Synaffix BV
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Expanding the therapeutic index of ADCs

Synaffix's site-specific–conjugation and payload-enhancement technologies are improving the safety and efficacy of antibody-drug conjugates (ADCs) without the need for antibody engineering.

The antibody-drug conjugate (ADC) landscape has evolved rapidly since Adcetris (brentuximab vedotin) and Kadcyla (ado-trastuzumab emtansine) received marketing approval. First-generation technologies used random conjugation methods that produced ADCs with a heterogeneous drug-to-antibody ratio (DAR) and variable stability, both of which compromise the therapeutic index (TI). To improve safety and efficacy, ADC technologies today focus on site-specific conjugation methods involving antibody engineering.

Synaffix is pioneering a nongenetic, engineering–free approach to ADCs by using the naturally occurring antibody glycan as an anchor point for the payload. The company's proprietary technology platform comprises GlycoConnect, a chemoenzymatic technology for site-specific ADC generation, and HydraSpace, a payload-enhancing spacer technology, which further improves the TI, design and manufacturing of ADCs. (**Fig. 1**).

The company's founders, Floris van Delft (chief scientific officer) and Sander van Berkel (director of R&D operations), pioneered the advancement and application of best-in-class, metal-free click chemistry probes during their earlier academic work, and continue to do since the inception of Synaffix in 2010. The company now focuses exclusively on ADCs.

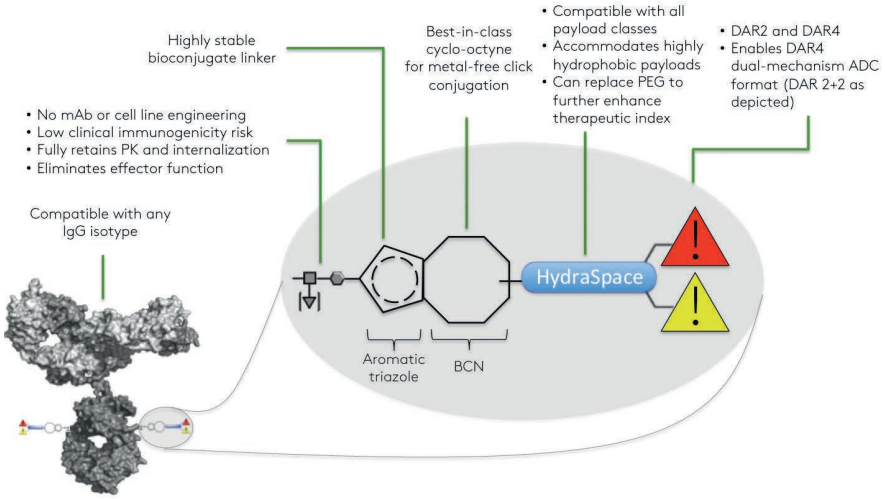

Figure 1: The GlycoConnect ADC-technology platform develops targeted cancer therapeutics with an improved therapeutic index. mAb, monoclonal antibody. Pharmacokinetics (PK); Polyethylene glycol (PEG)

Site-specific antibody conjugation

GlycoConnect makes use of the native N-linked glycosylation site that is present at approximately the same position on all monoclonal antibodies. Because various glycoforms can result from recombinant expression, the first step in GlycoConnect involves a proprietary enzyme that trims the glycan down to its core monosaccharide. Next, a proprietary azide-containing substrate is enzymatically installed onto the core sugar; this step is followed by the fully site-specific conjugation of the payload via proprietary metal-free click chemistry.

Studies both *in vitro* and *in vivo* have demonstrated superior safety and efficacy profiles for ADCs constructed with GlycoConnect. For example, *in vivo* data from a mouse patient-derived xenograft model show that a GlycoConnect ADC based on trastuzumab and maytansine (constructed as DAR2) is significantly more potent than the marketed ADC Kadcyla1 (average DAR of 3.5). Moreover, no dose-limiting toxicities were seen after the administration of a single 40 mg kg^{-1} dose of the GlycoConnect ADC, which compares favorably with Kadcyla's reported maximum tolerated dose of 20 mg kg^{-1}.

Conjugation to the native glycan site has no detectable effect on the pharmacokinetic profile. Moreover, Synaffix has also shown that using the native glycan as an anchor point maintains high ADC stability and achieves the best efficacy profile compared to alternate, engineered glycosylation sites[1]. "This gives us the confidence to continue using the native glycosylation site for ADC generation and maintain our engineering-free approach," said van Delft.

HydraSpace & dual-warhead ADCs

Synaffix has expanded its platform portfolio with payload-enhancing spacer technology called HydraSpace, which complements GlycoConnect by further improving the efficacy and manufacturability of ADCs.

HydraSpace is a short, polar spacer that improves conjugation efficiency, which enables the attachment of any payload—including highly hydrophobic toxins, such as PBD (pyrrolobenzodiazepine) dimers—to an antibody. In fact, HydraSpace allows the attachment of payloads that would require unacceptable stoichiometries or in-process aggregation with alternative approaches. Studies have shown that HydraSpace improves the conjugation efficiency of the payload, as well as reduces the aggregation propensity and expands the TI of the final ADC product.

HydraSpace enables the efficient attachment of multiple payloads to each glycan, without the need to engineer additional glycosylation sites into the antibody. Importantly, the branching format available with HydraSpace also enables the first-in-industry attachment of two different payload types to an antibody by a single conjugation event, thereby creating a dual-warhead ADC (DAR2+2) that is able to deliver two different drug mechanisms—with a potentially synergistic effect—to the same cell.

Synaffix's intellectual-property portfolio comprises 15 patents and applications covering GlycoConnect, HydraSpace and metal-free click chemistry. The process is linearly scalable to a multigram scale, and yield from the antibody starting material is typically >75%. The estimated cost of goods sold is commercially viable, and the process demonstrates compatibility with any immunoglobulin-γ (IgG) isotype.

Development timelines are reduced because the GlycoConnect process does not require genetic modification of the antibody. Proof-of-concept ADCs for nonclinical use can be generated from existing antibody material in just weeks. Synaffix can support its partners to initiate first-in-human studies with glycan-conjugated ADCs within one year of proof of concept.

Synaffix aims to be the preferred ADC technology partner, its technology is available for evaluation under a material-transfer agreement and can be licensed on a target-specific basis. "We can build a GlycoConnect ADC that contains HydraSpace, allow potential partners to test it and let the properties of the ADC speak for themselves," said Anthony DeBoer, director of business development at Synaffix.

1. van Geel, R. *et al. Bioconjug. Chem.* **26**, 2233–2242 (2015).

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