Next Generation Antibody Drug Conjugates:

Multi-Payload Conjugates targeting multiple mechanisms of cell killing

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Introduction

Antibody-Drug-Conjugates (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.

CatenaBio's novel Multi-Payload Conjugates (MPCs)™ with well-defined DAR of each payload have shown groundbreaking early results.

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

This approach is now being applied to multiple additional antibodies demonstrating the versatility of the conjugation platform. The ability to leverage multiple sites of attachment to produce optimal DAR for each payload has been shown to yield significantly 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. Unfortunately, the maleimide approach cannot attach multiple kinds of payloads with defined DAR to the same antibody, moreover, they often undergo rapid degradation in blood serum, resulting in sub-optimal stability.

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	✓	✓

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Introducing the CysTyr™ Platform

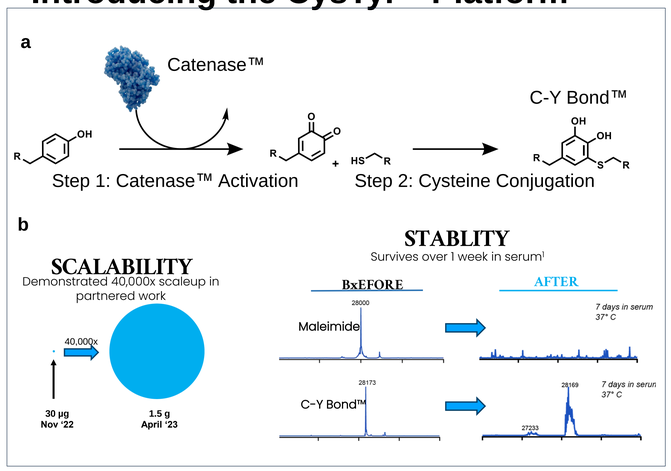

Figure 1: a) Catenase™ reaction scheme and b) Key performance metrics for Catenase ™ conjugations from previous work in model systems

TROP2 MPCs and DAR 4+2

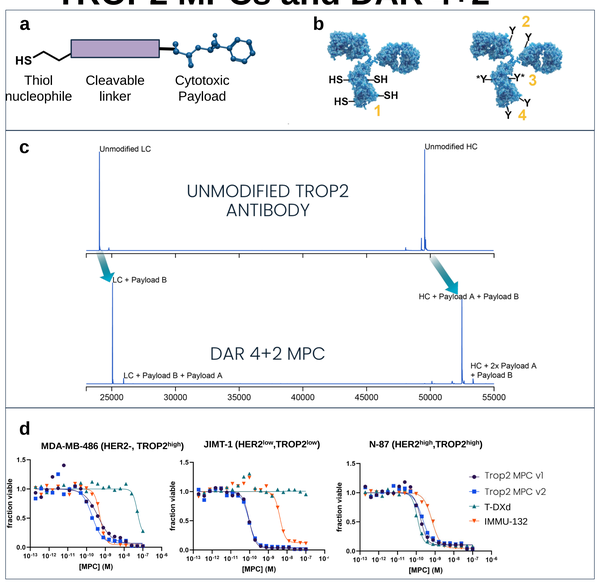

Figure 2: Conjugation of multiple cytotoxic payloads to the same antibody DAR 4+2. a) representative diagram for active payloads containing a terminal thiol, cleavable linker, and active payload b)Illustration of the 4 unique reaction sites accessible through CysTyr™ conjugation: cysteine residues, heavy and light chain termini, and native loop tyrosines. c) 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. d) in vitro activity assay on BT474, JIMT-1 and MDA-MB-468 cell lines comparing T-DXd (teal) and IMMU-132 (Sacituzumab govitecan, orange) to Catena's MPC

MPCs surpass T-DXd and Sacituzumab govitecan in vivo

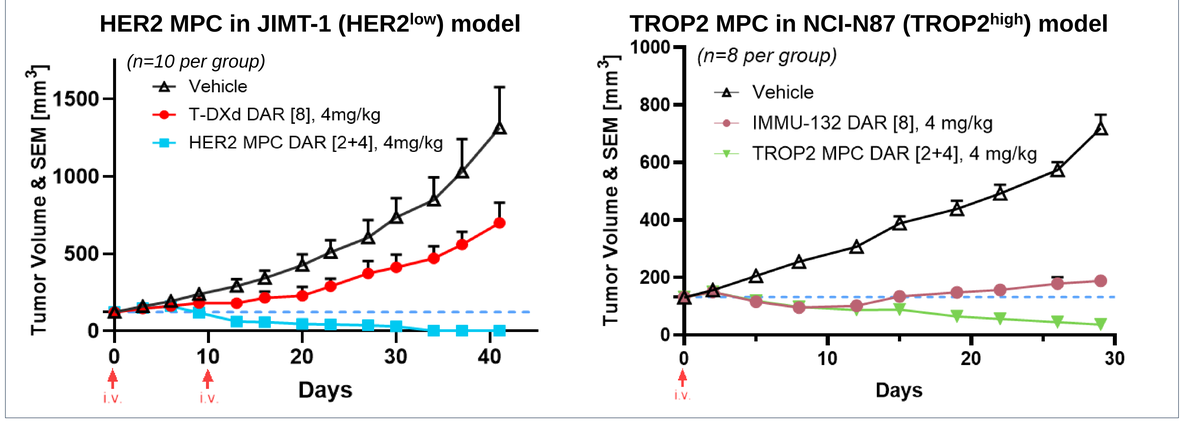

Figure 3: Comparison of Catena's MPCs T-DXd and Sacituzumab govitecan. MPCs combining Tubulin and Topo1 inhibitors were tested on xenograft models in SCID Beige mice. a) HER2 targeted MPC compared to trastuzumab Deruxtecan (T-DXd, or DS 8201a) in JIMT-1 xenograft models (HER2low n=10 mice per group). Mice were treated on days 0 and 10 via tail vein injection and tumors and bodyweight were measured for the duration of the study. T-DXd shows rapid tumor growth with DAR=8 of the Topo1 inhibitor, in contrast Catena's HER2-MPC shows full tumor elimination with DAR=2+4, combining tubulin and Topo1 inhibitors. b) TROP2 MPC compared to IMMU-132 (Sacituzumab govitecan) tested in NCI-N87 xenograft models (TROP2high, n=8 per group). Mice were treated on day 0 via tail vein injection and monitored for tumor volume and bodyweight. This study is still in progress.

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 2b) 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 (DS8201a) and Sacituzumab govitecan (IMMU-132), Catena's HER2 and TROP2 targeted MPCs show significant increases in total inhibition vs standard of care (Figure 2d).

Finally, murine models of breast cancer were established in SCID Beige mice using the JIMT1 (HER2low) or NCI-N87 (TROP2high) cell lines. After tumors reached 100 mm^3 mice were dosed with either MPC™ or the ADC of choice at the listed relative dosage(Figure 3). These results show that Catena's MPCs are highly effective despite containing half the relative payload of T-DXd.

Conclusions

- MPCs show superior efficacy in JIMT-1 and N-87 tumor models compared to T-DXd and Sacituzumab govitecan
- 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 significantly improved efficacy, with tunable DAR leading to optimal performance
- All studies to date demonstrate excellent tolerability as demonstrated by stable bodyweight across dosing levels

Next Steps

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

References:

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