Enhancing ADCC Efficacy of an Anticancer mAb Therapeutic by Increasing % of Afucosylated Glycans through Cell Culture Process Optimization

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Abstract

The poster describes how to enhance antibody-dependent cellular cytotoxicity (ADCC) of an anticancer monoclonal antibody overexpressed in Chinese hamster ovary (CHO) cells through cell culture process optimization to increase the afucosylated glycan population. The antibody is a monoclonal humanized IgG1 isotype that selectively binds to the extracellular receptor of cancer cells to induce antibody-dependent cellular cytotoxicity, leading to the death of cells that express the cancer target. The mechanism of action of the therapeutical antibody is in part related to ADCC. Afucosylated antibodies lacking the core fucose residue from Fc N-glycans exhibit strong ADCC at lower concentrations with much higher efficacy compared to fucosylated counterparts and can evade the inhibitory effect of serum immunoglobulin G (IgG) on ADCC through high binding to gamma receptor IIIa (FcγRIIIa). We enhanced the ADCC effect by increasing the % of afucosylated species through optimization of the cell culture process using a design of experiments (DOE) approach.

Optimization of cell culture process parameters in the 2 L scale-down model

Cell culture process parameters were identified with the potential to impact quantity of afucosylated species in final culture. These included dissolved oxygen (DO%) setpoint, process temperature and pH ranges. Design of experiment (DOE) studies were designed using JMP® (SAS) and executed in qualified 2 L scale-down models to characterize production stage DO % setpoint, process temperature, and pH range. Parameter setpoints and ranges incorporated into the DOE for evaluation are outlined in the table below. Experimental results from the DOE studies were input into JMP® to generate fixed-effect, least-square regression statistical models to quantitate the effect ofparameter ranges on quality attributes including %G0 glycan, SEC %Main, SEC %HMW, CEX %Main, and CEX %Acidic. Additionally, response surface prediction profiler graphs generated from the regression model were used to predict mean and confidence intervals of performance and quality attribute output values as functions of the input parameter values, and the predicted values were evaluated against the set of acceptance ranges.

Parameter	Study Setpoint or Range	Operating Setpoint or Range
Temperature (°C)	33, 33.5, 34, 34.5, 35, 36	35
DO (%)	15, 30, 45	30
pH Range	6.7 – 7.2	6.8 – 7.1
pH Range (Post-Shift)	6.6 – 7.0	6.7 – 6.9

The model prediction profiler using either 35 °C or 33.5°C and all other parameter inputs set to their operating ranges are shown in Figures 1 and 2.

For %G0, the predicted mean at 35.0°C is 3.7% with a confidence interval of 3.2% to 4.3% (Figure 1). In comparison, the predicted mean at 33.5°C is 4.1% with a confidence interval of 3.8% to 4.3% (Figure 2). Thus, performing the process at 33.5°C has a higher probability of achieving high %G0.

Figure 1 Temperature Characterization Profile at 35°C Setpoint

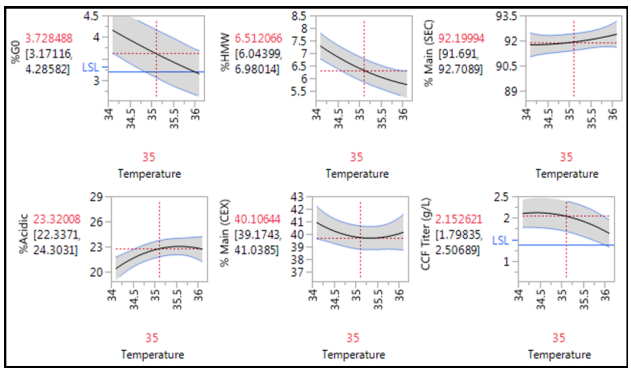
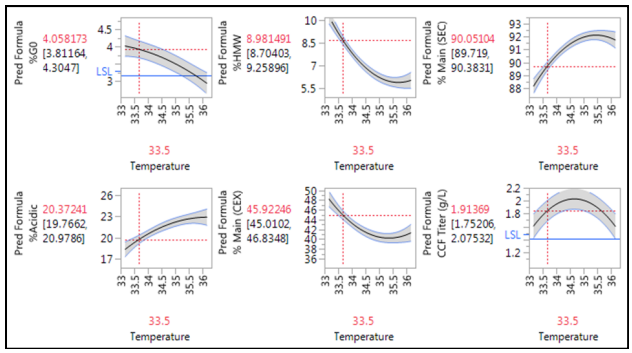


Figure 2 Temperature Characterization Profile at 33.5°C Setpoint



For SEC %HMW, the predicted mean at 35.0°C is 6.5% with the confidence interval of 6.0% to 7.0% (Figure 1). In comparison, the predicted mean at 33.5°C is 9.0% with the confidence interval of 8.7% to 9.3% (Figure 2).

For SEC %Main, the predicted mean at 35.0°C is 92.2% with a confidence interval of 91.7% to 92.7% (Figure 1). In comparison, the predicted mean at 33.5°C is 90.1% with a confidence interval of 89.7% to 90.4% (Figure 2). However, downstream operations have the potential to decrease SEC %HMW. See Figure 7, the lowered bioreactor temperature setpoint at 1000L scale did not have negative impact on the product purity.

For CEX %Acidic, the predicted mean at 35.0°C is 23.3% with a confidence interval of 22.3% to 24.3% (Figure 1). In comparison, the predicted mean at 33.5°C is 20.3% with the confidence interval of 19.8% to 21.0% (Figure 2). Therefore, operating at the temperature setpoint of 33.5°C would yield improved charge heterogeneity impurity profile compared to that at 35.0°C. For CEX %Main, the predicted mean at 35.0°C is 40.1% with a confidence interval of 39.2% to 41.0% (Figure 1). In comparison, the predicted mean at 33.5°C is 45.9% with the confidence interval of 45.0% to 46.8% (Figure 2). Therefore, operating at the temperature setpoint of 33.5°C would yield improved charge heterogeneity impurity profile compared to that at 35.0°C.

For titer, the predicted mean at 35.0°C is 2.2 mg/ml with a confidence interval of 1.8 mg/ml to 2.5 mg/ml (Figure 1). In comparison, the predicted mean at 33.5°C is 1.9 mg/ml with the confidence interval of 1.8 mg/ml to 2.1 mg/ml (Figure 2)

Based on results from the regression models, operating the process at 33.5°C instead of 35.0°C improves %G0. Because %G0 is a critical quality attribute and downstream operations have the potential of improving product related impurities assessed by SEC, operating at 33.5°C is preferred. Due to the increase in % G0 glycan at lower process temperature, 33.5°C is used as the temperature setpoint for subsequent productions at 1000L scale.

The following results at 1000L scale show product quality attribute comparisons of pre-change (35°C temperature setpoint) and post-change (33.5°C temperature setpoint).

Released glycan analysis by hydrophilic interaction liquid chromatography (HILIC)

The first method to evaluate glycan heterogeneity is by the examination of glycans released from the proteins and separated by hydrophilic interaction liquid chromatography (HILIC). In general, glycoproteins are first denatured, then treated with PNGase F to release the N-glycans. The released glycans are labeled with a fluorescent dye. The labeled glycans are then separated according to hydrophilicity by a mobile phase gradient of increasing aqueous buffer on a HILIC column with fluorescence detection.

As shown in Figure 3, the total afucosylation level was significantly increased from 8-9% in the pre-change lots to 9.3-10.5% in the post-change lots, demonstrating the effect of the lowered bioreactor temperature setpoint at 1000L scale.

Figure 3 Relative abundance (% Peak Area) of Afucosylated Glycans increased in post-change lots.

Glycopeptide Analysis by LC-MS/MS

This method for determining glycan structure is by identifying the residue site of glycosylation and quantifying the glycopeptides generated by tryptic digestion of test material. It allows more sensitive detection and quantitation of certain very minor glycans, such as those including sialic acids. Total Afucosylated glycans by Glycopeptide LC-MS/MS analysis shows an increase at 33.5°C process temperature.

As shown in Figure 4, the total afucosylation level was significantly increased from 7.09 -8.15 % in the pre-change lots to 8.93-9.86 % in the post-change lots, demonstrating the effect of the lowered bioreactor temperature setpoint at 1000L scale.

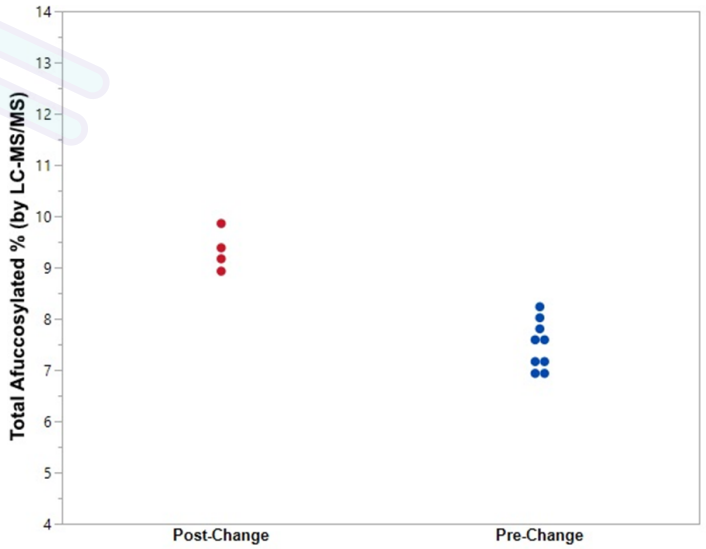


Figure 4 Relative abundance (% Peak Area) of Afucosylated Glycans increased in post-change lots.

Primary NK Cell ADCC Assay

Fc receptor-mediated ADCC is an important mechanism of action (MoA) by which anticancer antibody targets cancer cells for elimination. This study evaluated the anticancer mAb ADCC activities, using a cell line SKBR3 as target and an NK cell line (FcγRIIIa V158 variant) as effector.

As shown in Figure 5, the relative NK cytotoxicity level was significantly increased from 72.2 - 150.8 % in the pre-change lots to 96.2 - 180.1 % in the post-change lots, demonstrating the effect of increased afucosylated glycans in the post-change lots.

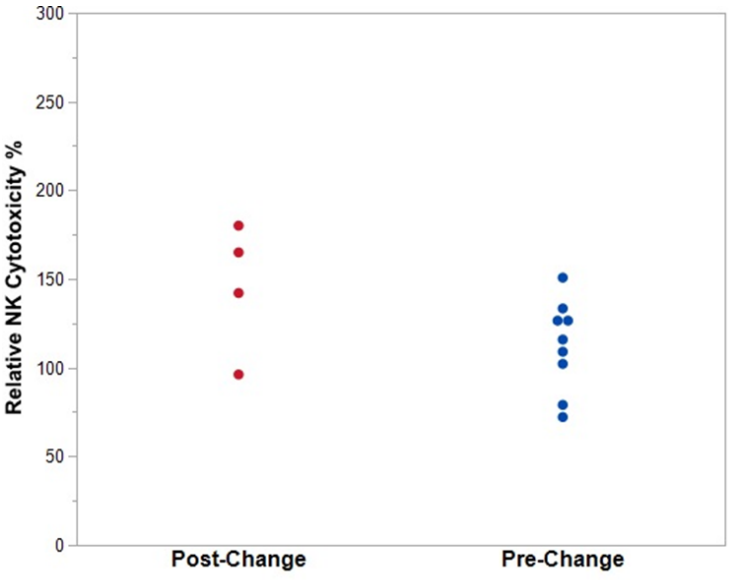


Figure 5 Primary NK cell ADCC activity increased in post-change products.

FcyRIIIa (158V) Binding by AlphaLISA

The effector function of antibody-dependent cellular cytotoxicity (ADCC) depends on both the receptor binding through the Fab domain of the antibody, and binding to FcγRIIIa (CD16a) on Natural Killer (NK) cells through the Fc domain. The Fc portion of the IgG antibody can engage various Fcγ receptors, FcRn (neonatal Fc receptor) and complement components. Binding to various Fcγ receptors and FcRn for the anticancer mAb was evaluated by AlphaLISA and SPR assays (data not shown).

Figure 6 shows the comparison of FcγRIIIa (158V) AlphaLISA Relative Potency Binding of post-change and pre-change products, the relative potency binding level was significantly increased from 92.9-114.7 % in the pre-change lots to 108.5-145.0 % in the post-change lots, FcγRIIIa (CD16a) binding result is consistent with the enhanced ADCC and the increased afucosylated glycans population by glycan analysis

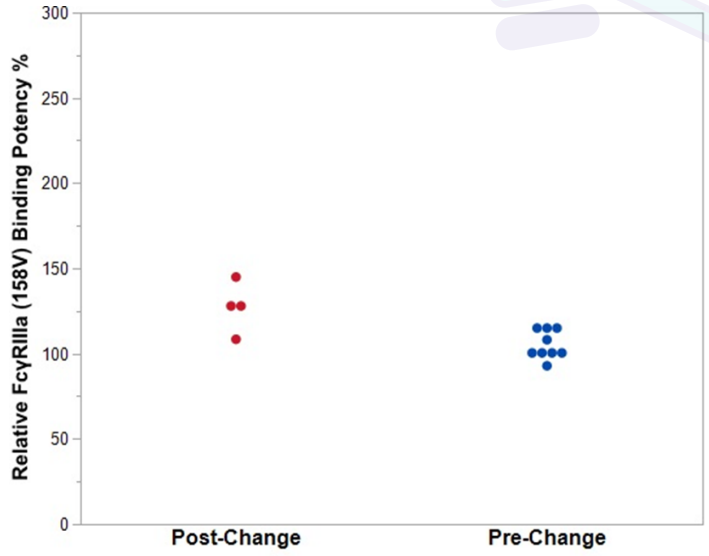


Figure 6 Comparison of FcγRIIIa (158V) AlphaLISA Relative Potency Binding of post-change and pre-change products.

Size Heterogeneity by Size Exclusion Chromatography with UV Detection (SEC)

Size exclusion chromatography (SEC) separates molecules based on their size by filtration through a gel. The gel consists of spherical beads containing pores of a specific size distribution. Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access most or all pores. Thus, large molecules elute first, smaller molecules elute later, while molecules that can access all the pores elute last from the column. Therefore, this method was used to determine the percent purity of the product.

Figure 7 shows SEC Results of Size Heterogeneity of pre-change and post-change products, demonstrating the lowered bioreactor temperature setpoint at 1000L scale did not have negative impact on the product purity.

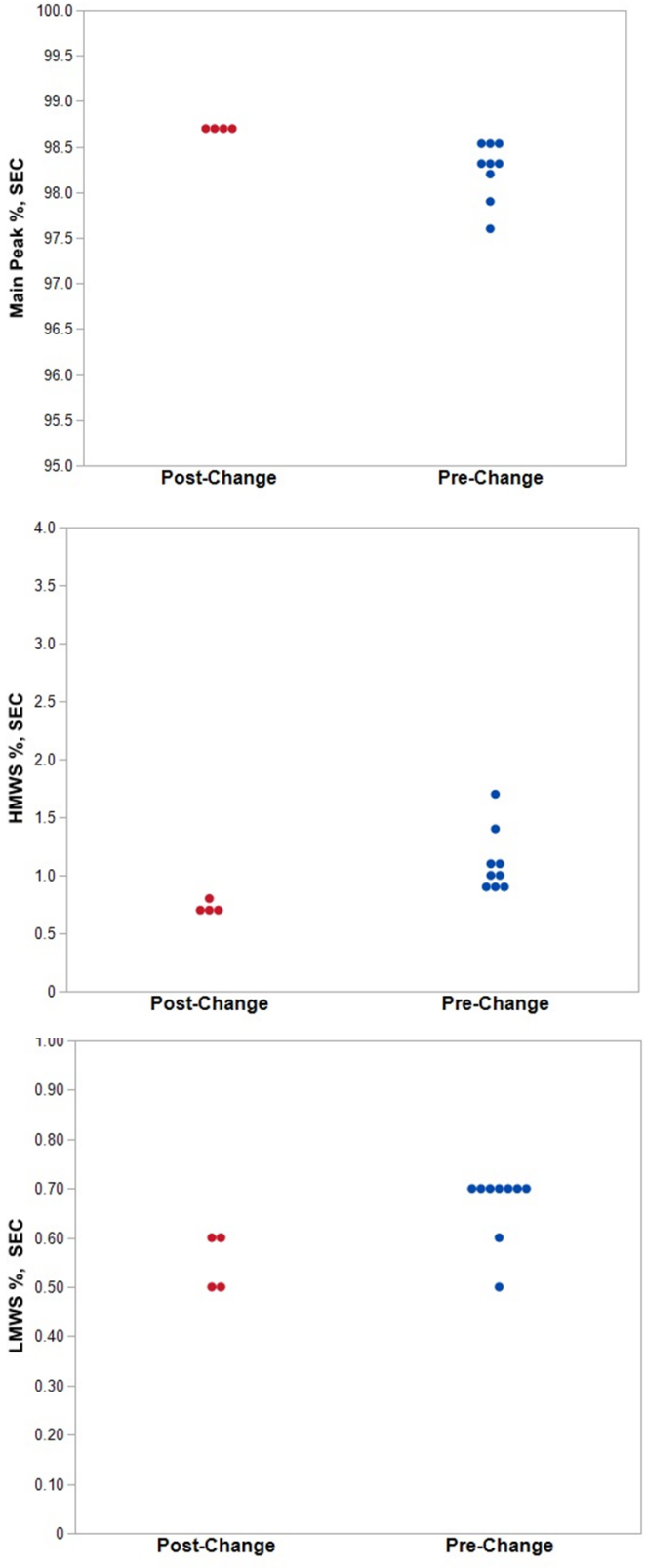


Figure 7 SEC Results of Size Heterogeneity of pre-change and post-change products

Protein Charge Heterogeneity by Cation Exchange Chromatography (CEX)

Cation exchange HPLC is used to characterize the charge heterogeneity of the antibody based on separation of protein molecules by their interaction with a negatively charged stationary phase. Proteins bind to cation exchange column at lower pH and salt concentration, and elute in the order of acidic, main and basic fractions as pH and salt concentration of the mobile phase increases.

Figure 8 shows the comparisons of % main peak, % total acidic peaks and % total basic peaks. The post-change lots had lower acidic and basic species than the pre-change lots.

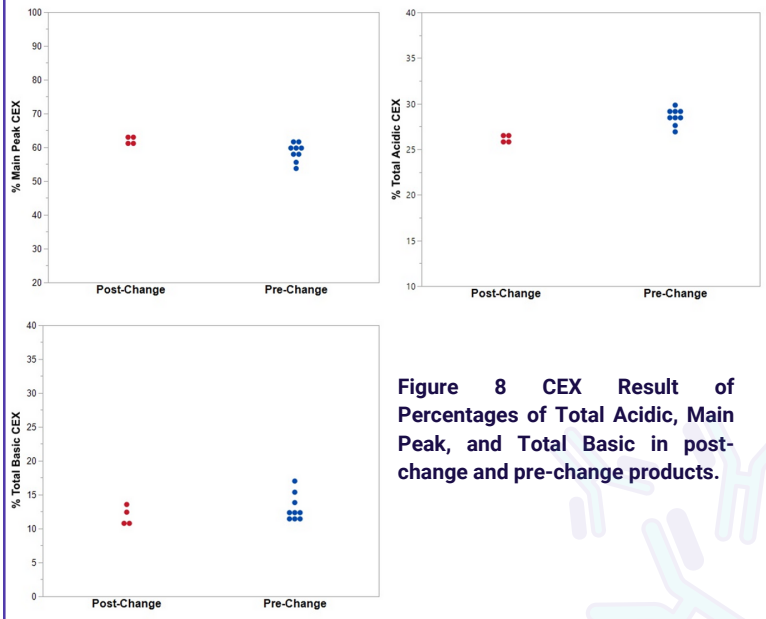


Figure 8 CEX Result of Percentages of Total Acidic, Main Peak, and Total Basic in post-change and pre-change products.

Discussion and Conclusion:

It is important to maximize the efficacy of therapeutic antibodies which can lower the cost by potentially reducing the dose or duration of treatment. Increasing afucosylation % of a monoclonal antibody by optimizing process parameters has the potential to enhance the efficacy of anticancer antibody therapeutics. The study showed operating the process temparature at 33.5°C instead of 35.0°C improved afucosylated glycan %, therefore, enhanced ADCC activity.

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