T Cell Targeting with PD-1-Selective Immune Cell Engagers Based on GlycoConnect™ Technology Show Favorable Efficacy and Tolerability

Remon van Geel, Mick Verhagen, Willem Vugs, Sorraya Popal, Sander S. van Berkel, Floris L. van Delft
Synaffix BV, Kloosterstraat 9, 5349 AC, Oss, the Netherlands. E-mail: remon.vangeel@lonza.com

Poster # 4077, AACR Annual Meeting 2024

Introduction

Engagement of T cells and NK cells to harness a patient's immune system is a promising approach in immuno-oncology. IL-15 is a key immunostimulatory cytokine that can induce expansion and activation of CD8+ T cells and NK cells by binding to IL-15Rα receptor expressed on antigen-presenting cells followed by binding to IL-2/IL-15Rβγ receptor. In contrast to IL-2, IL-15 does not stimulate Tregs.

While various therapies based on IL-15 are currently under clinical evaluation, the short half-life and systemic activation of the immune system results in reduced clinical activity and safety concerns. A promising approach to overcome these issues is fusion of IL-15 to an antibody targeting a tumor antigen or tumor-infiltrating T cell. However, these immunocytokines require careful design with respect to stoichiometry (CDR:IL-15), spacer length and conjugation site. Unfortunately, to avoid loss of function and/or stability and to retain expression titers, genetic engineering may be highly challenging.

We here demonstrate how GlycoConnect™ technology, *i.e.* the attachment of a functional modality to the native antibody glycan,[1] can be applied for the generation of immune cell-engaging antibodies *without* requiring recombinant DNA technology. This plug-and-play approach can be used to generate Fc-silent immune cell engagers with tailored stoichiometry (2:1 and 2:2) and variable spacer length. In particular we demonstrate how a PD-1-IL-15 conjugate can be used to activate PD-1+ tumor-infiltrating T cells via cis-binding, while avoiding systemic activation of peripheral T and NK cells, leading to a significantly improved therapeutic index in rodent models.

Clinical-Stage GlycoConnect™ Technology

We have reported[1] that chemoenzymatic attachment of payloads to the native antibody glycan (GlycoConnect™ technology) affords stable, homogeneous ADCs with tailored drug-to-antibody ratio (DAR) and excellent therapeutic index. Various ADC programs are currently employed in the clinic, including ADCT-601, ADCT-701, XMT-1660, MRG004A and IBI343.

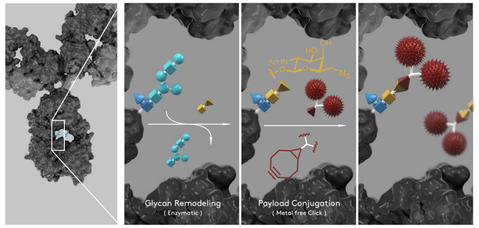

Figure 1. GlycoConnect™ technology: Two-stage approach to ADCs by (a) enzymatic glycan trimming & transfer of azidosugar, and (b) metal-free click attachment of BCN-modified linker-drug.

Drug-to-antibody ratio can be readily tailored to DAR4 (Figure 1) or DAR2 (not depicted), depending on payload potency, and can even be expanded to DAR12[2] or DAR8 (see poster 2614).

[1] Wijdeven *et al. mAbs* **2022**, *14*, *doi*:10.1080/19420862.2022.2078466.
[2] de Bever *et al. Bioconj. Chem.* **2023**, *34*, 538–548.

GlycoConnect™ Immune Cell Engagers

GlycoConnect™ technology is readily adapted to non-genetic generation of immune cell-engaging antibodies by selective attachment of IL-15 (Figure 2). GlycoConnect™ Immune Cell Engagers are Fc-silent but retain binding to FcRn. Ratio of CDR to IL-15 can be tailored to 2:2 (structure 3) by conventional approach or to 2:1 (structure 5) through glycan cross-linking via a trivalent BCN structure. Furthermore, a variable spacer length can be introduced using either PEG or peptide-based spacers.

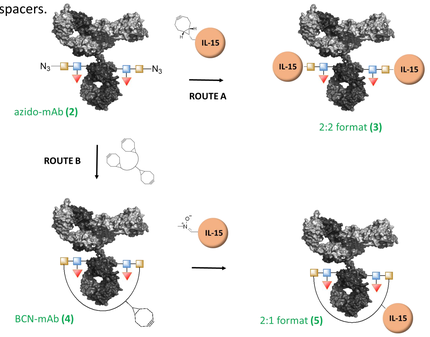

Figure 2. Strategy for generation of GlycoConnect™ Immune Cell Engagers with tailored stoichiometry. IL-15 is either modified with BCN (for 2:2 format) or nitrone (for 2:1 format).

Chemical Generation of Nitrone or BCN-tagged IL-15

Strain-promoted alkyne-nitrone (SPANC) has been reported for site-specific modification of peptides and proteins with exceptionally fast reaction kinetics.[3] A nitrone functionality is readily installed onto IL-15 with N-terminal serine via a straightforward one-pot, two-step chemical approach (Figure 3, top). The intermediate nitrone-modified IL-15 can subsequently be used for generation of a 2:1 GlycoConnect™ Immune Cell Engager. Alternatively, nitrone-IL-15 can be converted into BCN-IL-15 via reaction with bis-BCN (Figure 3, bottom), enabling 2:2 GlycoConnect™ Immune Cell Engagers.

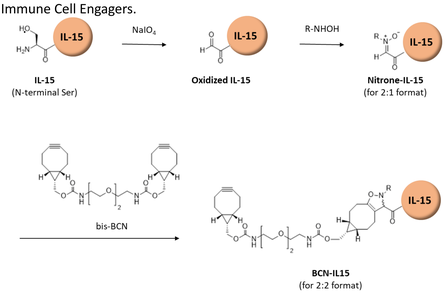

Figure 3. Scheme showing the generation of nitrone- and BCN-modified IL-15.

[3] Ning *et al. Angew. Chem. Int. Ed.* **2010**, *49*, 3065–3068.

Cis-Activation of Tumor-Infiltrating T Cells via PD-1

(Targeted) IL-15 therapies often lead to severe toxicities due to systemic activation of the immune system. Dose-limiting toxicities are caused by activation of peripheral T and NK cells, which is unfortunate as it is not required for anti-tumor efficacy. Previously, we have shown how IL-15 in the TME can be activated using tumor-targeting antibodies in combination with cleavable IL-15. Here, we demonstrate how GlycoConnect™ Immune Cell Engagers can selectively activate tumor-infiltrating T cells via cis-binding to PD-1 and IL-15Rβγ (Figure 4). Importantly, IL-15 conjugated to the antibody glycan via GlycoConnect™ technology does not activate T or NK cells in the absence of PD-1.

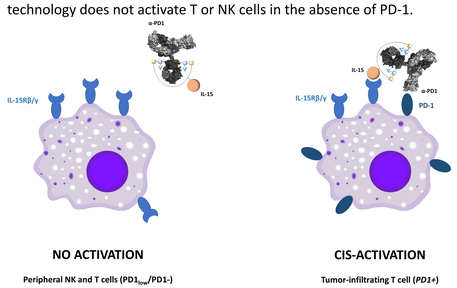

Figure 4. Schematic representation of selective activation of PD-1+ T cells using GlycoConnect™ technology.

In Vitro Activity in PD-1-Negative T cells

Binding and activation of endogenous IL-2/IL-15Rβγ was interrogated with a bioluminescent cell-based assay using PD-1 negative T cells expressing IL-15Rα and IL-15Rβγ (Figure 5). Conjugation of IL-15 to the antibody glycan via a short $(G_4S)_3$-spacer resulted in near complete loss of activity in target-negative cells (Figure 5, 1.2 and 10.1-fold induction for GlycoConnect™ Immune Cell Engagers and free IL-15, respectively). Activity can be fine-tuned via spacer length as demonstrated using the extended $(G_4S)_8$-spacer (3.1-fold induction).

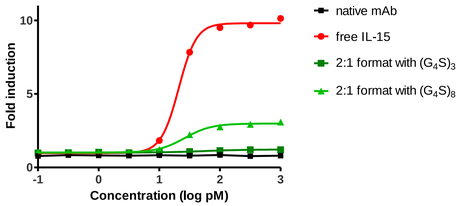

Figure 5. Activation of endogenous IL-2/IL-15Rβγ was evaluated using a bioluminescent cell-based assay. GlycoConnect™ Immune Cell Engagers with variable spacer length were compared to native antibody (negative control) and free IL-15.

In Vivo Efficacy

Anti-tumor efficacy of mPD-1-targeting GlycoConnect™ Immune Cell Engagers with 2:2 or 2:1 molecular format were evaluated in MC38 syngeneic mice model (Figure 6). The 2:1 format showed superior efficacy (90% TGI on day 16) compared to the 2:2 format at identical dose level (59% TGI on day 16). No body weight reduction or signs of toxicities were observed for both formats at this dose level (data not shown).

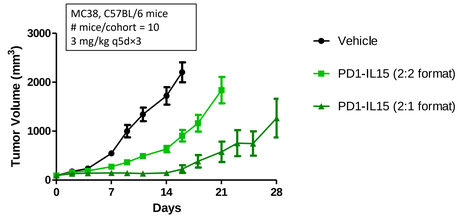

Figure 6. In vivo efficacy of IL-15-based GlycoConnect™ immunocytokines in C57BL/6 mice with MC38 murine colon carcinoma model.

The high affinity of IL-15 for IL-15Rα (low picomolar) will likely result in preferred binding of GlycoConnect™ Immune Cell Engagers to IL-15Rα-expressing cells over PD-1-expressing T cells. Therefore, we next evaluated a GlycoConnect™ Immune Cell Engagers with IL-15 mutant lacking IL-15Rα-binding. Efficacy was compared to the corresponding GlycoConnect™ Immune Cell Engagers with wildtype IL-15 using the same MC38 syngeneic mice model (Figure 7). The GlycoConnect™ Immune Cell Engagers with mutated IL-15 was more efficacious (80% TGI vs. 55% TGI on day 19). While for mPD-1-IL-15 all mice eventually showed tumor-regrowth, mPD-1-IL-15$_{mut}$ showed prolonged survival and even a complete response for 3/10 mice (Figure 7, bottom). No signs of toxicities were observed (data not shown).

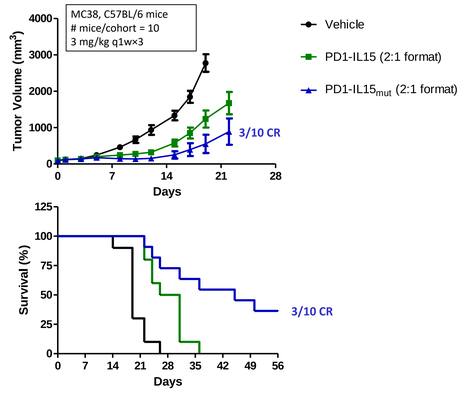

Figure 7. In vivo efficacy of GlycoConnect ™ immunocytokines C57BL/6 mice in MC38 murine colon carcinoma model. Graphs showing the mean tumor volume (top) and survival (bottom).

In Vivo Tolerability

While mPD-1-IL-15$_{mut}$ with 2:1 format was well tolerated at the effective dose of 3 mg/kg in tumor-bearing mice, the MTD was further interrogated by dosing mPD-1-IL-15 and mPD-1-IL-15$_{mut}$ at 12 mg/kg in non-tumor bearing balb/c mice (Figure 8). It was found that mPD-1-IL-15$_{mut}$ showed moderate BW loss on day 5-7 after which all mice recovered, indicating an MTD of ~12 mg/kg. While the MED still needs to be established, this indicated a promising TI of ≥4. Interestingly, the mPD-1-IL-15 showed no signs of toxicity at the 12 mg/kg dose level.

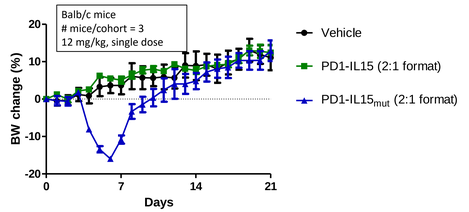

Figure 8. Tolerability in Balb/c mice. Mice (n=3) were dosed *s.c.* on day 0.

With an MTD of ~12 mg/kg or even higher both variants compare favorably to various clinical stage IL-15 based therapies such as N-803, SAR445710 and PF-072099601[4] (MTD of 1, 3 and 5 mg/kg, respectively). Importantly, only the more potent mPD-1-IL-15$_{mut}$ shows a durable response at a 4-fold lower dose level in the MC38 syngeneic mice model (3/10 CR). Overall, mPD-1-IL-15$_{mut}$ seems to be more promising for selective T cell targeting as it shows both the desired efficacy and tolerability.

[4] Note: PF-072099601 was discontinued

Conclusions

- GlycoConnect™ technology enables controlled attachment of IL-15 to the antibody glycan to generate immune cell-engagers with tailored spacer and stoichiometry
- GlycoConnect™ Immune Cell Engagers targeting PD-1 could selectively activate PD-1+ T cells, thereby minimizing systemic toxicities while achieving promising efficacy in a syngeneic mouse model
- Efficacy could be further enhanced by applying the 2:1 molecular format and an IL-15 mutant lacking IL-15Rα-binding. This PD-1-IL-15$_{mut}$ variant showed a good safety profile (MTD ~12 mg/kg) and a promising TI of ≥4

About Synaffix

Synaffix BV is a clinical-stage biotechnology company with best-in-class antibody conjugation technology. The business model comprises technology out-licensing of the IP portfolio, with granted claims that provide end-to-end patent protection through at least 2035. Synaffix has entered into target-specific license agreements with ADC Therapeutics, Mersana Therapeutics, Shanghai Miracogen, Innovent, ProfoundBio, Kyowa Kirin, Genmab, MacroGenics, Amgen, Hummingbird, CKD, ABL Bio and Sotio. Five GlycoConnect® ADCs have entered the clinic, with up to 20 ADCs rapidly advancing through preclinical development.

For more information, contact bd@synaffix.com

Synaffix
A Lonza Company