Characterization of First-In-Class Translation Inhibitor ADCs to Overcome SOC Payload Resistance

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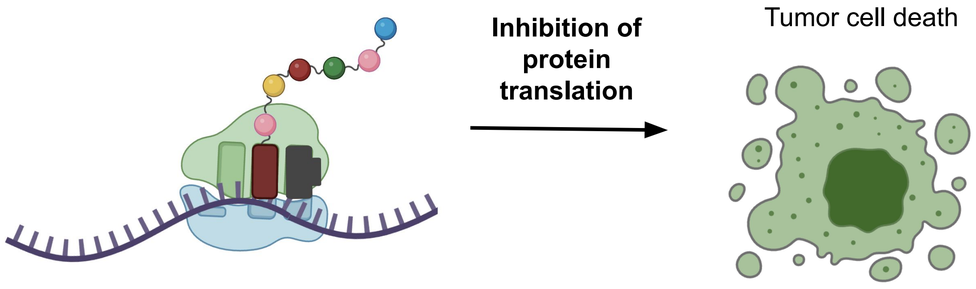
1. We Have Identified Multiple Protein Translation Inhibitors (PTI) With A MoA Orthogonal to Existing Classes of ADC Payloads



Tumors likely to be most sensitive to protein translation inhibition are those that:

- Rely on continuous supply of oncogenic drivers
- Overexpress oncogenic proteins with short half-lives
- Express elevated levels of translational machinery to sustain their oncoproteome

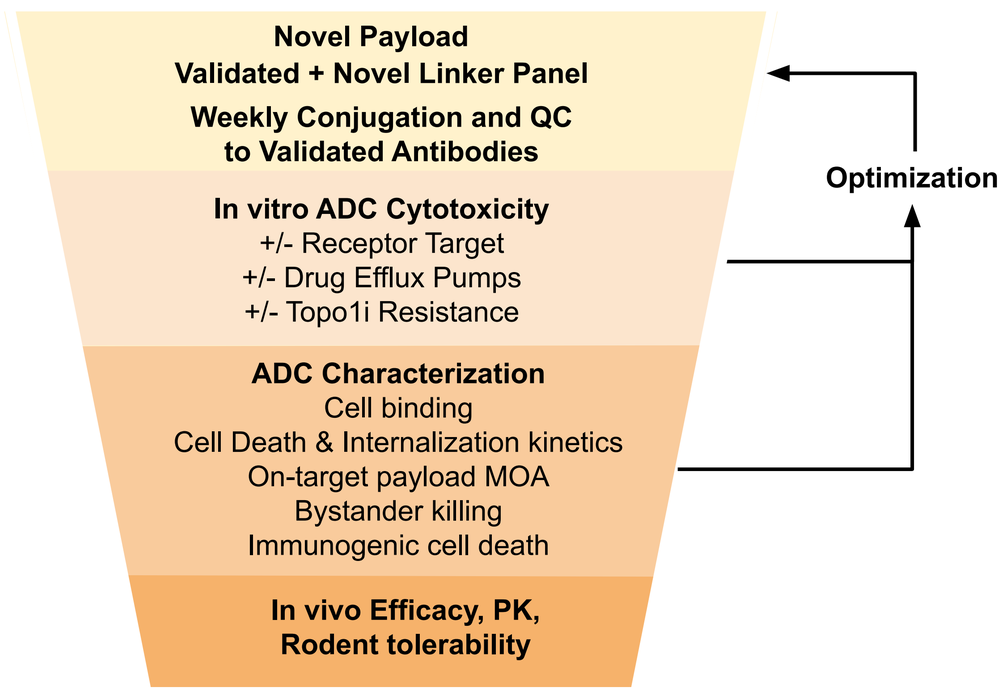
Cancer cell lines (n=103)	PTI 1	PTI 2	Tubulin inhibitor MMAE	Topo-I inhibitor Dxd
Bladder				
Bowel				
Breast				
Cervix				
Esophagus				
Kidney				
Lung				
Lymphoid				
Myeloid				
Ovarian				
Pancreas				
PNS				
Prostate				
Uterus				

Median IC50

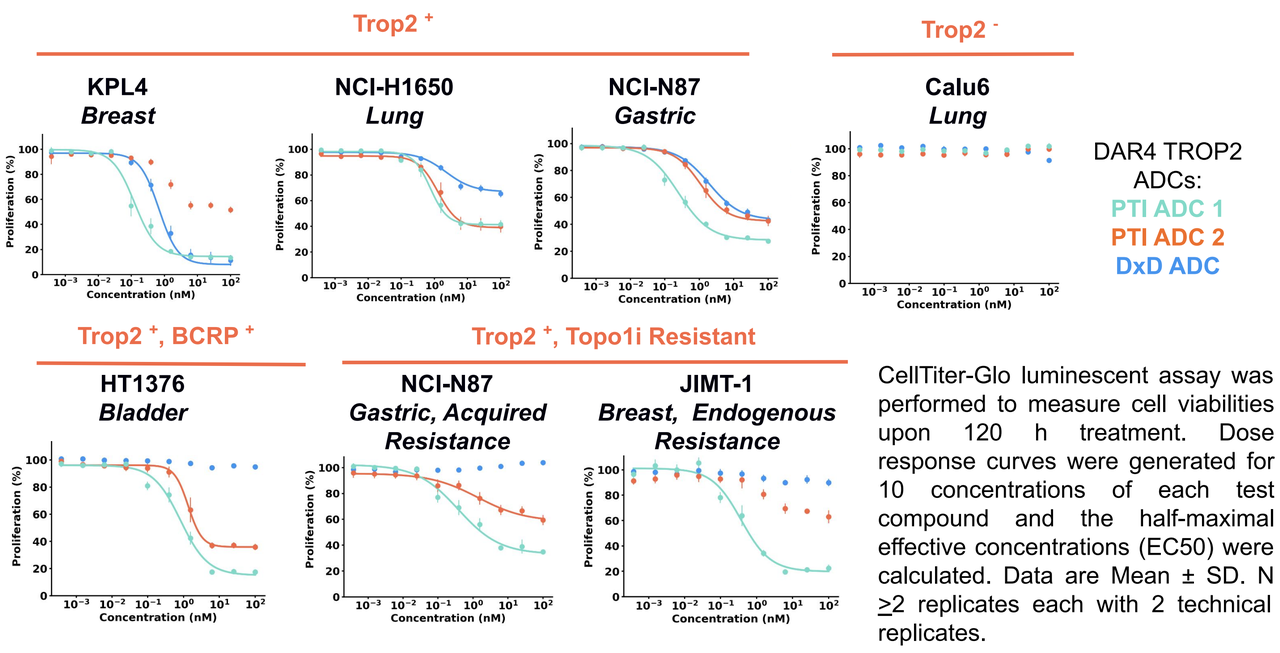
0-15 nM
15-100 nM
> 100 nM

CellTiter-Glo luminescent assay was performed to measure cell viabilities upon 72h treatment (N=2-3 biological replicate, N=2-3 technical replicates). Dose response curves were generated for 9 concentrations of each test compound and the half-maximal inhibitory concentration (IC50) was calculated. The authors would like to thank Crown Bioscience for their OmniScreen cell based screening services to generate the profiling data.

2. A High-Throughput Screening Pipeline Rapidly Identifies Novel Payloads and Advances Promising ADC Candidates

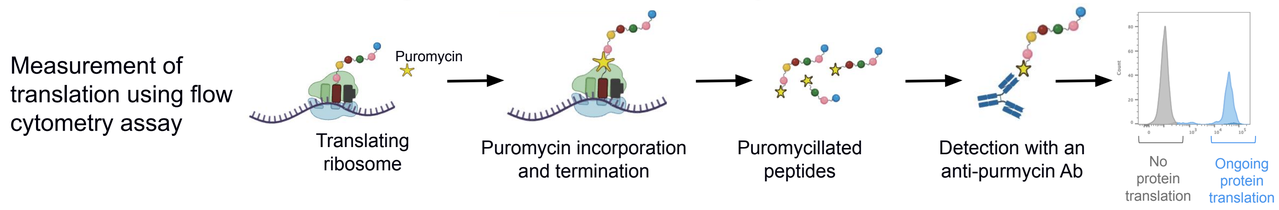


3. Protein Translation Inhibitor ADCs Demonstrate In Vitro Cytotoxicity Across Diverse Cell-Lines Including Drug Efflux Pump-Expressing and Topo1i Resistant Cells

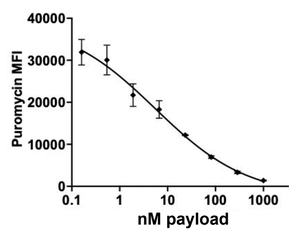


CellTiter-Glo luminescent assay was performed to measure cell viabilities upon 120 h treatment. Dose response curves were generated for 10 concentrations of each test compound and the half-maximal effective concentrations (EC50) were calculated. Data are Mean ± SD. N ≥2 replicates each with 2 technical replicates.

4. On-target Translation Inhibition Is Demonstrated In Vitro and In Vivo Using Fluorescent Puromycin Pulse Chase

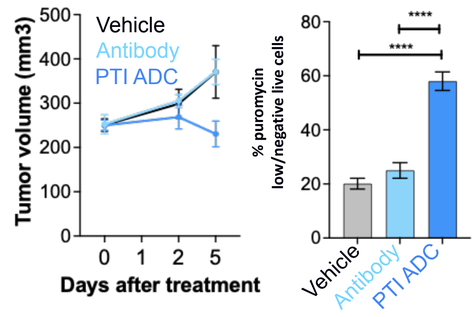


In vitro translation:



Cells were treated with PTI payload for 4 hours, washed and incubated with puromycin for 0.5 hour. Following puromycin removal, cells were stained with a viability dye and an anti-puromycin antibody, then analyzed by flow cytometry.

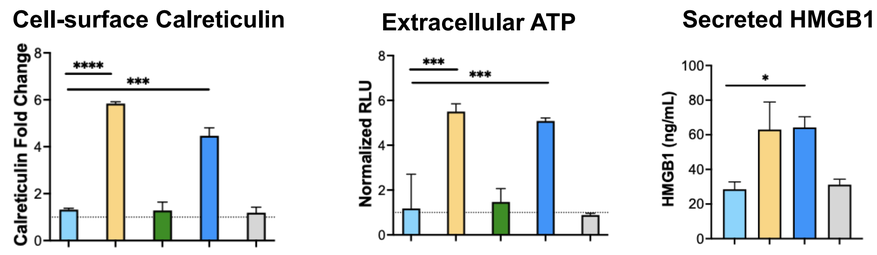
In vivo translation:



KPL4 tumors were harvested 5 days after dosing, dissociated, and pulsed with puromycin. Tumor cells were stained for viability, tumor receptor expression, and puromycin by flow cytometry. Data are +/- SEM, ****p<0.0001 by One Way ANOVA. (N=4 mice/group).

5. Protein Translation Inhibitor ADC Elicit Hallmarks of Immunogenic Cell Death

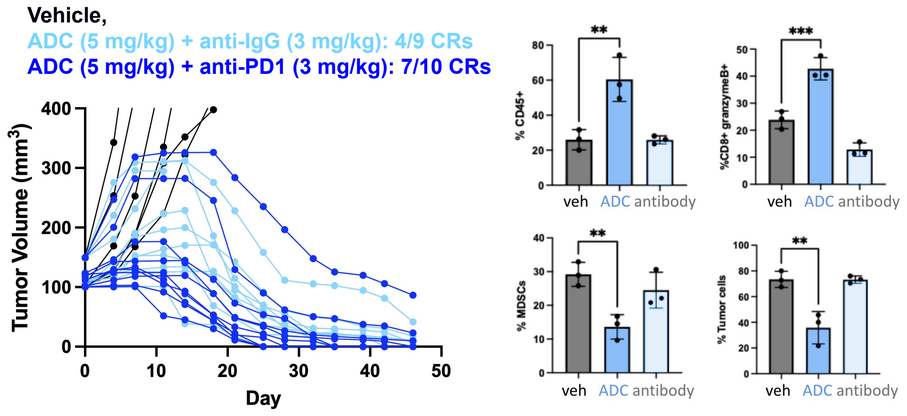
Trop2 ADCs:
Antibody (control)
MMAE
DxD
PTI
PBS (control)



Fold change in surface calreticulin, normalized ATP release and concentration of HMGB1 (ng/mL) in the supernatant after 48-168 hours of treatment, with the indicated ADCs or control naked antibody. Data are mean +/- SD (N=1-2 biological replicates, N=2-3 technical replicates). p values based on One way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

6. Protein Translation Inhibitor ADC Reduces Immunosuppression in the Tumor Microenvironment In Vivo

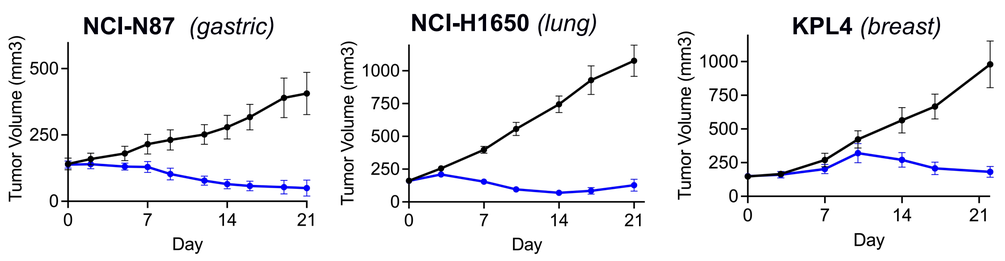
Vehicle,
ADC (5 mg/kg) + anti-IgG (3 mg/kg): 4/9 CRs
ADC (5 mg/kg) + anti-PD1 (3 mg/kg): 7/10 CRs



Tumor-bearing hTrop2-CT26 mice were treated with a single 5 mg/kg dose of Trop2 PTI ADC in combination with anti-PD1 or isotype control when tumors reached a volume of 100-200 mm^3. At day 7, tumors were harvested and processed for immune phenotyping. For rechallenge efficacy, mice were treated with ADC, anti-PD1 or isotype bi-weekly for 2 weeks and 4 weeks respectively. N=7-8 mice/group for efficacy; N=4 mice/groups for flow cytometry. Data are Mean ± SEM.

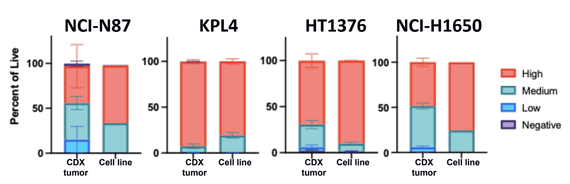
7. ADCs With Protein Translation Inhibitor Payload Demonstrate Durable Complete Responses In Vivo and Anti-Tumor Activity in Topo1i Resistant Models

PTI ADC demonstrates anti-tumor activity in multiple preclinical models with various levels of Trop2 expression.



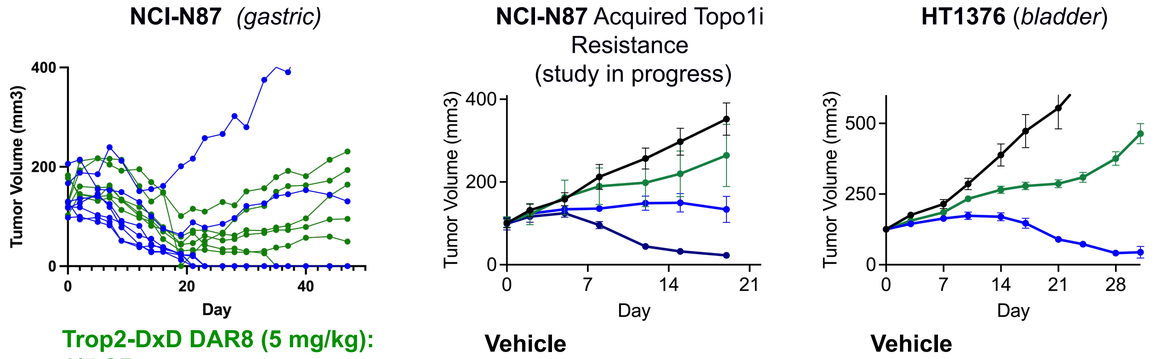
Vehicle
Trop2- PTI DAR4 (5-10 mg/kg)

Trop2 Expression Validation Across CDx Models:



Mouse tumors were excised when they reached ~275 mm^3. After tumor dissociation, Trop2 expression was evaluated by flow cytometry. Trop2 receptor was detected on CD45-negative EpCAM-positive tumor cells and compared to corresponding in vitro cultured cells.

PTI ADC demonstrates durable and complete responses and anti-tumor activity in preclinical models with acquired Topo1i resistance and BCRP expression driven resistance (HT1376).



Trop2-DxD DAR8 (5 mg/kg): 1/7 CRs
Trop2- PTI DAR4 (5 mg/kg): 5/7 CRs

Vehicle
Trop2-Dxd DAR4 (5 mg/kg)
Trop2-PTI DAR4 (5 mg/kg)
Trop2-PTI DAR4 (10 mg/kg)

Vehicle
Trop2-Dxd DAR4 (5 mg/kg)
Trop2-PTI DAR4 (5 mg/kg)

The in vivo therapeutic efficacy of ADCs was evaluated in the treatment of xenograft models as indicated. Indicated cell-lines were inoculated subcutaneously into mice (NCI-N87, HT1376, and NCI-H1650 in BALB/c nude mice, and KPL4 in NSG mice). Mice were dosed intravenously with indicated test articles at day 0 and 7 when tumors reached mean tumor volume of 100 or 175 mm3 (NCI-N87 acquired resistance, NCI-N87), or a range of 100-200 mm3 (KPL4, NCI-H1650, and HT1376). Data are Mean ± SEM (N=7-8 mice/group).