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Targeting product quality: Where systems biotechnology and process design meet

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Product quality is a result of the entire production process including protein sequence, host cell, media and process parameters. Many of the desired product properties are defined by posttranslational modifications with impact on biological activity, immunogenicity, half-life or stability. In-depth understanding of the host cells capabilities as well as of the process interactions enables the targeted modulation of product quality attributes by rational selection of host cells and design of bioprocesses. This is valuable for new biological molecules in order to improve efficacy, reduce side effects, access new patient populations. For biosimilars this allows developing into defined quality attribute profiles. The identification of suitable host cells, process parameters and media compositions to modulate quality attributes is challenging due to the complexity of the cell and the bio-processes. Here, we want to present two aspects of how we approach this challenge: First, by a global RNAseq-driven analysis that reveals distinct differential expression patterns of genes contributing to recombinant antibody glycosylation (Könitzer & Müller et al., 2015) and second by comprehensive data analysis, in-depth characterization and high-throughput screening of process parameters and media compounds impacting glycosylation.

To characterize different host cells a global analysis was performed on glyco-pattern and gene expression level. Six different monoclonal antibody projects with over 550 analyses were reviewed concerning their glyco-pattern distribution based on ESI-MS and HPLC data. Additionally, nearly 200 RNAseq gene expression data were used for a pathway-oriented analysis of the glycosylation-associated transcripts. Gene expression levels were compared between the three potential host cell lines as well as for host versus producing cell line. We identified with our new NGS pipeline 278 transcripts in our database. Expression patterns were host cell specific and depended on whether a mAb was expressed or not. For example, the expression of Sialyltransferase 10 (St3gal6) and B4galt6 (β 1,4-galactosyltransferase 6) could only be observed in the CHO-K1 host cell line while Cmah was only detectable in CHO-DG44 cells. Interestingly, St6gal1 was switched-on in mAb producing CHO-DG44 cells but at a very low level. this explains why normally only relatively low sialylation is observed with products produced in this cell line, and, since both the Sialyltransferase 10 and the CMP-Neu5Ac Hydroxylase activities are needed for constitution of with Neu5Gc sialic acid glycosylated antibodies, by lacking of the St3gal6 (CHO-DG44 cells) or the Cmah gene (CHO-K1 cells) mainly the non-immunogenic Neu5Ac sialic acids are predominant in CHO cells. Such data improve future production clone selection and process development strategies for better steering but may also support selection of critical quality attributes.

The impact of cell culture conditions and media compounds on the glycosylation pattern was assessed by an integrated screening approach. Initially a database was created including process and analytical data from twelve projects. Data sets of more than 2500 fed-batch processes with 6300 analytical data sets enabled a cross-project analysis and correlation of process parameters with product quality attributes. Additionally, multi parallel small scale bioreactors, robotics based product capture and high throughput analytics were combined to minimize hands-on-time to gain data for correlation analysis. Said setups supported the identification of numerous media supplements and upstream process conditions that were applied for rational modulation of glycosylation patterns. Moreover, case studies focusing on the optimization of glycan patterns and antibody dependent cellular cytotoxicity by using metal ions as media supplements will be shown.

Knowledge-driven selection of a host cell already gives direction to the product quality space to be expected with a certain molecule in clone selection. After gap analysis, process parameters can be chosen for application in process development to finally achieve the set quality target product profile.

Könitzer, Jennifer D and Müller, Markus M, et al. "A global RNA-seq-driven analysis of CHO host and production cell lines reveals distinct differential expression patterns of genes contributing to recombinant antibody glycosylation." Biotechnology Journal 10.9 (2015): 1412-23.