GlycoConnect™ ADC toolbox expansion with high DAR technology

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Introduction

In recent years topoisomerase 1 inhibitors (TOP1i) have gained significant attention as cytotoxic payloads for ADCs based on the clinical success of camptothecin-based ADCs trastuzumab deruxtecan (DXd) and sacituzumab govitecan (SN-38). In contrast to the majority of other approved ADCs, both trastuzumab deruxtecan and sacituzumab govitecan are featured with an average of 7–8 payloads per antibody. Obviously, such a high drug-to-antibody ratio (DAR) effectively delivers more drug to the tumor per internalization event, which is key in driving the clinical success of these ADCs with payloads of relatively mediocre potency.

Following these observations, we developed a straightforward approach to high DAR ADCs, with complete control of payload loading, by a simple extension of our clinical-stage ADC technology (GlycoConnect™)[1] and polar spacer technology (HydraSpace®)[2].

We here exhibit the stability and modularity of the new technology by assessment of efficacy and tolerability of a set of DAR8 ADCs based on TOP1i exatecan, as well as other payloads of lower potency.

GlycoConnect™ and HydraSpace® Technologies

We have reported[1] that chemoenzymatic attachment of payloads to the antibody glycan (GlycoConnect™ technology) affords stable, homogeneous ADCs with tailored drug-to-antibody ratio (DAR) and excellent therapeutic index. Various ADC programs are currently progressing through the clinic, including ADCT-601 and ADCT-701 (DAR2), XMT-1660 (DAR6), MRG004A (DAR4) and IBI343 (DAR4).

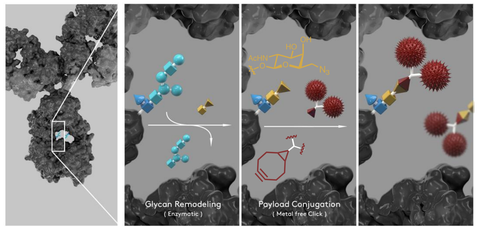


Figure 1. GlycoConnect™ technology: Two-stage approach to ADCs by (a) enzymatic glycan trimming & transfer of azidosugar, and (b) metal-free click attachment of BCN-modified linker-drug.

Incorporation of a short and polar spacer moiety (HydraSpace®) enables ADCs with further enhanced TI.[2]

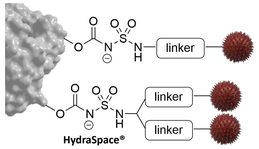


Figure 2. HydraSpace® technology, based on acylated sulfamide, due to its ionic nature at physiological pH, leads to improved manufacturability, efficacy and safety (data not shown).

[1] (a) van Geel *et al. Bioconj. Chem.* **2015**, *26*, 2233–2242. (b) Wijdeven *et al. Mabs* **2022**, 14:1, doi:10.1080/19420862.2022.2078466.
[2] Verkade *et al. Antibodies* **2018**, *7*, 12, doi:10.3390/antib7010012.

toxSYN® Platform

In order to match the ADC payload with the anticipated clinical indication, a toxSYN® platform is offered alongside GlycoConnect™ and HydraSpace® technologies, which comprises cytotoxic molecules that span MOAs from microtubule inhibition (MMAE, MMAF, maytansinoid) to DNA-damaging (based on calicheamicin or PNU) to topoisomerase 1 inhibition (exatecan).

1	SYNtecan E™	Topoisomerase 1 inhibitor	Camptothecin-based
2 3	SYNeamicin D™ SYNeamicin G™	DNA damaging agent	Calicheamicin-based
4	SYN-PNU™	DNA damaging agent	Nemorubicin-based
5 6	SYNstatin E™ SYNstatin F™	Microtubule inhibitors	Auristatin-based
7	SYNtansine™	Microtubule inhibitor	Maytansine-based

While the above represent the most commonly employed payloads in marketed ADCs, we are evaluating antibody-oligonucleotide conjugates and antibody-cytokine conjugates (based on IL-15) for immuno-oncology application (see poster 4077). Besides, we are actively expanding the toxSYN® repertoire with other well-validated cytotoxic payloads, including PBD dimers.

DAR Modularity Depending on Choice of Payload

GlycoConnect™ and HydraSpace® technologies enable drug-to-antibody ratios (DAR) of 2 or 4, most typically employed in clinical ADCs. However, for ADCs with ultra-potent payloads (*e.g.* calicheamicin, amanitin) the expected clinical dose is <<1 mg/kg, which compromises optimal biodistribution. In such case, a DAR1 format could potentially be preferred. See Figure 3 for illustrations of the different DAR species.

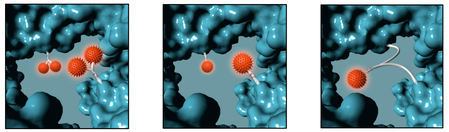


Figure 3. Illustration of DAR4, DAR2 and a DAR1 ADCs based on GlycoConnect™ technology.

In contrast to the majority of other approved ADCs, both trastuzumab deruxtecan and sacituzumab govitecan feature an average of 7–8 payloads per antibody. We recently developed a DAR8 technology (Figure 4) by reacting enzymatically remodeled antibody glycans with an orthogonal linker, which can subsequently be connected via an ultra-fast click reaction with any payload of choice of a branched linker-payload construct.

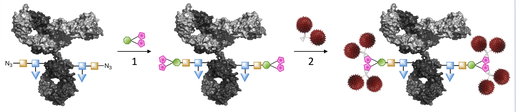


Figure 4. Two-stage strategy for a modular DAR8 approach. After remodeling, an orthogonal linker (1) is conjugated followed by linker-payload addition (2).

High DAR Technology by Modular Approach

We synthesized and evaluated branched spacers for initial reaction with with azido-modified antibody, consisting of an azide-reactive click moiety and (at least) two azide-compatible click probes for subsequent reaction with a BCN-based linker-payload. The modularity of the approach allows for a plurality of azido-orthogonal groups in the spacer, for combination with either a linear or a branched BCN-linker-payload, to generate ADCs with a DAR of 6, 8 or 12 (Figures 4 and 5).

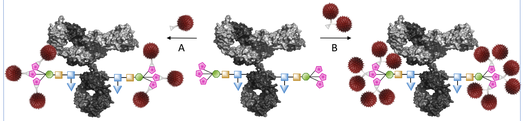


Figure 5. Schematic representation of the orthogonal linker. Depending on the number of orthogonal groups attached to the antibody in the first step, the linkage of a branched or a linear linker payload in the second step can generate DAR6, DAR8 or DAR12.

In vitro cytotoxicity assessment with NCI-N87 cells with a model linker-payload (LP) showed a clear DAR-dependent response (Figure 6).

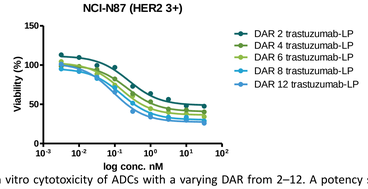
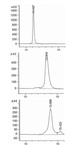


Figure 6. In vitro cytotoxicity of ADCs with a varying DAR from 2–12. A potency shift (IC50) for each DAR species is observed. LP = linker-payload (undisclosed).

Optimal Characteristics and Stability of High DAR ADCs

Based on the above strategy, an exatecan-based DAR8 ADC was generated and compared to trastuzumab-deruxtecan in terms of polarity and stability. HIC indicate a smaller relative retention time for GlycoConnect™ DAR8 with exatecan versus trastuzumab-deruxtecan.



Construct	Relative retention time
trastuzumab	1.00
trastuzumab-exatecan	1.20
trastuzumab-deruxtecan	1.25

Figure 7. HIC profiles of DAR8 exatecan-based ADC compared to naked antibody and trastuzumab-deruxtecan.

Stability of the GlycoConnect™ exatecan-based DAR8 ADC was corroborated versus trastuzumab-deruxtecan (Figure 8).

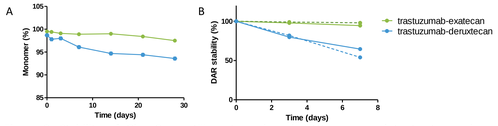


Figure 8. Stability of GlycoConnect™ exatecan-based ADCs compared to trastuzumab-deruxtecan over time in physiological conditions (A) and in either mouse (dotted line) or human (solid line) plasma over time (B).

In Vivo Evaluation of GlycoConnect™ DAR8 ADCs

Trastuzumab-based DAR8 and DAR4 ADCs were compared head-to-head in syngeneic mice grafted with hHER2-transfected EMT6 tumor cell line (QW*2, Figure 9), showing near identical efficacy at equal payload dose.

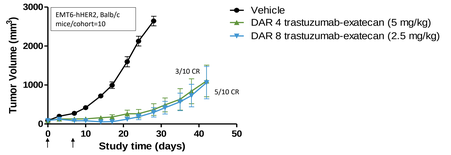


Figure 9. Ability to induce tumor regression of DAR4 and DAR8 trastuzumab-based ADCs at equal payload dose levels.

A second evaluation in the same model (QW*2, Figure 10) indicated a superior performance of the trastuzumab-exatecan DAR8 ADC versus DAR8 trastuzumab-deruxtecan at equal dose levels (2 mg/kg). Complete tumor regression could be achieved for GlycoConnect™ DAR8 ADC with exatecan payload at a dose level of 6 mg/kg.

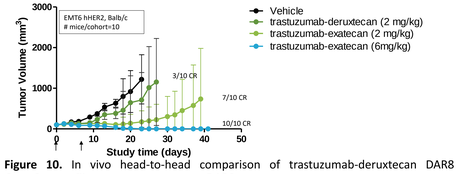


Figure 10. In vivo head-to-head comparison of trastuzumab-deruxtecan DAR8 with GlycoConnect™ trastuzumab-exatecan DAR8.

In Vitro Evaluation of Novel Payloads

Following exatecan, we produced DAR8 ADCs of various payloads non-typical for ADC application. ADCs and the corresponding free payloads were tested in vitro on HER2-positive (BT-474) and HER2-negative (MDA-MB-231) cells (Figure 11).

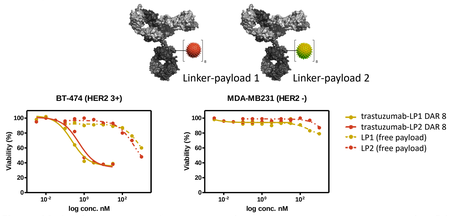


Figure 11. In vitro potency of two non-typical payloads and the corresponding DAR8 ADCs.

The novel payloads demonstrate low micromolar potency, whereas the corresponding ADCs showed a decrease in IC_{50} to sub-nanomolar potency when incubated with HER2-positive cells. The HER2-negative cell line showed that this efficacy was target-selective since only negligible cell killing was observed even at the highest ADC concentration.

Tolerability of DAR8 ADCs

Trastuzumab-exatecan was evaluated for tolerability in CD-1 mice (Figure 12). Single dose escalation was performed starting at 60 mg/kg, and increasing to 80 and even 100 mg/kg.

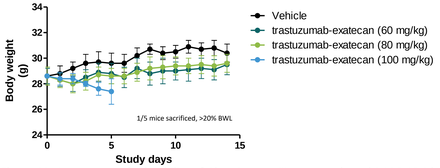


Figure 12. Assessment of tolerability of GlycoConnect™ exatecan-based DAR8 ADC by body weight in CD-1 mice (single dose, 5 mice/group).

Based on body weight, an MTD of 80 mg/kg was determined for DAR8 trastuzumab-exatecan, which corresponds closely, in terms of correction for payload dose, to previous studies with DAR4 trastuzumab-exatecan (MTD of 160 mg/kg). In the CDX model tested (Figure 9) it was shown that the same efficacy was reached using the same payload dose level (and hence double dosing level for DAR4 compared to DAR8), thereby indicating the TI of the DAR8 ADC to be highly similar to that of the DAR4 ADC which was already proven to be efficacious and safe: clinical evaluation of IBI343 indicated promising response in patients and the MTD was not reached yet at 10 mg/kg.

Taken together, next to the excellent stability of the DAR8 ADCs, the in vivo studies showed a durable response at low dose levels and a promising safety profile.

Conclusions

- GlycoConnect™ & HydraSpace® technologies enable attachment of any linker-payload to the antibody glycan
- Payload loading can now be increased to DAR8, thereby enabling full tailoring of payload loading to its potency (DAR1–DAR8)
- Favorable HIC profile and stability of DAR8 ADCs
- Efficacy and tolerability of DAR8 ADCs with exatecan is equal to DAR4 ADC based on same payload dose
- Potency assessment of the newly developed high DAR ADC technology broadens the repertoire of payloads with mediocre potency

About Synaffix

Synaffix BV is a clinical-stage biotechnology company with best-in-class antibody conjugation technology. The business model comprises technology out-licensing of the IP portfolio, with granted claims that provide end-to-end patent protection through at least 2035. Synaffix has entered into target-specific license agreements with ADC Therapeutics, Mersana Therapeutics, J&J, Shanghai Miracogen, Innovent, ProfoundBio, Kyowa Kirin, Genmab, MacroGenics, Amgen, Hummingbird CKD, ABL Bio and Sotio. Five GlycoConnect™ ADCs have entered the clinic, with up to 20 ADCs rapidly advancing through preclinical development.

For more information, contact bd@synaffix.com

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