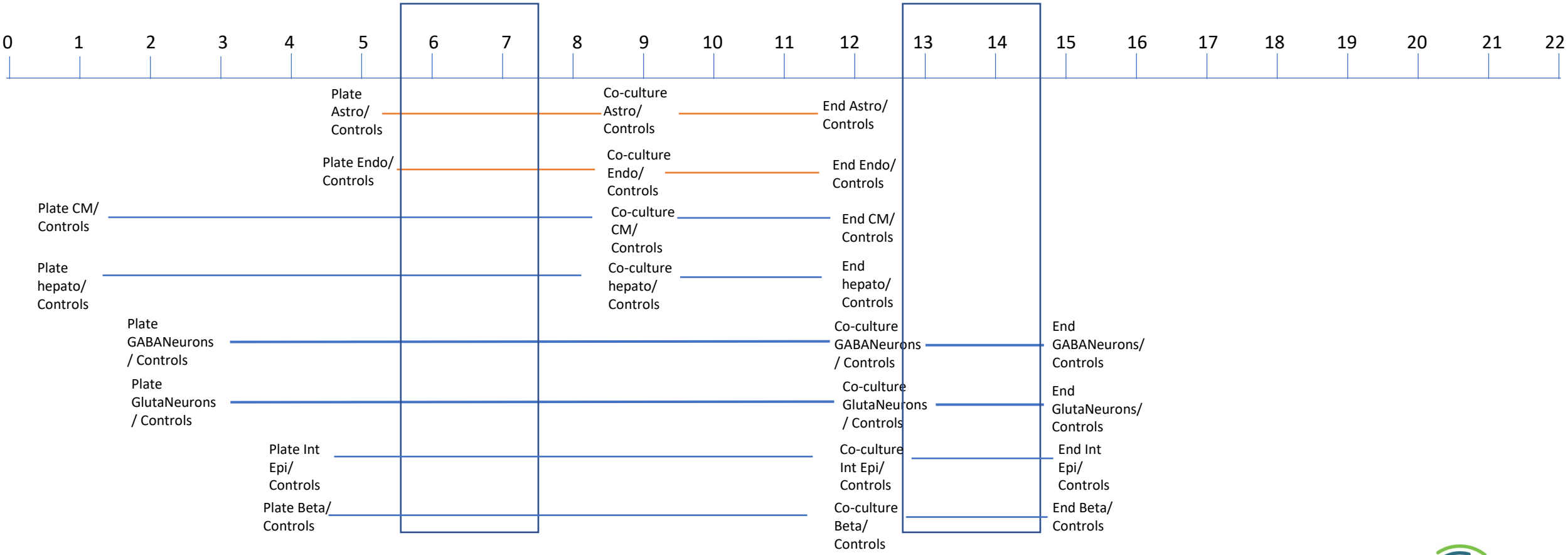


Pilot Study/Qualification

- Co-culture of CAR-T or negative control T-cell with positive and negative control cells to evaluate and verify T-cell killing of positive control cells in our system/workflow
- Elements of pilot study can include:
  - 1-3 effector:target ratios examined
  - Confirmation of expression of any reporter genes and target epitope in control cells
  - Plate binding kinetics of control cells
  - Readouts: impedance, cytokine release, cytotoxicity
  - Controls: target cells alone, effector cells alone, co-culture with untransduced control T-cell, positive control cells (express target antigen), negative control cells (do not express target antigen), medium only, total lysis controls (treated with detergent)
- Any method optimization for a specific CAR-T would happen during pilot work

Main Study Logistics

Example of logistics for pilot screen of 8 cell types



Study Plan

- Pilot study/qualification
- Main study – primary screen
- Follow-up analyses – western blot/flow cytometry/immunohistochemistry to see if target is expressed by any cell types if there is an unexpected hit and how much expression is there
- Additional co-cultures of target cells with CAR-Ts to tease out sensitivity and timing of effect (more ratios/more time points)
- Emphasis on weight of evidence approach/orthogonal data sets

Questions and Considerations

- Before receipt of study materials, we will need to have all cells to be used approved by our Institutional Biosafety Committee. There is a registration form that we will complete that includes detailed information about all cell lines as well as any viral or non-viral mechanisms for genetic modification. We will send a form where all relevant information can be collected. Please be prepared to include vector maps for any viral or plasmid vectors.
- If there is already an established killing assay for control cells, please share it with us so that we can use this as a starting point for assay optimization in our lab.
- Other considerations?

NCI Experimental Therapeutics (NExT) Program provides resources, not funding or money, for approved studies – NCI Experimental Therapeutics (NExT) (cancer.gov)

Free consultation services provided by NCI subject matter experts on development of experimental cancer agents – NCI Developmental Therapeutics Program