Next Generation Antibody Drug Conjugates:

Multi-Payload Conjugates targeting multiple mechanisms of cell killing

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Introduction

ADCs have had tremendous impact on patient outcomes and are now second line therapy for metastatic HER2 positive breast cancer. However, many patients fail to respond or relapse after treatment due to tumor heterogeneity and resistance to ADC payloads. Delivery of combination payloads via a single antibody could address this unmet need.

Our novel platform of Multi-Payload Conjugates (MPCs)™ with well-defined DARs has shown promising initial results.

We screened various combinations of payloads with different MOAs to optimize tumor cell killing, then optimized the DAR of each payload. These targeted combination ADCs demonstrated robust activity in both HER2 high and low expressing tumor cell lines *in vitro* and *in vivo*.

This approach has now been extended to include multiple antibodies showing the versatility of our conjugation platform. The ability to leverage multiple sites of attachment to produce optimal DAR for each payload has been shown to yield superior tumor regression across multiple cell lines when compared to current standards of care.

Current Challenges in ADCs

Over 80% of ADCs in the clinic today use *cysteine-based maleimide bonds* to attach payloads to antibodies. However, Maleimide bonds often undergo rapid degradation in blood serum, resulting in sub-optimal stability and most importantly, lack the ability to attach multiple kinds of payloads to the same antibody.

As a result, ADCs have remained effectively monotherapies in oncology where combination approaches have been established for decades as the norm.

CATENABIO	Maleimide	Trans-glutaminase	Artificial Amino Acids
Multiple Payloads	✓	X	X	–
Payloads of any Complexity/Size	✓	X	X	X
Scalability	✓	✓	–	X
Stability	✓	X	✓	✓

Introducing the CysTyr™ Platform

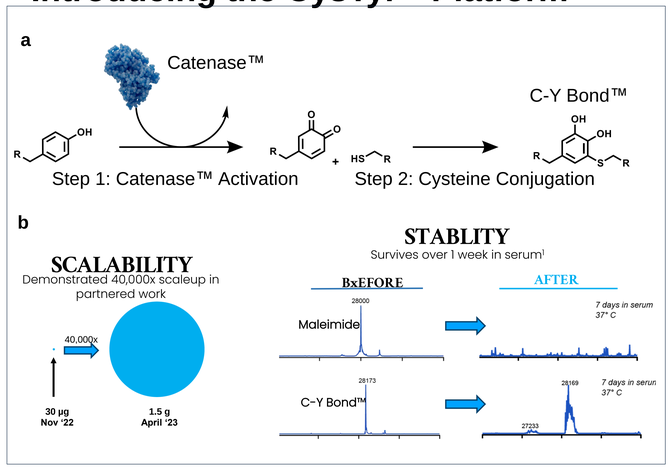

Figure 1: a) Catenase™ reaction scheme and b) Key performance metrics for Catenase ™ conjugations from previous work in model systems

HER2 MPC vs T-DXd in vivo

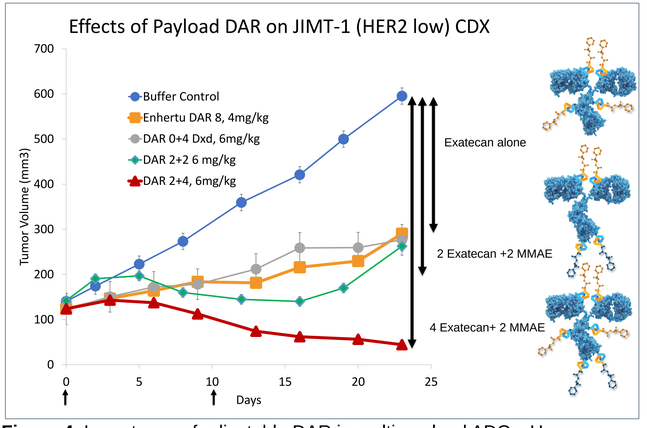

Figure 4: Importance of adjustable DAR in multi-payload ADCs. Here we show combined data from two separate studies where mouse xenograft models using JIMT-1 cells are treated (n=10/group, days 0 and 10) with MPCs of varying DAR. We show that optimal response is achieved with DAR 2+4 MPCs.

Catena's Differentiated Science

Catena's CysTyr™ platform with its novel C-Y Bond™ offers the revolutionary benefit of attaching two or more different payloads to the same antibody with tunable DAR, 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 Bond™[2]**, the CysTyr platform has potential to unlock new modalities in therapeutic design.

The C-Y Bond™ 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]**

HER2 MPCs and DAR 2+2

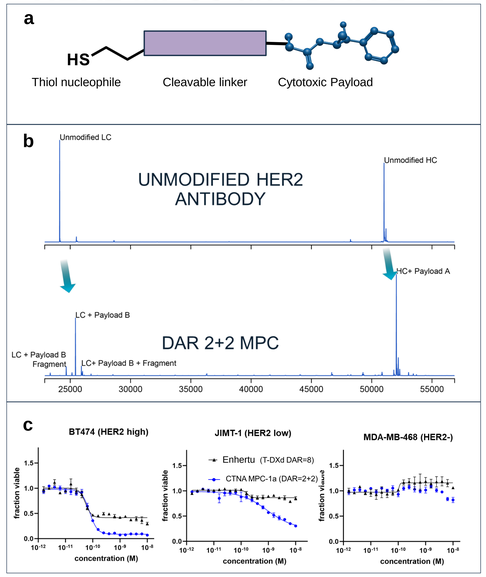

Figure 2: Conjugation of multiple cytotoxic payloads to the same antibody at a DAR of 2+2. a) representative diagram for active payloads containing a terminal thiol, cleavable linker, and active payload, b) LC-TOF Mass spectrum of starting antibody compared to final MPC after conjugation. Light chain mass shows over 93% conjugation of Tubulin inhibitor after reaction, while heavy chain after modification shows >95% modification of Topo1 inhibitor. c) in vitro activity assay on BT474, JIMT-1 and MDA-MB-468 cell lines comparing Enhertu (T-DXd, black) to Catena's MPC (blue)

TROP2 MPCs and DAR 4+2

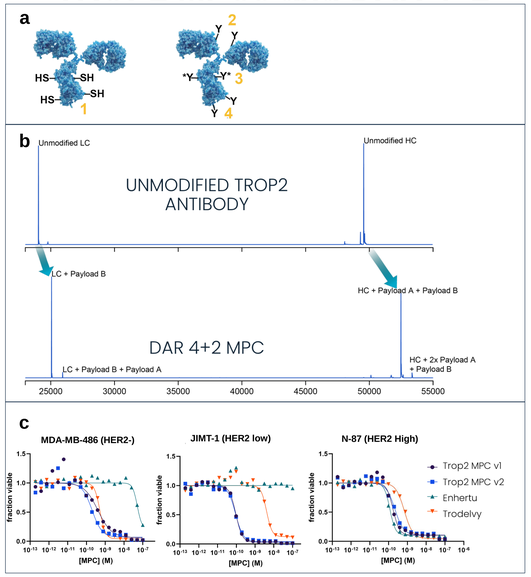

Figure 3: Conjugation of multiple cytotoxic payloads to the same antibody DAR 4+2. a)Illustration of the 4 unique reaction sites accessible through CysTyr™ conjugation: cysteine residues, heavy and light chain termini, and native loop tyrosines. b) LC-TOF Mass spectrum of starting antibody compared to final MPC after conjugation. Light chain mass shows over 93% conjugation of 2 Tubulin inhibitors after reaction, while heavy chain after modification shows >95% modification of 4 Topo1 inhibitors. c) in vitro activity assay on BT474, JIMT-1 and MDA-MB-468 cell lines comparing Enhertu (T-DXd, teal) and Trodelvy (orange) to Catena's MPC

Methods and Results

A library of MPCs™ 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 Catenase™ 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 T-DXd (Enhertu) and Trodelvy, Catena's HER2 and TROP2 targeted MPCs show significant increases in total inhibition vs standard of care (Figure 2c, Figure 3c).

Finally, murine models of breast cancer were established using the JIMT-1 (HER2 low) cell line. After tumors reached 100 mm^3 mice were dosed with either MPC™ or Enhertu (T-DXd) on day 0 and day 10 at the listed relative dosage(Figure 4). These results show that Catena's MPCs are highly effective despite containing half the relative payload of Enhertu.

Conclusions

- MPCs show superior efficacy in JIMT-1 tumor models compared to standard of care
- Catena has successfully produced a modular library of MPCs against various targets using the CysTyr ™ Conjugation platform
- MPCs™ tested in vivo and in vitro across multiple HER2+ cell lines showed improved efficacy, with tunable DAR leading to optimal performance
- The novel C-Y Bond™, using only naturally occurring amino acids confers enhanced stability in serum, which will potentially confer enhanced safety in addition to superior efficacy

Next Steps

- Advanced PK/tox studies
- Launch clinical development/ CMC with lead molecule(s)

References:

[1]Lobba, et al. 2020. "Site-Specific Bioconjugation through Enzyme-Catalyzed Tyrosine-Cysteine Bond Formation." ACS Central Science 6 (9): 1564–71.

[2]Cao, W., Maza, J. C. et al. (2023). Modification of Cysteine-Substituted Antibodies Using Enzymatic Oxidative Coupling Reactions. Bioconjugate Chemistry, 34(3), 510–517.

[3]Maza, J. C., et al. (2022). "Tyrosinase-Mediated Synthesis of Nanobody–Cell Conjugates." ACS Central Science .