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# The CHO cell line as a potentially safer host for cetuximab therapeutic antibody production than the Sp2/0 cell line

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**Abstract:** The host cell line influences the quality of the recombinant protein produced within it. In the case of monoclonal antibodies, the most frequently chosen hosts are hamster- and mouse-derived cell lines. However, the glycosylation profile of the mAbs generated using these cell lines is expected to be different. In this study, we used the cetuximab protein obtained from the Sp2/0 and CHO cell lines to analyse the content of fucosylated and non-fucosylated glycoforms, as well as structures containing N-glycolylneuraminic acid, N-acetylneuraminic acid and Gal-Gal structures. Our results confirm literature data suggesting a higher level of fucosylated glycoforms and a higher level of N-acetylneuraminic acid in CHO- over Sp2/0-derived cetuximab. We also confirm the lack of Gal-Gal structures in the CHO-derived antibody. Moreover, we have demonstrated the Sp2/0- and

CHO-derived IgG may have a comparable non-fucosylated glycoforms content. This is a significant find, since non-fucosylated species are the triggers of the biological activities of many antibodies.

**Keywords:** antibodies; antibody production; bioanalysis; biosimilars; biotechnology; cetuximab; cell lines; Chinese hamster ovary cell line; glycoproteins; glycosylation.

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## 1 Introduction

### *1.1 Overview of techniques for obtaining monoclonal antibodies*

Monoclonal antibodies are the fastest growing group of therapeutics in the world. Their history began in 1975, with the development of muromonab-CD3, which was licensed as Orthoclone OKT3 in 1986. It is a monoclonal mouse IgG2a antibody used in the prevention of kidney transplant rejection (Liu, 2014). However, it can cause acute side effects, which result from the release of human anti-mouse antibodies (HAMA) by the patients' organisms. In 1975, Köhler and Milstein developed a method for the immortalisation of B lymphocytes, which are responsible for the production of antibodies for a specific epitope. Short lived B lymphocytes were immortalised by fusing them with myeloma cells (Kunert and Reinhart, 2016). This approach – hybridoma technology – resulted in the development of an immortalised cell line producing antibodies with human characteristics (Parray et al., 2020). Initially, hybridoma technology was dedicated for the production of murine antigens. However, over the years, its application has been

broadened to include a wide range of antigens from different species (e.g., rabbit, human, chicken, goat, rat) (Parray et al., 2020). While hybridoma technology is widely used and easy to implement, it must be remembered that hybridoma clones obtained by fusing cells from different species are genetically unstable and it is then necessary to generate B lymphocytes by immunising laboratory animals with the target antigen (Parray et al., 2020). These limitations preclude the use of this technology for the production of human therapeutic antibodies. Naturally, some mAbs of human origin have been developed using hybridoma technology for the treatment of HIV, Chikungunya, and dengue, among others. Despite this, the instability of hybridoma clones presents multiple challenges from the point of view of both drug safety and the repeatability of the manufacturing process. Attempts to obtain natural human fusion partners have failed. In the following years, research on hybridoma technology led to the development of a combined ex-vivo immunisation strategy based on purified B and T cells cultured in the presence of antigen and growth factor and then fused with myeloma cells and screened for the production of antibodies against the antigen (Parray et al., 2020).

The next step in the development of cell lines offering stable and consistent production was the cloning of variable heavy (VH) and variable light (VL) chains from unstable hybridomas into vectors that could be used to introduce a genetic construct into mammalian cells (Parray et al., 2020). The genetic construct can also be obtained by chemical synthesis of a defined nucleotide fragment encoding the desired protein and this is currently the most widely used approach in the pharmaceutical industry.

Other technologies include the clustered regularly interspaced short palindromic repeats (CRISPR) system, associated with the use of caspase-9 (Cas9) from *Streptococcus pyogenes* and the phage display method. The CRISPR/Cas9 system allows for targeted sequence recognition and rearrangement (Choi and Meyerson, 2014).

In this study, we used mammalian cell lines transfected with an in-house genetic construct including DNA fragments coding for the cetuximab antibody.

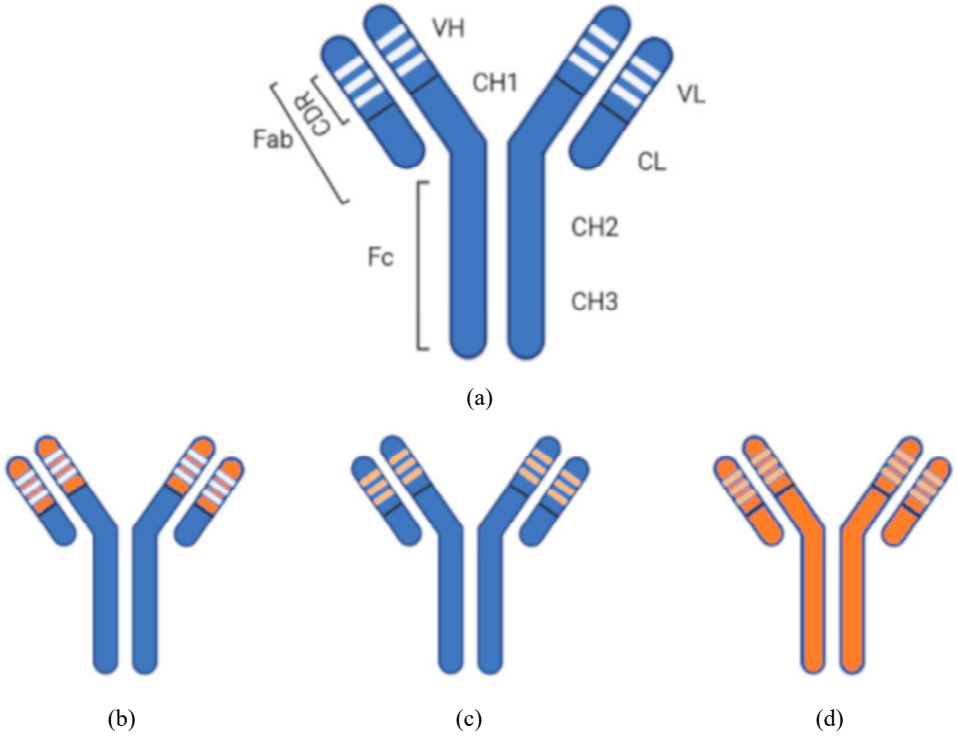
### *1.2 Monoclonal antibody structure*

The construction of therapeutic antibodies can vary. We can distinguish four main types of monoclonal antibodies based on the origin of their fragments (Figure 1):

- murine: entirely derived form a murine source
- chimeric: combining variable regions of murine origin with a human constant region
- humanised: derived from a human source but with a murine fragment responsible for binding to the target (complementary-determining region – CDR)
- human: entirely derived from a human source (Parray et al., 2020).

In this study, we worked with cetuximab, a chimeric monoclonal IgG1 antibody, which binds to the extracellular domain of the epidermal growth factor receptor (EGFR). Cetuximab inhibits the dimerisation of the receptor, thereby blocking tumour growth and metastasis (Blick and Scott, 2007; Chung et al., 2008).

**Figure 1** Schematic overview of antibody types: from human (blue domains) to murine antibodies (orange domains), (a) the human monoclonal antibody (b) the chimeric monoclonal antibody (c) the humanised monoclonal antibody (d) the murine monoclonal antibody (see online version for colours)




### *1.3 Expression systems*

Regardless of the complexity of the mAb and the way the genetic construct is obtained, an essential part of the entire system is the host cell line, which influences the quality characteristics of a protein. The selection of a suitable expression system has a key impact on productivity and the physicochemical characteristics of the protein of interest, and thus also on its biological activity, safety, pharmacokinetics and pharmacodynamics (Goh and Ng, 2017; Gomes et al., 2016). A range of expression systems are used in industry, depending on their application: bacteria, yeast, filamentous fungi, insect, plant or mammalian cells (Gomes et al., 2016).

Prokaryotes can generally be regarded as the most suitable expression systems for the production of non-glycosylated proteins. However, for glycosylated proteins, mammalian cell lines are preferred due to the differences in the glycosylation profile of proteins obtained from different expression systems (Schmidt, 2004). It is also important to note that the type of mammalian expression system influences the glycosylation profile of the desired protein. Most often, mammalian cell lines are divided into non-human and human systems.

Among non-human expression systems, the most widely used is the Chinese hamster ovary (CHO) cell line, which is used for large-scale production of mAbs. Systems based

on various CHO cell types (e.g., DUXB11, DG44, CHOK1) are used to obtain over 60% of currently approved biotherapeutics (Goh and Ng, 2017; Li et al., 2010; Parray et al., 2020). CHO cells are well characterised, easily transfected and robust. Most importantly, CHO cells are able to produce human-compatible glycosylation (Goh and Ng, 2017).

Other non-human mammalian expression systems include murine cells such as the NS0 myeloma or Sp2/0 hybridoma cell lines. However, murine cell lines employ a different glycosylation mechanism from the CHO expression system and attach potentially immunogenic glycoforms to the desired protein (Goh and Ng, 2017; Li et al., 2010).

Human cell lines (e.g., HEK293, BHK-21, PER.C6 or HT-1080) are capable of adding fully human post-translational modifications to the protein (Goh and Ng, 2017). While these human cell lines can be a promising alternative to non-human host cells, they also have disadvantages. The most important one is the capacity to produce sialyl-Lewisx (sLex) antennary fucosylation. This can influence the distribution and pharmacokinetics of the drug through binding to areas of inflammation (Goh and Ng, 2017).

In this study, we focused on the influence of non-human cell lines on the glycosylation of an IgG1 monoclonal antibody. During experiments presented in this paper the cetuximab antibody was expressed in CHO and Sp2/0 cell lines and analysed. Basing on previous studies, it was anticipated that three key differences in the glycosylation profile of the resulting molecules may exist between the two different mammalian expression systems.

### *1.3.1 Level of core fucosylation/non-fucosylation*

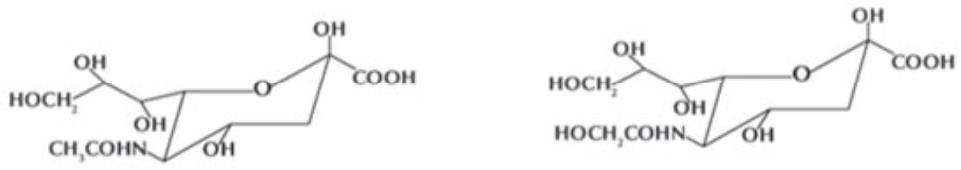
CHO-derived antibodies are known to contain high levels of core fucosylation (more than 95%) (Li et al., 2010; Raju, 2003), while mouse-derived antibodies often contain higher amounts (10%–40%) of non-fucosylated oligosaccharides (Raju, 2003). The content of fucosylated/non-fucosylated glycoforms has the greatest impact on the antibody-dependent cellular cytotoxicity (ADCC) of the antibody. The total non-fucosylation level increases the affinity of IgG1 to FcγRIIIa, thereby increasing ADCC activity, while the presence of fucosylated glycoforms inhibits IgG binding to FcγRIIIa (Lee et al., 2017; Kyoung et al., 2018; Liu, 2015).

### *1.3.2 Sialic acid structures*

Most non-human mammalian cell lines attach N-glycolylneuraminic acid (NGNA) to the glycosylation site of the protein (Goh and Ng, 2017). CHO cells can express low levels of NGNA (Li et al., 2010), while the main sialic acid form produced by the CHO cell line is N-acetylneuraminic acid (NANA) (Raju, 2003). The only difference between NANA and NGNA is the presence of an additional oxygen atom in NGNA (Figure 2). The presence of NGNA was initially ignored in drug development but it has been revealed that marketed cetuximab drug is prone to forming immune complexes with human anti-NGNA antibodies in vitro (Ghaderi et al., 2010). Such complexes promote cetuximab clearance (Liu, 2015), which leads to a decrease in drug efficiency. Moreover, it is hypothesised that NGNA may have a role in the pathogenesis of carcinomas, as its presence was indicated in various human cancers (Padler-Karavani et al., 2008). However, it has been confirmed that small amounts of NGNA are present in food and can

be incorporated into human tissues with no short-term effects (Kyoung et al., 2018; Li et al., 2010; Raju, 2003).

**Figure 2** Structure of (a) NANA and (b) NGNA




*Source:* Li et al. (2010)

### *1.3.3 Gal-Gal structure*

Mouse-derived cell lines, including Sp2/0, can form Galα1,3Gal (Gal-Gal) structures by attaching antigenic alpha-Gal onto recombinant proteins and producing galactose-α-1,3-galactose linkages (α-Gal) (Ayoub et al., 2013; Goh and Ng, 2017; Kyoung et al., 2018; Li et al., 2010). This is made possible by the presence of 1,3-galactosyltransferase, which mediates the transfer of Gal residues from UDP-Gal to the internal and/or exposed Gal residues (Raju, 2003). In contrast, glycans with Gal-Gal structures are not produced in CHO host cells (Li et al., 2010). This is important, as humans have been shown to express anti-α-Gal antibodies against Gal-Gal epitopes (Goh and Ng, 2017; Kyoung et al., 2018; Li et al., 2010; Raju, 2003). Chung et al. (2008) reported that Erbitux® had an immunogenic effect in almost one-third of cetuximab-treated subjects and the effect was correlated with the presence of anti-cetuximab, Gal-Gal specific antibodies in the patients' serum before treatment.

## 2 Materials and methods

### *2.1 Materials*

Twenty lots of IgG1 therapeutic antibody obtained from different expression systems were used:

- ten lots from the Sp2/0 cell line
- ten lots from the CHO cell line.

### *2.2 Methods*

All analytical methods used to generate results were developed and verified according to EMA guidelines [ICH Q2(R1), ICH Q6B].

### 2.2.1 N-linked glycan analysis

Glycosylation profile analysis was performed using N-glycans released from antibody and labelled with RapiFluor-MS (RFMS). Glycans from the Fc and Fab region were isolated using RapidPNGase F (New England Biolabs, Warsaw, Poland) and then labelled with an RFMS labelling agent (Waters, Warsaw, Poland). RFMS-labelled glycans were purified using a BioZen N Glycan Clean-Up Microelution SPE plate (Phenomenex, Warsaw, Poland). HILIC-UHPLC was performed to separate labelled glycans using an ACQUITY UPLC BEH Amide column (130 Å, 1.7 µm, 2.1 mm × 150 mm; Waters, Warsaw, Poland). The elution of RFMS-labelled glycans was recorded with a fluorescence detector using an excitation wavelength of 265 nm and an emission wavelength of 425 nm. The LOQ of the method was determined to be S/N = 10/1. LOQ was defined separately for each glycoform depending on signal value.

### 2.2.2 Glycan identification using mass spectrometry

Mass spectrometry was chosen to identify glycans. The RFMS-labelled glycans were separated using HILIC-UHPLC (Section 2.2.1) and identified using a Q Exactive Plus mass spectrometer (Thermo Scientific). A comprehensive list of molecular weights obtained from m/z values of possible glycan structures was compiled and compared with the MS data (full MS in positive ion mode, polarity within the range 760–2,500 m/z). The precision with which masses were obtained and identified using a Q Exactive Plusmass spectrometer was assumed to be 0.03 Da. Using a mass filter, the identified glycans were assigned to peaks observed in the total ion chromatogram (TIC).

### 2.2.3 Data evaluation and statistical analysis

The glycosylation profiles of the Sp2/0 and CHO host cell line products, including the fucosylation level, non-fucosylation level, sialic acid structure (NGNA and NANA) and Gal-Gal structure content were compared. The result for each group of glycoforms was calculated as a % content in the entire pool of glycosylated IgG.

To verify whether the Sp2/0- and CHO-derived antibodies differ significantly, a one way ANOVA test with a significance level of $\alpha = 0.05$ was performed for each glycostructure described. Statistically significant differences were confirmed for p values of $\leq 0.05$.

Differences among the glycosylation profiles of molecules obtained using different host cell lines were calculated by subtracting the lowest value from the highest with the direction of subtraction depending on the glycostructure (Table 1).

Additionally, the coefficient of variation (CV) was calculated for each host-specific group of glycoforms to verify the consistency of the results.

## 3 Results

A summary of the results presented as a % content of analysed glycostructures in the entire pool of mAbs obtained from Sp2/0 and CHO expression systems is presented in Table 1 and Figure 3.

**Table 1** Summary of the fucosylated, non-fucosylated, NGNA, NANA and Gal-Gal structure content in the entire IgG1 pool obtained from Sp2/0 and CHO expression systems

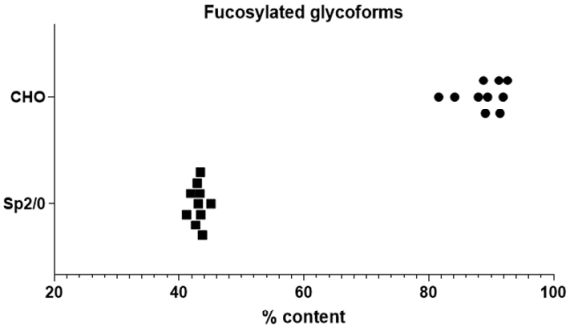
| *Lot no.* | *Fucosylated content (%)* | *Non-fucosylated content (%)* | *NGNA content (%)* | *NANA content (%)* | *Gal-Gal structure content (%)* |
|---|---|---|---|---|---|
| *Sp2/0* | | | | | |
| 1 | 42.63 | 6.34 | 14.69 | < LOQ | 35.64 |
| 2 | 42.87 | 5.71 | 14.48 | < LOQ | 36.94 |
| 3 | 41.83 | 6.87 | 13.94 | < LOQ | 36.01 |
| 4 | 43.73 | 5.82 | 15.01 | < LOQ | 35.77 |
| 5 | 43.45 | 5.40 | 13.32 | < LOQ | 36.31 |
| 6 | 41.18 | 6.50 | 16.27 | < LOQ | 36.27 |
| 7 | 43.06 | 5.81 | 15.35 | < LOQ | 35.52 |
| 8 | 43.30 | 5.66 | 15.88 | < LOQ | 23.84 |
| 9 | 43.39 | 5.57 | 16.10 | < LOQ | 23.84 |
| 10 | 45.08 | 6.23 | 15.23 | < LOQ | 22.84 |
| Mean Sp2/0 | 43.05 | 5.99 | 15.03 | < LOQ | 32.30 |
| CV Sp2/0 | 2.46 | 7.85 | 6.32 | X | 18.85 |
| *CHO* | | | | | |
| 1 | 92.62 | 3.91 | 5.54 | 29.78 | < LOQ |
| 2 | 89.05 | 4.64 | 4.10 | 25.66 | < LOQ |
| 3 | 84.16 | 4.02 | 5.13 | 22.25 | < LOQ |
| 4 | 81.59 | 4.37 | 4.89 | 20.36 | < LOQ |
| 5 | 87.92 | 5.37 | 5.89 | 21.55 | < LOQ |
| 6 | 91.92 | 5.02 | 6.00 | 22.45 | < LOQ |
| 7 | 88.75 | 7.46 | 4.06 | 16.47 | < LOQ |
| 8 | 89.42 | 7.26 | 4.51 | 18.57 | <LOQ |
| 9 | 91.41 | 4.99 | 4.00 | 25.06 | < LOQ |
| 10 | 91.23 | 4.95 | 3.90 | 25.09 | < LOQ |
| Mean CHO | 88.80 | 5.20 | 4.80 | 22.72 | < LOQ |
| CV CHO | 3.97 | 23.61 | 16.83 | 16.92 | X |
| Difference between means | 2.06× higher values in CHO | 1.15× higher values in Sp2/0 | 3.13 × higher values in Sp2/0 | Species present only in CHO | Species present only in Sp2/0 |
| ANOVA p value | < 0.0001 | 0.1867 | < 0.0001 | < 0.0001 | < 0.0001 |

*Source:* Data generated by HILIC-UHPLC

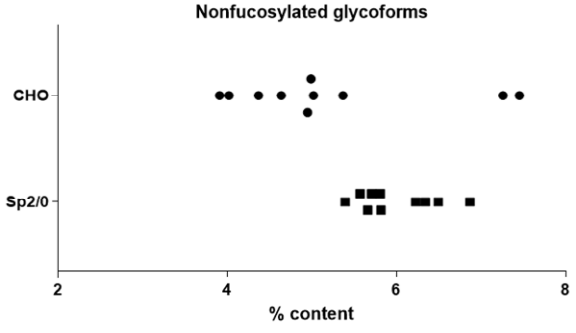
As presented in Table 1 and Figure 3, we have observed that in CHO-derived antibody pools the percentage content of fucosylated oligosaccharides was higher than in Sp2/0-derived antibody and was between 81.6% and 92.6% vs. 41.2%–45.1% in Sp2/0-derived IgG. The content of non-fucosylated glycoforms was comparable in CHO and Sp2/0-derived cetuximab (3.91%–7.46% in CHO-derived antibody vs. 5.40%–6.87% in Sp2/0-derived antibody). The content of NGNA in CHO-derived antibody was lower (mean value 4.8%) than in Sp2/0-derived antibody (mean value 15.0%), while NANA

was at the level of 16.5%–29.8% in CHO-derived antibody and in Sp2/0-derived antibody it was below the limit of quantitation. Gal-α-1,3Gal (Gal-Gal) structures were not found in CHO-derived antibody, while Sp2/0-derived IgG contained 22.8%–36.9% of Gal-Gal structures.

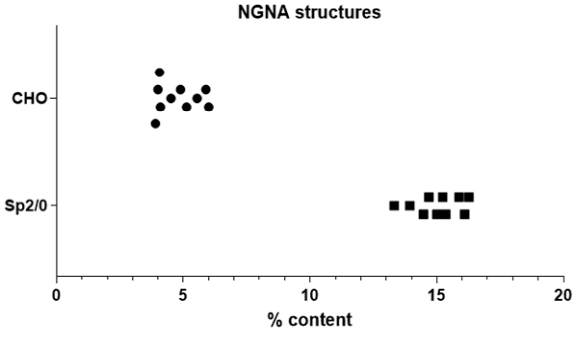
**Figure 3** Graphical summary of fucosylated and non-fucosylated glycoforms and NGNA structure content in the IgG1 obtained from the Sp2/0 (squares) and CHO (circles) expression systems




(a)




(b)




(c)

Note: NANA and Gal-Gal structures are not presented as their level was below LOQ in the molecules obtained from Sp2/0 and CHO, respectively.

*Source:* Data generated by HILIC-UHPLC method

## 4 Discussion

Our findings indicate that host cell line selection is a critical step in the production process of a monoclonal antibody. As most monoclonal antibodies have specific glycosylation patterns, mammalian cell lines appear to be the best choice of host cells for their production (Goh and Ng, 2017; Gomes et al., 2016; Schmidt, 2004). However, even between mammalian cell lines the glycosylation profile of the resulting proteins can vary significantly (Goh and Ng, 2017; Li et al., 2010).

In this study, we focused on two non-human mammalian cell lines commonly used for IgG production, namely Sp2/0 and CHO, to produce cetuximab. The glycoforms present on the molecules were identified using chromatography (HILIC-UHPLC) and mass spectrometry, and their content was calculated for the entire pool of glycoforms.

Our data confirms that the same IgG expressed in different cell lines demonstrates a different glycosylation profile (Goh and Ng, 2017; Li et al., 2010; Schmidt, 2004). Based on literature, it was expected that the CHO-derived antibody would present a higher level of fucosylation (up to 95%) (Li et al., 2010; Raju, 2003), a higher level of NANA content (Raju, 2003), and a lack of Gal-α-1,3Gal (Gal-Gal) structures, unlike the Sp2/0-derived antibody (Goh and Ng, 2017; Hmiel et al., 2015; Kyoung et al., 2018; Li et al., 2010). In contrast, the Sp2/0-derived antibody was expected to demonstrate a higher level of non-fucosylated oligosaccharides (10%–40% vs. 0%–10% in CHO) (Raju, 2003), and a higher level of NGNA content (Li et al., 2010).

These differences were confirmed in the present study with the one exception. The level of non-fucosylated glycoforms in Sp2/0- and CHO-derived cetuximab differed from literature data. Our results show that it is possible to obtain comparable levels of non-fucosylated glycoforms in both CHO- and Sp2/0-derived proteins. This conclusion is extremely important from the clinical point of view, due to the fact that non-fucosylated glycoforms are responsible for the main mechanisms of biological activity of many monoclonal antibodies (Bartusik-Czubek et al., 2021; Chung et al., 2008; Iida et al., 2009; Mori et al., 2007). It has been confirmed that the content of non-fucosylated glycoforms almost completely defines ADCC activity of the IgG (Jefferis, 2005; Liu, 2015; Sha et al., 2016). The total level of non-fucosylated species increases ADCC activity, while the presence of fucosylated glycoforms inhibits antibody binding to FcγRIIIa, which is responsible for ADCC (Liu, 2015). Studies carried out on the CHO cell line that did not add fucose to the oligosaccharide core of the synthesised IgG showed that the non-fucosylated antibody showed a 50-fold increase in binding to the ADCC-responsible receptor compared to the fucosylated analogue (Liu, 2015). Cetuximab can also induce ADCC by recruiting immune cells to tumour cells it has marked (Brand et al., 2011). By looking at both the mechanism of cetuximab activity and the content of non-fucosylated glycoforms of the antibody obtained in CHO and Sp2/0 cells analysed in our study, it can be concluded that using CHO as cells expressing cetuximab may result in obtaining a protein with not only a higher level of safety connected with its lower NGNA content and a lack of Gal-Gal structures, but also with increased ADCC activity (defined by non-fucosylated glycoforms) in relation to the protein obtained from the Sp2/0 cell line.

It is also important to emphasise that the results are consistent within each group of ten Sp2/0- and CHO-derived cetuximab lots. The %CV values were < 20% for each group of glycoforms from the same origin. However, the %CV value for non-fucosylated oligosaccharide content in the CHO-derived antibody was 23.61%. This discrepancy may be caused by two lots of CHO-derived IgG with unexpectedly high levels of non-fucosylated glycoforms (Figure 3). Nevertheless, this is an interesting observation.

Moreover, the difference in the levels of specific glycoforms (expressed as a % content) between murine- and hamster-derived antibodies were defined:

- The fucosylation level in CHO-derived antibodies was more than two-times higher than that observed in Sp2/0-derived antibodies. However, the content of fucosylated oligosaccharides itself does not have a significant effect on the biological activity of the antibodies (Loos et al., 2011).
- Non-fucosylated glycoforms content, in relation to the average, was slightly higher (1.15×) in murine-derived antibody, however the difference is not statistically significant (ANOVA p value > 0.05). A similar level of non-fucosylated oligosaccharides is especially important with regard to biological activity of the IgG. The total level of non-fucosylation increases the affinity of IgG1 to FcγRIIIa thereby increasing ADCC activity (Lee et al., 2017; Kyoung et al., 2018; Liu, 2015).
- The non-fucosylated glycoforms content in the murine-derived antibody was lower than suggested in the literature: 5.4%–6.5% compared to 10%–40% (Raju, 2003). However, the level of non-fucosylation is antibody-specific and can be sufficient for the biological activity of cetuximab (Chung et al., 2008; Mori et al., 2007; Raju, 2003).
- NGNA levels were more than three times higher in the Sp2/0-expressed antibody than in the one expressed in CHO. As mentioned in Section 1.3, NGNA leads to an immune response which promotes cetuximab clearance (Liu, 2015) and leads to drug efficiency decrease.
- NANA was not present in the Sp2/0-derived antibody, but was found at a mean level of 22.72% in the CHO-derived antibody. NANA naturally occurs in the human body (for example, more than 50% of proteins isolated from human milk include NANA) and therefore does not cause immune reactions and does not affect the drug half life time (Wang and Brand-Miller, 2003).
- Gal-Gal structures were absent in the hamster-derived antibody, but were found at a mean level of 32.30% in the murine-derived antibody. As mentioned in Section 1.3, humans have been shown to express anti-α-Gal antibodies against Gal-Gal epitopes and thus show immunological response to Gal-Gal structures (Chung et al., 2008; Goh and Ng, 2017; Kyoung et al., 2018; Li et al., 2010; Raju, 2003).

A summary of the differences observed in the levels of specific glycoforms in CHO- and Sp2/0-derived cetuximab antibody analysed in this study as well as their potential in vitro and in vivo relevance is presented in Table 2.

**Table 2** Summary of differences in the levels of specific glycoforms in CHO- and Sp2/0-derived cetuximab antibody analysed in this study and their potential in vitro and in vivo relevance

| | *Fucosylated glycoforms* | *Non-fucosylated glycoforms* | *NGNA glycoforms* | *NANA glycoforms* | *Gal-Gal structure* |
|---|---|---|---|---|---|
| In vitro relevance | Inhibits binding to FcγRIIIa causing ADCC decrease | Increases affinity to FcγRIIIa causing ADCC increase | No influence | No influence | No influence |
| In vivo (clinical) relevance | Lower activity | Higher activity (safety level has to be defined) | Stimulation of anti-NGNA antibodies production and decrease of drug efficiency through clearance | No influence | Stimulation of anti-α-Gal antibodies production and increase of drug immunogenicity |
| Difference observed in CHO- and Sp2/0-derived cetuximab | More than two times higher in CHO-derived antibody than in Sp2/0-derived antibody | Comparable in CHO and Sp2/0-derived cetuximab antibody | More than three times higher in the Sp2/0-expressed antibody than in those from CHO | Absent in the Sp2/0-derived antibody. Present at a mean level of 22.72% in the CHO-derived antibody | Absent in CHO derived antibody; present at a mean level of 32.30% in the Sp2/0-derived antibody |

The observed differences may of course be IgG-specific and have to be verified individually for each molecule. Despite this, our findings indicate the levels of host cell dependency that can be expected and serve as a guide when selecting a protein expression host. The observation that Sp2/0 and CHO-derived therapeutic proteins demonstrate similar levels of non-fucosylated glycoforms is important. In addition, the fact that CHO-derived antibodies demonstrate a lack of Gal-Gal and less than one third of the NGNA of Sp2/0-derived antibodies, defined as potentially immunogenic structures (Goh and Ng, 2017; Kyoung et al., 2018; Li et al., 2010; Raju, 2003) indicates that it is possible to obtain therapeutic antibodies with the same biological activity but greater safety using the CHO line.

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## Abbreviations

*ADCC* – antibody-dependent cellular cytotoxicity, *ANOVA* – analysis of variance, *Cas9* – caspase-9, *CDR* – complementary determining region, *CHO cells* – Chinese Hamster ovary cells, *CRISPR* – clustered regularly interspaced short palindromic repeats, *Fab* – fragment antigen-binding of monoclonal antibody, *Fc* – fragment crystallisable of monoclonal antibody, *Gal* – galactose, *HAMA* – human anti-mouse antibody, *HILIC* – hydrophilic interaction chromatography, *IgG* – immunoglobulin G, *LOQ* – limit of quantitation, *mAb* – monoclonal antibody, *NANA* – N-acetylneuraminic acid, *NGNA* – N-glycolylneuraminic acid, *scFVs* – single-chain variable fragments, *sLex* – sialyl-Lewisx antennary fucosylation, *S/N* – signal to noise ratio, *UDP-Gal* – urine diphosphate galactose, *UHPLC* – ultra-high performance liquid chromatography, *VH* – variable region of the heavy chain of monoclonal antibody and *VL* – variable region of the light chain of monoclonal antibody.