

SUMMARY ~~OF THE INVENTION~~

During the past years, many efforts have been made to fundamentally understand and technically control protein galactosylation process in mammalian cells, such as CHO cells. Until now there are several general strategies which can be used to influence the extent of protein glycosylation, where the effects are often cell-type and product specific (Hossler et al., *Glycobiology* 19(9) (2009) 936-949; Hossler, *Genomics and Systems Biology of Mammalian Cell Culture* 127 (2012) 187-219): 1) improving activity of glycosyltransferases, 2) improving availability and activity of nucleotide sugar transporters for nucleotide sugar transfer, 3) increasing availability of nucleotide sugar substrates; and 4) reducing glycosidases on extracellular glycan degradation. Crowell et al. (*Biotechnol Bioeng* 96(3) (2007) 538–549) showed that supplementation of CHO cell cultures with manganese, the preferred co-factor of both the oligosaccharyltransferase complex and β 1,4-galactosyltransferase, increased site occupancy and β 1,4-galactosylation of rhEPO N-glycans in late stage culture. Gramer et al., (*Biotechnol Bioeng.* 108(7) (2011) 1591-602)) reported that a synergistic combination of uridine, $MnCl_2$, and galactose significantly increased mAb galactosylation quantitatively with respect to the total UMG concentration supplied. Some approaches using over-expression and/or knockdown of glycosyltransferases and nucleotide sugar transporters (Jeong et al., *J Microbiol Biotechnol* 18(12) (2008) 1945–1952; Weikert et al., *Nat Biotechnol* 17(11) (1999) 1116–1121) have also been reported. Nucleotide sugar precursor feeding has been reported as a possible strategy to control glycosylation of recombinant proteins (Wong et al., *Biotechnology and Bioengineering* 107.2 (2010) 321-336). Specifically, the addition of galactose, glucosamine, and N-acetylmannosamine to cell culture media has been proven to increase intracellular nucleotide sugar levels. However, the effects of increased intracellular nucleotide sugar levels on glycosylation gene expression have not been well characterized. Moreover, the increase in intracellular nucleotide sugar levels did not necessarily lead to an improvement in glycosylation of the recombinant proteins. Some studies employed the strategy of knocking down cellular glycosidases (Ngantung et al., *Biotechnol Bioeng.* 95(1) (2006) 106-19) with the aim to improve protein glycosylation. However, this approach worked only from case to case. In summary, there is still a need for fundamental studies to investigate how various external factors influence intracellular galactosylation process.

As described herein, antibody galactosylation depends on the concentration of UDP-galactose present in cells as these sugar molecules act as substrates required for galactosylation. By this

means, increase in UDP-galactose content has been found to be associated to higher galactosylation and sialylation of the antibody expressed in CHO cells. Varying UDP-galactose levels in the cells can have significant implications on glycan heterogeneity.

In this context, the UDP-glucose and UDP-galactose conversion pathway comprising two identified enzymes, uridine diphosphate α -D-glucose epimerase (UDP-Glc-E) and UDP- α -D-glucose: α -D-galactose-1-phosphate uridylyltransferase (UDP-Gal-T), was found to play a crucial role within the UDP-glucose and UDP-galactose conversion pathway in mammalian cells, such as CHO cells.

In this regard, UDP-Gal-T (EC 2.7.7.12) are a very special class of enzymes which can be regulated with several simple sulfhydryl molecules, such as L-cysteine, glutathione, 2-mercaptoethanol and DTT. In as early as 1966, Mayes and Hansen (Methods Enzymol. 9 (1966) 708-713) found out that L-cysteine can be used to activate and stimulate the enzymatic activity of partially purified UDP-Gal-T from calf liver. In the following years, Mayes (Arch. Biochem. Biophys. 172 (1976) 715-720) carried out extensive studies with completely purified UDP-Gal-T from calf liver and further confirmed this activation function of L-cysteine. Similar studies have also been carried out on UDP-Gal-T from other organisms. Saito et al. (J. Biol. Chem. 242 (1967) 2362-2368) demonstrated that L-cysteine stimulates the purified *E. coli* UDP-Gal-T to reach its maximum activity. Chowdhury (Indian J. Biochem. Biophys. 16 (1979) 273-277) also confirmed this observation with purified UDP-GAL-T from *E. coli*.

Thereby, it has been found by the present ~~invention~~disclosure that it is possible to regulate and control intracellular UDP-galactose content and galactosylation of recombinant proteins, such as antibodies, during manufacturing process with sulfhydryl compounds or their derivatives. For example, it has been found by the present ~~invention~~disclosure that it is possible to regulate and control N-glycan processing of recombinant monoclonal antibody during manufacturing process with sulfhydryl compounds or their derivatives to successfully and specifically manipulate the cell culture process for fine tuning of antibody galactosylation.

One peculiarity of the present ~~invention~~disclosure is the use of the simple sulfhydryl molecules or compounds, such as L-cysteine, to modulate UDP-Gal-T *in vivo* activity in mammalian cell lines, such as CHO K1 and its derivative cell lines for producing a recombinant protein having monogalactosylated (G1) or digalactosylated (G2)glycans. In other words, the present ~~invention~~disclosure provides new approaches to regulate gluco- and galacto-configured UDP-sugars *in vivo*, particularly through modulating the newly identified and specified conversion pathway

with two enzymes, UDP-Gal-T and UDP-Glc-E, in mammalian cells, such as in CHO K1 and its derivative cell lines, for producing a recombinant protein having monogalactosylated (G1) or digalactosylated (G2) glycans. As shown in the Examples herein, it has been demonstrated that, by adjusting the relative media concentrations of certain sulfhydryl compounds or their derivatives, galactosylation of different antibodies can be precisely controlled in a high-yield, batch or fed-batch production process, with minimal impact on other glycoforms, other product quality attributes, or cell culture performance. Moreover, these data show that it is possible to precise regulation and control of a complex, dynamic cellular process at production scale for the definition of recombinant antibody product molecular heterogeneity and bioactivity. This enables an understanding of key effector interactions underpinning the knowledge-based design of cell culture media or feed composition to achieve a specified level of antibody galactosylation, whilst maximizing cell proliferation and productivity.

In ~~particular~~one aspect, the present ~~invention~~disclosure relates to an anti-CD20/anti-CD3 bispecific antibody having monogalactosylated (G1) and digalactosylated (G2) glycans, the anti-CD20/anti-CD3 bispecific antibody comprising a first antigen binding domain, and a second antigen binding domain, wherein the first antigen binding domain comprises a heavy chain variable domain (VH) comprising

- (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22,
 - (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and
 - (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; and
- a light chain variable domain (VL) comprising
- (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 25,
 - (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 26, and
 - (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 27;

wherein the second antigen binding domain comprises a heavy chain variable domain (VH) comprising

- (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 34,
 - (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 35, and
 - (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 36; and
- a light chain variable domain (VL) comprising
- (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 37,
 - (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 38, and
 - (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 39; and

- (b) a first light chain of SEQ ID NO: 33, a second light chain and a third light chain of SEQ ID NO: 44; or
- (c) the first heavy chain and the second heavy chain as defined in (a) and the first light chain, the second light chain and the third light chain as defined in (b).

EquallyIn another aspect, the present ~~invention~~disclosure relates to an anti α -synuclein antibody having monogalactosylated (G1) and digalactosylated (G2) glycans, the anti α -synuclein antibody comprising a heavy chain variable domain (VH) comprising

- (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10,
 - (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 11, and
 - (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; and
- a light chain variable domain (VL) comprising
- (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13,
 - (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and
 - (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and

wherein the anti α -synuclein antibody has 17.2-48.0% (w/w) of G1 and 3.1-15.0% (w/w) of G2 per total glycan; preferably 25.4-48.0% (w/w) of G1 and 3.5-15.0% (w/w) of G2 per total glycan; preferably 27.2-47.0% of G1 and 4.4 to 15.0% of G2 per total glycan; preferably 40.0-46.0% (w/w) of G1 and 8.4-15.0% (w/w) of G2 per total glycan; more preferably 41.0-45.0% (w/w) of G1 and 9.5-14.0% (w/w) of G2 per total glycan and most preferably 42.1-43.9% (w/w) of G1 and 10.6-13.3% (w/w) of G2 per total glycan.

Preferably, the anti α -synuclein antibody comprises

- (a) a VH sequence of SEQ ID NO: 16;
- (b) a VL sequence of SEQ ID NO: 17; or
- (c) the VH sequence as defined in (a) and the VL sequence as defined in (b).

More preferably, the anti α -synuclein antibody comprises

- (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16;
- (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17; or
- (c) the VH sequence as defined in (a) and the VL sequence as defined in (b).

Even more preferably, the anti α -synuclein antibody comprises a heavy chain of SEQ ID NO: 20 and a light chain of SEQ ID NO: 21.

Preferably, the the monogalactosylated (G1) and digalactosylated (G2) glycans of the anti-CD20/anti-CD3 bispecific antibody or the anti α -synuclein antibody as disclosed in the context of the ~~invention~~disclosure are associated with N-acetylglucosamine.

Equally, the present ~~invention~~disclosure relates to a cell culture medium for producing an antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans in a mammalian cell, the cell culture medium comprising a concentration of more than 4.0 mM and less than 10.0 mM of sulfhydryl group(s) from one or more sulfhydryl compound(s) and a concentration of at least more than 3.0 g/L glucose.

Preferably, the cell culture medium is a chemically defined medium, preferably a serum-free, protein-free and/or oligopeptide-free cell culture medium, more preferably a chemically defined medium, more preferably a serum-free, protein-free and oligopeptide-free cell culture medium.

Equally, the present ~~invention~~disclosure relates to a mammalian cell producing the anti-CD20/anti-CD3 bispecific antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans comprising a polynucleotide comprising a sequence having 80% identity to a polynucleotide encoding a first antigen binding domain comprising a first and a second heavy chain variable domain (VH) as disclosed in the context of the ~~invention~~disclosure, or comprising a sequence having 80% identity to a polynucleotide comprising a sequence encoding a second antigen binding domain comprising a first and a second light chain variable domain (VL) as disclosed in context of the ~~invention~~disclosure, or

one or more vectors comprising such polynucleotides, wherein said cell is cultivated in a cell culture medium as disclosed in the context of the ~~invention~~disclosure.

Preferably, the mammalian cell further comprises a sequence having 80% identity to a polynucleotide encoding a first and a second heavy chain as disclosed in the context of the ~~invention~~disclosure, or comprising a sequence having 80% identity to a polynucleotide comprising a sequence encoding a first and a second light chain as disclosed in context of the ~~invention~~disclosure, or

one or more vectors comprising such polynucleotides, wherein said cell is cultivated in a cell culture medium as disclosed in the context of the ~~invention~~disclosure.

Equally, the present ~~invention~~disclosure relates to an anti α -synuclein antibody having monogalactosylated (G1) and digalactosylated (G2) glycans comprising a polynucleotide

comprising a sequence having 80% identity to a polynucleotide encoding a heavy chain variable domain (VH) as disclosed in the context of the ~~invention~~disclosure, or a polynucleotide comprising a sequence having 80% identity to a polynucleotide encoding a light chain variable domain (VL) as disclosed in the context of the ~~invention~~disclosure, or one or more vectors comprising such polynucleotides, wherein said cell is cultivated in a cell culture medium as disclosed in the context of the ~~invention~~disclosure.

~~Equally~~In another aspect, the present ~~invention~~disclosure relates to a method of producing an anti-CD20/anti-CD3 bispecific antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans, said method comprising:

- (a) cultivating a mammalian cell ~~as disclosed in the context of the invention~~ in a cell culture medium ~~as disclosed in the context of the invention~~, wherein a concentration of at least more than 4.0 and less than 10.0 mM of the sulfhydryl group(s) from one or more sulfhydryl compound(s) and at least more than 3.0 g/L glucose in the cell culture medium is maintained for at least 3, more preferably for at least 4 and even more preferably for at least 5 days,
- (b) isolating said antibody.

Preferably, maintaining the concentration is effective for the production of an anti-CD20/anti-CD3 bispecific antibody having 19.0-29.0% (w/w) of G1 and 1.3-2.8% (w/w) of G2 per total glycan; preferably 20.0-28.0% (w/w) of G1 and 1.4-2.7% (w/w) of G2 per total glycan; more preferably 21.0-28.0% (w/w) of G1 and 1.5-2.7% (w/w) of G2 per total glycan and most preferably 21.0-27.4% (w/w) of G1 and 1.5-2.6% (w/w) of G2 per total glycan.

Preferably, the cultivation of the mammalian cell results in an increased titer of said antibody by at least 20%, preferably at least 30%, more preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and most preferably at least 80%

relative to the titer in a corresponding cultivation of the mammalian cell without maintaining the concentration of at least more than 4.0 and less than 10.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s) in the cell culture medium. As another example, the cultivation of the mammalian cell results in an increased titer of said antibody between 30 and 75 % relative to the titer in a corresponding cultivation of the mammalian cell without maintaining the concentration of at least more than 4.0 and less than 10.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s) in the cell culture medium.

~~Equally~~In another aspect, the present ~~invention~~disclosure relates to a method of producing an anti α -synuclein antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans, said method comprising:

- (a) cultivating a mammalian cell ~~as disclosed in the context of the invention~~ in a cell culture medium ~~as disclosed in the context of the invention~~, wherein a concentration of at least more than 4.0 and less than 10.0 mM of the sulfhydryl group(s) from one or more sulfhydryl compound(s) and at least more than 3.0 g/L glucose in the cell culture medium is maintained for at least 3, more preferably for at least 4 and even more preferably for at least 5 days,
- (b) isolating said antibody.

Preferably, the concentration is effective for the production of an anti α -synuclein antibody having 17.2-48.0% (w/w) of G1 and 3.1-15.0% (w/w) of G2 per total glycan 25.4-48.0% (w/w) of G1 and 3.5-15.0% (w/w) of G2 per total glycan; preferably 27.2-47.0% of G1 and 4.4 to 15.0% of G2 per total glycan; preferably 40.0-46.0% (w/w) of G1 and 8.4-15.0% (w/w) of G2 per total glycan; more preferably 41.0-45.0% (w/w) of G1 and 9.5-14.0% (w/w) of G2 per total glycan and most preferably 42.1-43.9% (w/w) of G1 and 10.6-13.3% (w/w) of G2 per total glycan.

Preferably, the cultivation of the mammalian cell results in an increased titer of said antibody by at least 10%, preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, even more preferably at least 80% and most preferably at least 100% relative to the titer in a corresponding cultivation of the mammalian cell without maintaining the concentration of at least more than 4.0 and less than 10.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s) in the cell culture medium.

Preferably, the cultivation of the mammalian cell results in the anti α -synuclein antibody having monogalactosylated (G1) and digalactosylated (G2) glycans characterized by

- (i) increased protein target binding by at least 10%, preferably at least 20%, more preferably at least 35%, even more preferably at least 45% and most preferably at least 50%;
- (ii) increased neonatal Fc receptor (FcRn) binding by at least 10%, preferably at least 20%, more preferably at least 33%, more preferably at least 40% and most preferably at least 45%; and/or;
- (iii) increased Fc γ RIIa binding by at least 10%, preferably at least 20%, more preferably at least 36%, even more preferably 45% and most preferably 50%,

relative to the non-monogalactosylated (G1) and non-digalactosylated (G2) forms of the anti α -synuclein antibody in a corresponding cultivation of the mammalian cell without maintaining the concentration of the at least more than 4.0 and less than 10.0 mM of sulfhydryl group(s) from the one or more sulfhydryl compound(s) in the cell culture medium.

The methods as disclosed in the context of the ~~invention~~disclosure preferably comprise cultivation of the mammalian cell in a starting concentration of at least more than 3.0 and less than 10.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s). Preferably, the methods further comprise the step of pre-cultivating of the mammalian cell in a cell culture medium prior to said cultivating. Preferably, the concentration is maintained for at least 5 days, preferably for at least 7 days, more preferably for at least 10 days, even more preferably for at least 12 days and most preferably for at least 14 days.

Preferably, the sulfhydryl group(s) from the one or more sulfhydryl compound(s) in the cell culture medium as disclosed in the context of the ~~invention~~disclosure are contained in the reduced and/or oxidized form, more preferably the concentration of the reduced form of said sulfhydryl ranges between more than 4.0 mM and less than 10.0 mM and/or the concentration of the oxidized form of said sulfhydryl ranges between more than 2.0 mM and less than 5.0 mM.

Preferably, the methods as disclosed in the context of the ~~invention~~disclosure further comprise harvesting the anti-CD20/anti-CD3 bispecific antibody or the anti α -synuclein antibody.

Preferably, the methods as disclosed in the context of the ~~invention~~disclosure further comprise measuring a level of the monogalactosylated (G1) and digalactosylated (G2) glycans of the anti-CD20/anti-CD3 bispecific antibody or the anti α -synuclein antibody. Preferably, the methods further comprise formulating the anti-CD20/anti-CD3 bispecific antibody or the anti α -synuclein antibody into a drug product.

Preferably, the cell culture medium as disclosed in the context of the ~~invention~~disclosure comprises at least more than 4.0 mM and equal or less than 9.0 mM, preferably at least more than 4.0 mM and equal or less than 8.0 mM, more preferably at least more than 4.0 mM and equal or less than 7.0 mM and even more preferably at least more than 4.0 mM and equal or less than 6.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s).

Preferably, the cell culture medium as disclosed in the context of the ~~invention~~disclosure comprises a concentration of at least 5.0 mM and less than 10.0 mM, preferably at least 5.0 mM and equal or less than 9.0 mM, more preferably at least 5.0 mM and equal or less than 8.0 mM, even

more preferably at least 5.0 mM and equal or less than 7.0 mM and most preferably at least 5.0 mM and equal or less than 6.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s).

Preferably, the cell culture medium as disclosed in the context of the ~~invention~~disclosure comprises at least more than 2.0 g/L glucose, preferably at least more than 3.0 g/L glucose and more preferably at least more than 4.0 g/L glucose.

Preferably, the cell culture medium comprises at most 13.0 g/L glucose, preferably at most 8.0 g/L, more preferably at most 7.0 g/L, even more preferably at most 6.0 g/L and most preferably at most 5.0 g/L glucose.

Preferably, the cell culture medium comprises between more than 2.0 g/L and at most 13.0 g/L glucose, preferably between more than 2.0 g/L and at most 8.0 g/L glucose, more preferably between more than 2.0 g/L and at most 7.0 g/L glucose, even more preferably between more than 2.0 g/L and at most 6.0 g/L glucose and most preferably between more than 2.0 g/L and at most 5.0 g/L glucose.

Preferably, the one or more sulfhydryl compound(s) of the cell culture medium as disclosed in the context of the ~~invention~~disclosure are selected from the group consisting of cysteine, cystine, succimer, methimazole, cysteamine, azathioprine, mercaptopurine, S-methylcysteine, selenocysteine, S-phosphocysteine, 4'-phosphopantetheine, butyrylthiocholine, carbocysteine, N-sulphocysteine, alethine, acetylcysteine, dimercaprol, coenzyme M, sodium aurothiomalate, pantethine, bucillamine, methylselenocysteine, dimercaptosuccinic acid, acetylcysteine amide, thioglycolic acid, 2,3-dimercaptopropanol, O-methylmercaptoethanol, mercaptoacetic acid, fl-mercaptopropionic acid, methylmercaptan, S-methylmercaptoethanol, glutathione, glutathione derivatives, and a combination thereof. More preferably, the one or more sulfhydryl compound(s) are selected from the group consisting of cysteine, cystine, and a combination thereof. Even more preferably, the one or more sulfhydryl compound(s) is cysteine, and wherein the cysteine concentration in the cell culture medium is more than 4.0 mM and less than 10.0 mM, preferably the cysteine concentration is at least 5.0 mM and equal or less than 6.0 mM. Equally preferably, the one or more sulfhydryl compound(s) is cystine, and wherein the cystine concentration in the cell culture medium is more than 2.0 mM and less than 5.0 mM, preferably the cystine concentration is at least 3.0 mM and equal or less than 4.0 mM.

Preferably, cultivation of the mammalian cell of the method as disclosed in the context of the ~~invention~~disclosure is in a large-scale format bioreactor, preferably in a 10,000 L bioreactor.

Equally, the present ~~invention~~disclosure relates to an anti-CD20/anti-CD3 bispecific antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans obtainable by the method or the mammalian cell as disclosed in the context of the ~~invention~~disclosure.

Equally, the present ~~invention~~disclosure relates to an anti α -synuclein antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans obtainable by the method or the mammalian cell as disclosed in the context of the ~~invention~~disclosure.

Preferably, the ~~invention~~disclosure relates to an anti-CD20/anti-CD3 bispecific antibody as disclosed in the context of the ~~invention~~disclosure having monogalactosylated (G1) and digalactosylated (G2) glycans for use as a medicament. More preferably, the anti-CD20/anti-CD3 bispecific antibody as disclosed in the context of the ~~invention~~disclosure ~~is~~ is for use in the treatment of patients with B-cell associated cancers, preferably for use in the treatment of patients with chronic leukemia and lymphoma.

In another aspect, the present disclosure relates to the anti-CD20/anti-CD3 bispecific antibody of the disclosure for use in the treatment of patients with B-cell associated cancers, preferably chronic leukemia and lymphoma.

In another aspect, the present disclosure relates to use of the anti-CD20/anti-CD3 bispecific antibody of the disclosure in the manufacture of a medicament for the treatment of patients with B-cell associated cancers, preferably chronic leukemia and lymphoma.

Equally preferably, the ~~invention~~disclosure relates to an anti α -synuclein antibody having monogalactosylated (G1) and digalactosylated (G2) glycans for use as a medicament. More preferably, the anti α -synuclein antibody as disclosed in the context of the ~~invention~~disclosure is for use in the treatment of patients with Parkinson's disease.

In another aspect, the present disclosure relates to the α -synuclein antibody of the disclosure for use in the treatment of patients with Parkinson's disease.

In another aspect, the present disclosure relates to use of the α -synuclein antibody of the disclosure in the manufacture of a medicament for the treatment of patients with Parkinson's disease.

CLAIMS

1. An anti α -synuclein antibody having monogalactosylated (G1) and digalactosylated (G2) glycans, the anti α -synuclein antibody comprising a heavy chain variable domain (VH) comprising
 - (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10,
 - (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 11, and
 - (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; anda light chain variable domain (VL) comprising
 - (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13,
 - (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and
 - (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; andwherein the anti α -synuclein antibody has 17.2-48.0% (w/w) of G1 and 3.1-15.0% (w/w) of G2 per total glycan; preferably 25.4-48.0% (w/w) of G1 and 3.5-15.0% (w/w) of G2 per total glycan; preferably 27.2-47.0% of G1 and 4.4 to 15.0% of G2 per total glycan; preferably 40.0-46.0% (w/w) of G1 and 8.4-15.0% (w/w) of G2 per total glycan; more preferably 41.0-45.0% (w/w) of G1 and 9.5-14.0% (w/w) of G2 per total glycan and most preferably 42.1-43.9% (w/w) of G1 and 10.6-13.3% (w/w) of G2 per total glycan.
2. The α -synuclein antibody of claim 1, wherein the anti α -synuclein antibody comprises
 - (a) a VH sequence of SEQ ID NO: 16;
 - (b) a VL sequence of SEQ ID NO: 17; or
 - (c) the VH sequence as defined in (a) and the VL sequence as defined in (b)
3. The anti α -synuclein antibody of claim 1 or 2, wherein the anti α -synuclein antibody comprises a heavy chain of SEQ ID NO: 20 and a light chain of SEQ ID NO: 21.
4. An anti-CD20/anti-CD3 bispecific antibody having monogalactosylated (G1) and digalactosylated (G2) glycans, the anti-CD20/anti-CD3 bispecific antibody comprising a first antigen binding domain, and a second antigen binding domain, wherein the first antigen binding domain comprises a heavy chain variable domain (VH) comprising
 - (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22,

(b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and
(c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; and
a light chain variable domain (VL) comprising
(d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 25,
(e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 26, and
(f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 27;
wherein the second antigen binding domain comprises a heavy chain variable domain (VH) comprising

(a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 34,
(b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 35, and
(c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 36; and
a light chain variable domain (VL) comprising
(d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 37,
(e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 38, and
(f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 39; and
wherein the anti-CD20/anti-CD3 bispecific antibody has 19.0-29.0% (w/w) of G1 and 1.3-2.8% (w/w) of G2 per total glycan; preferably 20.0-28.0% (w/w) of G1 and 1.4-2.7% (w/w) of G2 per total glycan; more preferably 21.0-28.0% (w/w) of G1 and 1.5-2.7% (w/w) of G2 per total glycan and most preferably 21.0-27.4% (w/w) of G1 and 1.5-2.6% (w/w) of G2 per total glycan.

5. The anti-CD20/anti-CD3 bispecific antibody of claim 4, wherein
- (a) the first antigen binding domain comprises a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 29;
 - (b) the second antigen binding domain comprises a VH sequence of SEQ ID NO: 40 and a VL sequence of SEQ ID NO: 41; or
 - (c) the first and second antigen binding domain comprises a VH sequence as defined in (a) and a VL sequence as defined in (b).
6. The anti-CD20/anti-CD3 bispecific antibody of any of claims 4 or 5, wherein the anti-CD20/anti-CD3 bispecific antibody comprises
- (a) a first heavy chain of SEQ ID NO: 46 and a second heavy chain of SEQ ID NO: 45;

- (b) a first light chain of SEQ ID NO: 33 and a second light chain of SEQ ID NO: 44; or
 - (c) the first heavy chain and the second heavy chain as defined in (a) and the first light chain and the second light chain as defined in (b).
7. The anti-CD20/anti-CD3 bispecific antibody of any of claims 4 to 6, wherein the anti-CD20/anti-CD3 bispecific antibody comprises
- (a) a first heavy chain of SEQ ID NO: 47 and a second heavy chain of SEQ ID NO: 45;
 - (b) a first light chain of SEQ ID NO: 33, a second light chain and a third light chain of SEQ ID NO: 44; or
 - (c) the first heavy chain and the second heavy chain as defined in (a) and the first light chain, the second light chain and the third light chain as defined in (b).
8. A method of producing a α -synuclein antibody of any of claims 1 to 3 having monogalactosylated (G1) and digalactosylated (G2) glycans, said method comprising:
- (a) cultivating a mammalian cell in a cell culture medium, wherein a concentration of at least more than 4.0 and less than 10.0 mM of sulfhydryl group(s) from one or more sulfhydryl compound(s) and at least more than 3.0 g/L glucose in the cell culture medium is maintained for at least 3, more preferably for at least 4 and even more preferably for at least 5 days,
 - (b) isolating said antibody.
9. A method of producing an anti-CD20/anti-CD3 bispecific antibody of any of claims 4 to 7 having monogalactosylated (G1) and digalactosylated (G2) glycans, said method comprising:
- (a) cultivating a mammalian cell in a cell culture medium, wherein a concentration of at least more than 4.0 and less than 10.0 mM of sulfhydryl group(s) from one or more sulfhydryl compound(s) and at least more than 3.0 g/L glucose in the cell culture medium is maintained for at least 3, more preferably for at least 4 and even more preferably for at least 5 days,
 - (b) isolating said antibody.

10. The method of claim 8 or 9, wherein the concentration is maintained for at least 5 days, preferably for at least 7 days, more preferably for at least 10 days, even more preferably for at least 12 days and most preferably for at least 14 days.
11. The method of any of claims 8 to 10, wherein the cell culture medium comprises a concentration of at least 5.0 mM and less than 10.0 mM, preferably at least 5.0 mM and equal or less than 9.0 mM, more preferably at least 5.0 mM and equal or less than 8.0 mM, even more preferably at least 5.0 mM and equal or less than 7.0 mM and most preferably at least 5.0 mM and equal or less than 6.0 mM of the sulfhydryl group(s) from the one or more sulfhydryl compound(s).
12. The method of any of claims 8 to 11, wherein the cell culture medium comprises between more than 3.0 g/L and at most 13 g/L glucose, preferably between more than 3.0 g/L and at most 8.0 g/L glucose, more preferably between more than 3.0 g/L and at most 7.0 g/L glucose, even more preferably between more than 3.0 g/L and at most 6.0 g/L glucose and most preferably between more than 3.0 g/L and at most 5.0 g/L glucose.
13. The method of any of claims 8 to 12, wherein the one or more sulfhydryl compound(s) are selected from the group consisting of cysteine, cystine, and a combination thereof.
14. The method of any of claims 8 to 13, wherein the cell culture medium is a chemically defined medium, preferably a serum-free, protein-free and/or oligopeptide-free cell culture medium.
15. The anti-CD20/anti-CD3 bispecific antibody of any of claims 1 to 4 for use in the treatment of patients with B-cell associated cancers, preferably chronic leukemia and lymphoma.
16. The α -synuclein antibody of any of claims 5 to 7 for use in the treatment of patients with Parkinson's disease.

17. Use of the anti-CD20/anti-CD3 bispecific antibody of any of claims 1 to 4 in the manufacture of a medicament for the treatment of patients with B-cell associated cancers, preferably chronic leukemia and lymphoma.
18. Use of the α -synuclein antibody of any of claims 5 to 7 in the manufacture of a medicament for the treatment of patients with Parkinson's disease.