New Methods for MOA-Based Potency Testing and CAR-T Cell Characterization

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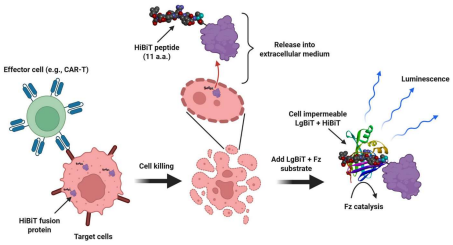
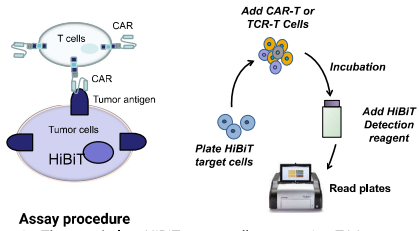
1. Introduction: HiBiT Technology Enables Novel Target Cell Killing (TCK) Assays:



- 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 Nanoluc® luciferase and emit light;
- Two formats:
  - Stable target cell lines
  - Transient mRNA transfection across diverse target cell backgrounds (adherent and suspension)

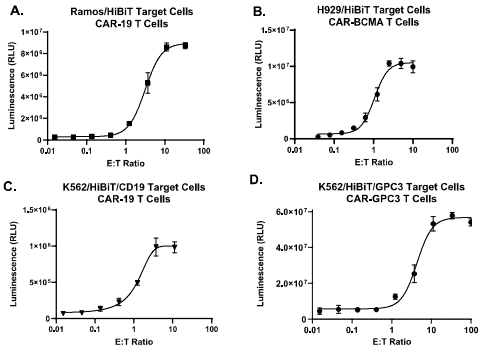
- Enables rapid, MOA-based potency testing for biologics and cell therapy products;
- Gain of signal from target cell alone;
- Simple, fast and sensitive

2. Assay Design and Workflow for TCK



Assay procedure

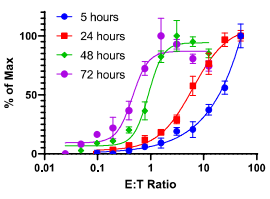
1. Thaw and plate HiBiT target cells expressing TAA
2. Add CAR-T or other types of effector cells
3. Add HiBiT Extracellular detection reagent
4. Read plates



Target antigens can be endogenous (**A, B**) or engineered (**C,D**). T Cells transduced with CAR-19 (**A,C**), CAR-BCMA (**B**), or CAR-GPC3 (**D**) lentivirus were combined with the indicated target cells, 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.

3. Extended Assay Incubation Times Indicate Serial Target Cell Killing

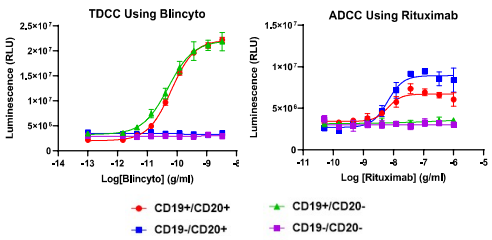
CAR-T Killing of Ramos/HiBiT Targets Over Time



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.

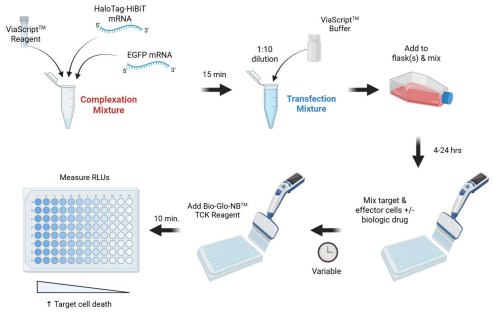
4. CD19/CD20 Knockout Panel Enables Testing of Tandem- and Dual-CAR T Cells



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.

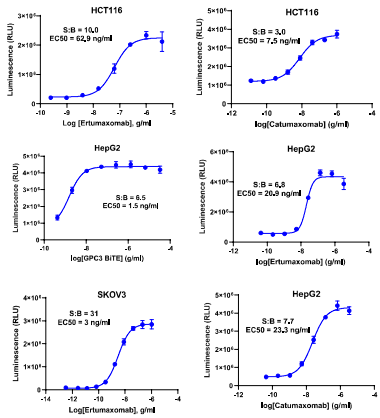
5. ViaScript™ TCK Bioassays

Extending the HiBiT TCK Bioassay beyond use of stable clones



- Efficient, low-toxicity transfection across diverse cell lines (adherent and suspension) using a novel mRNA transfection reagent
- 5MoU modified mRNA for increased intracellular stability; HaloTag-HiBiT expression is maintained for days
- mRNA transfection using ViaScript™ provides uniform & highly titratable expression (unlike plasmid DNA transfection)

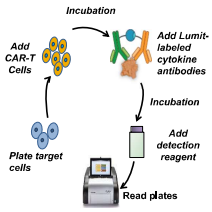
6. TCK with Adherent Target Cells



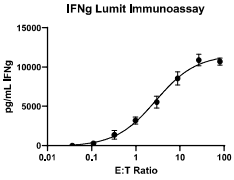
TDCC with Adherent Cells. Day 1, transfection of HaloTag-HiBiT mRNA using ViaScript™. Day 2, mixing of CD8+ T cells with target cells (10:1 E:T ratio) in the presence of varying concentrations of T cell engagers. Day 3, addition of Bio-Glo-NB™ TCK Reagent followed by luminescence measurement.

7. Homogenous Lumit® Immunoassays for Detection of Cytokines Secreted by CAR-T

Simple, fast and sensitive, no washing steps



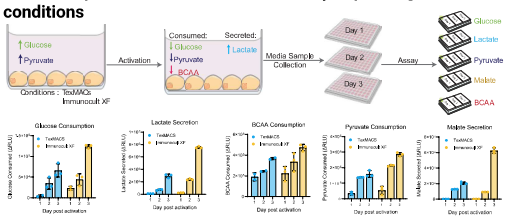
Assay procedure
1. Plate target cells
2. Add CAR-T Cells
3. Incubate 4-72 hours
4. Add Lumit antibodies
5. Incubate for one hour
6. Add detection reagent
7. Read plates



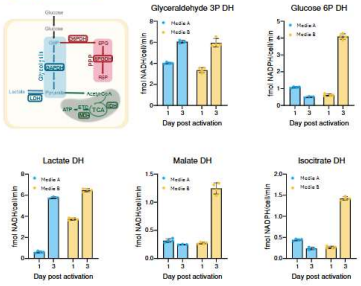
T Cells transduced with CAR-19 lentivirus were combined with Ramos target cells, at different E:T Ratios. After 24 hours incubation, Lumit Cytokine Immunoassays were used to quantify IFNg secretion.

8. Metabolic Measurements During T cell Activation

Metabolic profiles of activated T cells vary depending on culture conditions



Dehydrogenase activity varies in T cells activated in different media



9. Summary

MOA-based Potency Assays

1. **HiBiT Technology for Monitoring Target Cell Killing (TCK):**
   - For HiBiT TCK, target cells are engineered or transiently transfected to express a HiBiT-tagged protein, and release of this protein by dead/dying cells leads to complementation with the cell impermeable LgBiT.
   - This gain-of-signal approach enables precise measurements of target cell killing induced by various effectors, including CAR-T cells.
2. **Lumit® Immunoassays for Cytokine Detection:**
   Simple, fast, and sensitive, requiring no washing steps

Metabolic Measurements for Predicting Phenotypic Profiles of T cells

- A suite of metabolite assays allows for monitoring media nutrient consumption and dehydrogenase activities.
- Can be used as early screen tools for predicting phenotypic profiles of expanded T cells

Resources:

- For a complete listing of available TCK target cell lines, including target receptor KOs, visit: https://www.promega.com/products/reporter-bioassays/target-cell-killing-bioassays/
- For additional information on Promega Cell Therapy Solutions, visit: https://www.promega.com/applications/cell-and-gene-therapy/cell-therapy/

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