Rapid Empirical Screening of Antibody Payload Combinations via oYo-Link® Site-Specific Conjugation

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Introduction

Antibody-drug conjugates (ADCs) are an expanding class of targeted therapeutics with significant applications in cancer treatment. Their modular design allows them to be tailored to a wide range of cancer types.

The therapeutic efficacy of ADCs is governed by a complex interplay between the antibody, the cytotoxic payload, and the biological characteristics of the target cell. Because these interactions are often unpredictable based on binding affinity alone, identifying the most potent ADC configuration requires extensive empirical screening.

Given the many possible antibody-payload combinations, efficient methods are needed to prioritise the strongest candidates before advancing to pharmacokinetic and pharmacodynamic studies in animal models.

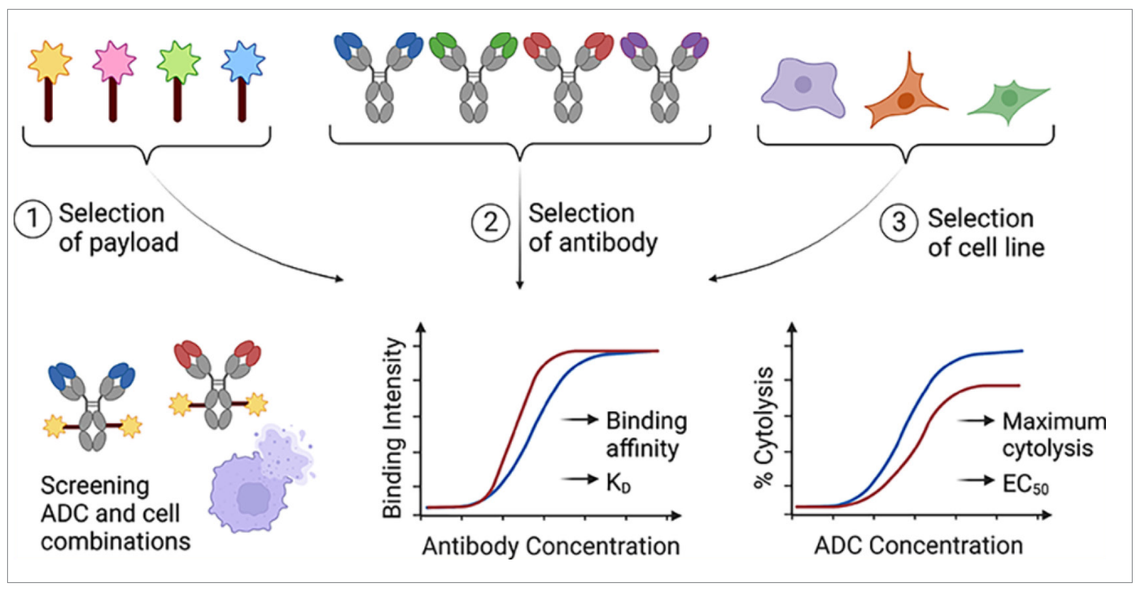


Figure 1. The process of identifying potent ADC configurations.

Method

In this study, we utilized oYo-Link® technology, a Light Activated Site-Specific Conjugation (LASIC) platform, to systematically evaluate a library of ADC variants. This approach allows for the rapid generation of ADCs with a drug-to-antibody ratio of ~2 with no subsequent purification required.

We conjugated four anti-EGFR antibodies (cetuximab, panitumumab, and clones 425 and 528) with four distinct tubulin-inhibitor payloads (DM1, DM4, VcMMAE, and VcMMAF) and screened them against three EGFR-expressing cancer cell lines.

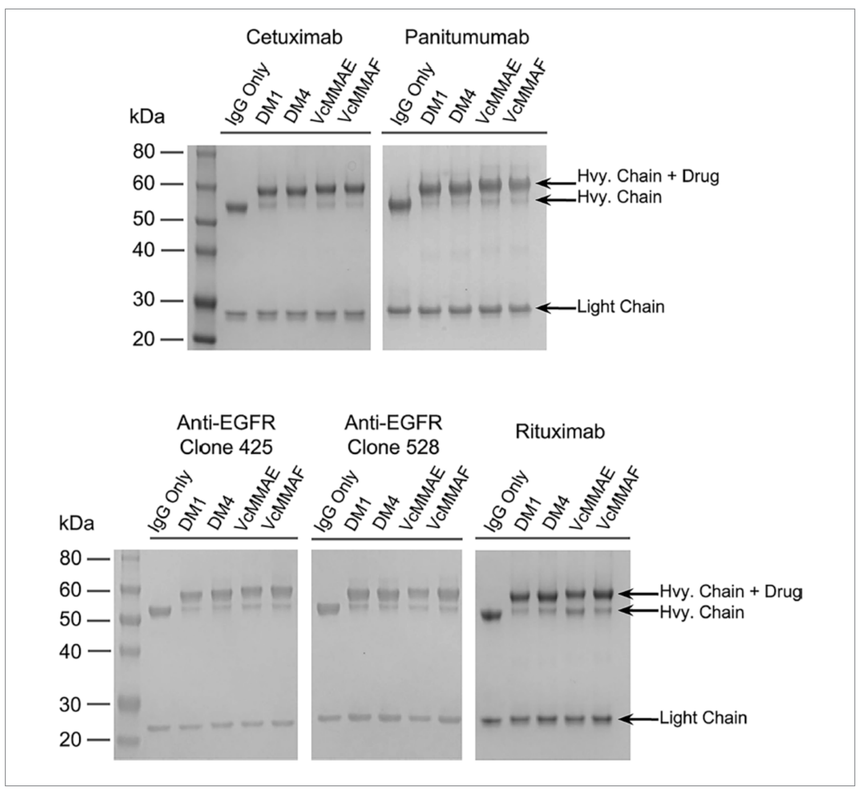


Figure 2. SDS-PAGE analysis of ADC cross-linking. Conjugation of oYo-Link to the Fc region produces an ~8 kDa upward shift in the antibody heavy chain. Effective cross-linking was achieved using antibody:oYo-Link ratios of 1 µg:1 µL (cetuximab, rituximab), 1 µg:2.5 µL (panitumumab), and 1 µg:3.5 µL (425, 528).

Results

Our results demonstrate that ADC potency is highly sensitive to the specific antibody-payload combination; for instance, lower-affinity antibodies often outperformed clinical benchmarks in specific resistant cell models.

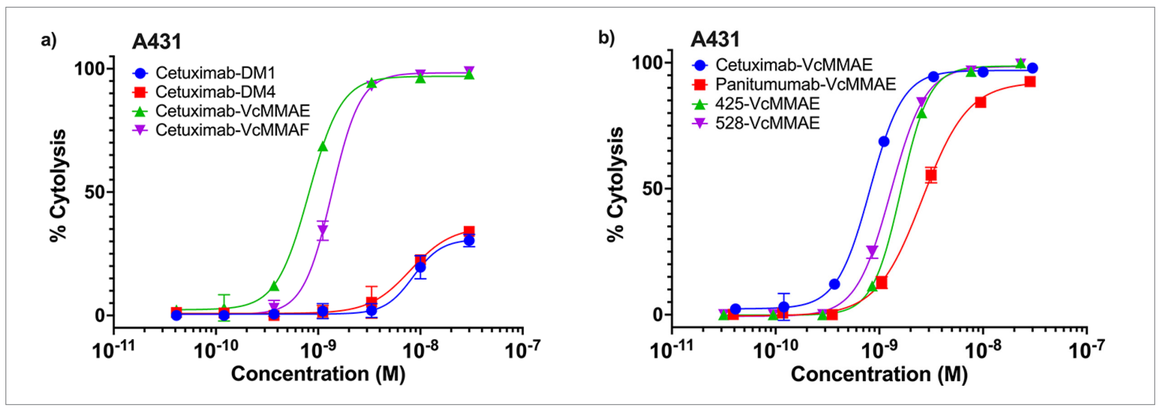


Figure 3. Dose-response curves from cytolysis assays comparing the efficacy of (a) different cytotoxic payloads cross-linked to cetuximab and (b) different anti-EGFR antibodies cross-linked to VcMMAE when delivered to A431 cells.

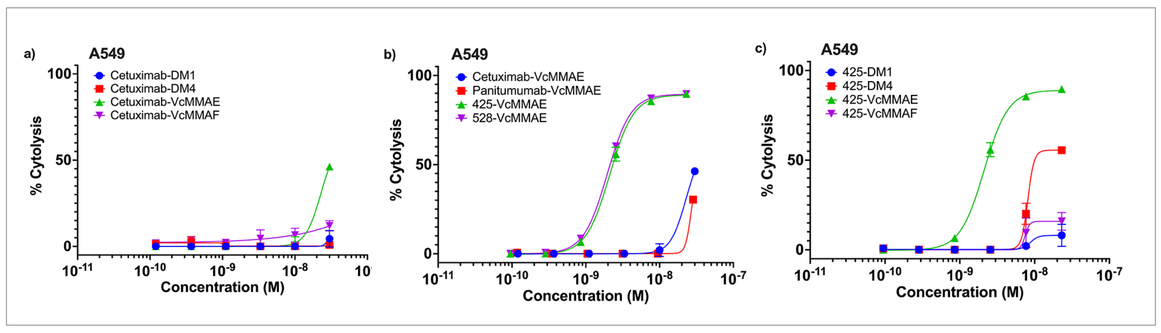


Figure 4. Dose–response curves from cytolysis assays comparing the efficacy of (a) different cytotoxic payloads cross-linked to cetuximab, (b) different anti-EGFR antibodies cross-linked to VcMMAE, and (c) different cytotoxic payloads cross-linked to 425 when delivered to A549 cells.

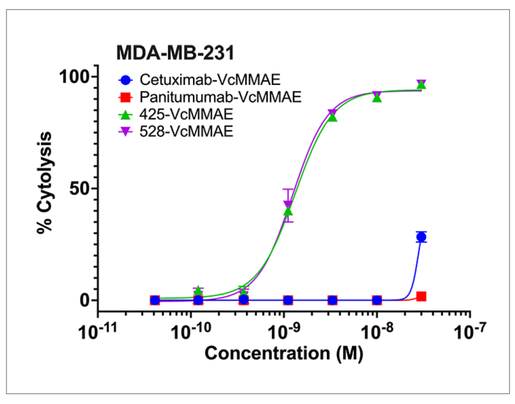
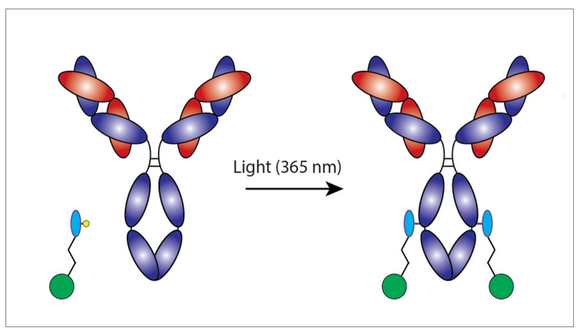


Figure 5. Dose–response curves from cytolysis assays comparing the efficacy of different anti-EGFR antibodies cross-linked to VcMMAE when delivered to MDA-MB-231 cells.

Discussion

Central to this high-throughput approach is the oYo-Link® platform, which provides several transformative advantages over traditional conjugation methods. By utilizing photoreactive antibody-binding domains, oYo-Link® enables the site-specific labeling of "off-the-shelf" native IgG antibodies without the need for complex protein engineering or sequence modifications. The conjugation process is exceptionally rapid and requires no subsequent purification, allowing for the efficient parallel production of uniform ADCs with a fixed drug-to-antibody ratio (DAR) of approximately 2. This uniformity ensures that differences in observed cytotoxicity are a direct result of the ADC composition rather than DAR heterogeneity.



By streamlining the transition from native antibody to precise conjugate, oYo-Link® facilitates the broad empirical screening necessary to identify optimal therapeutic candidates early in the drug development pipeline, ultimately accelerating the path toward more effective targeted biologics.

Acknowledgements

This work was supported in part by the NIH (NCI R01CA241661 and NCI R44CA221374).

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