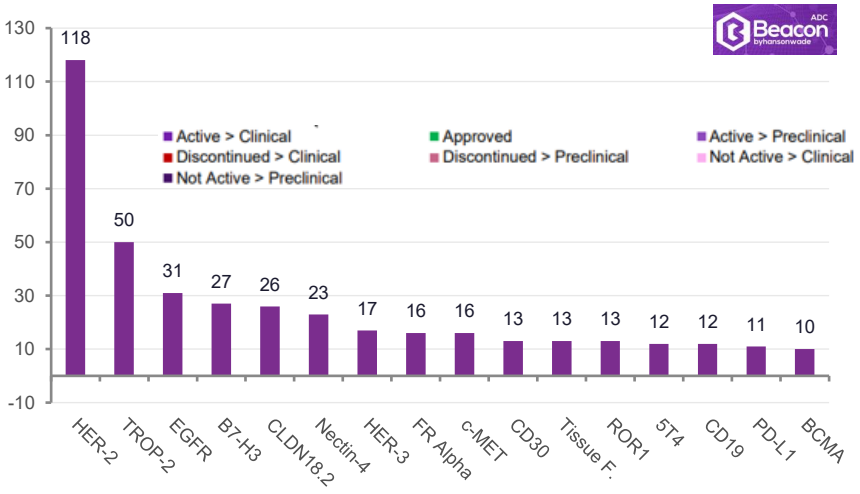
Quantifying the Unseen: Real-Time Assessment of ADC Bystander Killing Using HiBiT Technology

Gopal B. Krishnan Ph.D. MBA
Manager – Biologics & Proteomics
Promega

Top ADC Targets

ADC Assets by Target



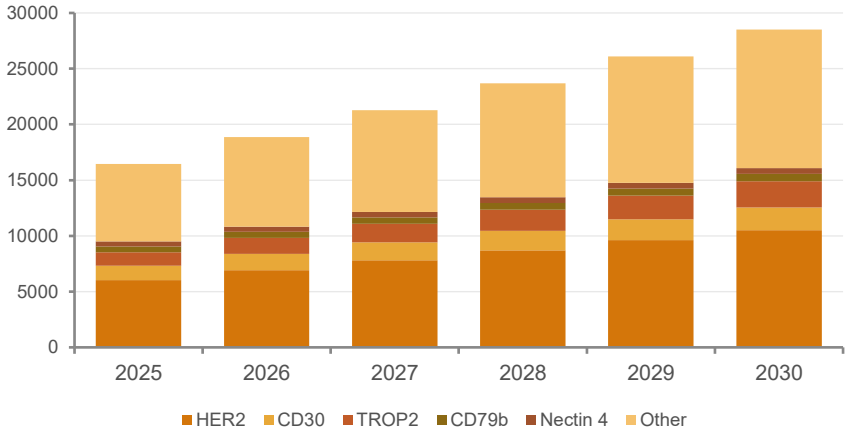
Source: Beacon ADC Database

Approved ADCs for Lung Cancer

1. HER2 — Trastuzumab deruxtecan (Enhertu): HER2-mutant lung cancer

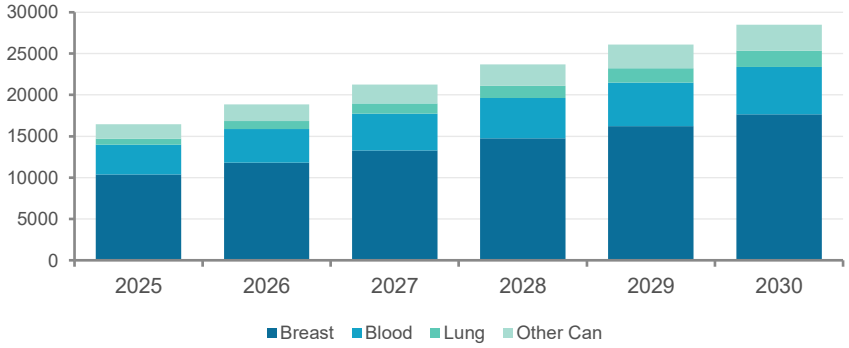
2. TROP2 — Sacituzumab govitecan (Trodelvy): Cancers with high TROP2 expression

Revenue Share by Year — Target



Source: Howe Sound Research, ADC Markets 2026-2030

Revenue Share by Year — Cancer Type

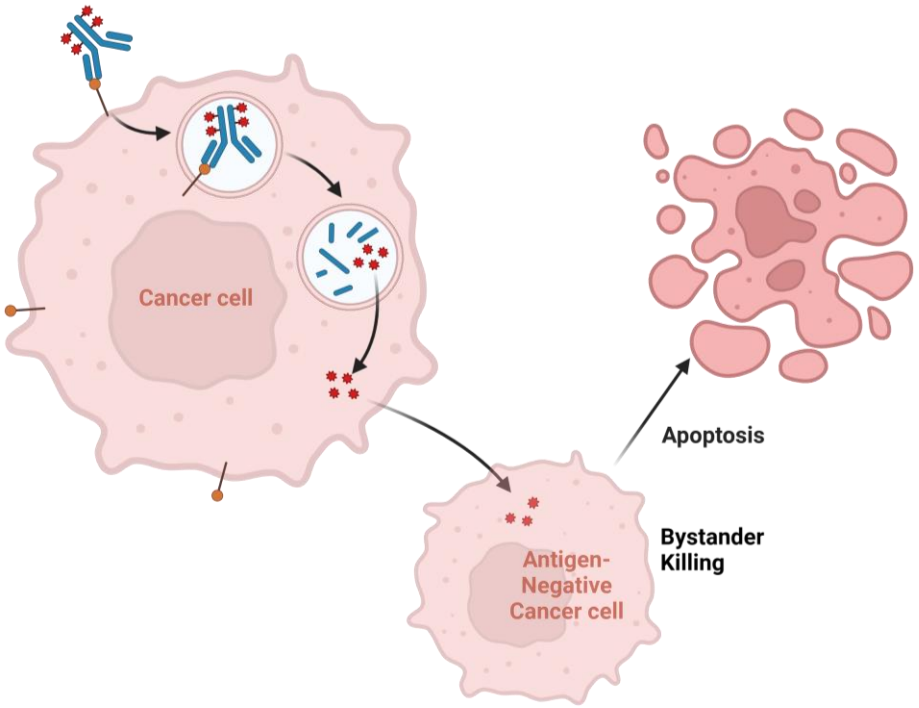


ADC Market Landscape • Sources: Howe Sound Research 2026, Beacon ADC Database

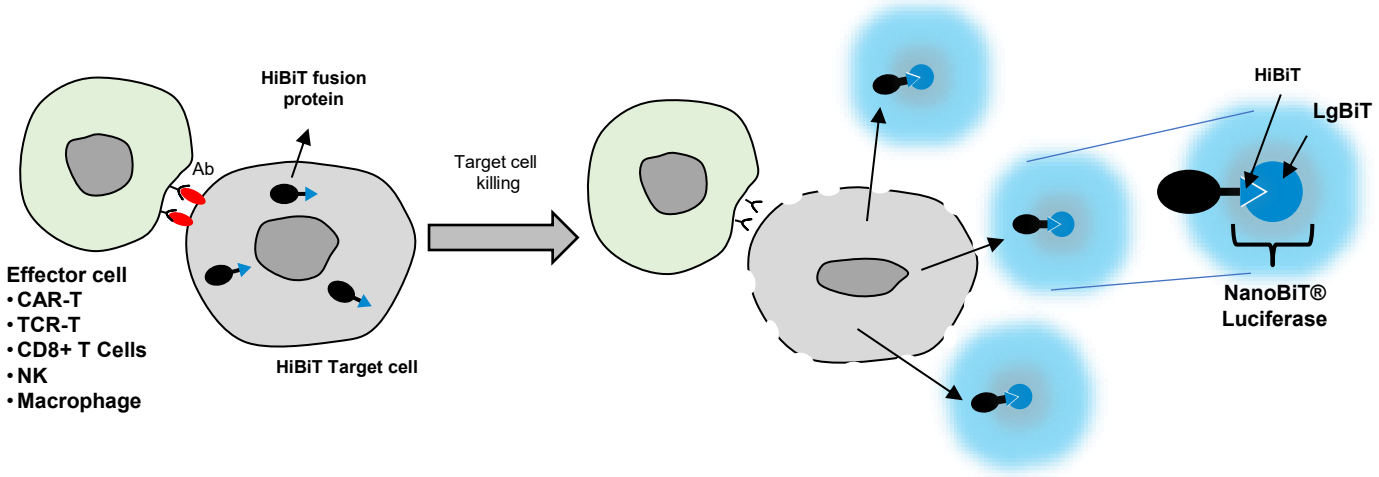
Bystander Cell Killing Effect

- Cleavable linkers allow payload to access nearby tumor cells.
- Prevents treatment failure/relapse due to tumor heterogeneity or antigen loss.

- **Must detect death of one cell population in mixed culture**



HiBiT Target Cell Killing (TCK)
Assay Principle

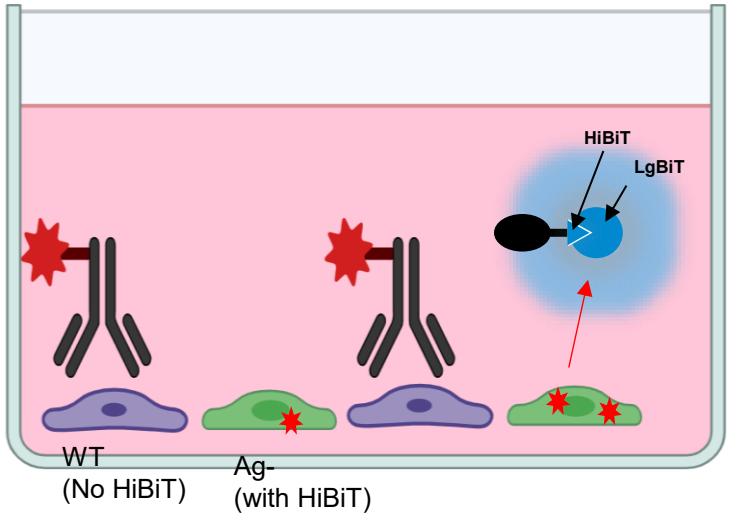


Assay Design
- Lysis of HiBiT target cells releases HiBiT into the medium
- HiBiT binds to LgBiT in the detection reagent, forms functional NanoBiT luciferase and emits luminescence

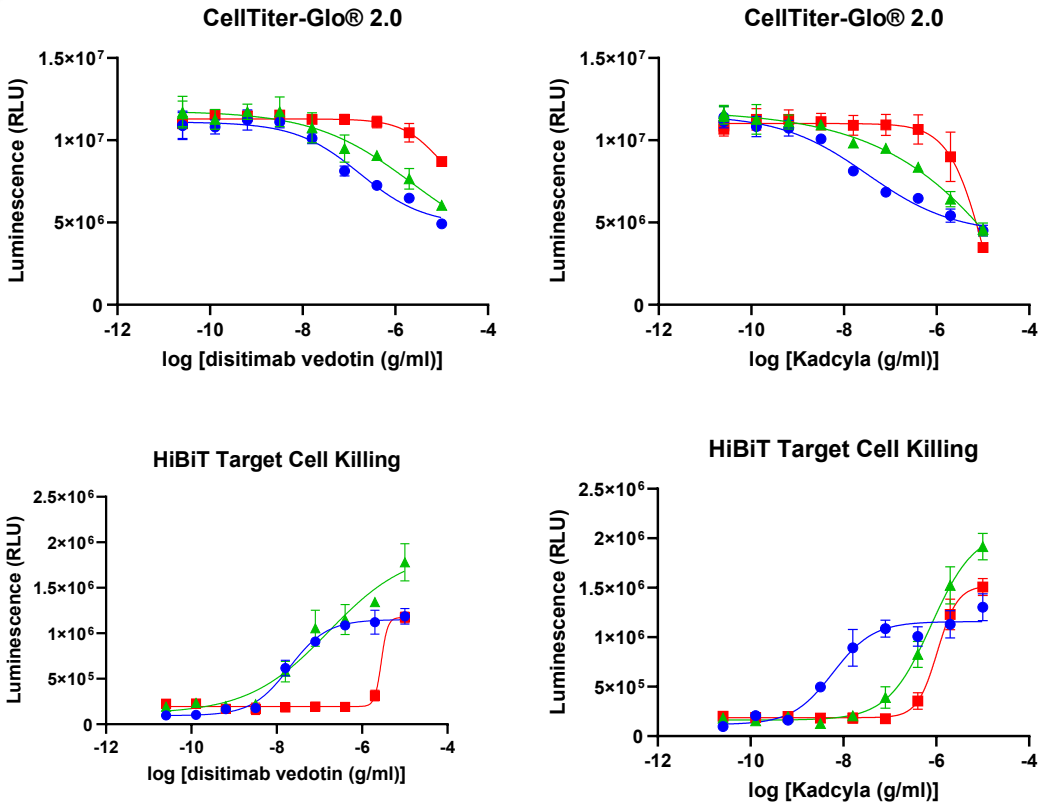
Features
- Measure target cell-specific killing
- Low spontaneous release
- Simple, homogenous
- No medium transfer required
- Measure assay response from hours to days

Bystander Cell Killing Assay Principle

- ADC binds **wild type** cell and payload is released
- Payload kills **HiBiT-expressing reporter** cell
- Luminescence is specific to bystander killing



Model System: SKOV3/HiBiT with anti-HER2 ADCs



- SKOV3 cells treated with:
  - Disitimab vedotin (HAS bystander effect)
  - Kadcyla (NO bystander effect)
- 96 hour assay
- WT SKOV3/HiBiT: direct cytotoxicity by ADC
- HER2KO SKOV3/HiBiT: off-target cytotoxicity
- 10:1 ratio of WT SKOV3 (no HiBiT) and HER2KO SKOV3/HiBiT: bystander killing

- CellTiter-Glo cannot distinguish which ADC has bystander killing
- With HiBiT TCK, green curve shifts left from red curve when bystander killing is present

Bystander Killing Assay | HiBiT TCK Summary

MODEL SYSTEM	CO-CULTURE	COMPARISON	READOUT
SKOV3/HiBiT HER2+ Ovarian Carcinoma	**10:1 Ratio** WT SKOV3 + HER2-KO/HiBiT	**Disitamab vedotin** vs. Kadcyla (non-cleavable)	**96-hour** Kinetic Luminescence

Key Assay Features

Specificity
HiBiT signal originates only from bystander (antigen-negative) cell death — not direct on-target killing

Quantitative
Luminescent readout proportional to cell death enables EC50 calculation for bystander killing potency

Kinetic
96-hour time-course measurement captures full bystander killing dynamics and onset timing

Discriminating
Clearly differentiates cleavable (bystander-competent) from non-cleavable linker ADCs

Simple Format

★ KEY DIFFERENTIATOR

CellTiter-Glo cannot distinguish bystander from direct killing. HiBiT TCK shows a clear **left-shift of EC50** for bystander-competent ADCs.

Applications

DISCOVERY

ADC Candidate Screening
Linker-payload optimization for bystander competence

COMPARABILITY

Head-to-Head Comparison
Rank ADC constructs by bystander killing potency

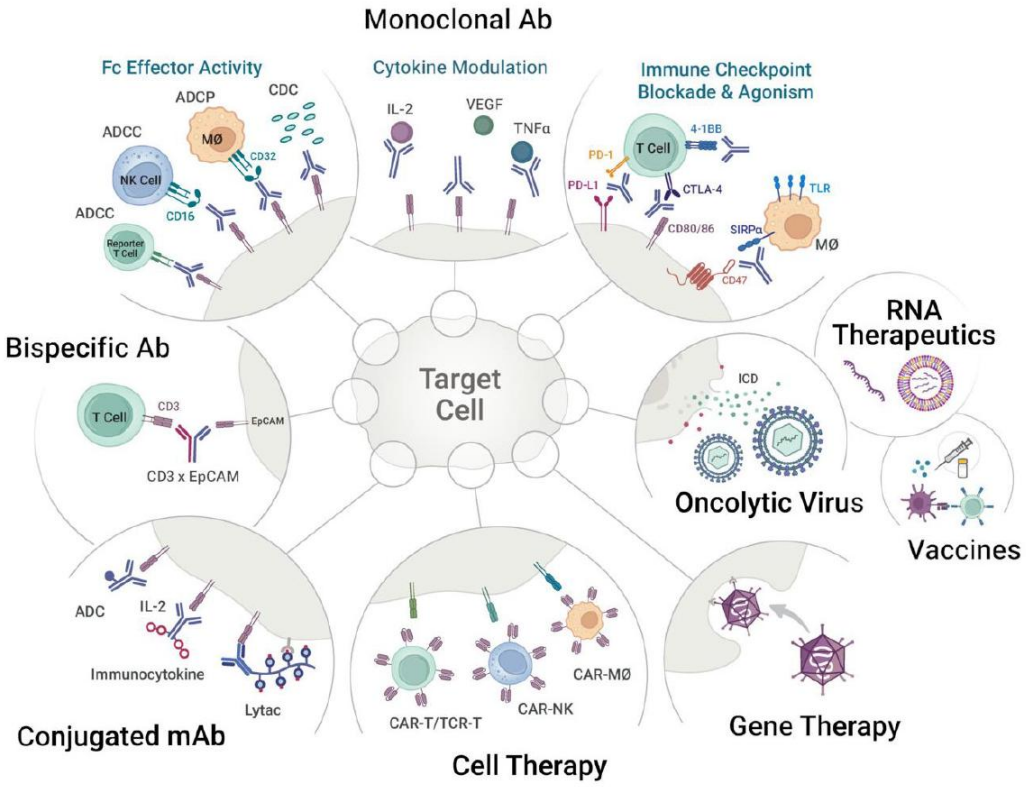
MOA STUDIES

Tumor Heterogeneity
Address antigen loss and heterogeneous expression

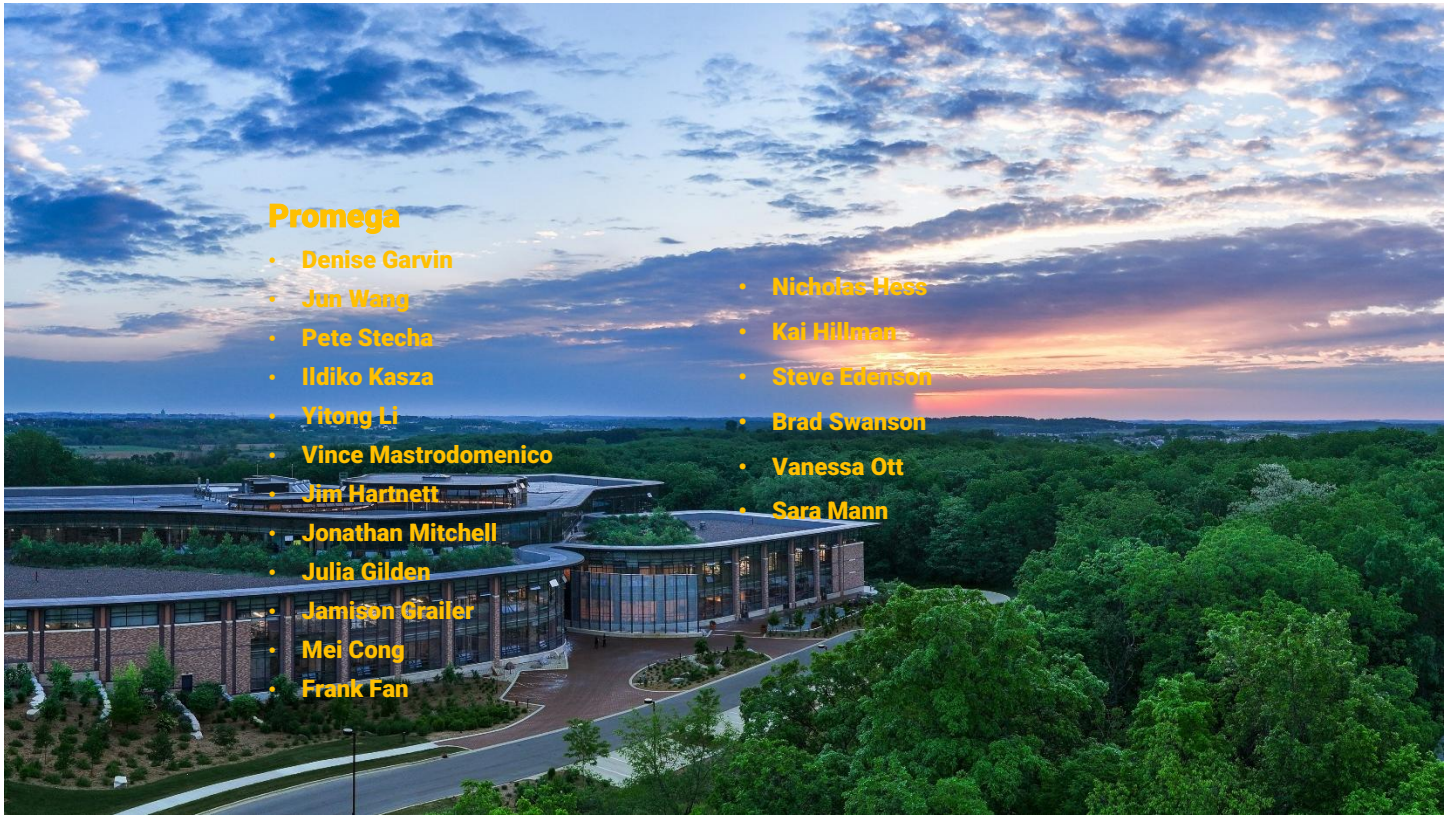
PLATFORM

Unified TCK Workflow
Same platform for ADCC, TDCC, ADCP, and CAR-T

Biologics Portfolio



Acknowledgements



Promega

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Thank You!