First-in-Class Non-Genetic Generation of DAR1 ADCs for Application with Ultra-Potent Payloads and Immune Cell Engagers

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Introduction

Conjugation of toxic payloads by chemoenzymatic remodeling–conjugation of the antibody native glycan (GlycoConnect™), combined with a highly polar spacer technology (HydraSpace™) provides ADCs with tailored DAR2 or DAR4 and with a higher therapeutic index (TI) versus mainstream technologies.[1,2] Key quality attributes include high linker stability and polarity, low ADC aggregation and enhanced solid tumor penetration. The native glycan as attachment site also contributes to the superiority of GlycoConnect™ ADCs: (a) no binding to Fc-γ receptors; (b) increased stability by shielding of the (hydrophobic) payload and (c) preventing systemic proteolysis of peptide-cleavable linkers.

Here we show that GlycoConnect™ technology can be readily extended to DAR1 ADCs for application with ultra-potent payloads. In addition, attachment of small protein formats to the antibody enables the generation of T cell and NK cell-engaging bispecific antibodies with tailored molecular format for application in immunotherapy.

GlycoConnect™ Technology

Chemoenzymatic attachment of toxic payloads to the globally conserved antibody glycan affords homogeneous and stable ADCs in high efficiency without requiring genetic engineering (GlycoConnect™ technology).[1]

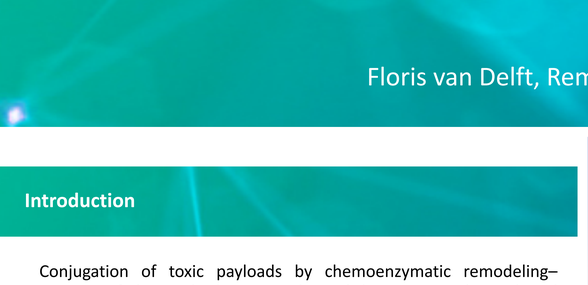


Figure 1. GlycoConnect™ technology: one-pot enzymatic glycan trimming and transfer of an azidosugar affords azido-modified antibody. Conjugation of payload by metal-free click chemistry with BCN-modified linker-drug affords ADCs with tailored DAR4 (depicted) or DAR2.

HydraSpace™ Technology

Incorporation of a short and highly polar spacer moiety (HydraSpace™ technology) enables ADCs with highly hydrophobic payloads and with tailored stoichiometry (DAR2 or DAR4).[2]

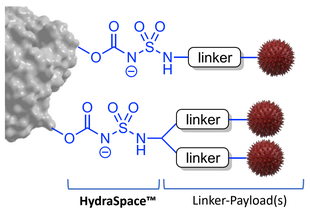


Figure 2. HydraSpace™ technology, based on acylated sulfamide, enables tailored DAR2 or DAR4 ADCs and due to its ionic nature at physiological pH leads to improved manufacturability, efficacy and safety (data not shown).

[1]Van Geel *et al*. *Bioconj. Chem.* **2015**, *26*, 2233–2242
[2]Verkade *et al. Antibodies* **2018**, *7*, doi:10.3390/antib7010012

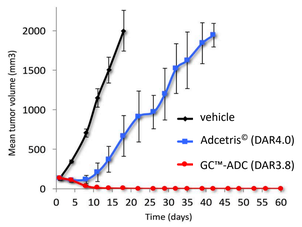
toxSYN™ Platform

The toxSYN™ platform comprises ADC payloads that span multiple MOAs, most recently expanded with topoisomerase inhibitors based on DXd (SYNtecan D™) and exatecan (SYNtecan E™). Each toxSYN™ linker-payload is functionalized for conjugation using GlycoConnect™ and HydraSpace™ technologies, readily tailored to various DAR formats.

SYNeamicin G™	Calicheamicin-based (DNA damaging agent)
SYNtecan D™ SYNtecan E™	Camptothecin-based (DNA topoisomerase 1 inhibitor)
SYN-PNU™	PNU-159,682-based (DNA damaging agent)
SYNstatin E™ SYNstatin F™	Auristatin-based (microtubule inhibitors)
SYNtansine™	Maytansine-based (microtubule inhibitor)
SYN-38™	SN-38-based (DNA topoisomerase 1 inhibitor)

Better than Adcetris®

An exemplary in vivo benchmarking study of a GC/HS™ ADC versus Adcetris® in rodents, based on same components brentuximab-vcPABC-MMAE and with same DAR, indicates that both efficacy and safety are significantly improved (Figure 3). A three-fold increase in HNSTD (3 ⟶ 9 mpk) was noted in cynomolgus monkey (data not shown).



Dose (mg/kg)	Adcetris® (DAR4.0)	GC-ADC (DAR3.8)
70	Not Tested	X
60		MTD
40		No DLTs
20	X	
15	MTD	

Figure 3. (left) Single dose administration of DAR4 GC-ADC (brentuximab-MMAE) or Adcetris® to Karpas-299 CDX mice (n = 7) at 1 mg/kg. (right) Determination of MTD in rats.

Binding to Fc-γ receptors and FcRn

The globally conserved mAb glycan is essential for effector function. However, circulating immune cells may deplete ADCs by Fc-γ receptor-mediated uptake, thereby compromising efficacy and/or induce dose-limiting toxicities.[3] We corroborated that binding of GC-ADCs to Fc-γ receptors I, IIa/b and IIIa is eliminated. Importantly, binding to (human) FcRn-receptor is retained (Figure 4).

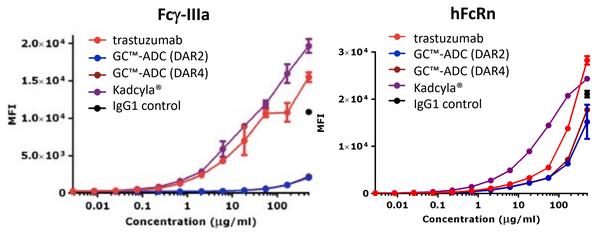


Figure 4. In vitro evaluation of binding to GC-ADCs (DAR2 and DAR4) and Kadcyla® to Fc-γ receptors (IIa depicted) and FcRn reveal abrogation and retention of binding, respectively.

[3]Mahalingaiah *et al*. *Pharmacol. Therap.* **2019**, *200*, 110–125

Tailoring of Drug-to-Antibody Ratio (DAR)

The most common drug-to-antibody ratio of ADCs in the clinic is 2–4, while a minority of ADCs (~six) are DAR 6–8. For ADCs with highly potent payloads (*e.g.* calicheamicin, PBD dimer, IGN, amanitin, PNU-159,682), a DAR2 is common, however the recommended dose in the clinic is typically restricted to levels <0.5 mg/kg, which may compromise PK and biodistribution, for example target receptor saturation may not be reached. In such case, one way to increase clinical ADC dose is to reduce the payload loading of the antibody, *i.e.* DAR1.[4]

We here present an approach for DAR1 ADCs that does not require antibody reengineering by cross-linking enzymatically remodeled antibody glycans with a bis-BCN-modified payload (ROUTE A) or by employing a trivalent BCN structure (ROUTE B, Figure 5).

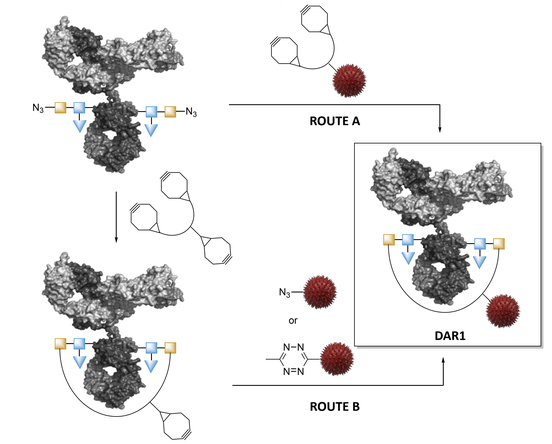


Figure 5. Two strategies for single payload conjugation through glycan cross-linking.

DAR1 ADCs

Based on the above strategy, model BCN_2-MMAE was directly conjugated to bisazido-trastuzumab (ROUTE A in Figure 5) to provide a DAR1 ADC with high efficiency (Figure 6). Other payloads (PBD dimer, PNU-159,682 were also successfully conjugated to provide DAR1 ADCs (not depicted).

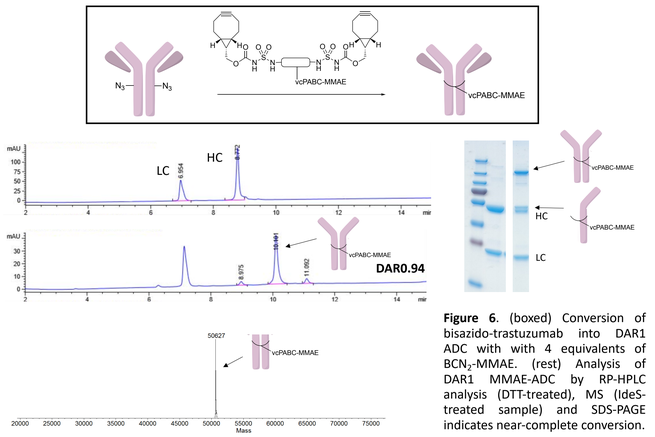


Figure 6. (boxed) Conversion of bisazido-trastuzumab into DAR1 ADC with with 4 equivalents of BCN_2-MMAE. (rest) Analysis of DAR1 MMAE-ADC by RP-HPLC analysis (DTT-treated), MS (IdeS-treated sample) and SDS-PAGE indicates near-complete conversion.

T Cell and NK Cell-Engagers

Engagement of T cell or NK cells to harness a patient's own immune system is a promising approach in immuno-oncology.

Here we present the adaption of GlycoConnect™ technology for the generation of immune cell-redirecting antibodies (Figure 7), without requiring prior protein reengineering. Instead, application of DAR1 approach to ADCs (ROUTE B) allows the conversion of any IgG isotype into an Fc-silent T cell-engager by attachment of anti-CD3 scFv or cytokine (IL-2/IL-15.

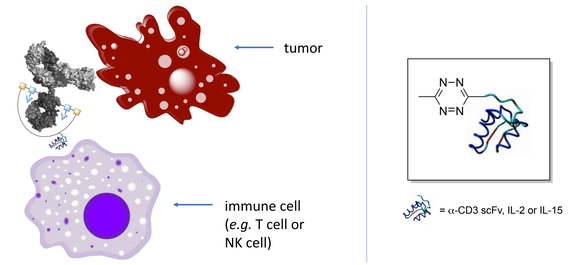


Figure 7. (left) Immune cell engagement by a GlycoConnect™-modified antibody harboring a CD3-binding scFv fragment. (right) Tetrazine-containing scFv or cytokine for metal-free click attachment to BCN-antibody (ROUTE B in Figure 5).

CD3 cell surface-binding was confirmed in T cell lymphoma cell line (Jurkat E6.1) for all formats (depicted for rituximab-hOKT3 in Figure 8).

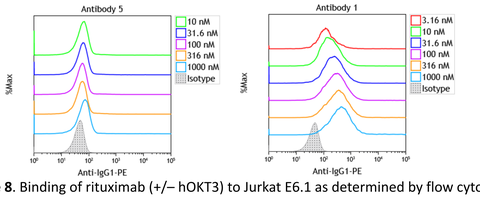


Figure 8. Binding of rituximab (+/– hOKT3) to Jurkat E6.1 as determined by flow cytometry.

Retention of FcRn binding was also established, as well as target-specific cell-killing potential by co-incubation of Raji cells with PBMCs and GlycoConnect™ bispecifics (not depicted).

Conclusions

GlycoConnect™ and HydraSpace™ technologies were extended to enable conjugation of a single payload to an antibody. Two alternative strategies were developed, one involving direct attachment, which was applied for the generation of DAR1 ADCs with ultrapotent payloads. The second approach, involving a two-stage introduction of payload, is most suitable for the attachment of small protein fragments, thereby offering a first-in-class technology for efficient, non-genetic generation of Fc-silent bispecific antibody formats with tailored 2:2 or 2:1 molecular format.

About Synaffix

Synaffix BV is a biotechnology company based in the Netherlands with best-in-class, clinical-stage antibody conjugation technology. The business model comprises technology out-licensing of our intellectual property portfolio, with granted claims that provide end-to-end patent protection on the platform through at least 2035. Synaffix has entered into non-exclusive, target-specific license agreements with ADC Therapeutics, Mersana Therapeutics and Shanghai Miracogen.

For more information, contact Anthony DeBoer: bd@synaffix.com.