Novel Linker-Payload (SYNtecan E™) based on Topoisomerase 1 Inhibitor Exatecan enables Potent ADCs with Promising In Vivo Efficacy, on Par with Deruxtecan Technology

Floris van Delft, Remon van Geel, Laureen de Bever, Inge Hurkmans, Jorin Hoogenboom, Sorraya Popal, Jorge Verkade, Jord van Schaik, Sander van Berkel
Synaffix BV, Kloosterstraat 9, 5349 AC, Oss, the Netherlands

Introduction

Camptothecins, *e.g* topotecan and irinotecan, form a powerful class of chemotherapy drugs. Based on their ability to inhibit topoisomerase I, camptothecins are also unique payloads for antibody-drug conjugates (ADCs), as exemplified by the recent market approval of Enhertu® and Trodelvy®. We have shown earlier that the native glycan of monoclonal antibodies is a priviliged conjugation site for ADCs (GlycoConnect™) and the highly polar spacer technology (HydraSpace™) enables the conjugation of any cytotoxic payload, providing ADCs with significantly expanded therapeutic index (TI) versus mainstream ADC technologies.

We here show that exatecan, a clinically validated and potent campothecin, is readily combined with HydraSpace™ technology to provide linker-payload (SYNtecan E™). Homogeneous and stable GlycoConnect™ ADCs were readily generated from trastuzumab and compared head-to-head to ADCs containing deruxtecan, the linker-payload also applied in Enhertu®. GlycoConnect™ ADCs were found to be of equal efficacy, both in vitro and in vivo. Most promisingly, complete tumor regression was observed in a mouse xenograft study (BT-474) after a single dose, thereby demonstrating the potential of SYNtecan E™ for application in ADCs for the treatment of cancer.

GlycoConnect™ Technology

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

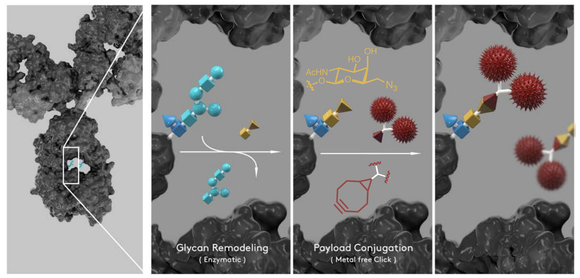


Figure 1. GlycoConnect™ technology: one-pot enzymatic glycan trimming and transfer of an azidosugar affords azido-modified antibody. Conjugation of payload by metal-free click chemistry with BCN-modified linker-drug affords ADCs with tailored DAR4 (depicted) or DAR2.

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]

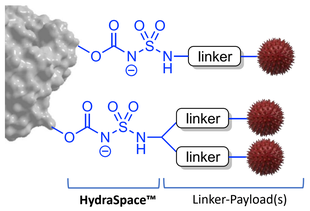
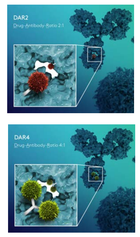


Figure 2. HydraSpace™ technology, based on acylated sulfamide, enables tailored DAR2 or DAR4 ADCs and due to its ionic nature at physiological pH leads to improved manufacturability, efficacy and safety (data not shown).

toxSYN™ Platform

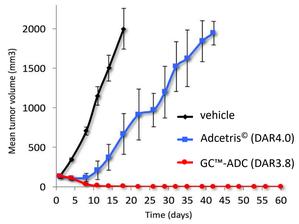
The toxSYN™ platform comprises ADC payloads that span multiple MOAs, including tubulin inhibitors and DNA damaging agents. Each toxSYN™ linker-payload is readily tailored to various DAR formats (DAR4, DAR2, DAR1) using HydraSpace™ polar spacer technology and suitably functionalized for combination with GlycoConnect™.

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.

Topoisomerase 1 Inhibitors

Camptothecin (Figure 4) is a naturally occurring, pentacyclic quinoline alkaloid that possesses high cytotoxic activity through the inhibition of topoisomerase 1, leading to cell death. However, despite its broad-spectrum antitumor activity and unique cytotoxic mechanism, poor solubility and hydrolysis under physiological conditions prevented clinical utilization of camptothecin itself. Therefore, various water-soluble analogues were developed that have received FDA approval: topotecan and irinotecan. Topotecan is used primarily in the treatment of advanced ovarian cancer, non-small cell lung cancer and cervical cancer. Irinotecan is a prodrug of SN-38, a more potent CPT analogue and is used primarily in the treatment of colorectal cancer. SN-38 is also used as payload in various ADCs.

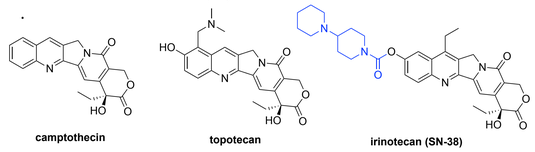


Figure 4. Chemical structures of camptothecin and its synthetic derivatives topotecan and irinotecan.

Clinical ADCs with Topoisomerase 1 Inhibitor Payloads

Two ADCs with camptothecin-based payloads have been approved, *i.e.* Trodelvy® and Enhertu®. Trodelvy®, a TROP2-targeting ADC with SN-38 as active catabolite, was approved in April 2020 for the treatment of TNBC. Enhertu®, an ADC approved late 2019 for the treatment of unresectable or metastatic HER2-positive breast cancer, consists of fam-trastuzumab conjugated to deruxtecan, which is stochastically conjugated to cysteine and releases active catabolite DXd, a synthetic derivative of exatexan (Figure 5). Exatecan is a potent campothecin that was evaluated in a phase III clinical trial but was discontinued due to lack of improvement over standard of care. Besides the two marketed drugs, six additional ADCs are under clinical development: four ADCs based on deruxtecan, one with SN-38 linker-drug (govetican) and one with belotecan payload.

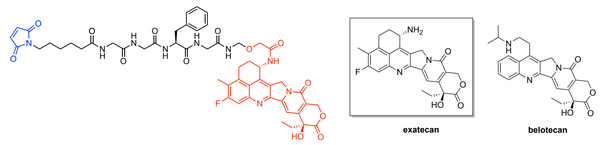


Figure 5. Chemical structures of deruxtecan (with DXd in red), exatecan and belotecan.

GlycoConnect™/HydraSpace™ ADCs with Exatecan Payload

We reasoned that exatecan itself would constitute a valuable payload for ADC development, due to its improved potency and higher membrane permeability (potential bystander effect) versus other camptothecins. Besides, exatecan is a poor substrate for the Pgp transporter that confers multidrug resistance. To this end, we synthesized various linker-payloads based on exatecan (SYNtecan E™ technology), featuring HydraSpace™ to accommodate exatecan's high hydrophobicity and dipeptide-PAB cleavable linker, which were conjugated to trastuzumab (Figure 6).

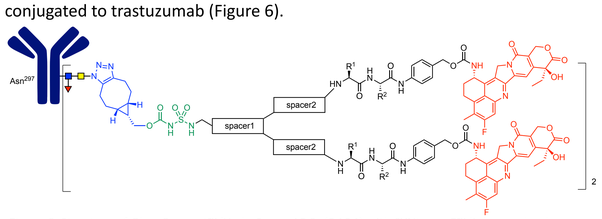


Figure 6. Structure of GlycoConnect™/HydraSpace ADCs (DAR4) with SYNtecan E™ linker-payload.

Structural analysis by HIC revealed the highly homogeneous nature of the GlycoConnect™ ADCs with SYNtecan E™ and the favorable relative retention time versus non-conjugated antibody (Figure 7).

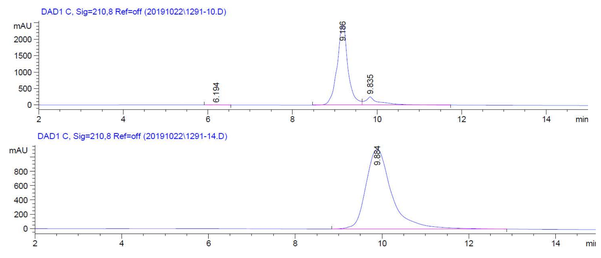


Figure 7. HIC profile of DAR4 SYNtecan E™ ADCs

In Vivo Comparison to Kadcyla and Deruxtecan-Based ADCs

SYNtecen E™ ADCs were evaluated in a mouse xenograft model, showing partial response at low dose (4 mg/kg) and full tumor regression after single dose administration of 12 mg/kg (Figure 8), while Kadcyla showed negligible effect (Figure 8). Target-specific killing was confirmed by inclusion of an isotype-control ADCs.

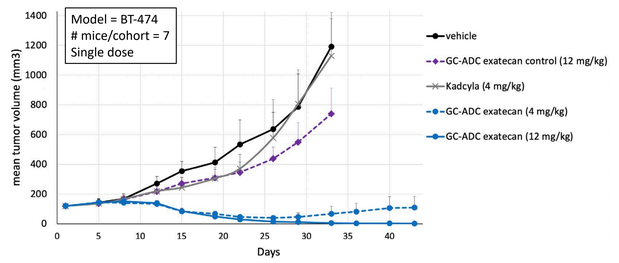


Figure 8. In vivo efficacy of SYNtecan E™ ADCs in BT-474 xenograft model.

In the same model, SYNtecan E™-based ADCs were also compared to similar ADCs based on trastuzumab and deruxtecan (prepared by ADC Biotechnology) at equivalent payload levels, showing near identical response both at low and high dose levels (Figure 9). Deruxtecan-based ADCs included both DAR4 and DAR8 format (equivalent to Enhertu®).

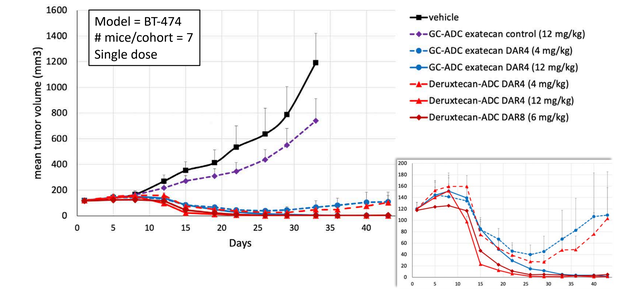


Figure 9. Comparison of SYNtecan E™ DAR4 ADCs to similar ADCs prepared from trastuzumab and deruxtecan, DAR4 or DAR8. Insert: blow-up of plot 0–200 mm³.

Conclusions

ADCs were successfully developed with GlycoConnect™ and HydraSpace™ technologies and SYNtecan E™, a novel linker-payload based on exatecan as active drug. The resulting SYNtecan E™ ADCs were found to be highly homogeneous and stable and showed promising in vivo efficacy, on par with ADCs based on deruxtecan technology.

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.

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