# Review of: "Challenges and Opportunities for the Large-Scale Chemoenzymatic Glycoengineering of Therapeutic N-Glycosylated Monoclonal Antibodies"

Qiang Yang[1]

1 University of Maryland, College Park

Potential competing interests: I am an employee and shareholder of GlycoT Therapeutics.

This nice review by Drs. Ivanova and Falcioni summarized the penitential advantages of chemoenzymatic glycoengineering to improve/develop antibody or antibody-based therapeutics and pointed out the potential hurdles of the application of this technology in the industrial setting. This technology was mainly developed by multiple academic groups, including Dr. Lai-Xi Wang's group from University of Maryland and Dr. Chi-Huey Wong's team from Academia Sinica (1). The core components of this technology platform are the patented glycosynthase and the glycan oxazoline donors. Currently the EndoS-D233Q and EndoS2-D184M glycosynthase (patented by University of Maryland) has been exclusively licensed to GlycoT Therapeutics (a start-up company based in Maryland, 2019) while EndoS2-T138Q glycosynthase developed by Wong group has been licensed to CHO Pharma. As the authors summarized, CHO Pharma has used this technology to produce afucosylated antibody that has been used in phase I human clinical trial. Although CHO Pharm has not disclose most of their technology development, successful initiation of Phase I human clinical suggested the company has developed the technology route that can produce protocol to produce hundred grams to kilogram scale of SGP from egg yolk powders. In 2020, Daiichi Sankyo has non-exclusively licensed the technology from GlycoT Therapeutics for the development of therapeutics. In the published patents from Daiichi Sankyo, it has established protocol to hundred-gram to kilogram scale of SGP (WO2014208742A1, WO2017110984A1). To facilitate the large-scale production of glycan oxazoline, it also isolated a heat-resistant endoglycosidase for the processing of SGP (WO2018101454A1). GlycoT Therapeutics has established the efficient product ten-gram scale of sialylated glycan oxazoline which can be readily scaled-up to produce hundred-gram level (unpublished data). Another Japanese company Fushimi has also established protocol to produce SGP in hundred-gram scale. All these efforts sufficiently demonstrated the scale-up potential of the chemoenzymatic approach. On the other hand, further cost of good analysis will be performed to evaluate if isolation and preparation of glycan oxazoline donors from natural source can support large scale Phase III trial and subsequent commercial manufactures. All those hurdles clearly listed out by the review authors must be fully addressed.

The commercialization potential of chemoenzymatic engineering for large scale production of glycol-engineered antibody is mainly determined by the cost consideration comparing to other platforms. For example, the potential to produce afucosylated antibody with enhanced ADCC activities, are very likely to be limited. From the perspective of cost of goods analysis, the method likely cannot compete with cell-based engineering approach. Nevertheless, the method may be helpful for the speedy production of multiple afucosylated antibodies (with stocks of oxazolines available) for the initial in

vitro and in vivo evaluation of candidate antibody. The main opportunity of chemoenzymatic engineering of antibody with native glycoforms is probably the improvement of Fc-sialylation, which is critical for the anti-inflammatory activity of antibodies, as evidenced in multiple studies using mouse models of inflammation or autoimmune disease. Recent clinical trial with Momenta preliminarily confirmed the efficacy of hypersialylated IVIG in patients with Immune Thrombocytopenic Purpura (*2019*). With chemoenzymatic method, Fc-sialylation of IVIG can be readily enhanced to > 95% (GlycoT, unpublished data). In contrast, sialylation enhancement with cell engineering based technology can only reach 30-40% (1). Such irreplicable advantage of chemoenzymatic approach may offset cost issue, warranting industrial large-scale applications.

The most promising application of chemoenzymatic engineering is the introduction of modified glycan which can't be achieved with cell engineering or metabolic incorporation approaches (1,2). Especially, glycan-specific antibody-drug conjugates is the most promising direction. The sialylated glycan prepared from SGP can be easily functionalized with azide or other active groups to form tagged glycan oxazolines in a simple one-pot reactions (3). After transglycosylation, linker-payload can be introduced to the functionalized antibody with mild click reactions (3-5). Such method offers several advantages comparing to other approaches to prepare site-specific ADCs, such as 1) no requirement of genetically engineering of antibody, 2) no interference to antigen-binding, 3) stable conjugation to an optimal site of antibody, 4) retaining affinity to FcRn receptor which is crucial for serum half-life of antibody. In a further development, a one-pot transglycosylation strategy with only wild-type EndoS2 to introduce synthesized azido-disaccharide oxazoline has been established, as the transformed product is highly resistant to EndoS2 drydrolase activity (5). All these developments represent the promise for the development of therapeutics based on Fc-glycan specific antibody-conjugation.

Reference

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