WHITE PAPER

CleanCut GS ADCC+ CHO

A Gene-Edited Host Cell Platform for Afucosylated Antibody Production with Maintained Productivity

Cell Line Development | Afucosylated Antibody Production | Designer CHO Host Platforms

At a Glance

CleanCut GS ADCC+ is designed to enable afucosylated antibody production while preserving the productivity profile of the CleanCut GS CHO platform.

85–89% Afucosylation observed across lead CleanCut GS ADCC+ clones	**5.7–5.8 g/L** High final titers in a 14-day fed-batch format
97.9–106.4% Day 14 Qp relative to CleanCut GS control	**2.0–4.8×** Increase in apparent ADCC potency in reporter assay

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Introduction

For therapeutic antibodies that rely on antibody-dependent cellular cytotoxicity (ADCC), Fc glycosylation can be a decisive product attribute. Core fucose on the Fc N-glycan reduces binding to FcγRIIIa on immune effector cells, limiting ADCC potency. Conversely, reducing or eliminating core fucose can strengthen FcγRIIIa engagement and improve immune-cell-mediated apoptosis of target cells.

Afucosylation is now an established antibody-engineering strategy for indications where stronger Fc-mediated effector function is desired, including oncology, inflammatory, and autoimmune diseases. One representative example is obinutuzumab, a humanized type II anti-CD20 antibody engineered with reduced core fucosylation to increase FcγRIIIa binding and enhance ADCC against CD20-positive B-cells, and is used to treat diseases such as Chronic Lympocytic Leukemia (1).

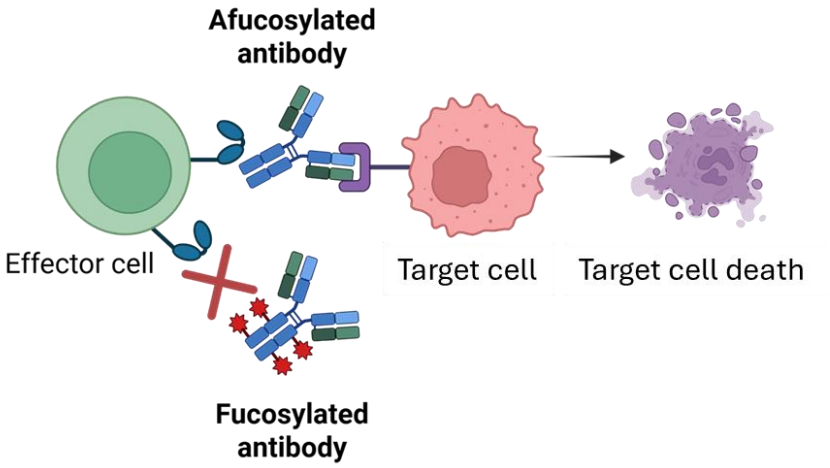

Figure 1: Afucosylated antibodies strengthen FcγRIIIa-mediated immune effector engagement, increasing ADCC against antigen-positive target cells. In contrast, Fc fucosylation weakens this interaction and can limit target-cell killing when ADCC is central to the mechanism of action.

Despite the biological value of afucosylation, implementation can introduce manufacturing and commercial tradeoffs. Some afucosylation approaches require post-production glycan modification, or platform licenses of CHO hosts with a knockout of α-1,6-fucosyltransferase (encoded by the FUT8 gene) that add operational complexity. The FUT8 gene encodes the key enzyme responsible for transferring a core fucose to the N-glycan of an antibodies Fc region. Liu et al. reported that several afucosylated monoclonal antibodies in commercial manufacturing were produced from Fut8 knockout CHO host cells but usually have impeded cell growth and lower production titers (2). Regarding post-production glycan modification, since harsh chemical treatments post-production can damage the antibody structure, the reduction of fucose is often achieved during production with the addition of costly metabolic inhibitors such as 2-fluorofucose (2-FF) which act as a decoy substrate for FUT8 (3).

Demeetra's CleanCut GS ADCC+ CHO host was developed to address that practical gap: enabling afucosylated antibody production in a high-performing CleanCut Glutamine Synthetase (GS) double-knockout background while maintaining growth, titer, and cell-specific productivity. The platform combines the CleanCut GS CHO foundation with a FUT8 knockout (4). As a proof of concept, Harbor-IN was used to stably integrate a trastuzumab expression construct with GS selection, enabling evaluation of afucosylation, productivity, and ADCC activity in the same workflow. Here we demonstrate CleanCut GS ADCC+ clones produced afucosylated trastuzumab, maintained Qp near that of the CleanCut GS control, and demonstrated enhanced ADCC potency in a reporter assay.

The CleanCut GS ADCC+ Platform

CleanCut GS ADCC+ is built on the CleanCut GS CHO host, a double GS knockout CHO-K1 platform engineered using Cas-CLOVER. The foundational cell line is a double glutamine synthetase (GS) knockout host designed to increase the stringency of GS-based selection by eliminating endogenous GS activity from both the primary GS locus and the GS-like (pseudogene) secondary locus. This creates a stronger dependence on the integrated GS expression cassette, thereby enriching for high-yielding, stable pools and clones. When paired with Harbor-IN to express trastuzumab, CleanCut GS CHO has supported high fed-batch titers, cell-specific productivity above 100 pg/cell/day, and confirmed production stability through 60 generations. The ADCC+ variant adds FUT8 knockout to this CleanCut GS foundation.

Analytical confirmation of afucosylation

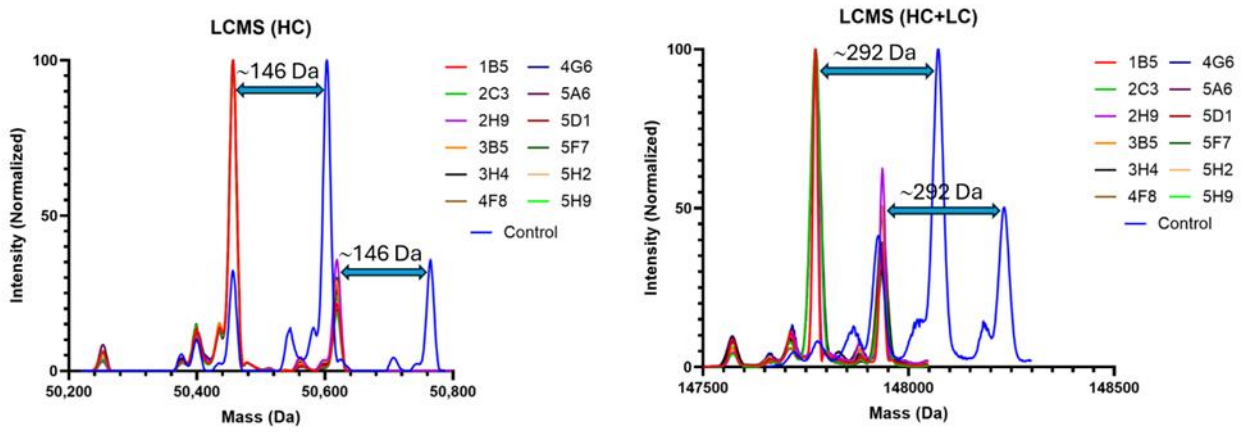

Figure 2: Trastuzumab from CleanCut GS ADCC+ clones and CleanCut GS (blue) were purified from a 14-day fed-batch experiment and analyzed by reduced and intact LCMS. Reduced LCMS showed a consistent –146 Da mass shift in the heavy chain from ADCC+ clones (left), and intact LCMS showed an approximately –292 Da mass shift relative to trastuzumab from the CleanCut GS control (right).

These results in Figure 2 are consistent with the loss of one core fucose (146 Da) per heavy chain, and therefore the loss of two fucose residues (292 Da) from intact IgG. Lead CleanCut GS ADCC+ clones produced trastuzumab with approximately 89%, 85%, and 85% afucosylation, compared with 3.5% in the CleanCut GS control, a greater than 24-fold reduction in core fucosylation. While complete elimination of core fucose is not consistently achieved in FUT8 knockout CHO systems, with residual fucosylation commonly reported in the literature, the levels observed here are well within the range associated with enhanced FcγRIIIa binding and ADCC activity. The levels may also be attributed to deconvolution/reporting constraints.

Cell-specific productivity maintained

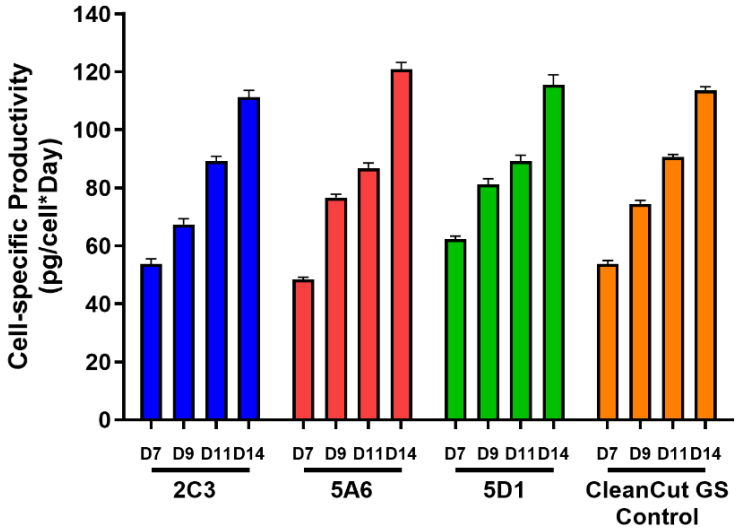

Figure 3: In a 14-day TubeSpin fed-batch study, FUT8 knockout clones 2C3, 5A6, and 5D1 reached Day 14 Qp values of 111.23, 120.85, and 115.61 pg/cell/day, respectively. The CleanCut GS control Qp was 113.59 pg/cell/day. On a relative basis, the ADCC+ clones achieved 97.9%, 106.4%, and 101.7% of the control Qp. Qp values were calculated based on global Day 0 to Day 14 values.

These results indicate that CleanCut GS ADCC+ can deliver the desired Fc glycan profile without imposing any apparent productivity penalty in the tested trastuzumab workflow. The lead ADCC+ clones maintained cell-specific productivity comparable to the parental non-FUT8-knockout CleanCut GS control, while also producing high fed-batch titers of approximately 5.7–5.8 g/L, supporting the platform's use for afucosylated antibody production without rebuilding the process around a lower-yielding host.

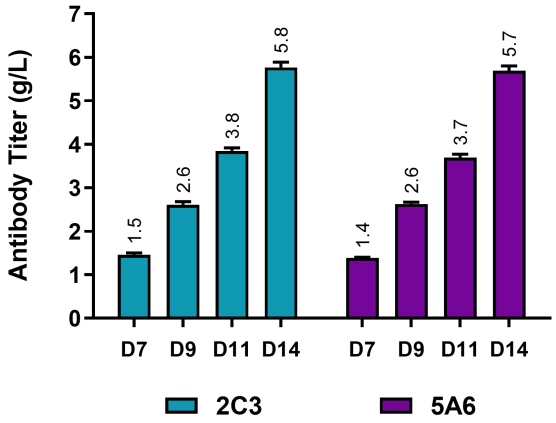

Figure 4. In the same 14-day fed-batch format used for Qp assessment, CleanCut GS ADCC+ clones 2C3 and 5A6 showed increasing antibody titers over time. Both clones reached high final titers by Day 14, with 2C3 at 5.8 g/L and 5A6 at 5.7 g/L.

Enhanced ADCC Potency

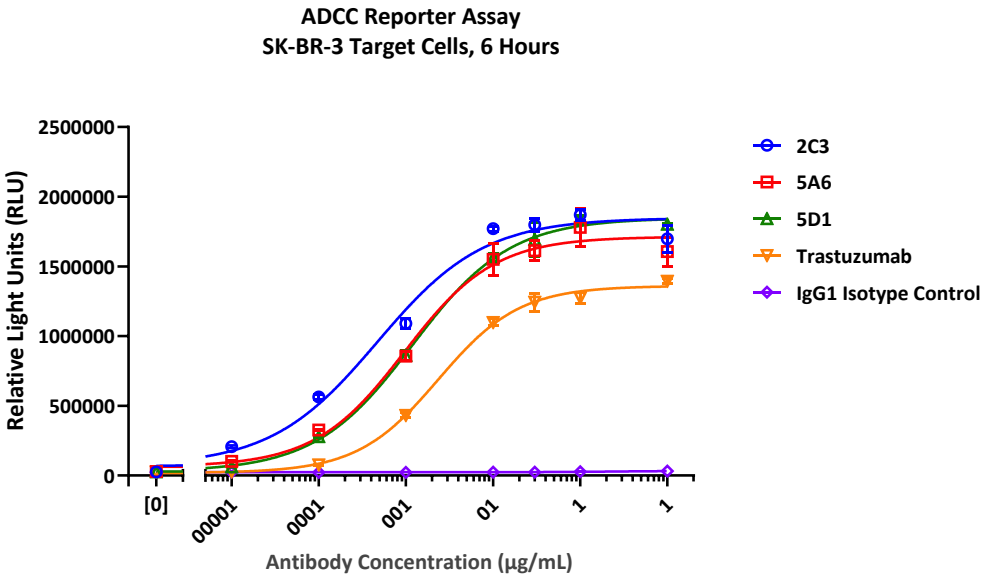

Figure 5: ADCC reporter bioassay (Promega) - increasing antibody concentrations are added to elicit ADCC against the human target expressing cell line SK-BR-3, using a reporter cell line engineered to express human FcγRIIIa-V158. ADCC readout is a luminescence signal from NFAT response element driving expression of firefly luciferase measured as relative light units (RLU).

In the ADCC reporter bioassay, afucosylated antibodies generated from clones 2C3, 5A6, and 5D1 shifted the response curves leftward and produced higher peak signals than the fucosylated CleanCut GS trastuzumab control, indicating stronger ADCC activity at lower antibody concentrations. The CleanCut GS control trastuzumab reached an EC50 of 0.024 µg/mL, whereas the ADCC+ antibodies reached EC50 values of 0.005 µg/mL for 2C3, 0.010 µg/mL for 5A6, and 0.012 µg/mL for 5D1. This represents a 50–79% reduction in EC50, or a 2.0–4.8× increase in apparent ADCC potency in this assay, with clone 2C3 showing the strongest dose-response shift.

Conclusion

Afucosylation is a validated strategy for improving Fc-mediated effector function, but its value to a drug developer depends on whether it can be implemented without adding manufacturing complexity, post-production glycan modification steps, or ongoing royalty obligations that compound over a program's commercial life.

CleanCut GS ADCC+ is built on a foundation that has already demonstrated high titer, stringent GS selection, not a new platform built around the FUT8 knockout, but an extension of a proven host. The trastuzumab data presented here provides a proof of concept; the expectation is that the same CleanCut GS productivity characteristics will carry forward into client molecules developed through this host.

Platform Access

For biopharma companies building their own CLD capabilities, CleanCut GS ADCC+ is available under a one-time GMP implementation fee with unlimited product use, no per-program royalties, no milestone-based access restrictions. For organizations that prefer a fully supported path, Demeetra offers CLD services using CleanCut GS ADCC+ with no royalty obligations on resulting cell lines, with an integrated route to clinical and GMP material available through Demeetra's partnership with NorthX Biologics.

The result is an afucosylation-capable host that fits inside a standard CLD workflow, with commercial terms designed to remain attractive as programs advance.

References

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