Site-Specific Glycan Conjugation Enables Robust Antibody–Drug Conjugates

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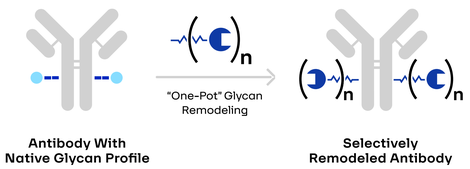
Abstract

Developing next-generation antibody–drug conjugates (ADCs) requires conjugation technologies that deliver precision, stability, and consistent performance across diverse payloads. Traditional cysteine-based methods often produce heterogeneous products with variable drug-to-antibody ratios (DARs) and increased aggregation, limiting reliability and efficacy. Glycan-directed conjugation is an enzymatic approach that remodels the conserved Fc glycan at Asn297 to introduce defined reactive handles for site-specific linker-payload attachment. This method preserves native antibody structure and function without disulfide reduction or protein engineering, achieving high conjugation efficiency, uniform DARs, and low aggregation.

Analytical characterization (SEC, HIC) confirmed improved monomer content and DAR homogeneity compared to maleimide-based conjugates. Glycan-conjugated ADCs demonstrated enhanced serum stability with minimal payload loss over seven days, while maintaining potent *in vitro* cytotoxicity and bystander activity.

These results establish glycan-directed conjugation as a robust, versatile, and high-performance platform for generating stable, homogeneous ADCs suitable for a wide range of antibody and payload combinations, supporting the development of next-generation therapeutics.

Glycan Remodeling



- Modifies the antibody's native Fc-region glycans to introduce precise payload attachment sites.
- Introduced sugars often contain reactive handles (e.g., azides, ketones) for selective conjugation.

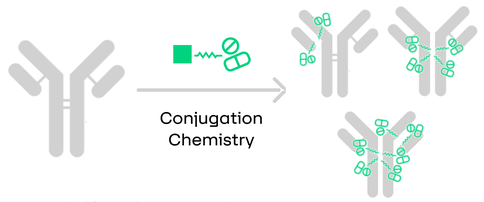
Advantages:

- Generates uniform, site-specific conjugation points leading to homogenous conjugates.
- Preserves antibody structure and antigen-binding activity.
- Enables formation of consistent and highly stable ADCs.

Conjugation Profile

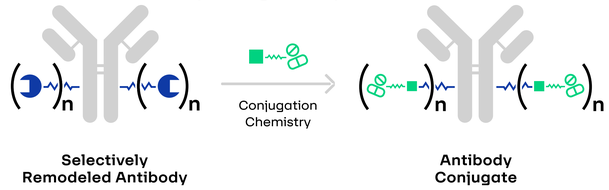
Cysteine–Maleimide Conjugation:

- Involves reduction of native disulfide bonds.
- Linker–payloads attach stochastically to exposed thiols.



Glycan Remodeling Approach:

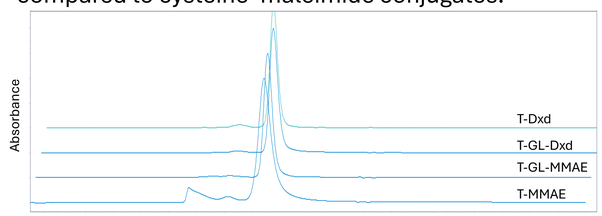
- Introduces specific reactive sites for controlled conjugation.
- Produces more homogeneous DARs controlled by the new glycan profile.
- Maintains correct pairing of bispecific antibodies.



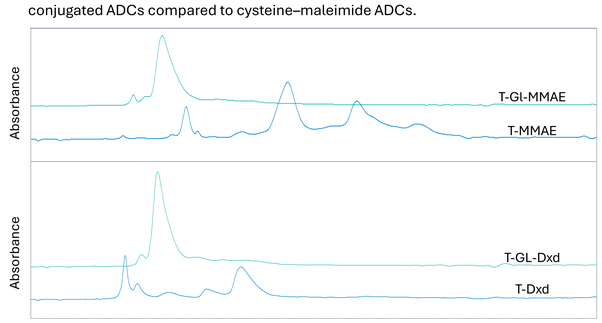
HPLC Profiling

Improved homogeneity and monomer content indicate enhanced conjugate stability and developability.

- Glycan conjugates exhibit a higher monomer percentage by SEC.
- HIC profiles of glycan conjugates are more homogeneous compared to cysteine–maleimide conjugates.



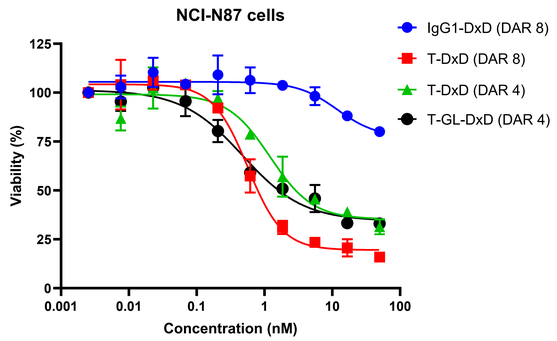
SEC comparison showing high percent monomer composition of glycan conjugated ADCs compared to cysteine–maleimide ADCs.

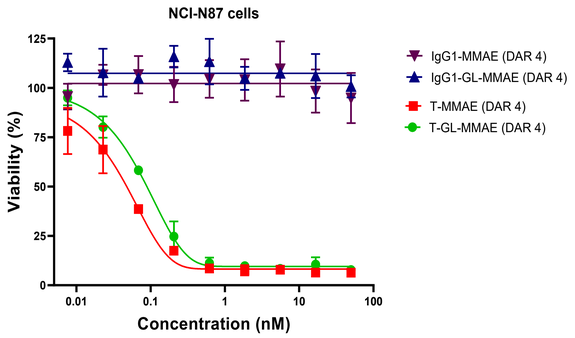


HIC comparison showing high homogeneity of glycan conjugated ADCs compared to cysteine–maleimide ADCs.

In vitro Studies

Glycan conjugates show comparable effects of glycan conjugation to cysteine-maleimide *in vitro* cytotoxicity assays.

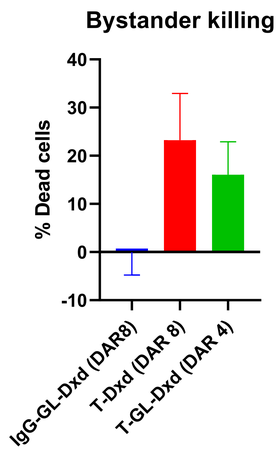




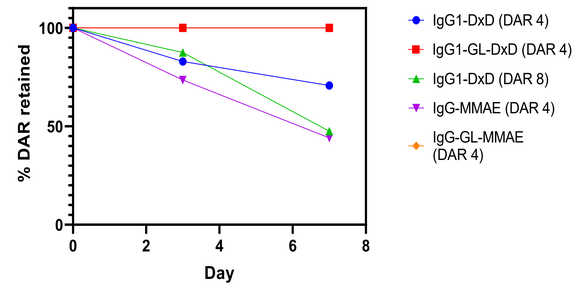
Bystander Effect

Bystander effects of Dxd containing ADCs see no significant effect.

HER2-positive NCI-N87 cells were co-cultured with HER2-negative MDA-MB-231 cells stably expressing a luciferase reporter at a 1:2 ratio. ADCs were added at a final concentration of 10 nM and incubated for 6 days. Luciferase activity from MDA-MB-231-luc cells was measured to assess bystander cytotoxicity.



Serum Stability



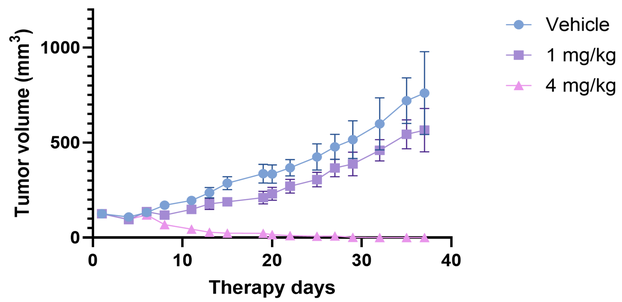
Stability Results (1-week study):

- Glycan-conjugated: minimal linker–payload loss
- Cysteine–maleimide: up to 50% linker–payload loss

Implications for Efficacy and Safety:

- Enhanced *in vivo* stability
- Reduced potential for off-target toxicity

In vivo



In vivo evaluation of glycan site-specific ADCs (DAR 4). Dose response of Exatecan ADCs were administered once at day at 1mg/kg and 4 mg/kg into mice with NCI-N87 tumors. Data points are presented as means ± SD

Conclusion

Glycan-directed conjugation preserves antibody structure and generates homogeneous ADCs with enhanced stability and consistent DAR control. Compared to cysteine–maleimide chemistry, this approach reduces linker-payload loss and improves serum stability, translating to better manufacturability and safety profiles.

These findings establish glycan remodeling as a robust, scalable platform for next-generation ADC design, supporting efficient process development and cost-effective GMP translation.