Highly potent, bispecific ADCs by application of clinical stage GlycoConnect® technology to bYlok® antibodies

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Introduction

Chemotherapy continues to suffer from poor Therapeutic Index (TI). Even for next-generation targeted therapies, drugs are generally still administered at the maximum tolerated dose. Antibody-drug conjugates (ADCs) are partially solving this problem. One way to achieve better targeting of ADCs is by employing bispecific antibodies as delivery vehicle: by targeting two (independently) upregulated receptors on tumor cells instead of one, better selective tumor uptake can be achieved, thereby having the potential to increase the TI.

We here exhibit the extension of our clinical-stage ADC technology (GlycoConnect®)[1] and polar spacer technology (HydraSpace®)[2] into the field of bispecifics. By combining the bYlok® bispecific pairing technology and GlycoConnect® technology we enable a fast and straightforward path towards best-in-class bispecific ADCs.

bYlok® bispecific antibodies

The number of bispecific molecules entering the clinic is still growing, as they afford many potential clinical benefits. However, bispecific antibodies can be a challenge to make. The main challenge is correct pairing of the heavy and light chains, as a theoretical yield of correctly paired bispecifics is only 12.5%.

The knobs-into-holes technology (KIH) provides a partial solution to the mispairing challenge and increases the theoretical yield of correctly paired species to 25%. By combining Lonza's bYlok® technology with KIH, the mispairing challenge might be solved (Figure 1).

Heterogeneous mixture of very similar species	Partial solution: Knob-into-Hole Technology	Complete solution: bYlok® technology

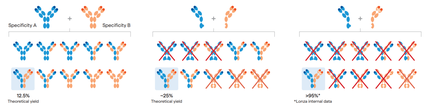

Figure 1. How bYlok® overcomes the limitations of existing bispecific pairing solutions.

In Lonza's bYlok® technology a disulfide bridge from the constant domain CH1/CL interface is moved to the variable region VH/VL interface. This minor change drives the formation of a heterodimer. bYlok® molecules are similar in structure to natural antibodies and can be purified using standard downstream processing method. The minor structural modification also reduces the predicted immunogenicity risk.

For the assessment of bYlok® GlycoConnect® ADCs, we first produced all possible bispecific variants of trastuzumab and panitumumab, see Figure 2. Analysis of all species, based on functionality, chain assembly, IgG purity and size variant profile suggested the best molecule to proceed with

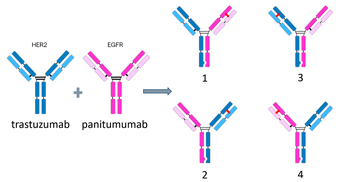

Figure 2. Set of four different combinations possible employing bYlok® technology on trastuzumab and panitumumab

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-701, XMT-1660, MRG004A, MGC026 and IBI343.

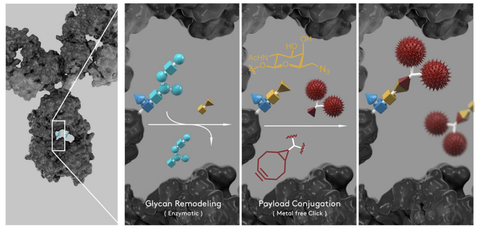

Figure 3. GlycoConnect® technology: Two-stage approach to ADCs by (a) enzymatic glycan trimming & transfer of azidosugar, and (b) metal-free click attachment of BCN-modified linker-drug.

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

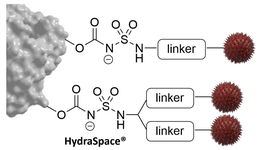

Figure 4. HydraSpace® technology, based on acylated sulfamide, due to its ionic nature at physiological pH, leads to improved manufacturability, efficacy and safety (data not shown).

[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 modes of action (MOAs) from microtubule inhibition (MMAE, MMAF, maytansinoid) to DNA-damaging (based on calicheamicin, PBD or PNU) to topoisomerase 1 inhibition (exatecan).

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

While the above represent the most commonly employed payloads in marketed ADCs, we are evaluating pro-PBD conjugates (Poster #334).

Efficient Remodeling and Conjugation of bYlok® bispecific

Remodeling of the bispecific containing one heavy chain and light chain from panitumumab and from trastuzumab was successful (shift observed in MS, Figure 5).

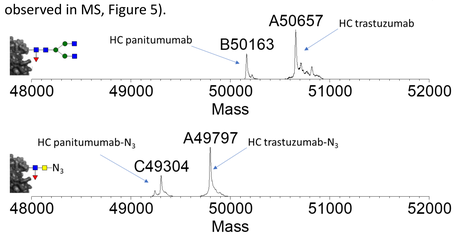

Figure 5. MS based analysis showed the expected mass change after trimming of the glycan (mostly G0F) and the addition of the azidosugar. The light chain did not change, data not shown.

The conjugation was performed with either SYNstatin E™ or SYNtecan E™. Standard conjugation conditions, as for monospecific antibodies, was applied to the bYlok® molecules. Yields and purity obtained were equal to conventional antibodies. DTT reduction before RP-UPLC measurement resulted in clear separation of all the peaks (Figure 6A). Conjugation with either SYNstatin E™ or SYNtecan E™ resulted in a shift and DAR of 3.7 for both linker payloads (Figure 6B and C).

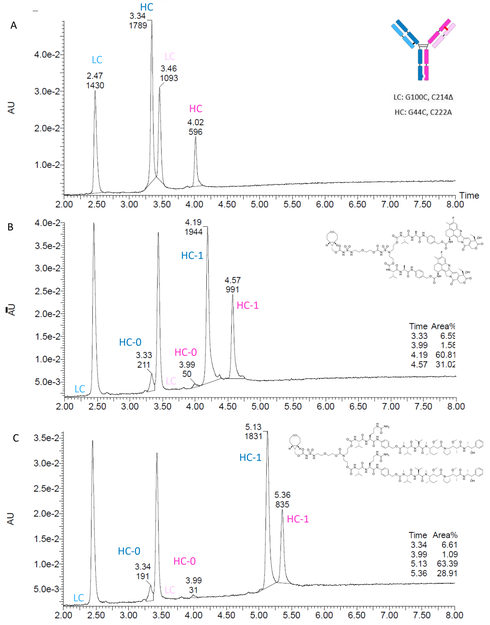

Figure 6. RP-UPLC spectra of bYlok® bispecific antibody (A) and conjugates with SYNtecan E™ (B) and SYNstatin E™ (C).

In Vivo Evaluation of bYlok® ADCs with SYNtecan E™

Two CDX cell lines, with medium expression of EGFR and HER2, were selected for in vivo efficacy studies. In both studies, dose-dependent tumor growth inhibition by the bispecific ADCs after single dose was observed. Model NCI-H358 showed tumor regression at a doses of 3 and 10 mg/kg (Figure 7A). In model A549, growth inhibition was observed with the highest dose level (Figure 7B).

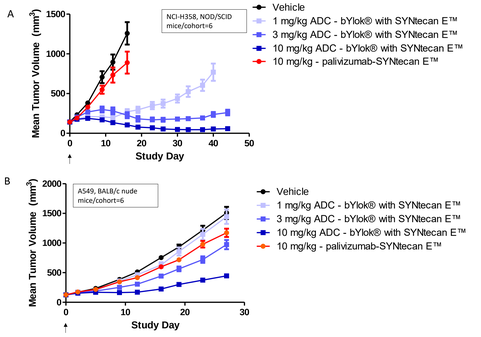

Figure 7. In vivo efficacy of bYlok® ADCs with SYNtecan E™ in CDX model NCI-H358 (A) and A549 (B) after single dose treatment.

In Vitro Evaluation of bYlok® ADCs with SYNstatin E™

In vitro evaluation was performed with a set of five different cell lines. These cell lines either have a high EGFR expression level or a high HER2 expression level, medium expression of both, or no expression for both targets (Table 1)

	EGFR expression level	HER2 expression level
Cell line	PE molecules/cell	
NCI-N87	27044	1567873
NCI-H520	125	122
NCI-H358	21008	24433
A549	46549	11199
A431	940209	33127

Table 1. Target expression level of five cell lines determined by FACS analysis.

In vitro toxicity assay with bYlok® with SYNstatin E™ showed varying IC50 and max cell killing based on the target expression of either EGFR and HER2 (Figure 8A). As a negative control for targeting, palivizumab-SYNstatin E™ was incubated with the same cell lines and showed hardly any toxicity (Figure 8B). As a positive control, cisplatin showed a similar toxicity to all cell lines, independent of target expression levels (Figure 8C).

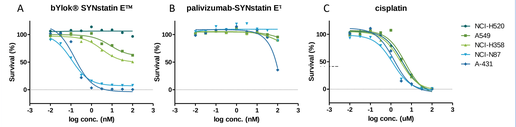

Figure 8. In vitro cellular cytotoxicity of bYlok® with SYNstatin E™ (A), palivizumab-SYNstatin E™ (B) and cisplatin (C).

In Vivo Evaluation of bYlok® ADCs with SYNstatin E™

The same two cell lines were used to test ADCs generated using the bYlok® technology in combination with SYNstatin E™ in vivo. Again a clear dose response relation was observed and due to higher potency of SYNstatin E™ compared to SYNtecan E™ a better efficacy was obtained at all dose levels. In model NCI-H358 tumor regression was observed up to the lowest dose (Figure 9A) and model A549 showed stasis and growth inhibiton for resp. 10 and 3 mg/kg (Figure 9B).

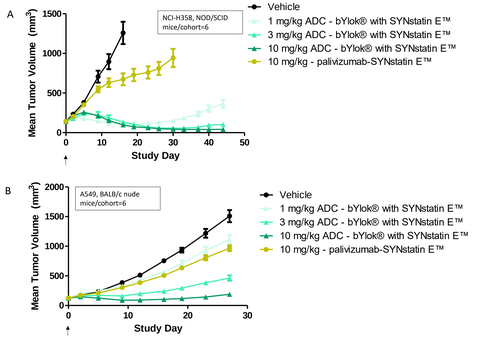

Figure 9. In vivo efficacy of bYlok® ADCs with SYNstatin E™ in CDX model NCI-H358 (A) and A549 (B) after single dose treatment.

Conclusions

- ADCs generated using the bYlok® technology were successfully produced by combining with GlycoConnect® technology
- Antibodies generated using the bYlok® technology show good in process stability
- Good overall characteristics in terms of DAR, monomer level and yield (comparable to conventional antibodies)
- In vitro and in vivo potency was demonstrated for ADCs containing either SYNstatin E™ or SYNtecan E™

About Synaffix

Synaffix BV is a biotechnology company with best-in-class antibody conjugation technology. Synaffix holds granted patents to its technology. The business model of Synaffix is target-specific technology out-licensing, as exemplified through its partnered pipeline. Synaffix has entered into target-specific license agreements with Mersana Therapeutics, J&J, Lepu Biopharma), Innovent, Kyowa Kirin, Kivu, Genmab, MacroGenics, Amgen, Hummingbird, CKD, Elevation Oncology, ABL Bio, Sotio, Boehringer Ingelheim, BigHat and Mitsubishi Tanabe Pharma. Our partners have moved five GlycoConnect® ADCs into the clinic, with up to 20 ADCs rapidly advancing through preclinical development.

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