

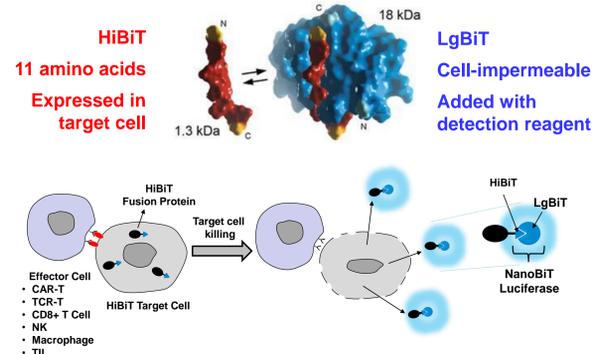
NanoBiT Technologies for CAR-T Characterization and Potency Testing

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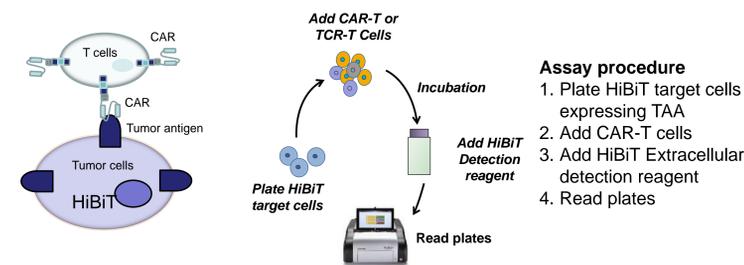


1. Introduction: NanoBiT™ Technology Enables Novel Target Cell Killing Assays:



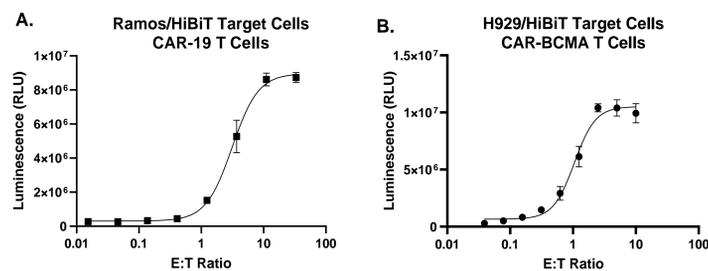
- Incubation of effector cells and HiBiT-expressing target cells leads to tumor-associated antigen (TAA) dependent lysis of target cells and release of HiBiT into the medium
- HiBiT binds to cell-impermeable LgBiT in the detection reagent to form functional NanoBiT™ luciferase and emit light
- Enables rapid, MOA-based potency testing for biologics and cell therapy products

2. Assay Design and Workflow for CAR-T Testing



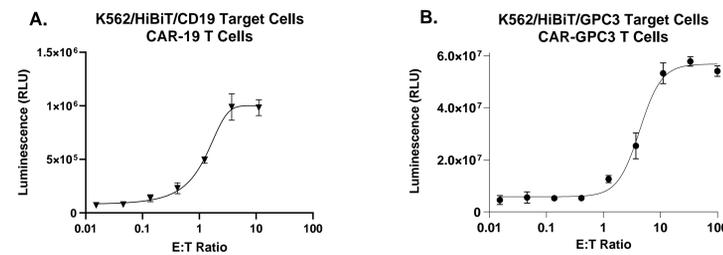
- Low spontaneous release (<10% maximum release)
- Signal comes from target cells only
- Simple, fast and sensitive

3. HiBiT TCK Assay with Endogenously Expressed Target Antigens



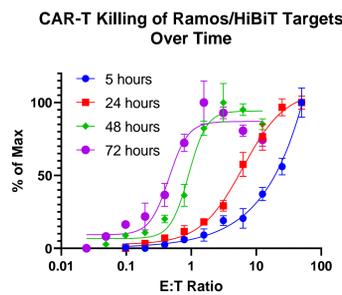
T Cells transduced with CAR-19 (A) or CAR-BCMA (B) lentivirus were combined with Ramos/HiBiT or H929/HiBiT target cells, respectively, at different E:T Ratios. After 24 hours incubation, Nano-Glo® HiBiT Extracellular detection reagent was added and luminescence was read on a Glomax Discover plate reader.

4. HiBiT TCK Assay with Ectopic Expression of Target Antigen



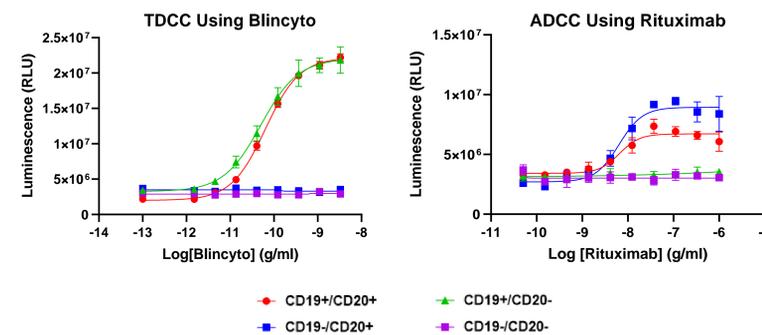
T Cells transduced with CAR-19 (A) or CAR-GPC3 (B) lentivirus were combined with K562/HiBiT Target Cells stably expressing CD19 or GPC3, respectively. After 24 hours incubation, Nano-Glo® HiBiT Extracellular detection reagent was added and luminescence was read on a Glomax Discover plate reader.

5. Extended Assay Incubation Times Indicate Serial Target Cell Killing



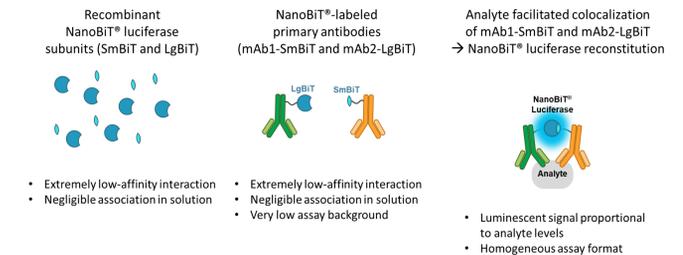
T Cells transduced with CAR-19 lentivirus were combined with Ramos/HiBiT target cells at different E:T Ratios. After incubation for the indicated times, Nano-Glo® HiBiT Extracellular detection reagent was added and luminescence was read on a Glomax Discover plate reader. The EC50 shifts left over time, indicating serial target cell killing at lower E:T ratios.

6. CD19/CD20 Knockout Panel Enables Testing of Tandem- and Dual-CAR T Cells



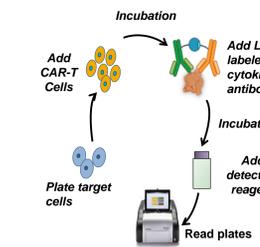
CRISPR was used to generate Raji/HiBiT Target Cells lacking expression of CD19, CD20, or both. A. When combined with activated CD8+ T Cells (TDCC Qualified) and a CD19xCD3 bispecific antibody (Blincyto), parental and CD20 knockout cells are readily lysed, while CD19 knockout and CD19/CD20 double knockout cells are protected from killing. B. The Raji/HiBiT panel was tested in a PBMC ADCC assay using a-CD20, Rituximab. Here, parental and CD19 knockout cells are killed in the presence of antibody, while CD20 knockout and CD19/CD20 double knockout are protected.

7. Homogenous Lumit Immunoassays for Cytokine Detection



- Extremely low-affinity interaction
- Negligible association in solution
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- Negligible association in solution
- Very low assay background

- Luminescent signal proportional to analyte levels
- Homogeneous assay format

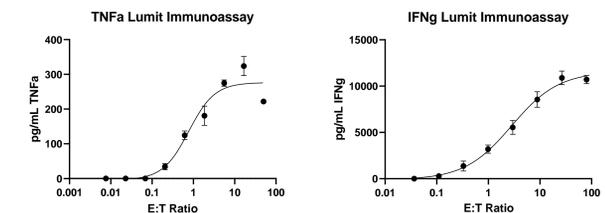


Assay procedure

1. Plate target cells
2. Add CAR-T Cells
3. Incubate 4-72 hours
4. Add Lumit antibodies
5. Incubate for one hour
6. Add detection reagent
7. Read plates

- Simple, fast and sensitive
- Homogenous, no washing steps

8. Lumit Measurement of Cytokine Secretion by CAR-19 T Cells



T Cells transduced with CAR-19 lentivirus were combined with Ramos target cells, at different E:T Ratios. After 24 hours incubation, Lumit Cytokine Immunoassays were used to quantify TNFa and IFNg secretion.

9. Available HiBiT Target Cell Lines

Target Cells	Cell Line Background	Endogenous Target Antigens	Cell Line	Ectopic Antigens
A549	Lung carcinoma	EGFR	CHO-K1	mTNFa
H929	MM	BCMA, CD38		Claudin 18.2
OVCAR3	Ovarian carcinoma	MSLN, HER2	K562	SARS-CoV-2-S
SK-BR-3	Breast cancer	HER2, EpCAM		TIGIT
SKOV3	Ovarian carcinoma	MSLN, MUC16, HER2, PD-L1	Raji	BCMA
Raji	B cell lymphoma	CD19, CD20, CD22, CD38		CD19
Ramos	B cell lymphoma	CD19, CD20, CD22, CD38	U937	GPC3
U937	Lymphoma	CD33, CLL-1		