

Introduction

ADCs have had tremendous impact on patient outcomes in breast cancer and are now second line therapy for stage IV HER2 positive metastatic breast cancer. However, many patients fail to respond or relapse after treatment with ADC therapies due to tumor heterogeneity and resistance to ADC payloads. Here we show a novel conjugation system capable of attaching distinct payloads at different sites to the same antibody, enabling the production of stable single-molecule targeted combination therapies, Multi-Payload Conjugates (MPCs)TM, with a well-defined drug-antibody ratio (DAR). We screened combinations of different payloads targeting several different mechanisms of cell division attached to a HER2 targeting antibody to optimize tumor cell killing. These targeted combination ADCs demonstrated robust killing in both HER2 high and low tumor cell lines.

Antibody Drug Conjugates have revolutionized the treatment of high HER2 positive breast cancer. More recently advances have been made in the design of ADCs to expand indications to include HER2 low. MPCsTM offer the next step in ADC design and allow for the combination of multiple mechanisms of action in a single therapeutic. These molecules offer the potential to circumvent tumor resistance pathways and deliver deeper and more durable responses.

Current Challenges in ADCs

Two key challenges facing ADC development are:

- Over 90% of ADCs in the clinic today use one of two bonds to attach payloads to antibodies: *lysine-based amide bonds*, OR *cysteine-based maleimide bonds*. Both bonds have significant limitations: Amide bonds result in heterogeneous therapeutics; while Maleimide bonds undergo rapid degradation in blood serum, resulting in sub-optimal stability.
- Inability to attach multiple distinct payloads to a single antibody, resulting in high rates of resistance and relapse in patients treated by ADCs.

Combination chemotherapies have been the standard of care for decades in oncology, but the industry has lacked a conjugation technology that allows multiple (two or more) payloads on the same antibody while allowing for stable conjugation of complex next-generation cargo. A comparison of Catena's approach to existing bonds can be found below:

	Cysteine-X Maleimide Bond (~75% of ADCs)	Lysine-X Amide Bond (~25% of ADCs)	Tyrosine-Cysteine C-Y Bond*
SIMPLICITY	X	X	✓ Native Amino Acids in Physiological Conditions
STABILITY	X	✓	✓ Highly Stable
SELECTIVITY	✓	X	✓ Highly Selective; Specific Conjugations
SCALABILITY	-	-	✓ High Yields & Throughput
ABLE TO ADD MULTIPLE PAYLOADS	X	X	✓ Unique ability to add warheads with different MOAs

Introducing the CysTyrTM Platform

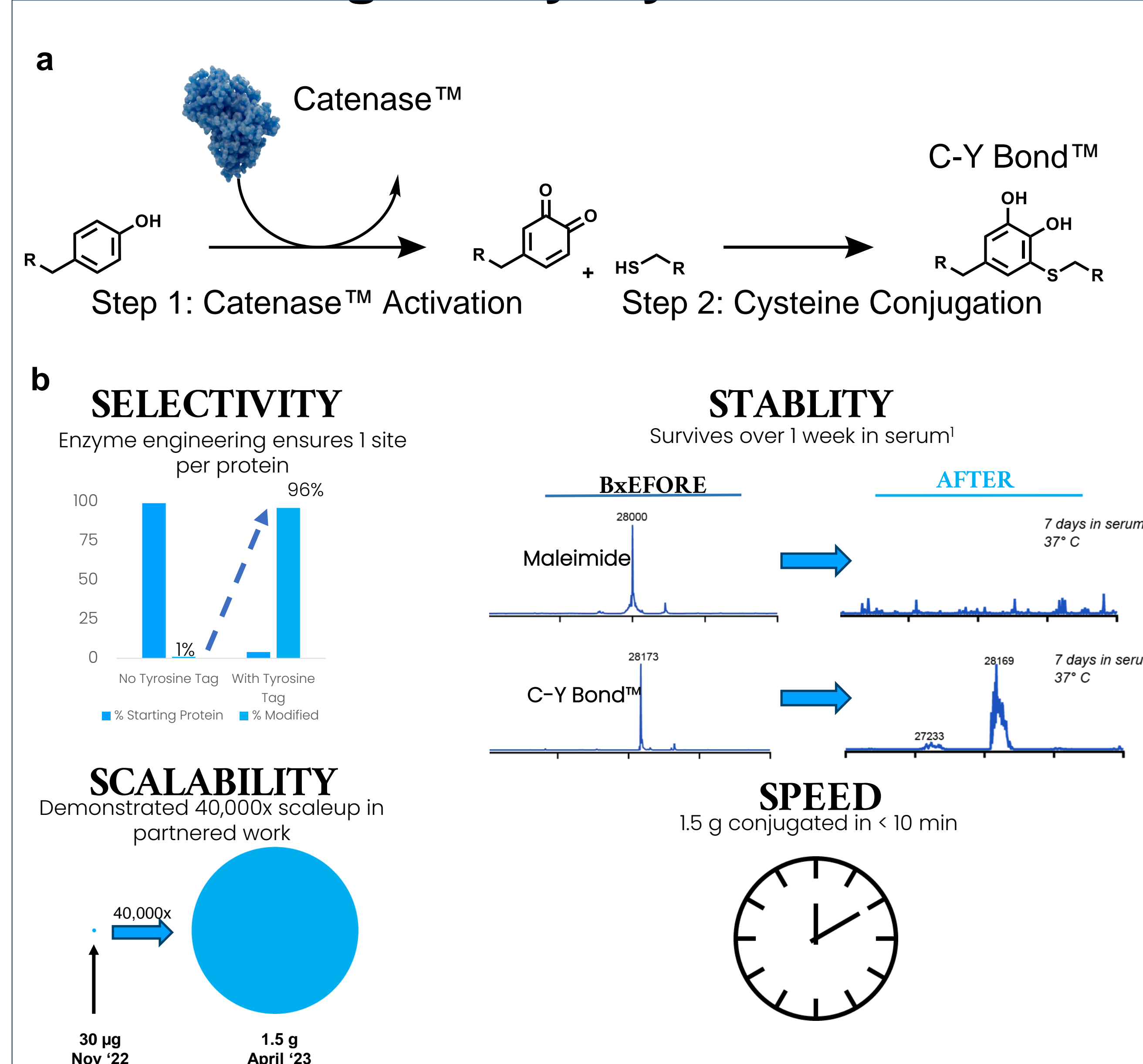


Figure 1: a) CatenaseTM reaction scheme and b) Key performance metrics for CatenaseTM conjugations from previous work in model systems

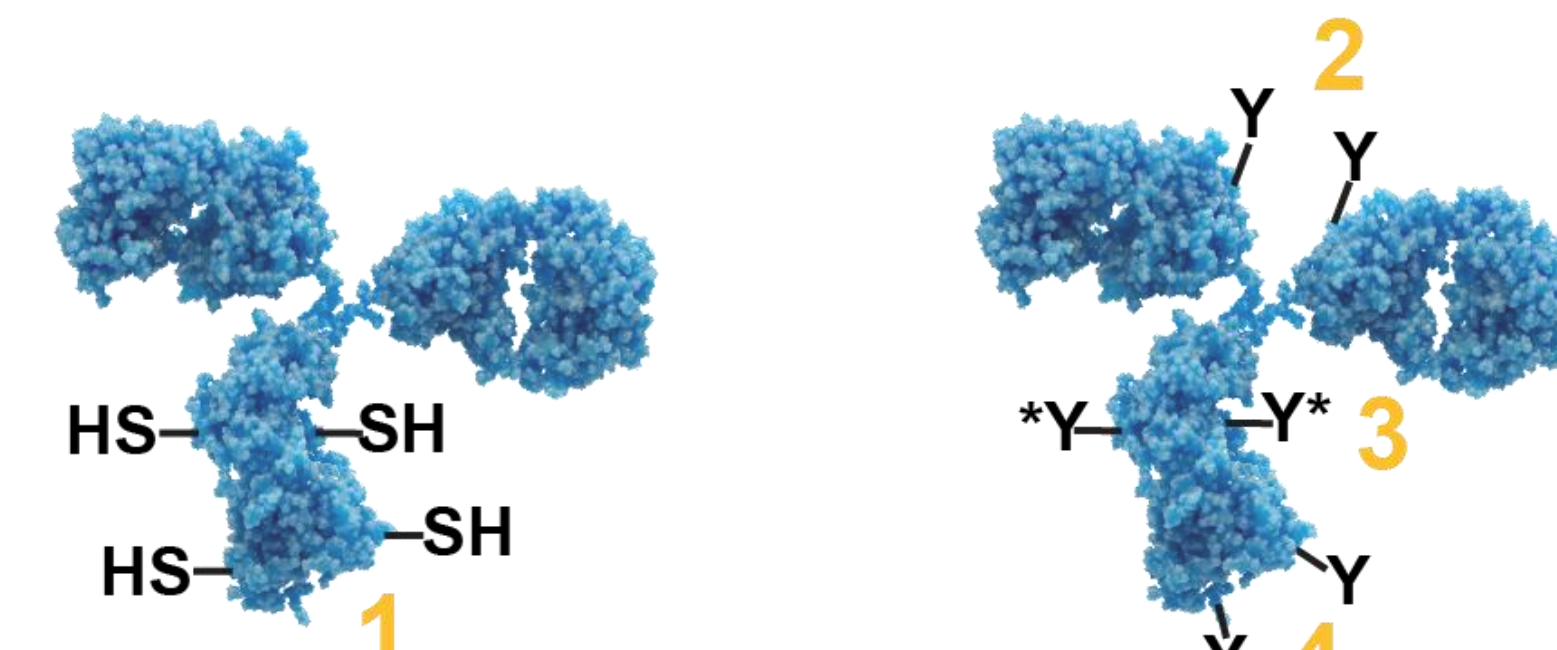


Figure 2: Illustration of the 4 unique reaction sites accessible through CysTyrTM conjugation, 1) cysteine residues, 2,4) heavy and light chain termini, and 3) native loop tyrosines

Catena's Differentiated Science

Catena's CysTyrTM platform with its novel C-Y BondTM offers the revolutionary benefit of attaching two or more different payloads to the same antibody, all while preserving geometric flexibility by allowing attachment to different sites. This is accomplished by selective conjugation to terminal or native loop tyrosines or the flexible cysteine mutants used in industry today. Coupled with superior stability, selectivity, and scalability of the C-Y BondTM,² the CysTyr platform has potential to unlock new modalities in therapeutic design.

By combining multiple payloads and thereby multiple mechanisms of action, Catena aims to produce Multi-Payload Conjugates (MPCsTM) that can offer superior efficacy by incorporating the benefits of combination therapies with targeted therapeutics.

The C-Y BondTM also allows attachment of payloads far beyond the small molecules used in current ADCs. To date, we have validated conjugation of a diverse array of compounds from toxins, isotope chelators, nucleic acids, peptides, enzymes, cytokines, nanobodies, and even whole cells.³

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Antibody Modification

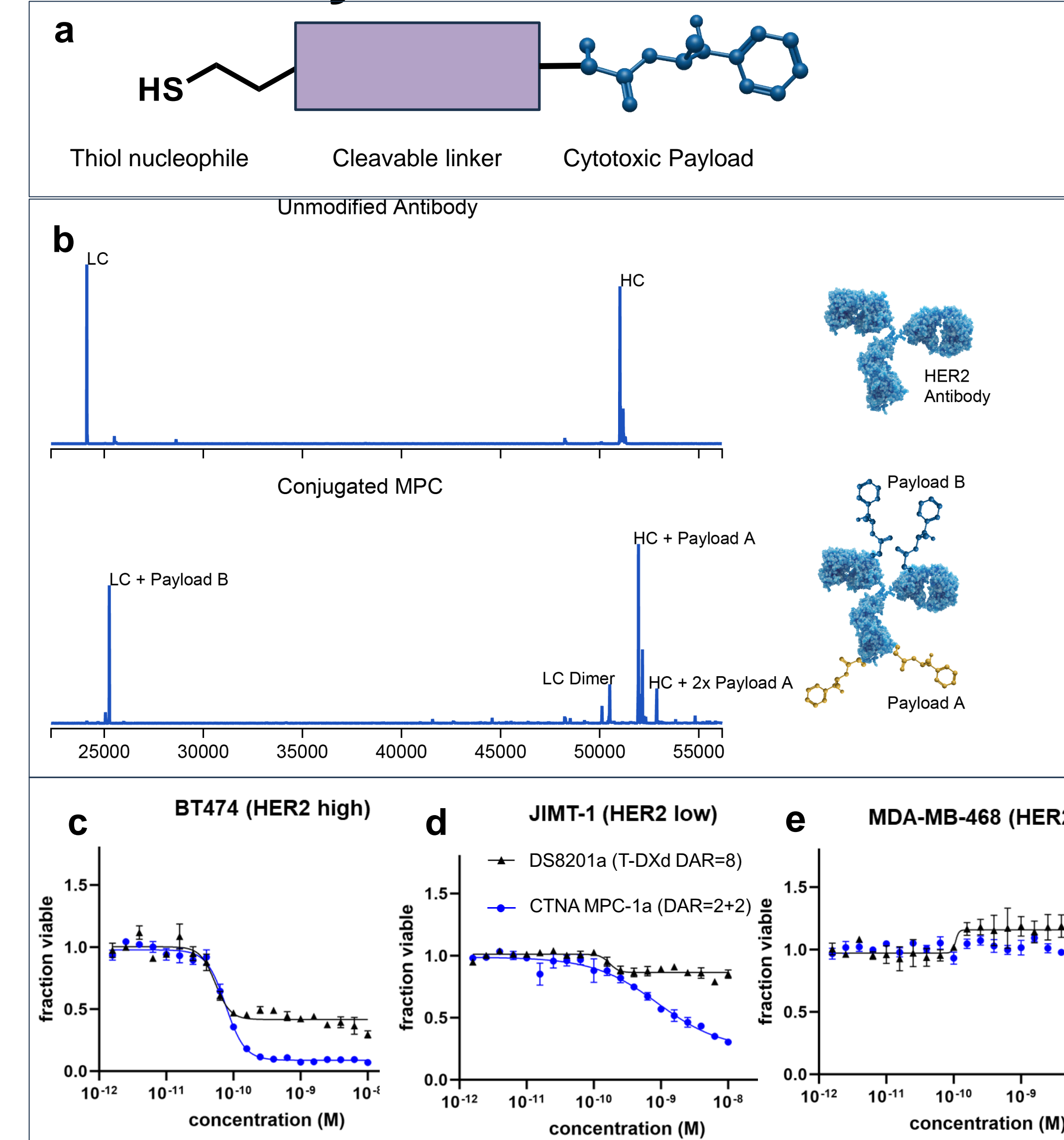


Figure 3: Conjugation of multiple cytotoxic payloads to the same antibody. a) representative diagram for active payloads containing a terminal thiol, cleavable linker, and active payload, b) LC-TOF Mass spectrum of starting antibody compared to final MPC after conjugation. Light chain mass shows over 93% conjugation of Tubulin inhibitor after reaction, while heavy chain after modification shows 99% modification of +1 or +2 of Topo1 inhibitor. c-e) in vitro activity assay on BT474, JIMT-1 and MDA-MB-468 cell lines comparing DS8201a (T-DXd, black) to Catena's MPC (blue) shows significantly greater impact on cell viability for the MPC molecule in both high and low HER2 expressing tumor lines.

Methods and Results

A library of MPCsTM was created through combinations of 6 different payloads in concurrent reactions and tested for activity across multiple cell lines. Antibodies were modified in a 2-step reaction: combining antibody and 1st payload of interest with CatenaseTM enzyme for an initial one-pot conjugation, followed by a simple buffer exchange to remove unreacted payload before conjugation with the 2nd payload of interest. All payloads coupled with high efficiency to tyrosine residues on the heavy or light chains (ex. Figure 3a) and tested in vitro against cell lines of interest. Cellular inhibition was measured via Alamar Blue Assay (Invitrogen) as recommended by the manufacturer across 20 concentration points for each compound. In head-to-head trials against Ds8201a (T-DXd) these show significant increases in total inhibition (Figure 3c-e, Figure 4 b,c).

Finally, murine models of breast cancer were established using the JIMT-1 (HER2 low) cell line. After tumors reached 100 mm³ mice were dosed with either MPCTM or DS8201a (T-DXd) at the respective quantities listed (Figure 4a).

in vivo comparison vs T-DXd

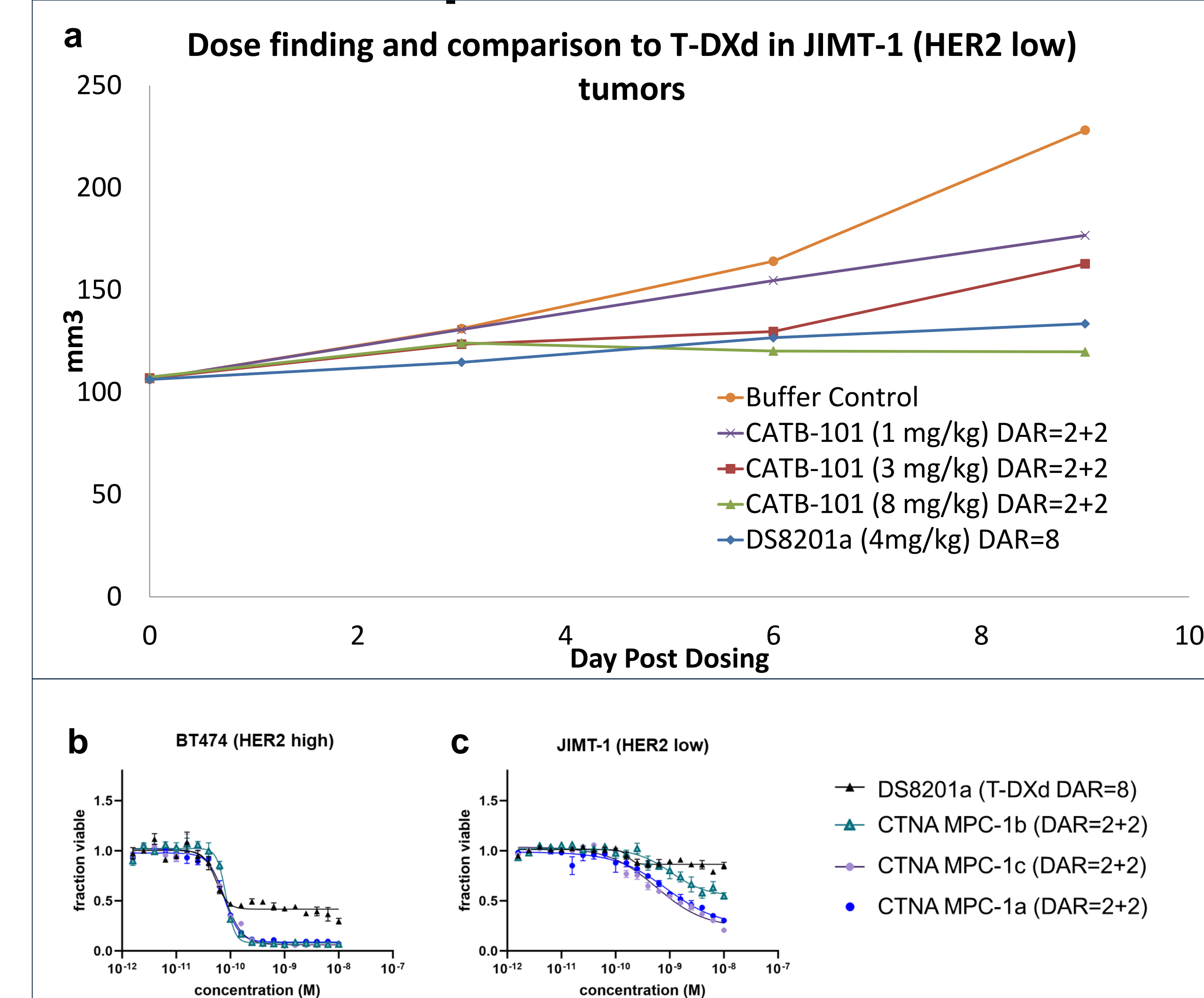


Figure 4: a) in vivo activity data for HER2 targeted MPC CATB-101 compared to DS8201a (T-DXd). CATB-101 shows equivalent or better activity compared to DS8201a despite containing half the DAR, showing the benefit of the MPC approach (b). MPCsTM library compared against BT474 (f) and JIMT-1 cells (c) show similarly efficient cell killing, with slight differences attributed to the specific payload combinations. Further study will identify optimal payload combinations.

Conclusions

- MPCs are active in HER2 low model of Breast Cancer in preliminary results
- Catena has successfully produced a modular library of MPCs using the CysTyrTM Conjugation platform
- MPCsTM tested in vitro across multiple HER2+ cell lines showed improved efficacy compared to DS8201a (T-DXd)
- The novel C-Y BondTM, using only naturally occurring amino acids confers enhanced stability in serum, which will potentially confer enhanced safety in addition to superior efficacy

Next Steps

- Generate additional combinations and positions including variable DAR (e.g. 4+2)
- Optimize payload combinations and therapeutic performance

Company Overview

CatenaBio was launched out of the Jennifer Doudna and Matt Francis labs at UC Berkeley, with a groundbreaking conjugation technology. Catena's novel approach generates never-before possible therapeutic structures by enabling rapid, selective, scalable, and stable conjugation of proteins using only native amino acids. Catena's initial pipeline focus is first-in-class 'Multi-Payload Conjugates' called MPCsTM.

References:

- Lobba, et al. 2020. "Site-Specific Bioconjugation through Enzyme-Catalyzed Tyrosine-Cysteine Bond Formation." ACS Central Science 6 (9): 1564-71.
- Cao, W., Maza, J. C. et al. (2023). Modification of Cysteine-Substituted Antibodies Using Enzymatic Oxidative Coupling Reactions. Bioconjugate Chemistry, 34(3), 510-517.
- Maza, J. C., et al. (2022). "Tyrosinase-Mediated Synthesis of Nanobody-Cell Conjugates." ACS Central Science.