

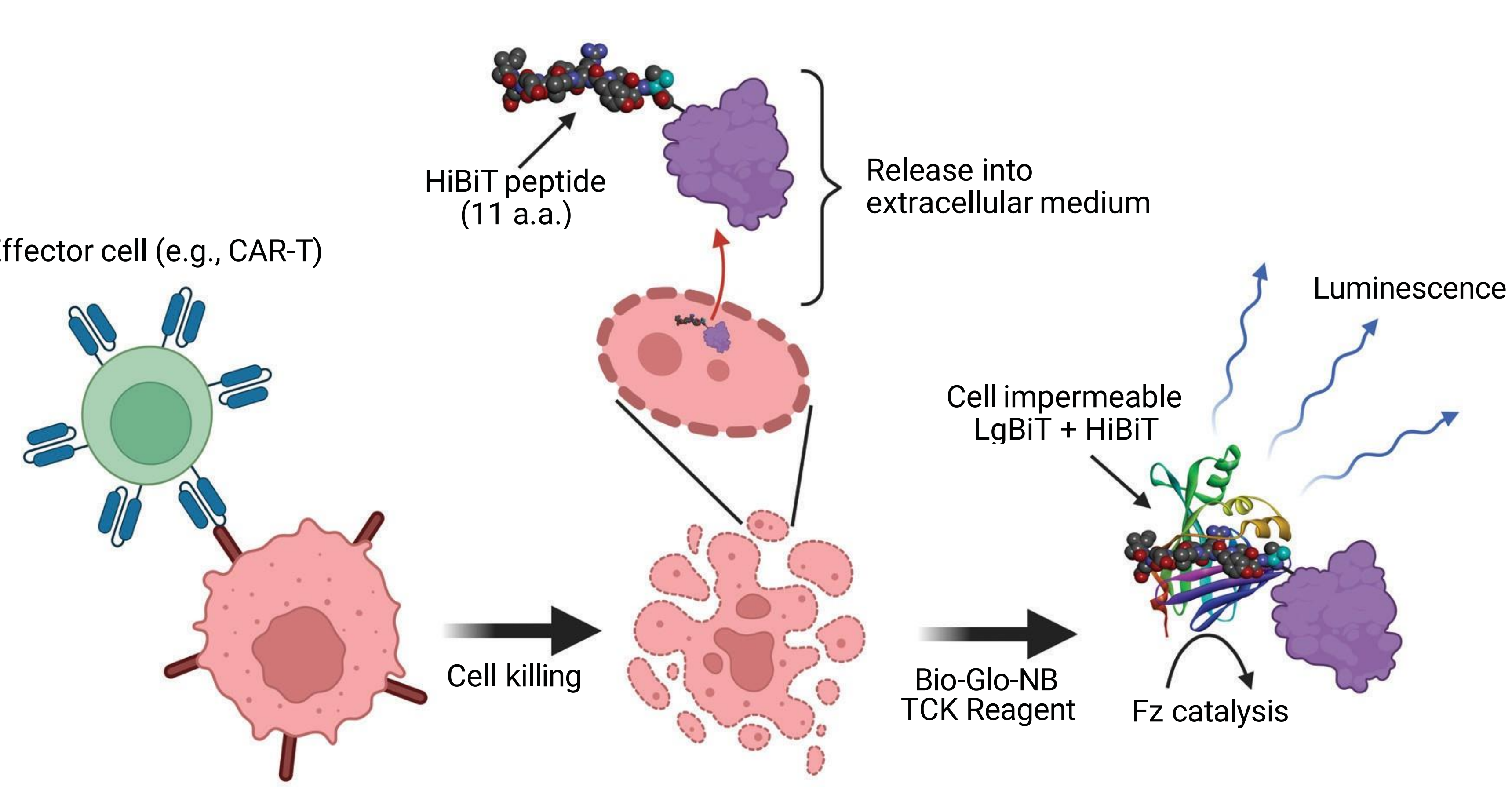
HiBiT Targeted Cell Killing (TCK) Bioassays For Characterization and Potency Testing

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1. Targeted Cell Killing (TCK) Technology

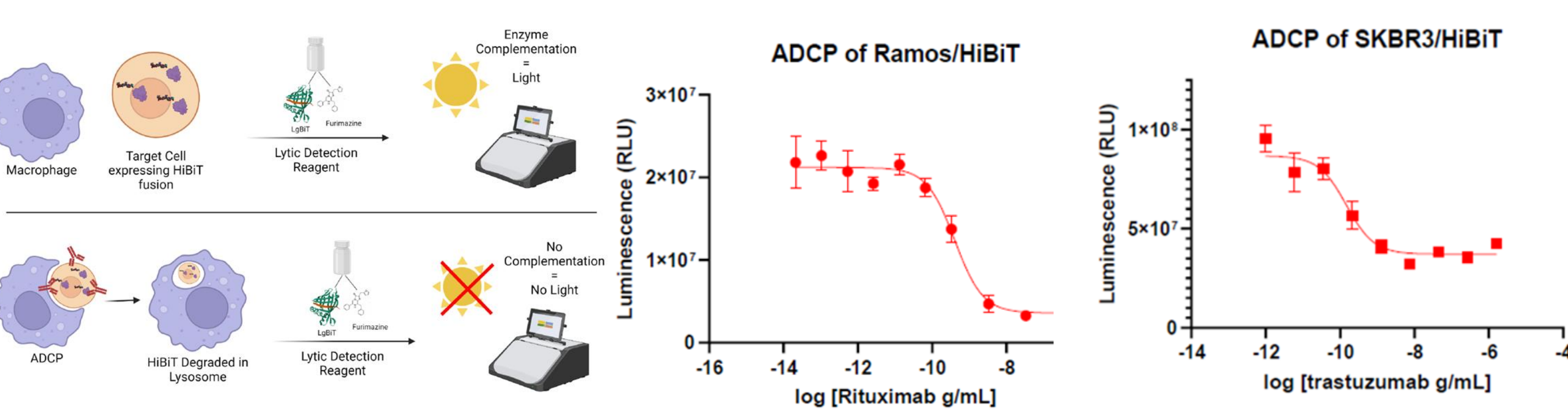


Representation of the HiBiT TCK Bioassay. TCK Cells stably expressing a HiBiT fusion protein are cocultured with effector cells (CAR-T, T cells, NK cells, etc.). Effector cell-mediated killing of TCK Cells leads to release of the HiBiT fusion protein into the extracellular medium. Cell impermeable LgBiT and Furimazine substrate (Fz) are added as components of the Bio-Glo-NB™ TCK Reagent. HiBiT complementation with LgBiT generates NanoBiT® luciferase, a bright, luminescent enzyme.

2. TCK Portfolio of Cell Lines

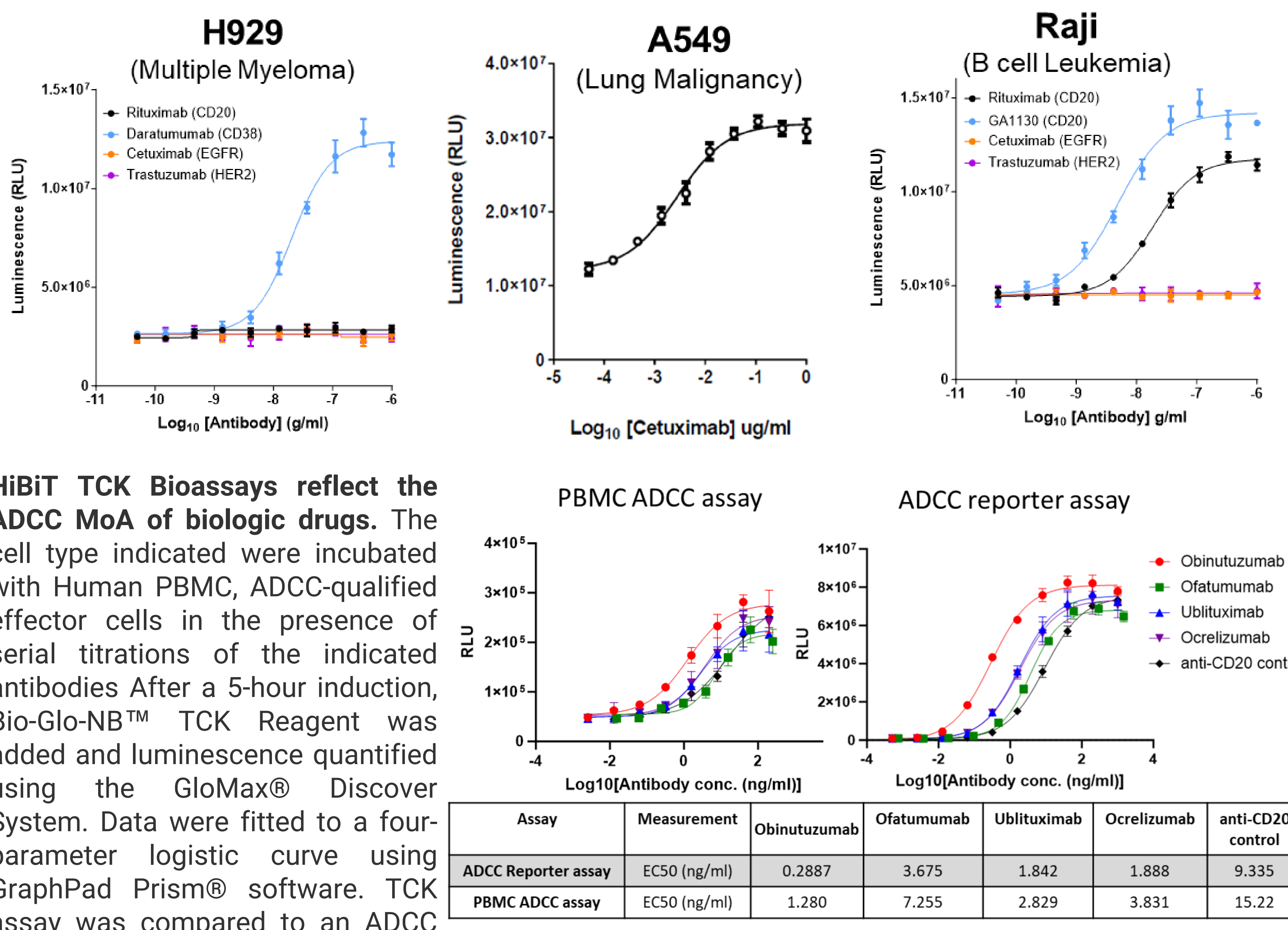
- Blood Cancer Targets**
- B cell Lymphoma/Leukemia lines (Raji & Ramos) expressing CD19, CD20 and CD22 and CD19-KO, CD20-KO, and CD19/20-KO lines
 - Myeloid Leukemia line (U937 & K562) expressing CD33 and CLL-1.
 - Multiple Myeloma line (H929) expressing BCMA and CD38
 - T cell Leukemia line (T2) expressing 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

3. ADCP Applications & Primary Macrophages

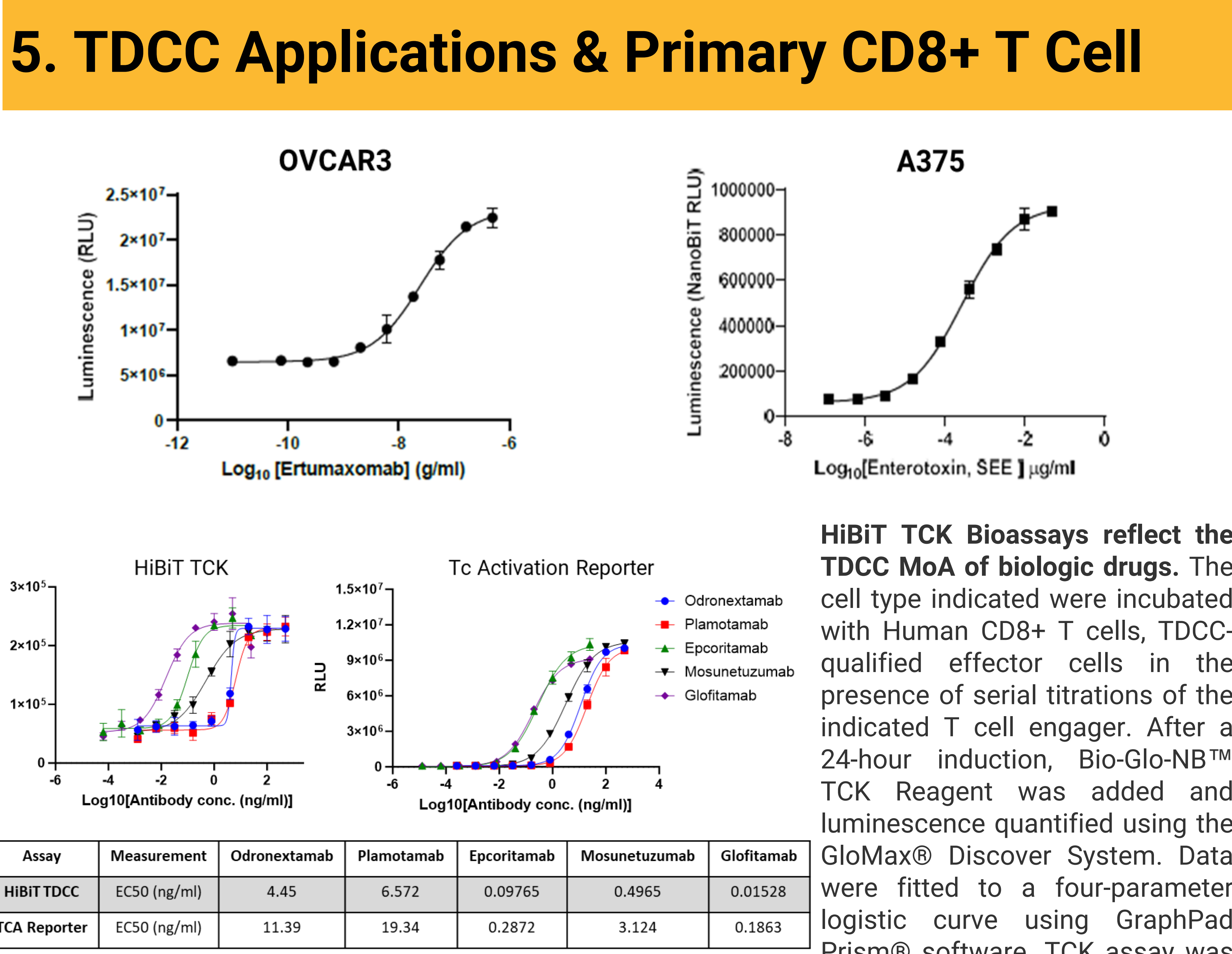


TCK Bioassays reflect the ADCP MOA of biologic drugs. The indicated TCK cells were incubated with Human Macrophages (ADCP-qualified) in the presence of serial titrations of the indicated antibody. After a 24-hour induction, Bio-Glo-NB Lytic Reagent was added and luminescence quantified using the GloMax Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism. Loss of signal reflects HiBiT degradation following phagocytosis of the TCK cell.

4. ADCC Applications & Primary PBMCs

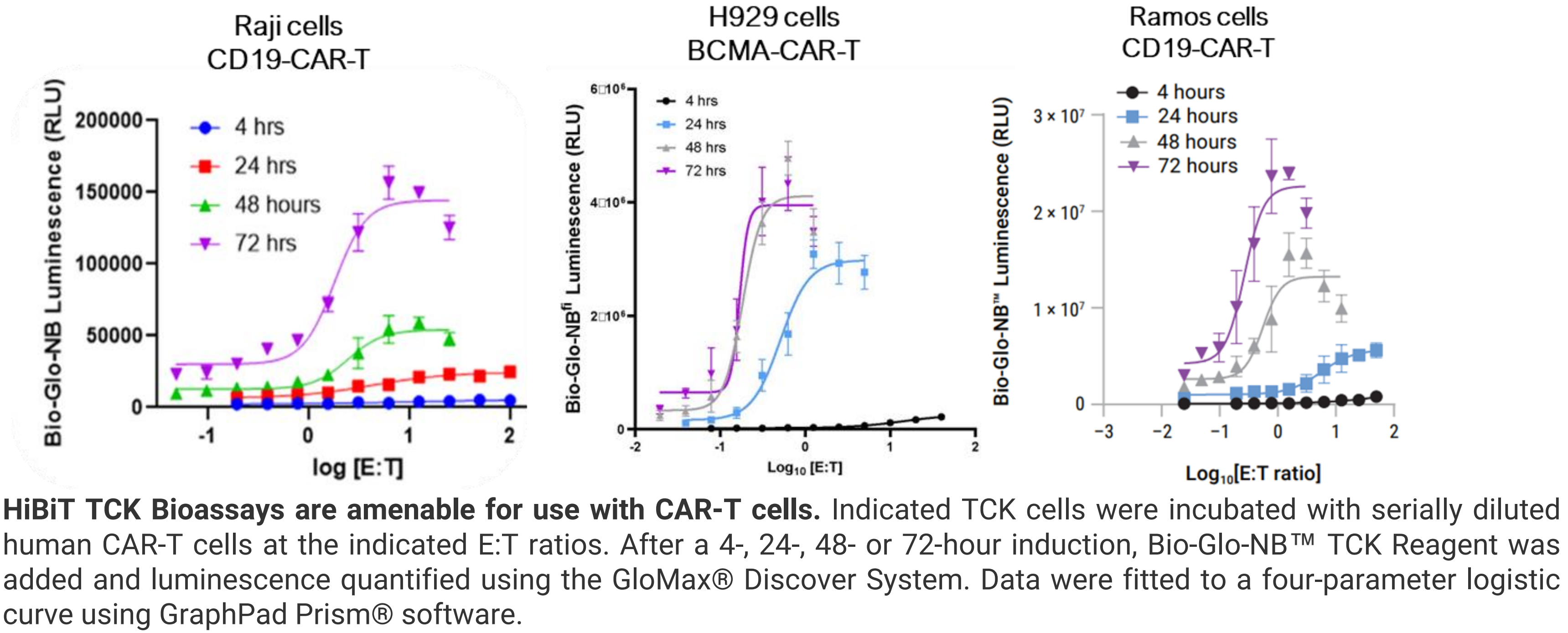


5. TDCC Applications & Primary CD8+ T Cell



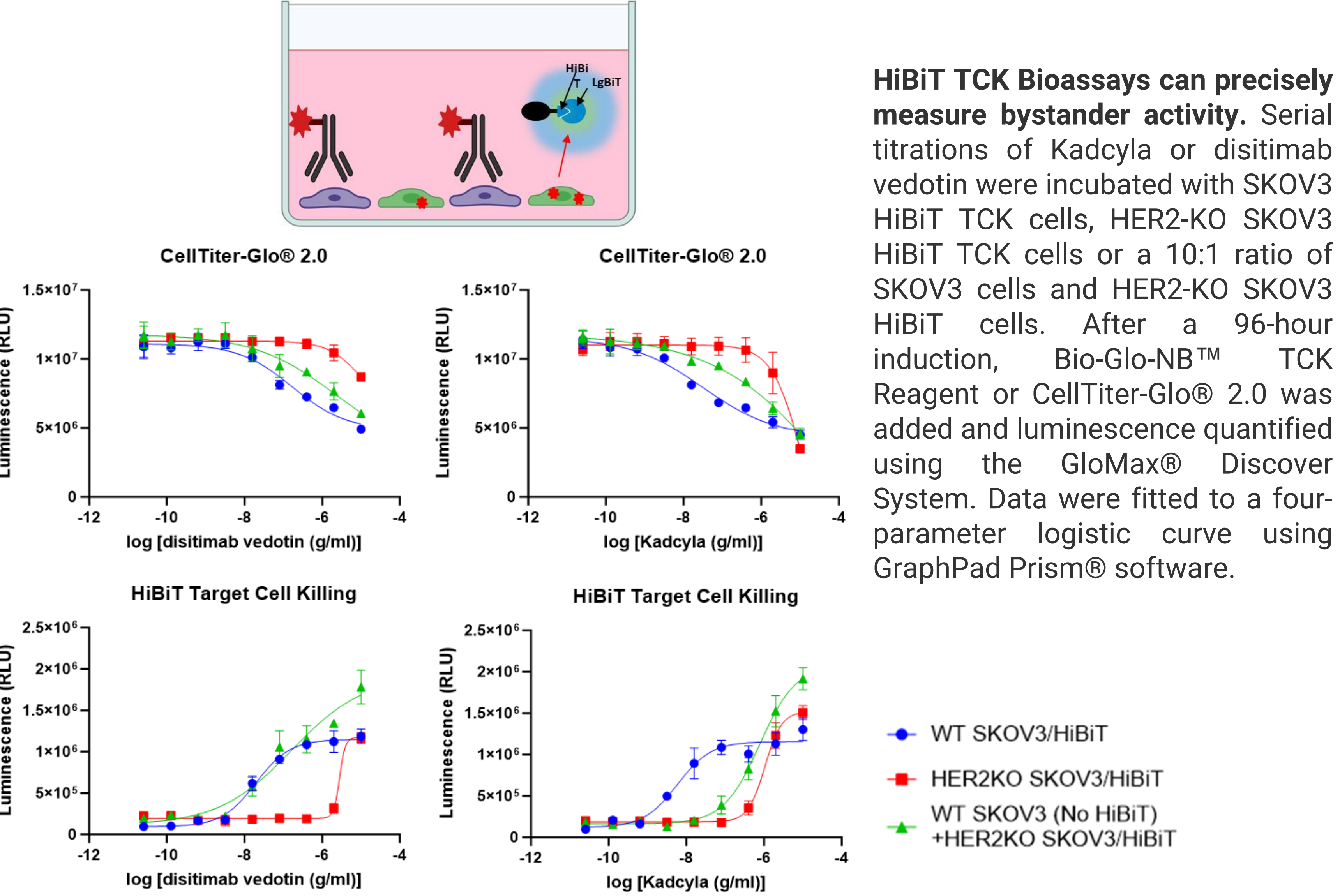
HiBiT TCK Bioassays reflect the TDCC MoA of biologic drugs. The cell type indicated were incubated with Human CD8+ T cells, TDCC-qualified effector cells in the presence of serial titrations of the indicated T cell engager. After a 24-hour induction, Bio-Glo-NB™ TCK Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. TCK assay was compared to an TDCC reporter assay with relative potencies to be in good agreement.

6. CAR-T Applications



HiBiT TCK Bioassays are amenable for use with CAR-T cells. Indicated TCK cells were incubated with serially diluted human CAR-T cells at the indicated E:T ratios. After a 4-, 24-, 48- or 72-hour induction, Bio-Glo-NB™ TCK Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software.

7. ADC Direct & Bystander Activity



8. Conclusions

HiBiT TCK Bioassay platform provides a simple, sensitive, and highly specific method to support screening, potency testing, and lot release of biologic therapeutics

- Robust selection of off-the-shelf HiBiT target cells expressing common immunotherapy targets
- Thaw-and-Use format eliminates the need for cell culture
- Flexible platform with a rapid and convenient workflow relative to existing methods like flow cytometry

HiBiT TCK Bioassays provide a biologically-relevant method for measuring cell killing across a variety of applications and biologic drug modalities:

- Antibody dependent cellular phagocytosis (ADCP)
- Antibody dependent cellular cytotoxicity (ADCC)
- T-cell Dependent cellular cytotoxicity (TDCC)
- CAR-T cell therapy
- Antibody-Drug Conjugates