MODULATING ANTIBODY GALACTOSYLATION THROUGH CELL CULTURE MEDIUM FOR IMPROVED FUNCTION AND PRODUCT QUALITY

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Introduction

The production of therapeutic antibodies (Abs) requires high product titers and excellent product quality to ensure efficient manufacturing and potent drug efficacy. Glycosylation, or the attachment of sugars to organic molecules, is a critical quality aspect that can significantly alter Ab binding, function, and therapeutic effect. Galactose is a key sugar of interest due to its significant impact on Ab function and the ability to control galactosylation through cell culture medium.

In this poster, media components including manganese, uridine, and galactose were assessed in their ability to modulate levels of galactose on a model therapeutic Ab produced in Chinese hamster ovary (CHO) fed-batch cultures. Various conditions were able to tune galactosylation levels without compromising cell growth and Ab titers. Furthermore, an *in vitro* assay was improved upon and utilized to evaluate the functional ability of the Abs to bind and activate complement-dependent cytotoxicity (CDC). Differences in galactosylation significantly altered the Abs' ability to induce cell cytotoxicity. Design of experiment analysis determined the optimal ratio of supplements to maximize galactosylation. This "Optimized Supplement" was verified and compared to other suppliers' galactosylation supplements.

Overall, media composition was able to modulate antibody galactosylation and consequently antibody function. The Optimized Supplement outperformed all other supplier supplements and resulted in the best overall cell growth, glycan profile, and functional Ab activity. The ability to evaluate different medium components, measure glycosylation levels, and evaluate antibody function provides cohesive control of antibody product quality.

Methods

Fed-Batch Cultures

- CHO cells expressing IgG1 Ab against CD20
- Culture vessel: 125 mL shake flasks
- Seeding density: 0.3 x 10^6 cells/mL
- BalanCD® CHO Growth A and 4% BalanCD® CHO Feed 4 on culture days 3-7
- Supplier feeds and supplements were used as directed

Culture Analysis

- Viable cell density and cell viability: Beckman Vi-Cell XR
- Ab titer: Pall FortéBio QK^e
- Glycan analysis: PerkinElmer LabChip GXII

CDC Assay

- Daudi B lymphoblast cells
- Normal human complement serum
- Promega CytoTox-Glo® cytotoxicity assay

Results

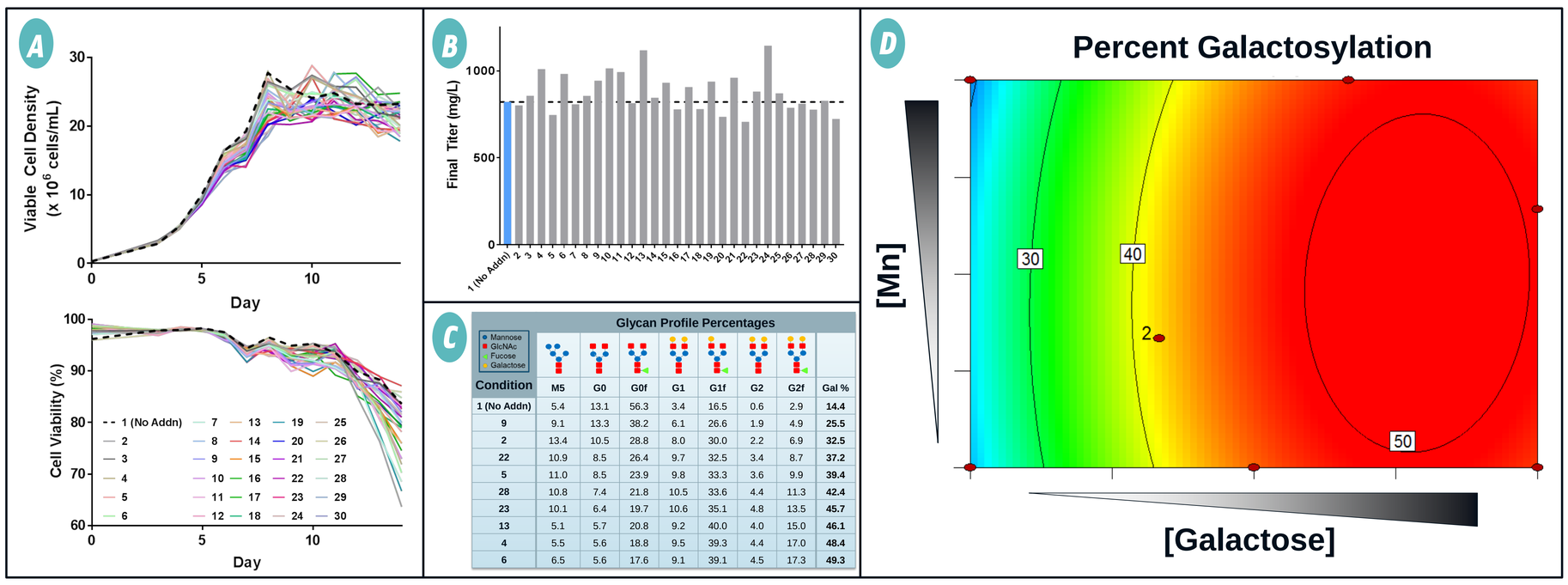

FIGURE 1. Design of experiment (DoE) study to identify key modulators of galactosylation. Various supplements were added to CHO fed-batch cultures expressing an IgG1 Ab and analyzed for impact on cell growth, Ab production, and product quality.
(A) Viable cell density and cell viability of the 30 DoE fed-batch conditions. Galactose-modulating supplements did not significantly impact cell growth as viable cell densities and cell viabilities were similar to the control (Condition 1: No Addn) which had no supplementation.
(B) Final Ab titer of the 30 DoE fed-batch conditions. A dashed line is provided to easily compare all the conditions against the titer of the control (Condition 1: No Addn; blue bar). Galactose-modulating supplements had a limited effect on Ab titer levels compared to the control.
(C) Percentage breakdown of the glycan profiles from a set of the DoE fed-batch conditions. Percent galactose is defined as the number of galactoses divided by the number of possible galactoses. The DoE components were able to increase Ab galactosylation and resulted in a wide ranged of percent galactose from a low of 14% to a high of 45%.
(D) DoE analysis of the effect of manganese at a fixed concentration, uridine, and galactose on Ab galactosylation. In the red area denoted at 50% galactosylation, a composition of galactose modulators was selected and denoted as the "Optimized Supplement." This Optimized Supplement was further verified and compared to galactose-modulating supplements from other suppliers in **Figure 2**.

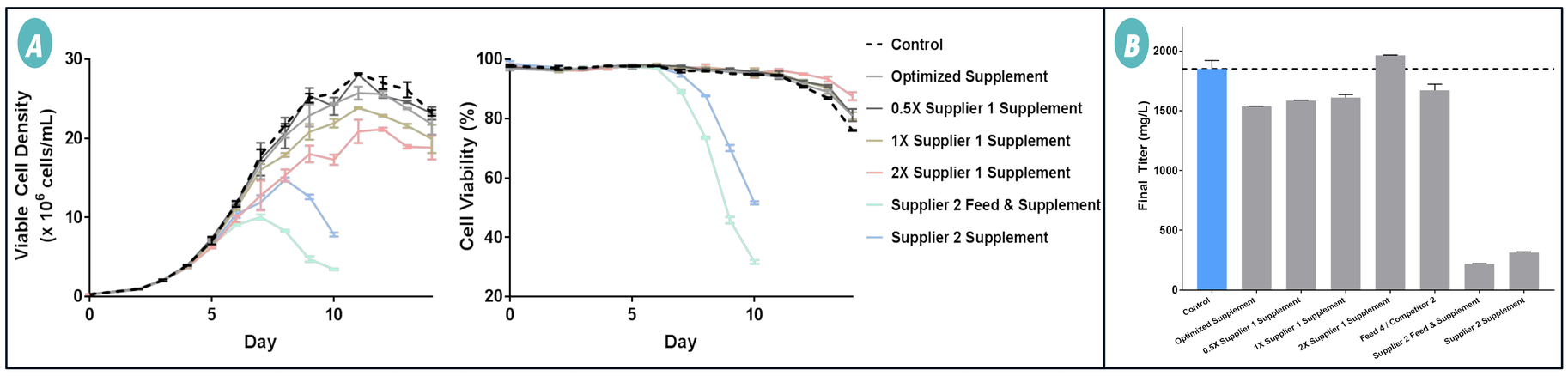

FIGURE 2. Verification of Optimized Supplement and comparison to supplements from other suppliers. Supplements were added to CHO fed-batch cultures according to manufacturer's instructions and analyzed for impact on cell growth and Ab production. **(A)** Viable cell density and cell viability of the cultures with and without galactose-modulating supplements. The Optimized Supplement and Supplier 1 Supplements did not significantly impact cell growth as viable cell density and cell viability were similar to the control. Supplier 2 Supplements significantly lowered viable cell density and cell viability; the cultures ended prematurely since they fell below 70% viability. **(B)** Final Ab titer of the cultures with and without galactose-modulating supplements. The Optimized Supplement and Supplier 1 Supplements did had a limited impact on Ab titer while Supplier 2 Supplements significantly decreased titers due to stunted cell growth and viability. Due to poor growth and titer levels, Supplier 2 was excluded from further studies.

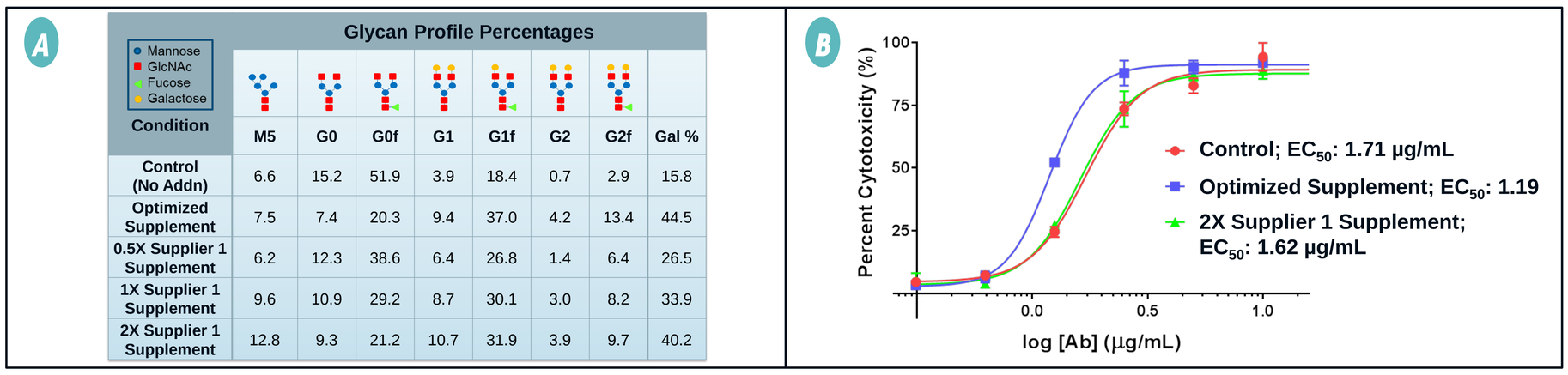

FIGURE 3. Glycan and CDC analysis of Abs. **(A)** Abs from cultures with high viable cell density, viability, and titers were further analyzed for their glycan profiles. The Optimized Supplement and 2X Supplier 1 Supplement resulted in Abs with galactose percentage levels of about 45%. The lower concentrations of Supplier 1's Supplements resulted in lower galactose percentages and were excluded from further studies. **(B)** Abs that passed the requirements of high viable cell density, viability, titer, and percent galactosylation were evaluated on their therapeutic effect and ability to induce CDC *in vitro*. Increased galactosylation by the Optimized Supplement resulted in more therapeutic Abs with lower EC_{50} values than the control. Conversely, the high galactosylation from the 2X Supplier 1 Supplement did not result in as low of an EC_{50} value compared to the Abs from the Optimized Supplement.

Summary: Optimized Supplements modulated galactosylation and improved Ab therapeutic efficacy.

	Max Viable Cell Density (x 10^6 cells/mL)	Estimated Cumulative Cell Density (x 10^6 cells/mL)	Final Titer (mg/L)	Percent Galactosylation (%)	Man 5 (%)	CDC EC_{50} (µg/mL)
Control, No Supplements	28.2	198	1850	15.8	6.6	1.71
Optimized Supplement	25.7	185	1538	44.5	7.5	1.19
2X Supplier 1 Supplement	21.1	148	1964	40.2	12.8	1.62

Presented at ESACT, May 2017

P/N 10740ICC Rev 0