Conjugation of toxic payloads by chemoenzymatic remodelling—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.12 Key quality attributes include high linker stability and polarity, low ADAG and enhancement of solid tumor penetration. The native glycan as attachment site also contributes to the superiority of GlycoConnect™ ADCs: (a) no binding to Fcγ 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 DAR4 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.

**Introduction**

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**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).8

**HydraSpace™ Technology**

Incorporation of a short and highly polar spacer moiety (HydraSpace™ technology) enables ADCs with highly hydrophobic payloads and with tailored stochiometry (DAR2 or DAR4).7

**toxSYN™ Platform**

The toxSYN™ platform comprises ADC payloads that span multiple MOA, most recently expanded with topoisomerase inhibitors based on Dtn (SYNstatin™) and saxitoxin (SYNtansine™). Each toxSYN™ linker-payload is functionalized for conjugation using GlycoConnect™ and HydraSpace™ technologies, readily tailored to various DAR formats.

**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 (1%) are DAR 6-8. For ADCs with highly potent payloads (e.g. calichecimine, PBD dimer, iKN, amantin, 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. DAR4.4

We here present an approach for DAR4 ADCs that does not require antibody reengineering by cross-linking enzymatically remodeled antibody glycans with a bi-BCN-modified payload (ROUTE A) or by employing a rivulant BCN structure (ROUTE B, Figure 5).

**Better than Ad cetris®**

An exemplary in vivo benchmarking study of a GCHS™ ADC versus Ad cetris® 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 HKSTD (3 → 9 mpk) was noted in cynomolgus monkey (data not shown).

**Conclusions**

The 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-target specific 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, Merana Therapeutics and Shanghai Micromed for more information, contact Anthony DeBoer: info@synaffix.com.