Non-Genetic Generation of Fc-Silent T Cell-Redirecting Bispecific Antibodies

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Introduction

Immune cell-redirecting antibodies have emerged as promising biological drugs in cancer treatment. New technologies are overcoming the limitations of 1st generation T cell-redirecting bispecifics, especially extending half-life to allow intermittent dosing, reducing immunogenicity and improving the safety profile, in particular related to cytokine release syndrome (CRS). Today, T cell-engagers exist with a wide variety of molecular architectures, typically Fc-silent IgG-type. However, nearly without exception, these bispecifics are generated by fusion of a cancer-binding and an anti-CD3 antibody, which inevitably requires genetic reengineering of an existing targeting antibody and/or extensive optimization of antibody format.

We have shown earlier that the native glycan of a monoclonal antibody provides a privileged site for controlled attachment of small-molecule cytotoxic drugs (GlycoConnect™ technology).[1] The resulting antibody-drug conjugates (ADCs) were found to display significantly improved therapeutic index versus ADCs prepared by conventional technologies, while improving manufacturability.

Here, we show that GlycoConnect™ technology is also suitable for attachment of small protein formats (scFv, interleukins) to an antibody without requiring engineering, and with concomitant abrogation of effector function (nihilation of Fc-γ receptor binding). Stoichiometry can be precisely controlled to 2:2 format but also 2:1 format, thereby generating T cell cell-engaging bispecific antibodies for application in immunotherapy with potentially improved safety profile.

Clinical Stage GlycoConnect™ Technology

Chemoenzymatic attachment of highly toxic payloads to the globally conserved antibody glycan at Asn-297 affords homogeneous and stable ADCs with high efficiency (>75%) without recombinant DNA technology.[1] GlycoConnect™ technology has been adapted by various ADC developers, and entered clinical phase early 2019 (ADCT-601), with a second program commencing 2020 (XMT-1592).

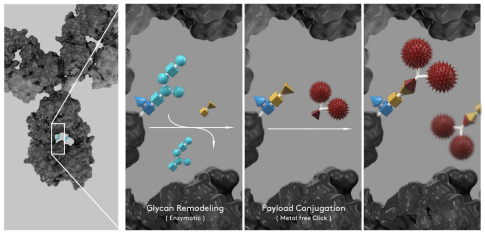

Figure 1. GlycoConnect™ technology: enzymatic glycan remodeling followed by conjugation of linker-drug based on metal-free click chemistry.

Drug-to-antibody ratio can be readily tailored to DAR4 (Figure 1) or DAR2 (not depicted), depending on payload potency[2], and can be expanded to controlled attachment of a single payload (DAR1) (This work).

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

Binding to Fc-γ receptors and FcRn

The globally conserved glycan is essential if mAb effector function is desired. However, circulating immune cells may also lead to drug depletion by Fc-γ receptor-mediated uptake, thereby compromising efficacy and/or inducing dose-limiting toxicities.[3] We corroborated that binding of GC™-ADCs to Fc-γ receptors IIIa is eliminated (Figure 2), as well as to Fc-γ receptors I, IIa/b (not depicted). Binding to FcRn-receptor is retained.

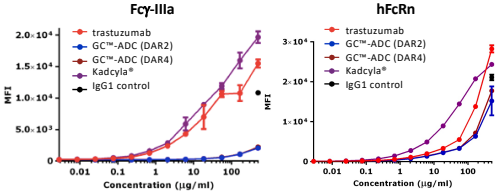

Figure 2. In vitro binding to GC™-ADCs (DAR2 and DAR4) and Kadcyla® to Fc-γ receptors (IIa depicted) and FcRn reveal abrogation and retention of binding, respectively.

DAR1 ADCs

While the majority of clinical ADCs feature a drug-to-antibody ratio between 3–8, for ADCs with highly potent payloads (*e.g.* PBD dimer), DAR2 is preferred. Nevertheless, low clinical dose for ADCs with ultrapotent payloads (<1 mg/kg) may compromise PK and biodistribution, *e.g.* by not reaching target receptor saturation.

Most recently, it was reported that reaction of a bismaleimide-functionalized PBD dimer with a cysteine-engineered antibody provides DAR1 ADCs.[4] We here present a generally applicable approach, not requiring antibody reengineering, by cross-linking antibody glycans with a bisBCN-modified modified payload (ROUTE A) or by employing a trivalent BCN structure (ROUTE B, Figure 3).

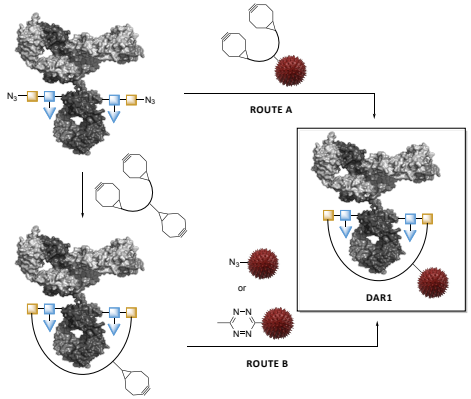

Figure 3. Two routes for generation of DAR1 ADCs through glycan cross-linking.

[3]Mahalingaiah *et al. Pharmacol. Therap.* **2019**, *200*, 110–125
[4]White *et al. mAbs* **2019**, *11*, 500–515

Fc-Silent Immune Cell-Engagers

Engagement of T cell or NK cells to harness a patient's immune system is a promising approach in immuno-oncology. Analysis of the clinical pipeline indicates that bispecific T cell-engagers feature a wide variety of molecular architectures, typically based on scFv or IgG, generated by genetic fusion of a cancer target-binding to an anti-CD3 fragment.

Here we present the adaption of GlycoConnect™ technology to the non-genetic generation of immune cell-redirecting antibodies (Figure 4, left) by selective attachment of anti-CD3 scFv (Figure 4, right). Ratio of CDR to anti-CD3 (or IL-2/15) can be tailored to 2:2 by conventional approach (Figure 1) or to 2:1 by application of DAR1 technology (ROUTE B in Figure 3), which may be of particular value to minimize cytokine release syndrome (CRS).

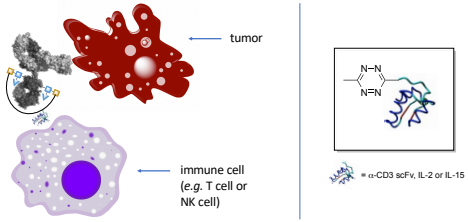

Figure 4. Immune cell engagement by a GlycoConnect™-modified antibody harboring a CD3-binding scFv fragment or IL-2/15 (left). Tetrazine-containing scFv or cytokine for metal-free click attachment to antibody (right).

Bispecific T Cell-Engagers

Bispecific T cell engagers were generated by conjugation of two (Figure 5, left) or a single (Figure 5, right) anti-CD3 scFv based on humanized OKT3.

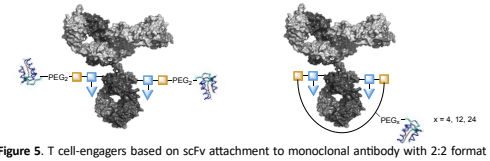

Figure 5. T cell-engagers based on scFv attachment to monoclonal antibody with 2:2 format (left) or 2:1 format (right).

Clean and controlled conjugation of rituximab (anti-CD20) to hOKT3 (anti-CD3) constructs with various spacer lengths was observed as judged by SDS-PAGE (Figure 6, left) and SEC (Figure 6, right).

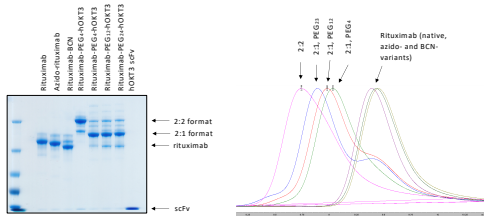

Figure 6. Analysis of rituximab-hOKT3 bispecifics by SDS-PAGE (left) and SEC (right).

Binding to FcRn and CD3

Retention of binding of rituximab-scFv conjugates to human FcRn was confirmed at pH 6.0 (Biacore), with absence of binding at pH 7.4, similar to naked antibodies (Table).

Antibody	pH	K_D (M)	R_{MAX} (RU)	Chi² (RU²)
Irrelevant IgG1 WT	6.0	1.74E-06	67	0.783
	7.4	-	-	-
Rituximab	6.0	1.57E-06	96.4	2.82
	7.4	-	-	-
Rituximab-(PEG2-hOKT3)2	6.0	2.16E-06	149.6	8.53
	7.4	-	-	-
Rituximab-PEG4-hOKT3	6.0	1.91E-06	122.9	5.36
	7.4	-	-	-
Rituximab-PEG12-hOKT3	6.0	1.90E-06	114.6	4.02
	7.4	-	-	-
Rituximab-PEG24-hOKT3	6.0	2.05E-06	123.5	5.47
	7.4	-	-	-
Rituximab-BCN	6.0	1.89E-06	89.8	2.01
	7.4	-	-	-

CD3 cell surface binding was confirmed in T cell lymphoma cell line (Jurkat E6.1) for all formats (depicted for 2:2 rituximab-hOKT3 in Figure 7).

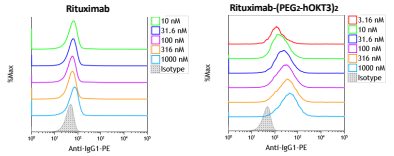

Figure 7. Binding of rituximab (+/– hOKT3) to Jurkat E6.1 as determined by flow cytometry.

Conclusions

- GlycoConnect™ technology enables controlled attachment of payloads to antibodies (4, 2 or 1 per antibody) without genetic engineering of the antibody
- Trimming the antibody glycan fully abrogates binding to Fc-γ receptors, thus nihilating effector function
- Based on the enhanced therapeutic index observed, 2 GlycoConnect™ ADCs have entered the clinic, with ≥4 ADCs rapidly advancing through preclinical development
- In addition to cytotoxic payloads, small protein fragments (like scFv) can be site-specifically attached to generate bispecific T cell engagers with tailored stoichiometry, where the 2:1 format may be of particular interest to minimize cytokine release syndrome (CRS)

About Synaffix

Synaffix BV is a clinical-stage biotechnology company based in the Netherlands with best-in-class 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.

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