Next Generation Antibody Drug Conjugates:

Multi-Payload Conjugates targeting multiple mechanisms of cell killing

Marco Lobba, Devin Trinter, Maxwell Nguyen, Daniel Gutierrez, Samantha Brady, Chanez Symister, Andrew Lau, Richard Kendall, Saurabh Johri, Matthew Francis*

Catena Biosciences, Inc. Berkeley, CA

*University of California, Berkeley

info@catenabio.com

Abstract PO5-27-10

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)®, 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. Multi-Payload Conjugates® offer the next step in ADC design and allow for the combination of multiple mechanisms of action in a single MPC. 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:

1. 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.
2. 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 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 CysTyr® Platform

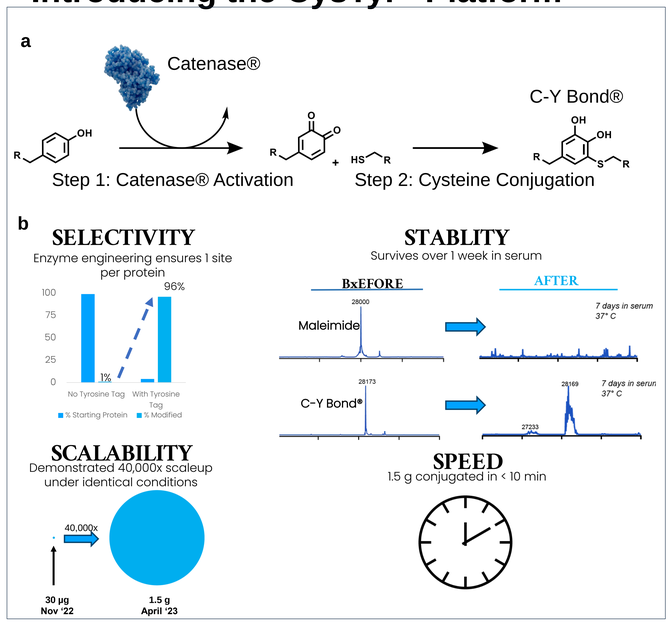

Figure 1: a) Catenase® reaction scheme and b) Key performance metrics for Catenase® conjugations

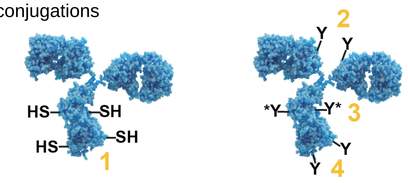

Figure 2: Illustration of the 4 unique reaction sites accessible through CysTyr® conjugation, 1) cysteine residues, 2,4) heavy and light chain termini, and 3) native loop tyrosines

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, 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.

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

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]**

Antibody Modification

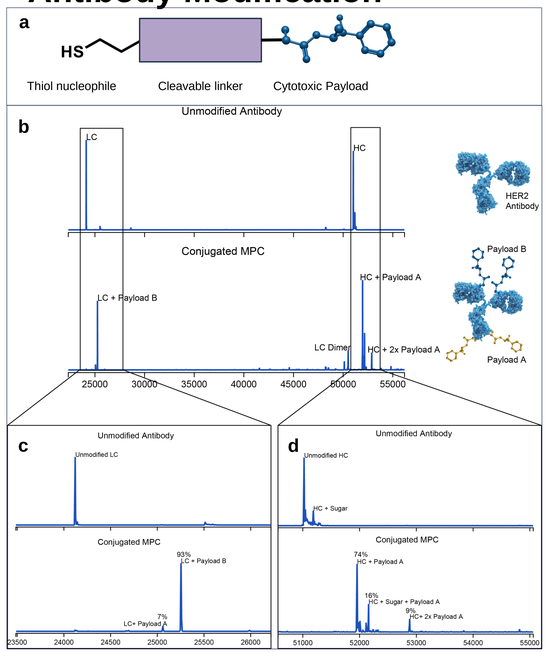

Figure 3: Conjugation of multiple cytotoxic payloads to the same antibody. a) representative diagram for active payloads containing a terminal thiol, cleavable linker, and active payload, b) LC-TOF Mass spectrum of starting antibody compared to final MPC after conjugation c) detailed view of light chain mass showing over 93% conjugation of desired payload after reaction, d) heavy chain after modification showing 99% conversion to +1 (90%) or +2 (9%) of payload A. The heavy chain contains 2 species based on the presence/ absence of a terminal sugar in the Fc region.

Methods and Results

A library of MPCs® was created through combinations of 5 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 (example spectra Figure 3) 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 (n=4, Figure 4). In head-to-head trials against Ds8201a (T-Dxd) these show significant increases in total inhibition. The greater stability of the C-Y Bond® and low, defined DAR of reaction offers the potential of incorporating higher potency payloads with differentiated MOAs to create more efficacious targeted therapies capable of bypassing common cancer resistance mechanisms.

In vitro comparison vs T-DXd

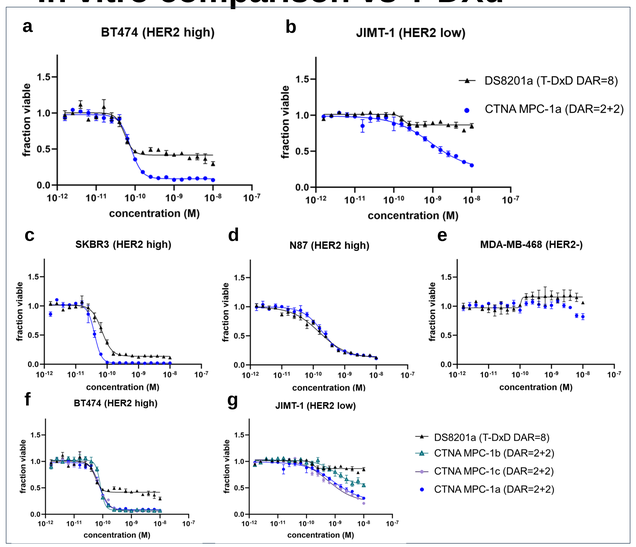

Figure 4: in vitro activity data for HER2 targeted MPC (blue) compared to DS8201a (T-DXd, black). Significant improvement in cell inhibition shown for high HER2 cell lines BT474 and SKBR3 (a, c) and low HER2 JIMT-1 (b) with equivalent activity in N87 cells (d). Both compounds show high selectivity through lack of inhibition in HER2 negative MDA-MB-468 cells (e). Multiple MPCs® compared against BT474 (f) and JIMT-1 cells (g) show similarly efficient cell killing, with slight differences attributed to the specific payload combinations. Further study will identify optimal payload combinations.

Conclusions

- Catena has successfully produced a modular library of MPCs using the CysTyr® Conjugation platform
- MPCs® tested in vitro across multiple HER2+ cell lines showed improved efficacy compared to DS8201a (T-DXd) the current standard of care
- 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

- in vivo activity and studies in resistant cell lines
- Generate additional combinations and positions including variable DAR (e.g. 4+2)

Company Overview

CatenaBio was spun 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 MPCs®.**

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 .