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Optimizing PK characteristics of a novel IgG_1 recombinant mAb biotherapeutic by increasing galactosylation and total sialic acid content using a fed-batch process in CHO

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Abstract

The majority of biotherapeutic proteins require the production cell line to synthesize unique glycan structures that are necessary for the biological activity of the molecules and are known to affect the pharmacokinetic/pharmacodynamic (PK/PD) characteristics of the proteins. Gibco™ GlycanTune™ B+ Total Feed is a single-part Advanced Granulation Technology (AGT™) feeding supplement designed to increase galactosylation at N-linked sites in recombinant proteins. Here we present a case study using GlycanTune B+ Total Feed during fed-batch production to improve the glycan profile and biological activity of an innovator IgG_1 mAb. The molecule contained exposed N-acetylglucosamine (GlcNAc) and mannose on an N-linked site on the kappa chain. The GlycanTune B+ feed was evaluated to replace Gibco™ EfficientFeed™ B+ Supplement used in the process. Using a fed-batch process with an existing CHO cell line, the performance of GlycanTune B+ feed was evaluated in shake flasks, 3 L benchtop bioreactors, and 1,000 L GMP bioreactors. Cell growth and productivity with GlycanTune B+ feed was comparable to that observed with EfficientFeed B+ Supplement. Purified IgG_1 from test fed batches at each scale was analyzed using capillary isoelectric focusing (cIEF) and LC-MS instrumentation. Analysis by cIEF demonstrated that the percentage of acidic species increased to ~98% with GlycanTune B+ feed, which indicated a significant increase in galactose and terminal sialic acid. Analysis by LC-MS showed that feeding with GlycanTune B+ feed shifted the light chain glycan profile to the fully galactosylated form with nearly complete sialylation. The glycan structures on the heavy chain were also analyzed and found to be relatively unchanged when GlycanTune B+ feed was used. Proteins from GMP-compliant 1,000 L bioreactor fed batches with GlycanTune B+ feed were shown to be 90–95% mono- and di-sialylated by LC-MS analysis. The total sialic acid content was determined to have increased from 0.33 moles/mole of IgG_1 mAb with EfficientFeed B+ Supplement to 2.08 moles/mole with GlycanTune B+ feed. In a mouse PK study, the mAb produced with GlycanTune B+ feed increased 5-fold in *in vivo* concentration relative to that produced with EfficientFeed B+ Supplement. This increase in concentration correlated with the increase in sialic acid content. The ability of GlycanTune B+ feed to dramatically increase galactosylation and the total sialic acid content of recombinant mAbs offers a novel way to modify the PK characteristics of biotherapeutics. Increasing the concentration of IgG_1 mAb in circulation *in vivo* could potentially help reduce dosage, material needs, and the cost of a biotherapeutic.

Introduction

The production of a novel IgG_1 mAb demonstrated an inadequate glycan profile and insufficient PK/PD characteristics when produced in a fed-batch process using CHO cells and EfficientFeed B+ Supplement. The goal of this work was to evaluate whether replacing EfficientFeed B+ Supplement with GlycanTune B+ feed could increase mAb galactosylation and sialyiation and improve PK characteristics. The process needed to achieve comparable or better cell growth and productivity, as well as an improved glycan profile, and scale-up to the GMP 1,000 L single-use bioreactor (S.U.B.) was evaluated using the existing CHO cell line and a similar optimal procedure with GlycanTune B+ feed.

Materials and methods

GlycanTune B+ feed was evaluated with a proof-of-concept shake flask study, a 3 L bioreactor study, and a confirmatory 1,000 L GMP-scale study. Gibco™ CD CHO Medium was used as the basal formulation for the test fed batches in each study. Supplementation was evaluated with GlycanTune B+ feed and with EfficientFeed B+ Supplement as the control feed. The 3 L bioreactor study also evaluated whether an initial feed at day 0 or day 4 was optimal for GlycanTune B+ feed.

The 1,000 L S.U.B. study was carried out using the optimized GlycanTune B+ feed process with 3.33% on days 4 and 7 and 1.67% on days 10 and 13. It was comparable to historical 1,000 L S.U.B. runs performed with EfficientFeed B+ Supplement using the same process.

The mAb titers were tested by HPLC protein A affinity chromatography. The percentage of acidic species was tested by cIEF, and sialic acid concentrations were determined by HPLC. Glycan profiles were evaluated by LC-MS. An *in vivo* mouse PK study was conducted by an outside laboratory to evaluate the performance of the mAbs produced with either GlycanTune B+ feed or the EfficientFeed B+ Supplement control. Antibody concentrations in the PK study were measured using an IgG (Fc-specific) ELISA.

Results

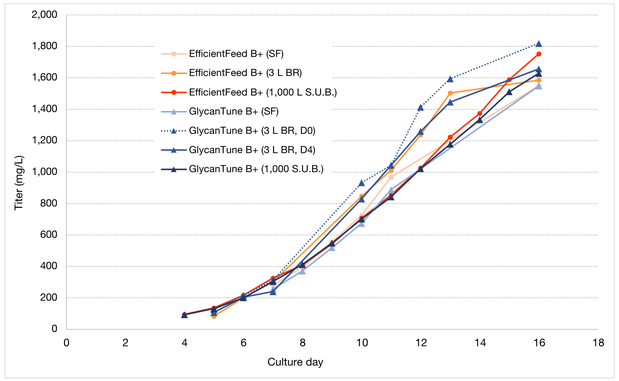

Figure 1. Evaluation of titers using GlycanTune B+ and EfficientFeed B+ feeds. GlycanTune B+ feed (blue) produced antibody titers that were comparable to those obtained in the control process performed with EfficientFeed B+ feed (orange) in the shake flask (SF), 3 L bioreactor (3 L BR), and 1,000 L GMP S.U.B. (1,000 L S.U.B.) studies. In the 3 L BR study the initial GlycanTune B+ feed on day 0 (D0) produced a slightly higher titer than the initial day 4 feed (D4); however, the improvement did not warrant changing the existing process from a regulatory perspective.

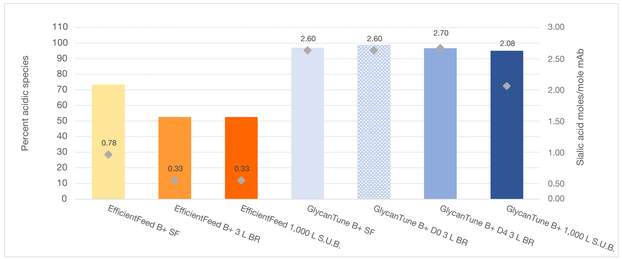

Figure 2. Percentage of acidic species and sialic acid concentrations. At the three production scales tested, cIEF analysis of the mAb showed a substantial increase in acidic species to nearly 100% with GlycanTune B+ feed (blue), relative to that produced with EfficientFeed B+ feed (orange), at approximately 50% to 70% acidic species. The higher percentage of acidic species observed with GlycanTune B+ feed indicated the feed likely increased mAb galactosylation. HPLC analysis of the sialic acid concentrations (secondary axis) indicated sialyiation increased from a range of 0.33 to 0.78 moles/mole mAb with EfficientB+ feed to a range of 2.08 to 2.70 moles/mole mAb with GlycanTune B+ feed.

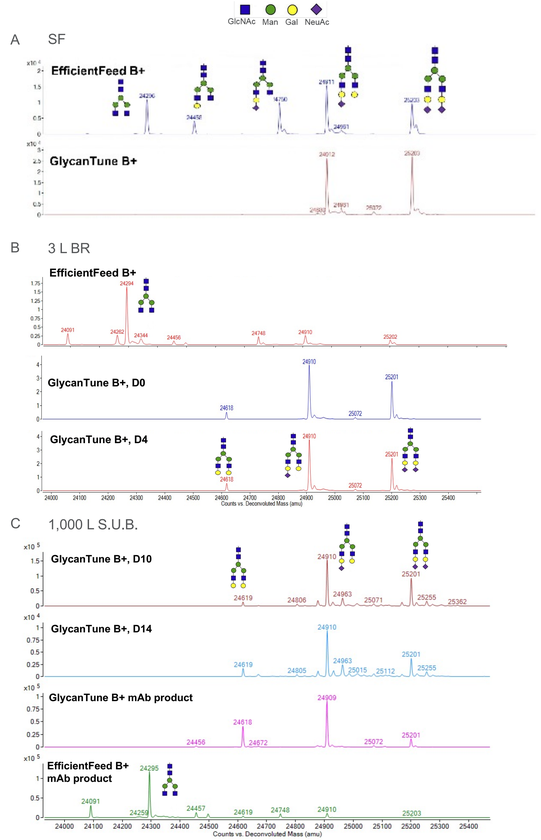

Figure 3. Glycan profiles. LC-MS analysis of the mAb light chain glycan structures confirmed that use of GlycanTune B+ feed resulted in improved profiles with full galactosylation and nearly complete sialyiation. **(A)** Shake flask (SF) glycan profiles obtained using GlycanTune B+ and EfficientFeed B+ feeds. **(B)** 3 L bioreactor (3 L BR) glycan profiles obtained using GlycanTune B+ and EfficientFeed B+ feeds. **(C)** 1,000 L GMP S.U.B. (1,000 L S.U.B.) glycan profiles on day 10 (D10) and day 14 (D14) obtained using GlycanTune B+ feed, and final mAb product glycan profiles obtained using GlycanTune B+ and EfficientFeed B+ feeds. The mAb heavy chain (non-sialylated) glycan profile was relatively unchanged using the GlycanTune B+ feed (data not shown).

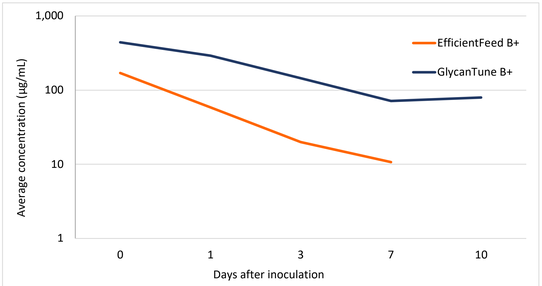

Figure 4. Mouse *in vivo PK* study. The results of the PK study in mice indicated that mAb production with the GlycanTune B+ feed process resulted in a 5-fold increase in the concentration of the biological molecule, on average. This increase correlated to a 6-fold increase in sialic acid levels, from 0.33 to 2.08 moles/mole mAb in the 1,000 L bioreactor, as shown in Figure 2.

Conclusions

The results of this case study demonstrate the relatively minimal development time required to effectively evaluate GlycanTune B+ feed as a replacement for EfficientFeed B+ Supplement to improve the glycan profile and resulting PK characteristics of a novel mAb. Progressive scale-up studies performed using shake flasks, 3 L bioreactors, and 1,000 L S.U.B.s were effectively complete within 2 months and were easily achieved while comparing GlycanTune B+ feed to an EfficientFeed B+ feed control. A GlycanTune B+ day 0 feed start was evaluated at the 3 L scale and provided a comparable titer. Therefore, the existing feed strategy with a day 4 feed start was maintained.

Throughout scale-up testing, GlycanTune B+ feed met requirements by enabling cell growth (data not shown) and antibody titers that were comparable to those obtained with EfficientFeed B+ control feed using the existing CHO cell line and feed strategy. Feeding with GlycanTune B+ supplement consistently and effectively improved the mAb light chain glycan structure, with full galactosylation and nearly complete sialylation. Higher sialic acid levels were correlated with a demonstrated 5-fold improvement in the *in vivo* mAb concentration in a mouse PK study. GlycanTune B+ feed has demonstrated the potential to dramatically increase mAb galactosylation and sialic acid content, as well as improve molecular PK characteristics. Such improvements in mAb profiles can potentially reduce therapeutic dosage requirements, manufacturing material consumption, and drug costs.

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