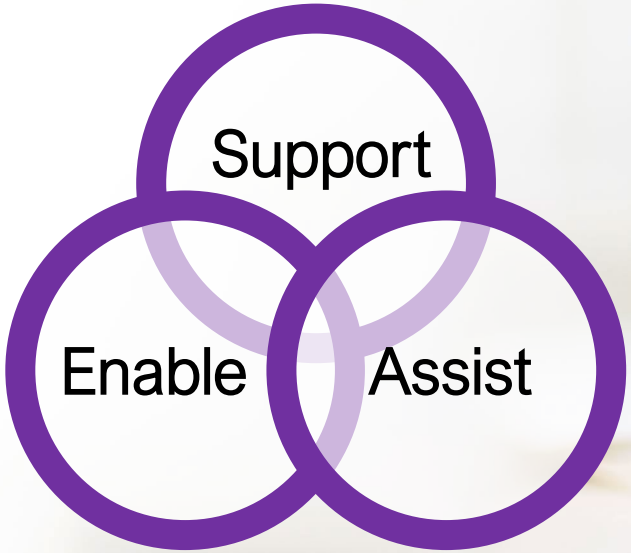
Utilizing HiBiT TCK Bioassays To Validate Cell Therapy & mAb-Mediated Immunotherapy Potency

Nicholas J. Hess
Associate Product Manager

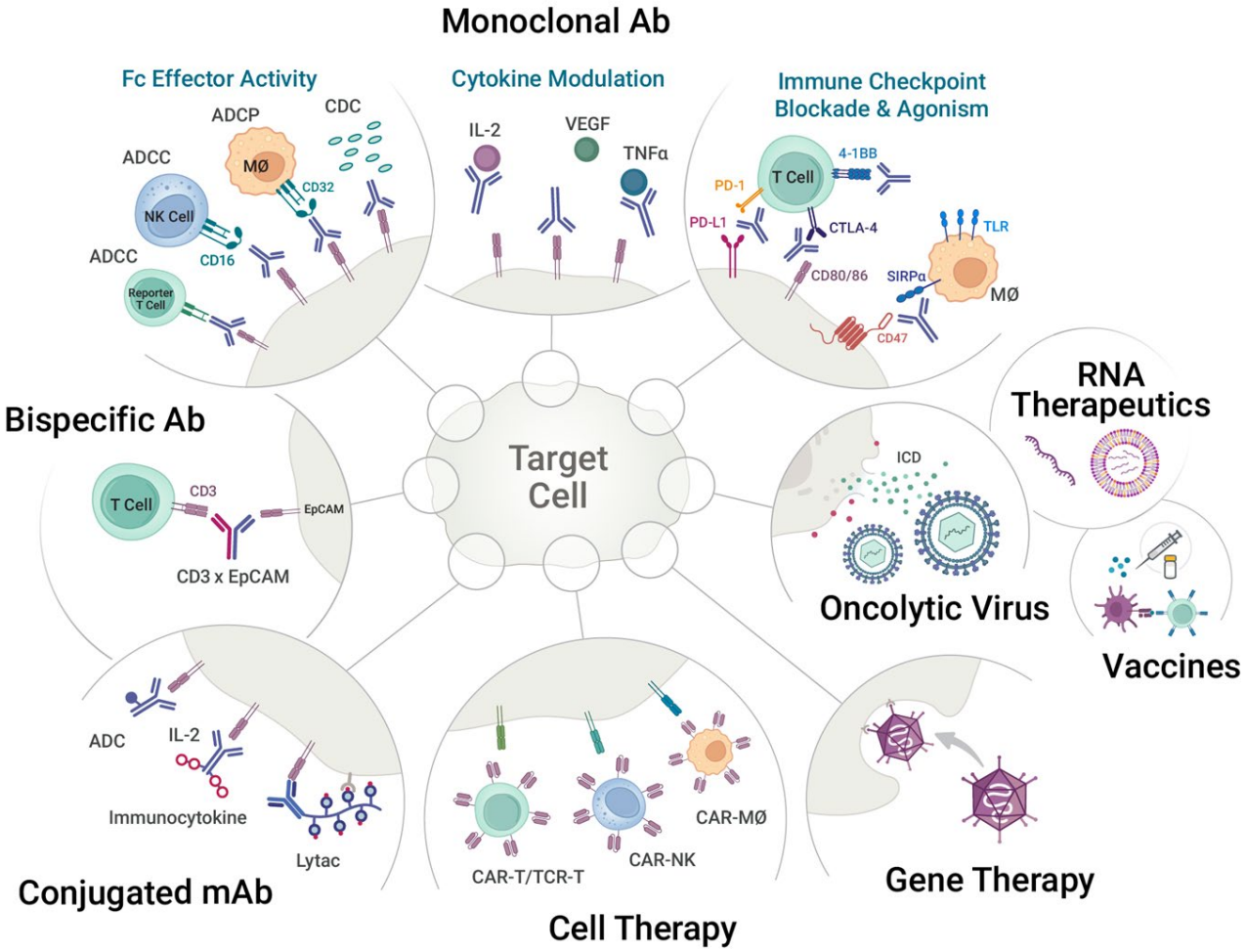
Our Mission

Provide innovative biological reagents and integrated systems used in research and applied technology worldwide.



The Promega Approach!

Portfolio of Products

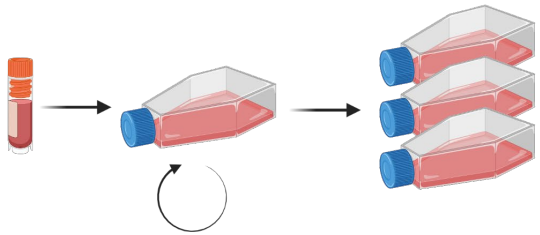


The Promega Approach!

Product Formats

Propagation Model

1 - 2 weeks



Standard Cell Culture

Cell Counting,
Centrifugation,
Resuspension,
Plating

Add assay,
Measure bioluminescence

T&U Model

3 - 24 hours

Thaw-And-Use Cells

~~Cell Counting,~~
~~Centrifugation,~~
Resuspension,
Plating

Add assay,
Measure bioluminescence

Challenges

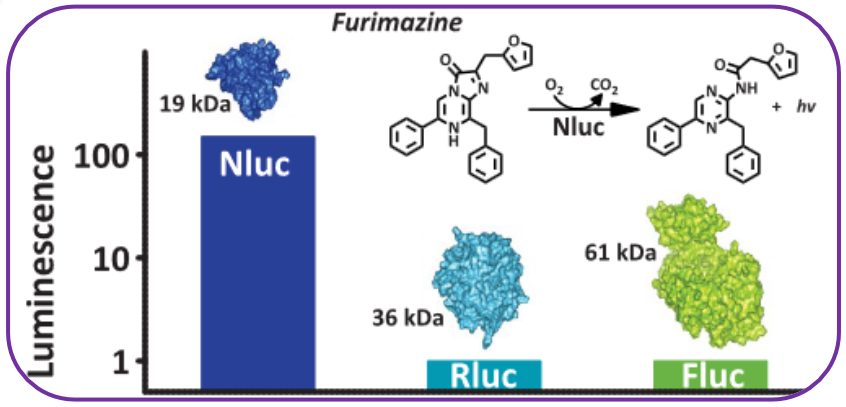
- ❖ *Cell culture is labor intensive and expensive*
- ❖ *Daily cell culture duties can lead to high run-to-run variation*

Benefits

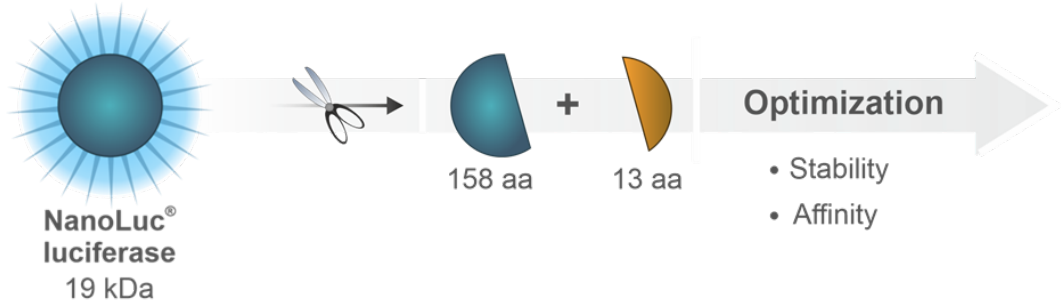
- ✓ *Simple & convenient*
- ✓ *Time & cost savings*
- ✓ *Consistent results*
- ✓ *Easy lab-to-lab transfer*
- ✓ *Automation-ready*

Functional Potency Testing

Luminescence Technology



Hall, M. P. et al. Engineered Luciferase Reporter from a Deep Sea Shrimp Utilizing a Novel Imidazopyrazinone Substrate. ACS Chem. Biol. 7, 1848–1857 (2012).

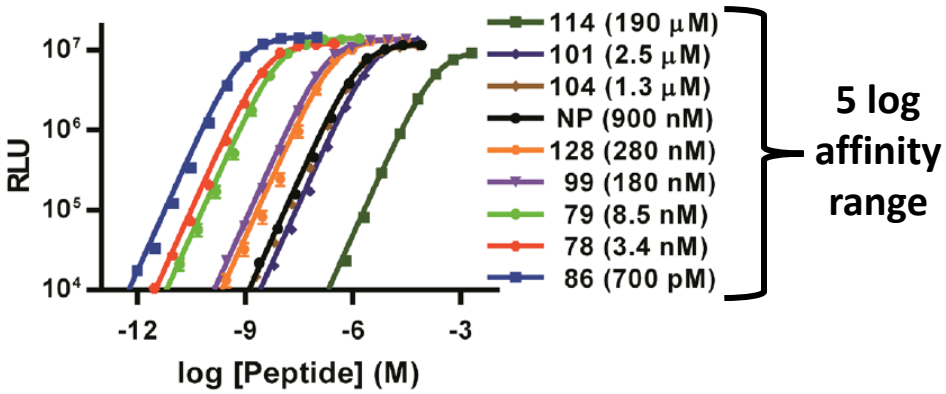


NanoLuc is a blue-emitting luciferase with <u>100X</u> brighter signal than Renilla/Firefly/Beetle luciferases

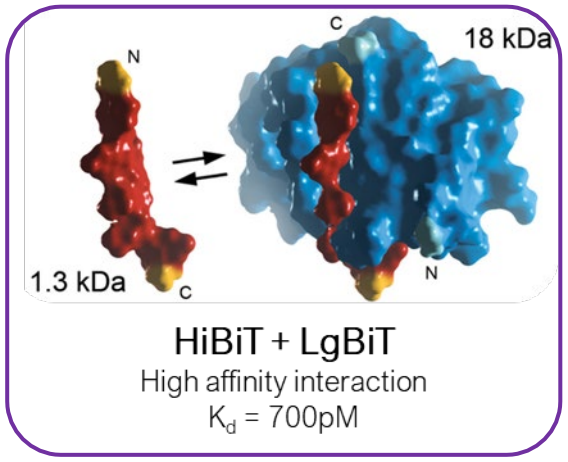
Through complementation studies, we have developed a split Nano-luc with a small, stable, high affinity tag

SmBiT: VTGYRLFEEIL **HiBiT:** VSGWRLFKKIS

Screen 350 peptides w/ LgBiT



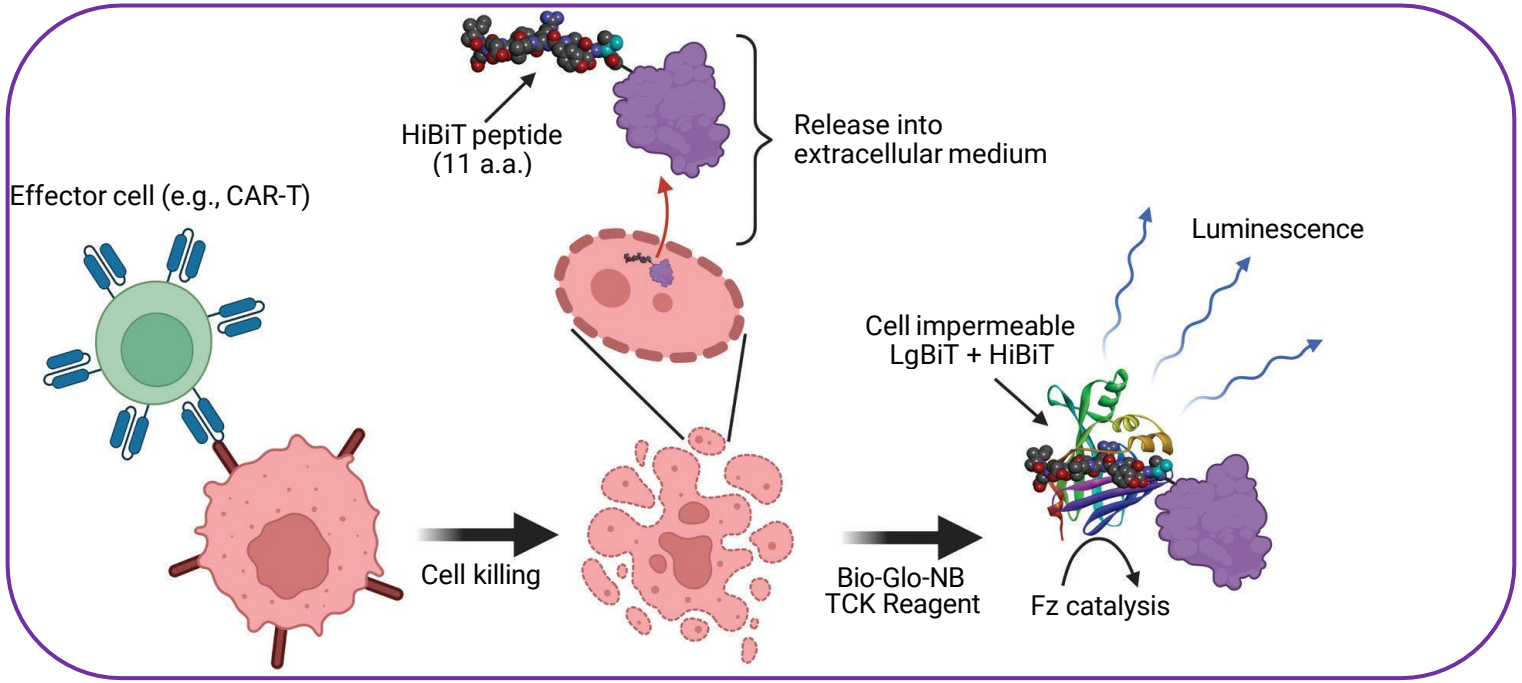
5 log affinity range



Dixon, A. S. *et al.* NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem Biol* **11**, 400–408 (2016).

Functional Potency Testing

Targeted Cell Killing (TCK) Concept, Workflow & Advantages

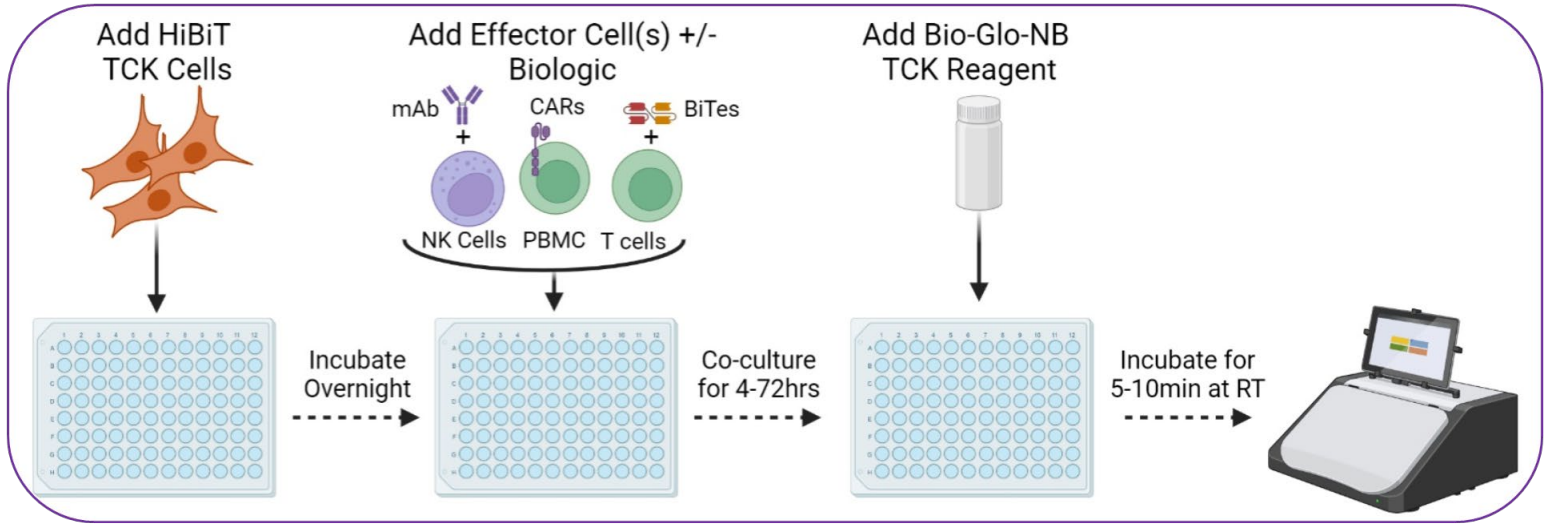


- HiBiT peptide stable in medium for >3 days
- Little/no background from HiBiT “leaking” from target cell
- HiBiT conjugated to HaloTag (HT) or N-terminus of LDH

Functional Potency Testing

Targeted Cell Killing (TCK) Concept, Workflow & Advantages

Comparing Cytotoxicity Assays	HiBiT TCK	Flow Cytometry	Fluore-scent Dyes	IncuCyte
Processing Time				
Signal: Noise		–		
Analysis				



- Gain-of-signal assay
- No loading, staining or washing steps
- Robust s/n ratio with as little as 2k target cells
- Luminescence measured using a standard luminometer
- Simple analysis to measure % lysis

Functional Potency Testing

TCK Portfolio

11 unique cell lines
+4 KOs

Blood Cancer Targets

B cell Lymphoma/Leukemia lines (**Raji** & **Ramos**)
expressing **CD19**, **CD20** and **CD22**
and **CD19-KO**, **CD20-KO,** and **CD19/20-KO** lines

Myeloid Leukemia line (**U937** & **K562**)
expressing **CD33** and **CLL-1**.

Multiple Myeloma line (**H929**)
expressing **BCMA** and **CD38**

T cell Leukemia line (**T2**)
expressing **CD5**, **CD7**, **CD30** and **CD52**

Solid Cancer Targets

Ovarian Carcinoma lines (**OVCAR3** & **SKOV3**)
expressing **HER2**, **MSLN**, **5T4**, **WT** and **MUC16**
and **MSLN-KO** line

Breast Adenocarcinoma line (**SK-BR-3**)
expressing **HER2** and **EpCAM**

Lung Carcinoma line (**A549**)
expressing **EGFR**

Melanoma line (**A375**)
expressing **HER2**, **CD70**, **B7-H3**

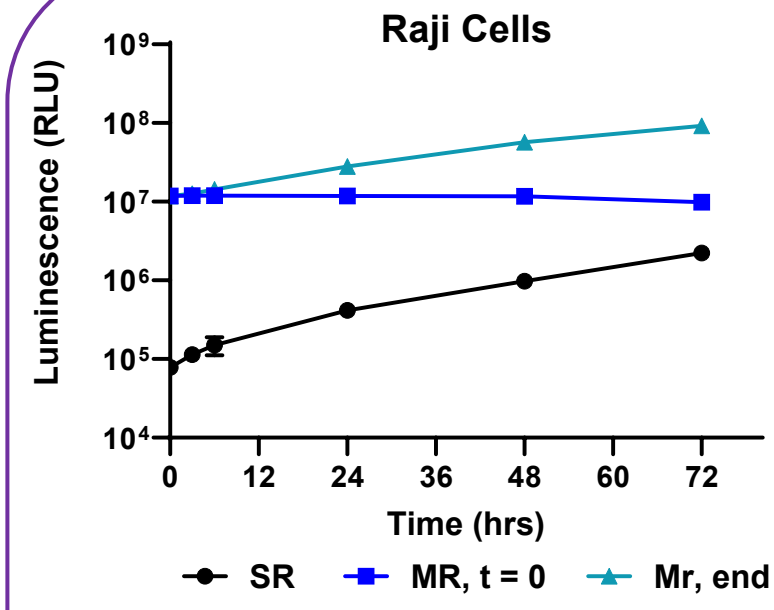
K562 expressing **CD19, BCMA, GPC3** and **MHC-II** (via CIITA insertion)
CHO-K1 expressing **Claudin 18.2**, **membrane TNFα**, **SARS-CoV-2 spike protein**

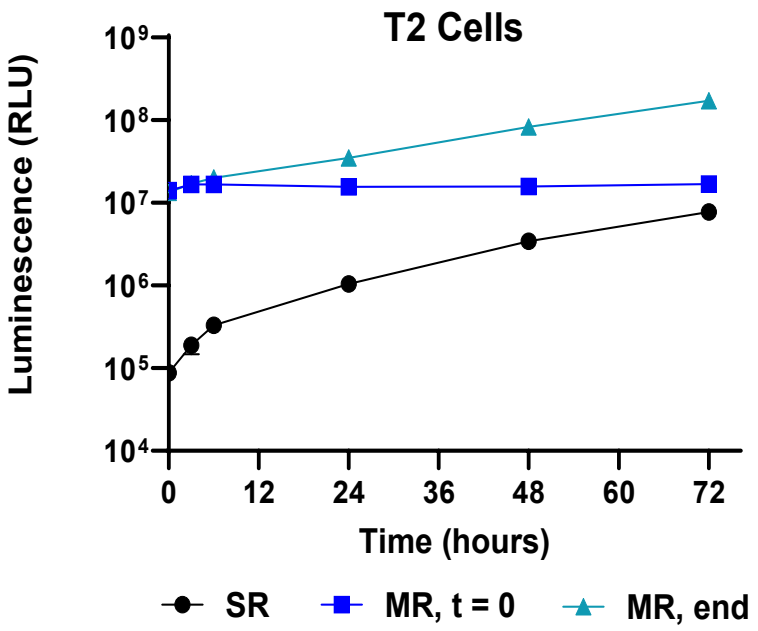
HiBiT Target Cell Killing Bioassay (promega.com)

Inquire To EA For Product Information

Functional Potency Testing

HiBiT TCK Controls





% Specific Lysis

- (RLU, sample – RLU, SR)/(RLU, MR – RLU, SR)X100

Spontaneous Release control (SR)

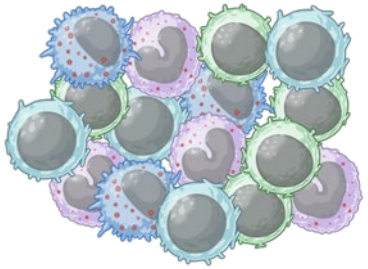
- Rate of SR depends on cell type & cell health

Maximum Release control (MR)

- Add digitonin for 100% lysis
  - MR, t = 0: Add digitonin at time zero
  - MR, end: Add digitonin with detection reagent

Functional Potency Testing

ADCC Applications Using PBMC

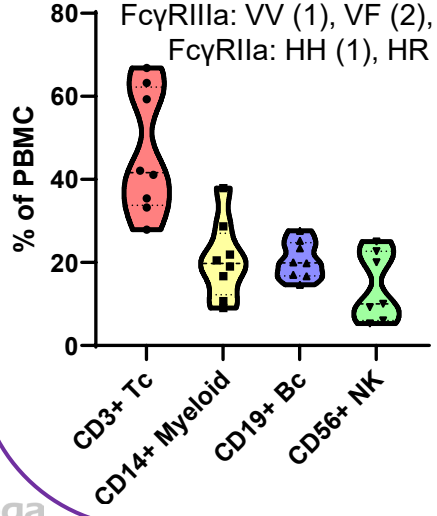


PBMC

5 Unique Donors

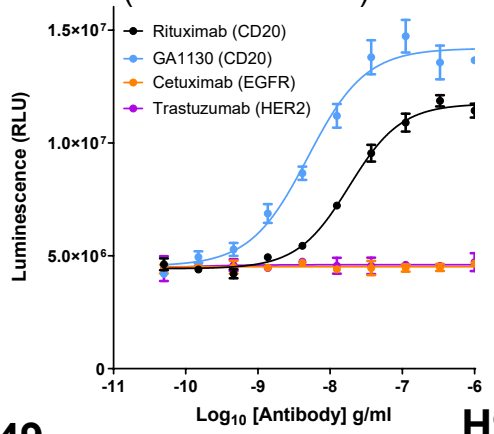
FcγRIIIa: VV (1), VF (2), FF (2)

FcγRIIa: HH (1), HR (4)



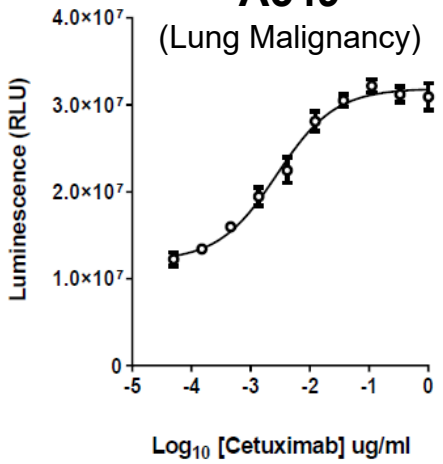
Raji

(B cell Leukemia)



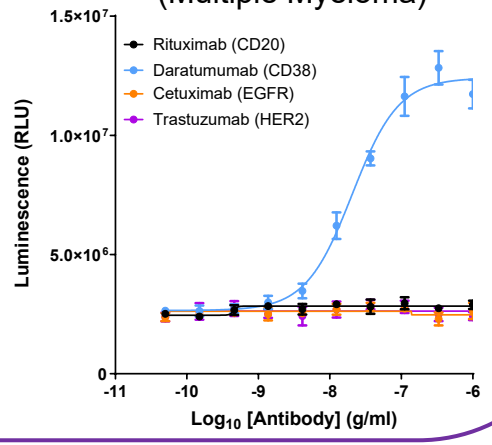
A549

(Lung Malignancy)



H929

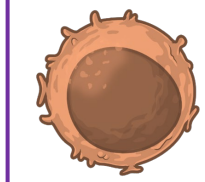
(Multiple Myeloma)



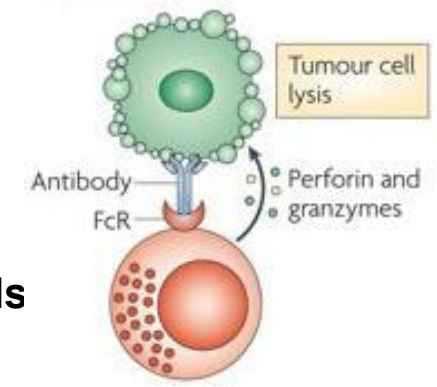
- We have 3-6 qualified donors at any given time
- PBMCs are not pooled
- PBMC are qualified against 6 different TCK lines
  - Raji/Ramos w/rituximab
  - H929 w/daratumumab
  - SKOV3/SK-BR-3 w/trastuzumab
  - A549 w/cetuximab
- Each donor is genotyped and characterized
- Allows user to measure human variation in biologic potency

Functional Potency Testing

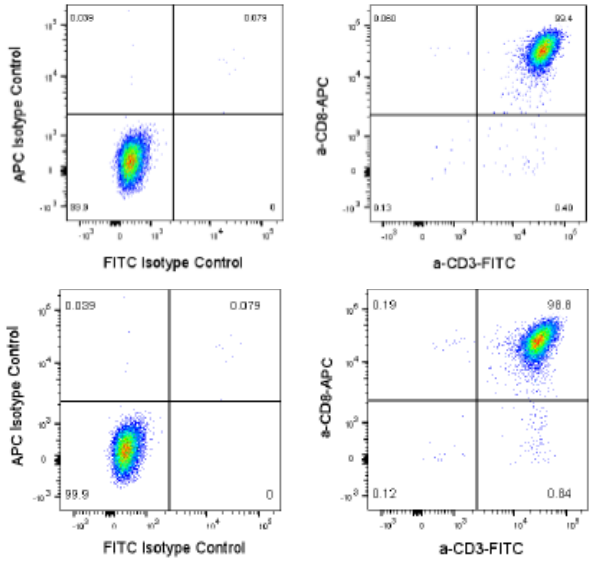
TDCC Applications Using CD8+ T cells

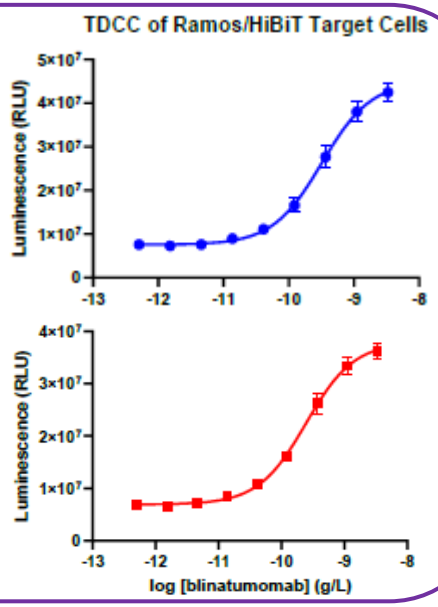


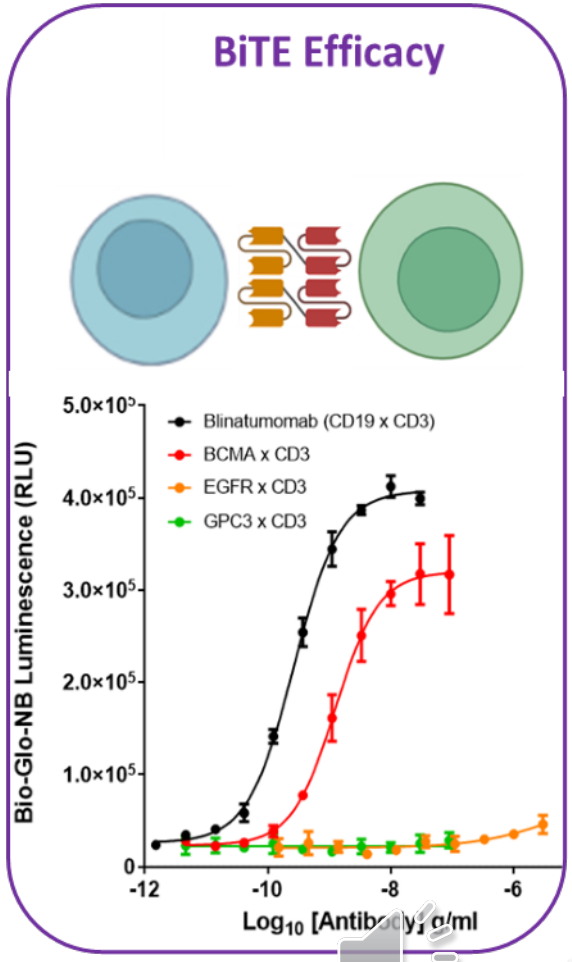
CD8+ T cells



TDCC



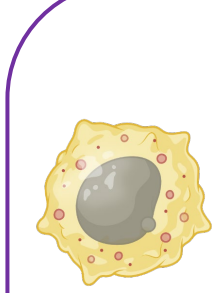




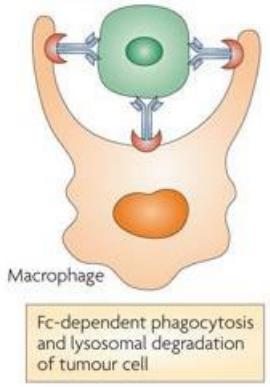
- We have 2-4 donors, qualified against 6 TCK lines at any given time
  - Raji/Ramos w/blinatumomab
  - H929 w/Teclistamab
  - SKOV3/SK-BR-3 w/Her-2 BiTe
  - A549 w/EGFR BiTe
- Each donor is characterized for purity following expansion

Functional Potency Testing

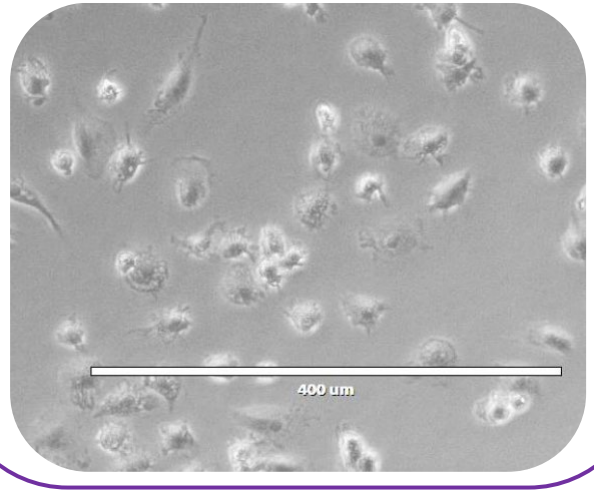
ADCP Applications Using Macrophages

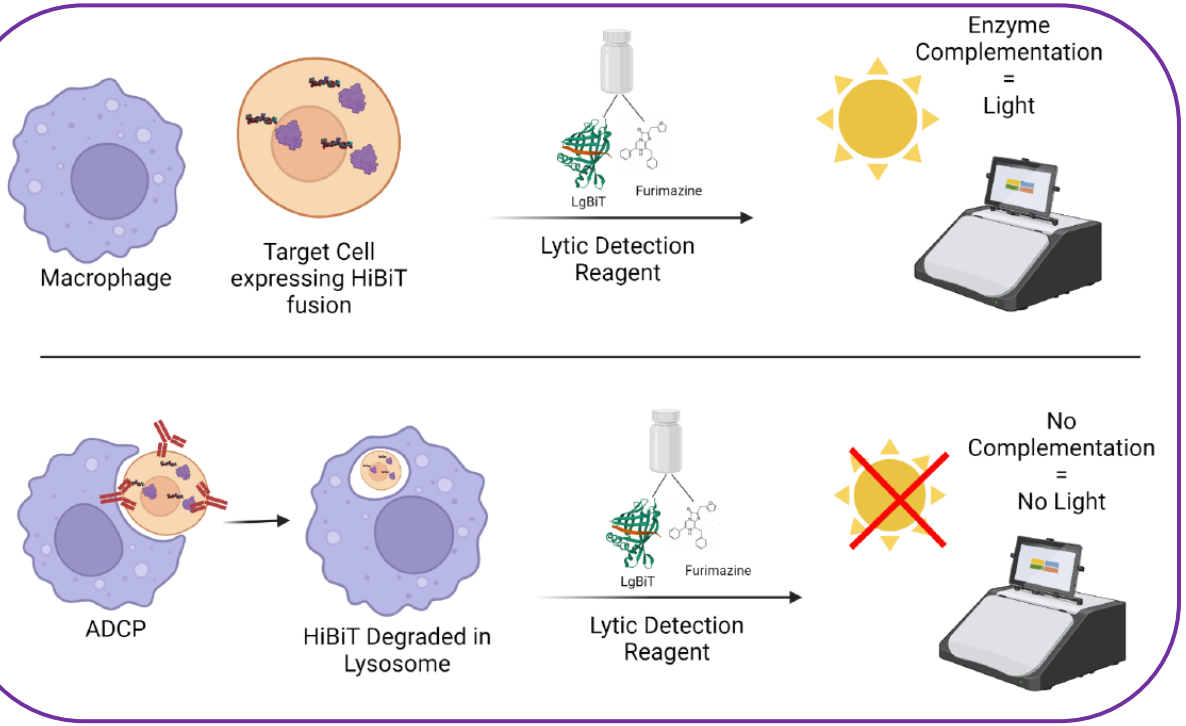


Mϕ



ADCP

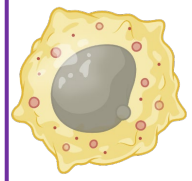




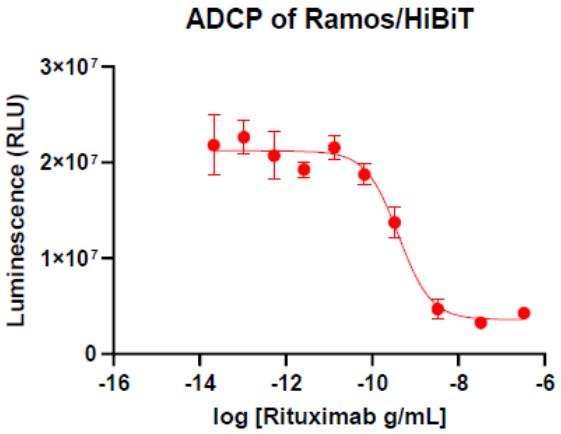
- Primary human monocytes are isolated and cultured for 5-7 days
- IFNγ used prior to assay to mature Mϕ into an M1 phenotype

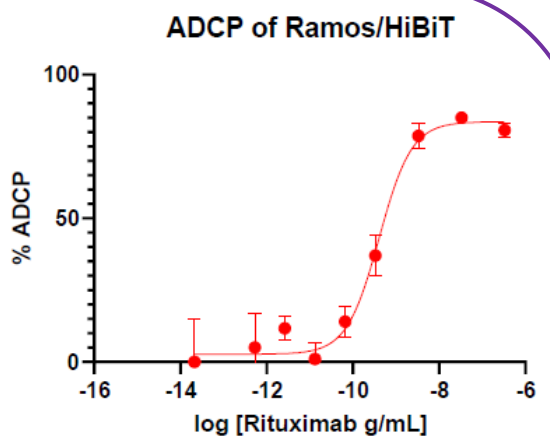
Functional Potency Testing

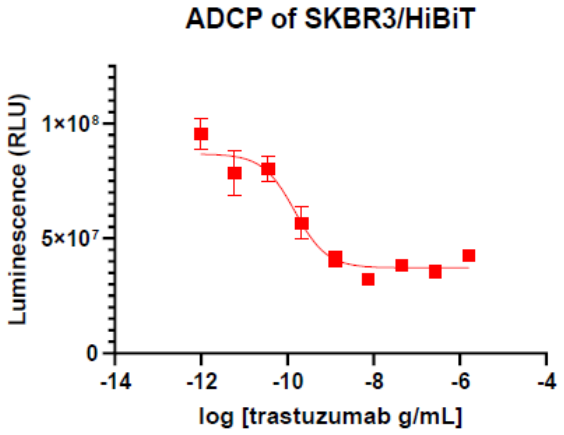
ADCP Applications Using Macrophages

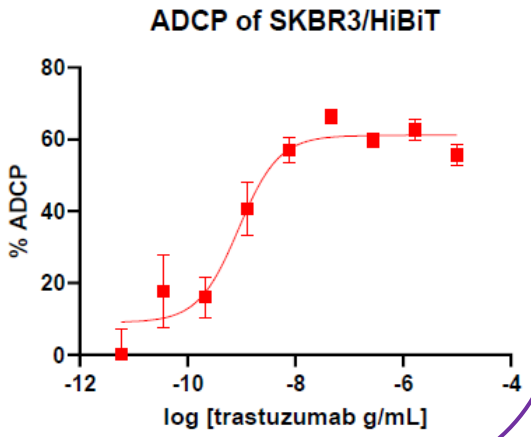


Mϕ









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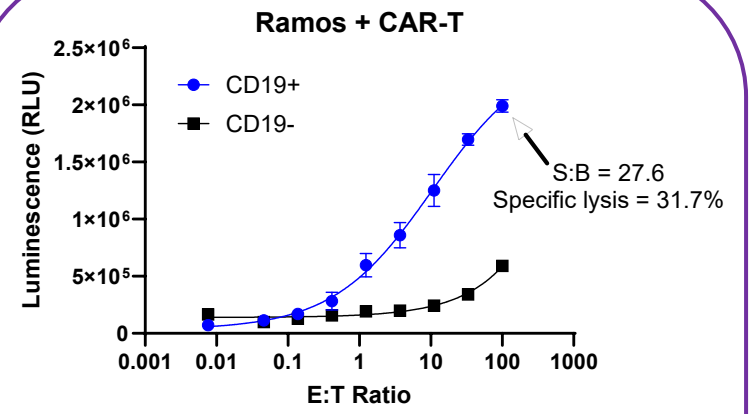
  - SKOV3/SK-BR-3 w/trastuzumab
  - A549 w/cetuximab

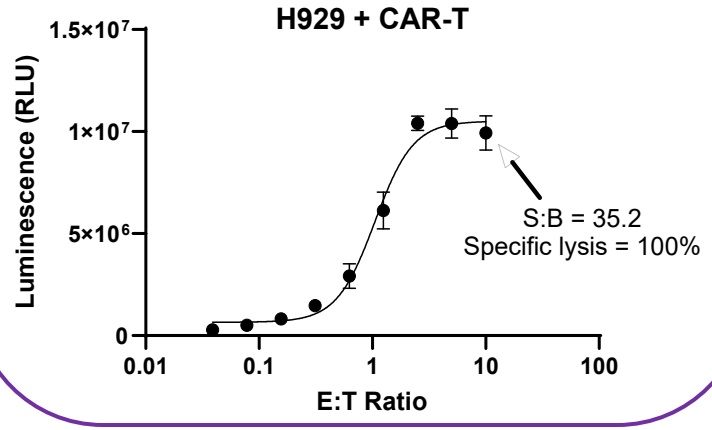
Functional Potency Testing

CAR-T Applications

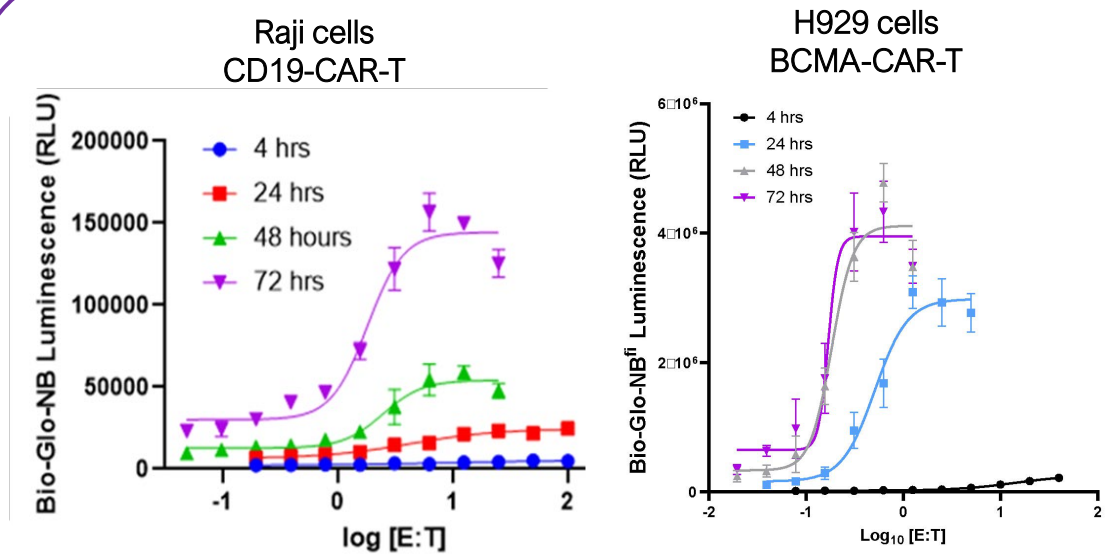
- Homebrew CAR-T (αCD19-41BB-CD3z)
- 2,500 target cells/well
- 24 hr incubation

- ProMab PM-CAR1031-1M (αBCMA-4-CD28-CD3z)
- 1,500 target cells/well
- 24 hr incubation



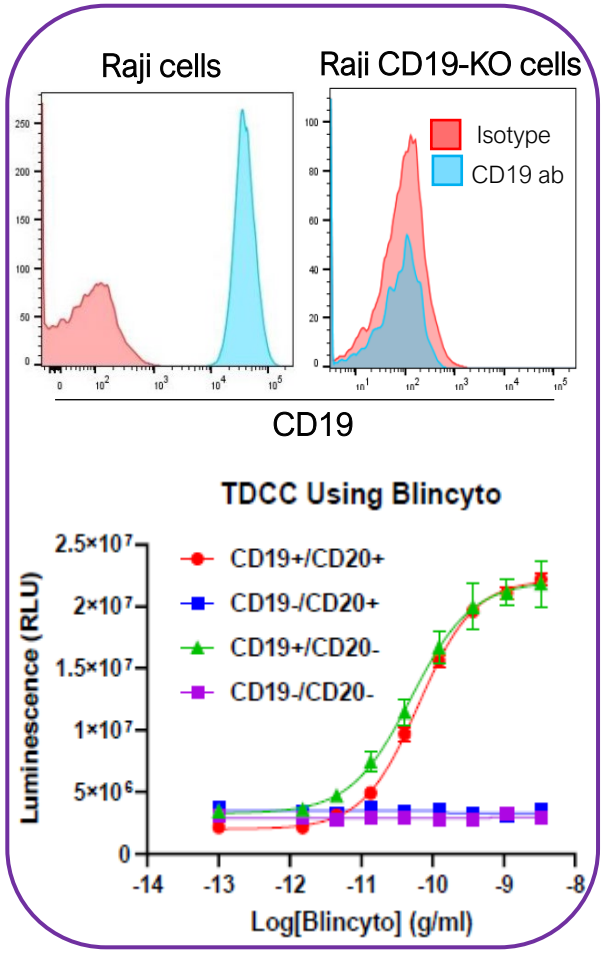


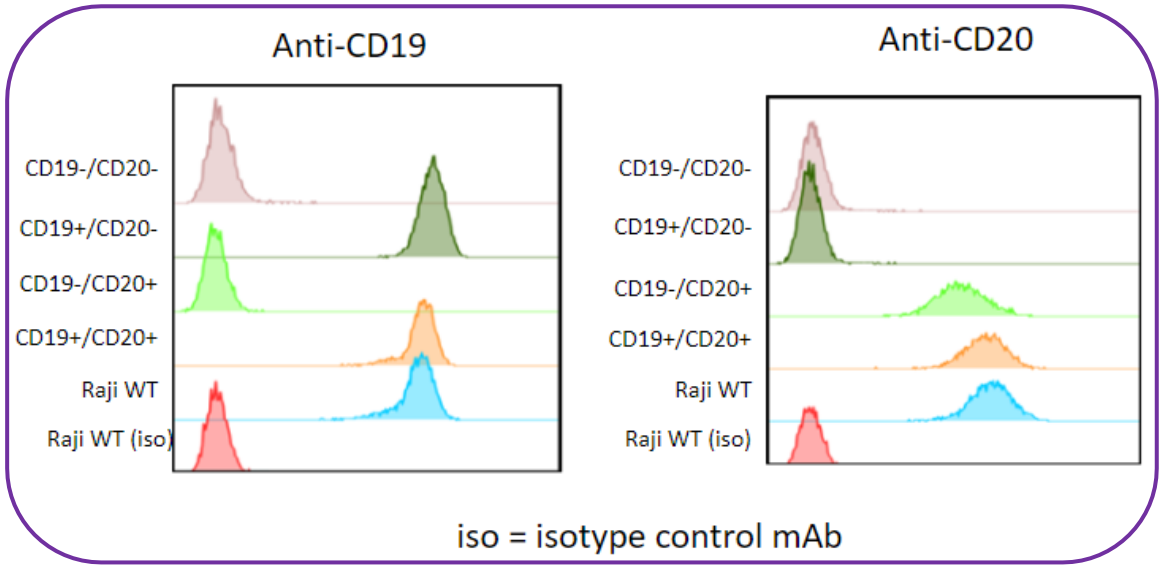
Suitable For Various Time Points



Functional Potency Testing

Testing Antigen Specificity





"… capacity of CAR T cells to secrete cytokines and mediate cytolysis should be restricted in an antigen-dependent manner, which can be tested by exposure to various cells that vary in their expression of the target antigen."

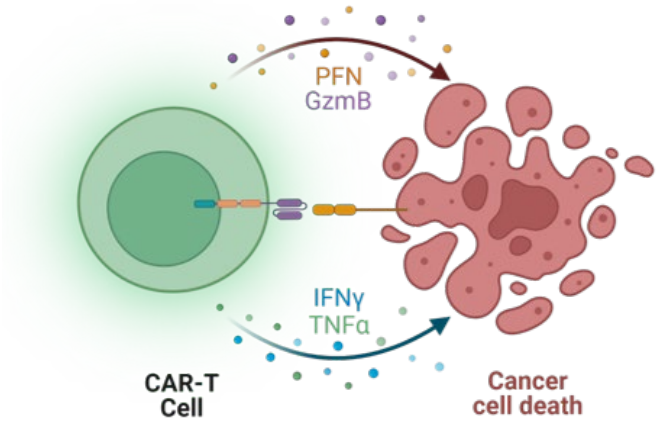
*FDA Guidance on Considerations for the Development of CAR T Cell Products, 2024

- Specificity of biologic can be determined by either:
  - Using mAb against antigen not expressed on line or;
  - KO-target antigen

Summary

HiBiT Target Cell Killing assay

- Gain-of-signal assay for target cell killing
- No signal contribution from effector cells
- Add-mix-read, non-lytic format
- Low numbers of target cells
- CRISPR KO cell lines to measure target-independent killing



Blood Cancer Targets

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Promega
Thank you!