

Fan et al., Biotech & Bioeng (2015) **112**(3) 521-535 found that, in fed-batch processes for CHO cells with two chemically defined proprietary media, the balance of glucose and amino acid concentration is important for cell growth, IgG titer and Fc situated N-glycosylation such that faster cell growth, higher integral for viable cells and IgG production – and more mature glycoproteins – could be obtained as a result of a more balanced amino acid concentration in the culture and higher glucose consumption. The authors decided that the effect of media and process optimisation on Fc glycosylation should be understood from case-to-case and results from the interplay of the protein processing rate, cell metabolism and expression and activity of Golgi resident proteins.

Huang et al., Anal. Biochem. **349** (2006) 197-207 analyzed the effect of glycosylation on antibody clearance of an anti-A β -IgG1 monoclonal antibody and found rapid clearance of N-acetyl glucosamine terminated biantennary complex type glycans.

St Amand et al., Biotechnol Bioeng (2014) **111**(10) 1957-70 found that each of manganese, galactose and ammonia when used as media supplements had significant effects on certain glycans and glycan distribution.

EP 1960428 is directed to antibodies against amyloid beta, with glycosylation in the variable region, to clear existing and prevent formation of new β -amyloid plaques in humans. The glycosylation in the V_H region is selected from a sugar structure of the biantennary complex type without core fucosylation; of the biantennary hybrid type; or of the biantennary oligomannose type, wherein the hybrid and oligomannose structures are considered minor and, combined, are present at 25% or less of the composition. The oligomannose sugar structures are characterised by containing Man4, Man5 or Man6 subunits, including those mannose units in the typical N-linked core structure. There is no disclosure of either the need, or methods, for regulating the relative high mannose content in the antibody glycoforms.

The present invention has been devised in light of the above considerations.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure relates to monoclonal antibody compositions in which the relative content of a particular glycoform, the high mannose glycoform, at the N-glycosylation site in the Fab region of the antibody, is regulated. Diagnosis and methods of treatment of diseases with such antibody compositions and the medical uses of such antibody compositions are also provided,

as well as methods for the preparation of such antibody compositions and the antibody compositions produced thereby.

The following are aspects of the inventiondisclosure in its broadest sense. Alternative and preferred embodimentsexamples, in which the nature of the features of the inventiondisclosure are more closely defined, follow in the detailed description.

Throughout the following disclosure, for the sake of brevity the term “antibody” will be used to encompass monoclonal antibodies, polyclonal antibodies, multispecific antibodies, including bispecific antibodies, and antibody fragments (as hereinafter defined) so long as they exhibit the desired antigen-binding activity.

~~In its first aspect, the invention~~The disclosure provides a composition comprising a monoclonal antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of glycosylated Fab in the composition, about 20% or less of Fab regions in the composition have an N-linked high mannose glycan.

In one aspect, the present disclosure provides a composition comprising a glycosylated monoclonal antibody, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, the antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of glycosylated Fab in the composition, about 20% or less of Fab regions in the composition have an N-linked high mannose glycan.

The composition of the inventiondisclosure may be a pharmaceutical composition. Alternatively, the composition of the inventiondisclosure may be a cell culture supernatant obtainable during and/or after recombinant production of the antibody.

~~In one aspect, the~~The present inventiondisclosure provides a method for reducing the rate at which an antibody clears from the circulation of an animal to which the antibody has been administered, which method comprises regulating the relative content of high mannose Fab glycoforms of a glycosylated monoclonal antibody in a composition comprising the antibody.

In another aspect, the present disclosure provides a method for reducing the rate at which a glycosylated monoclonal antibody, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, clears from the circulation of an animal to which the antibody has been administered, which method comprises regulating the relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody in a composition comprising the antibody.

~~In one aspect, the~~The present ~~invention~~disclosure envisages a method for regulating the relative content of high mannose Fab glycoforms of a glycosylated monoclonal antibody comprised in the composition of the ~~invention~~disclosure, which method comprises, over all or a part of the production phase of fermentation, optimising the concentration of glucose in a culture medium used for producing the glycosylated monoclonal antibody by fermentation therein of a eukaryotic cell expressing the monoclonal antibody.

In another aspect, the present disclosure provides a method for regulating the relative content of high mannose Fab glycoforms of a glycosylated monoclonal antibody in a composition, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, the method comprising, during the production phase of fermentation, optimising the concentration of a carbohydrate source for a eukaryotic cell in a culture medium used for producing the glycosylated monoclonal antibody by fermentation therein of the eukaryotic cell expressing the monoclonal antibody.

Optimising the concentration of a carbohydrate source for the eukaryotic cell in the culture medium during all or a part of the production phase comprises maintaining an average concentration of the carbohydrate source in the culture medium during all or a part of the

production phase that correlates with the desired relative content of high mannose Fab glycoforms resulting from the fermentation.

The method may further comprise a step of recovering the monoclonal antibody from the culture medium.

In the ~~invention~~disclosure the “relative” content means the content of high mannose Fab glycoforms in the composition in relation to the content of all other Fab glycoforms of the monoclonal antibody in the composition.

In one ~~embodiment~~example of the ~~invention~~disclosure, in the composition, the high mannose Fab glycoforms make up about 20% or less of the total Fab glycoforms of the monoclonal antibody. Thus, the ~~invention~~disclosure provides a monoclonal antibody composition, the composition comprising N-linked glycosylated Fab region(s), wherein about 20% or less of the N-linked glycosylated Fab region(s) are the N-linked high mannose glycoform.

In the methods of the ~~invention~~disclosure, to achieve about 20% or less of Fab high mannose glycoforms of the monoclonal antibody in the composition, glucose is the carbohydrate source and the concentration of glucose is optimised such that the average glucose concentration (optionally with the average calculated over day -7 to day 0 of the production phase) in the recombinant production of the monoclonal antibody is from about 0.50 g/L to about 18.00 g/L, preferably from about 1.50 g/L to about 14.00 g/L and more preferably from about 2.00 g/L to about 12.50 g/L.

In a further aspect, the present ~~invention~~disclosure provides a monoclonal antibody composition obtainable by a method set out above. The monoclonal antibody composition may be a cell culture supernatant, or may be a pharmaceutical composition.

~~In a further aspect, the~~The present ~~invention~~disclosure provides a method of treatment of a disease comprising administering to a patient suffering from the disease a composition comprising a monoclonal antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of N-glycosylated Fab regions in the composition, about 20% or less of Fab regions in the composition have an N-linked high mannose glycan.

~~In a further aspect, the~~The present ~~invention~~disclosure provides a composition comprising a monoclonal antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of N-glycosylated Fab regions in the composition, about 20% or less of Fab regions in

the composition have an N-linked high mannose glycan, for use in the treatment of a disease in an individual suffering therefrom.

In addition, the present ~~invention~~disclosure provides a composition comprising a monoclonal antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of N-glycosylated Fab regions in the composition, about 20% or less of Fab regions in the composition have an N-linked high mannose glycan for use in the diagnosis of a disease.

Where mentioned above~~In these aspects~~, the disease may be, for example, dementia, Alzheimer's Disease, motor neuropathy, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), scrapie, HIV-related dementia, Creutzfeld-Jakob disease (CJD), hereditary cerebral haemorrhage, Down's syndrome and neuronal disorders related to ageing; and cancer, such as metastatic colorectal cancer, metastatic non-small cell lung cancer, ovarian cancer and head and neck cancer.

In another aspect, the present disclosure provides a composition as described herein, for use in the diagnosis or treatment of a disease in an individual suspected of or suffering therefrom, optionally wherein the disease is dementia, Alzheimer's Disease, motor neuropathy, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), scrapie, HIV-related dementia, Creutzfeld-Jakob disease (CJD), hereditary cerebral haemorrhage, Down's syndrome and neuronal disorders related to ageing; and cancer, such as metastatic colorectal cancer, metastatic non-small cell lung cancer, ovarian cancer and head and neck cancer.

In another aspect, the present disclosure provides use of a composition as described herein in the manufacture of a medicament for the diagnosis or treatment of a disease in an individual suspected of or suffering therefrom, wherein the disease is dementia, Alzheimer's Disease, motor neuropathy, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), scrapie, HIV-related dementia, Creutzfeld-Jakob disease (CJD), hereditary cerebral haemorrhage, Down's syndrome and neuronal disorders related to ageing; and cancer, such as metastatic colorectal cancer, metastatic non-small cell lung cancer, ovarian cancer and head and neck cancer.

DESCRIPTION OF THE FIGURES

Figure 1 Fab Glycosylation Species. The species are summed up into several sum parameters as Sum Fab hybrid mannose (Hybrid man), Sum Fab Sialylation (Sial), Sum Fab Galactosylation (Gal), Fab high mannose (High man) and Fab mannose (Man).

Figure 2: Impact of glucose on Fab high mannose production, shown as area% Fab high mannose over average glucose concentration from day -7 to day 0 [g/L]. Available data from all representative runs of the given project shown (not only runs dedicated to glucose variations). Fermentation runs vary in scales, Roche production sites and minor process changes (e.g. agitation, aeration, cell bank, cell age). A glucose solution is added on demand via bolus or continuous addition.

Figure 3A & 3B: Impact of glucose on Fab high mannose production. Fab high mannose as sum and also the 3 parts of the sum (Fab mannose 5, 6 and 7) in area% over average glucose concentration from day -7 to day 0 [g/L] (Fig 3A). Comparison of different glucose average levels. One experimental setup dedicated for glucose variation, all other parameters are kept constant. Glucose is added on demand daily via bolus addition. Additionally, in Figure 3B, the calculation of the glucose average is explained: the calculation is done based on the daily measured glucose concentration from samples before the bolus addition and the calculated glucose concentration after the bolus addition.

CLAIMS

1. A composition comprising a glycosylated monoclonal antibody, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, the antibody having N-glycosylation in the Fab region(s) thereof, wherein relative to the total amount of glycosylated Fab in the composition, about 20% or less of Fab regions in the composition have an N-linked high mannose glycan.
2. The composition of claim 1 which is a pharmaceutical composition or a cell culture supernatant obtainable during or after recombinant production of the antibody.
3. A method for reducing the rate at which a glycosylated monoclonal antibody, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, clears from the circulation of an animal to which the antibody has been administered, which method comprises regulating the relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody in a composition comprising the antibody.
4. The method of claim 3 wherein regulating the relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody in a composition comprising the antibody comprises maintaining an average concentration of glucose in a culture medium used for producing the glycosylated monoclonal antibody by fermentation therein of a eukaryotic cell expressing the monoclonal antibody, during all or a part of the production phase of the fermentation.
5. A method for regulating the relative content of high mannose Fab glycoforms of a glycosylated monoclonal antibody in a composition, wherein said antibody is an anti-human Abeta antibody, a bi-specific antibody comprising an anti-human Abeta antibody, or a fragment of an anti-human Abeta antibody comprising a glycosylated Fab region and capable of binding Abeta, the method comprising, during the production phase of fermentation, optimising the concentration of a carbohydrate source for a eukaryotic cell in a culture medium used for producing the glycosylated monoclonal antibody by fermentation therein of the eukaryotic cell expressing the monoclonal antibody.
6. The method of claim 5 comprising maintaining an average concentration of glucose in the culture medium during all or a part of the production phase.

7. The method of claim 5 or claim 6 further comprising a step of recovering the antibody from the culture medium.
8. The method of any one of claims 3 to 7 wherein the high mannose Fab glycoforms make up about 20% or less of the total Fab glycoforms of the monoclonal antibody.
9. The composition of claim 1 or 2 or method of any one of claims 3 to 8 wherein the percentage of Fab regions having N-linked high mannose glycan is from about 0-20%, about 0-15%, about 0-12% or about 0-10%, optionally wherein the percentage of Fab regions having N-linked high mannose glycan is from about 2-20%, about 2-15%, about 2-12% or about 2-10% or about 4-10%, about 4-12%, about 4-15% or about 4-20%.
10. The composition or method of claim 9 wherein the high mannose glycan is one or a mixture of Man5, Man6 and Man7 glycoforms, optionally wherein the mannose residues in the high mannose glycan are Man5, Man 6 and Man7.
11. The composition or method of any one of claims 1 to 10 wherein the monoclonal antibody is Gantenerumab and comprises:
- (a) a V_H and a V_L CDR amino acid sequences as set out in SEQ ID Nos:1-6, above, a V_H and a V_L domain amino acid sequences of SEQ ID NO:7 and SEQ ID NO:8, or a heavy and light chain comprising the amino acid sequence of SEQ ID NO:9 and 10;
 - (b) a V_H CDR1 comprising the amino acid sequence of SEQ ID NO:1; a V_H CDR2 comprising the amino acid sequence of SEQ ID NO:2; a V_H CDR3 comprising the amino acid sequence of SEQ ID NO:3; a V_L CDR1 comprising the amino acid sequence of SEQ ID NO:4; a V_L CDR2 comprising the amino acid sequence of SEQ ID NO:5; and a V_L CDR3 comprising the amino acid sequence of SEQ ID NO:6;
 - (c) a V_H domain comprising the amino acid sequence of SEQ ID NO:7; and a V_L domain comprising the amino acid sequence of SEQ ID NO:8; or
 - (d) a heavy chain comprising the amino acid sequence of SEQ ID NO:9; and a light chain comprising the amino acid sequence of SEQ ID NO:10;
- said composition comprising about 20% or less of a high mannose glycoform of said antibody relative to the total amount of V_H glycosylated antibody in the composition, wherein said glycosylation is N-glycosylation at Asn52 in SEQ ID NO:9.
12. The composition or method of claim 11 wherein, when the monoclonal antibody is a bi-specific antibody, one of the binding specificities is for human A-beta and the other specificity is for the transferrin receptor.

13. The composition of method of claim 12, wherein the monoclonal antibody is a bi-specific antibody comprising:

- a heavy chain with the amino acid sequence of SEQ ID NO:9;
- a light chain with the amino acid sequence of SEQ ID NO:10;
- a heavy chain Fab fragment with an amino acid sequence of SEQ ID NO:11; and
- a light chain with an amino acid sequence of SEQ ID NO:12.

14. The method of any one of claims 3 to 13, wherein the average glucose concentration in the culture medium over the production phase in the recombinant production of the monoclonal antibody is from about 0.5 g/L to about 18 g/L.

15. The method of claim 14, wherein the concentration of glucose is averaged over day -7 to day 0 of the production phase.

16. The method of any one of claims 3 to 15 wherein the concentration of glucose is monitored and controlled in the culture medium to achieve an average concentration thereof over all or a part of the production phase, optionally over day -7 to day 0 of the production phase.

17. The method of any one of claims 3 to 16 wherein:

(a) the desired relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody resulting from the fermentation is about 3%, the average concentration of glucose in the culture medium between about day -7 and day 0 is between about 0 g/L and about 3 g/L;

(b) the desired relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody resulting from the fermentation is about 7%, the average concentration of glucose in the culture medium between about day -7 and day 0 is between about 3 g/L and about 6 g/L;

(b) the desired relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody resulting from the fermentation is about 10.5%, the average concentration of glucose in the culture medium between about day -7 and day 0 is between about 6 g/L and about 9 g/L;

(c) the desired relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody resulting from the fermentation is about 13%, the average concentration of glucose in the culture medium between about day -7 and day 0 is between about 9 g/L and about 11 g/L;

or

(d) the desired relative content of high mannose Fab glycoforms of the glycosylated monoclonal antibody resulting from the fermentation is about 15%, the average concentration of glucose in the culture medium between about day -7 and day 0 is between about 11 g/L and about 14 g/L.

18. A monoclonal antibody composition obtainable by the method of any one of claims 3 to 17.

19. A composition according to any one of claims 1 to 13, for use in the diagnosis or treatment of a disease in an individual suspected of or suffering therefrom, optionally wherein the disease is dementia, Alzheimer's Disease, motor neuropathy, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), scrapie, HIV-related dementia, Creutzfeld-Jakob disease (CJD), hereditary cerebral haemorrhage, Down's syndrome and neuronal disorders related to ageing; and cancer, such as metastatic colorectal cancer, metastatic non-small cell lung cancer, ovarian cancer and head and neck cancer.

20. Use of a composition according to any one of claims 1 to 13 in the manufacture of a medicament for the diagnosis or treatment of a disease in an individual suspected of or suffering therefrom, wherein the disease is dementia, Alzheimer's Disease, motor neuropathy, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), scrapie, HIV-related dementia, Creutzfeld-Jakob disease (CJD), hereditary cerebral haemorrhage, Down's syndrome and neuronal disorders related to ageing; and cancer, such as metastatic colorectal cancer, metastatic non-small cell lung cancer, ovarian cancer and head and neck cancer.

~~20~~21. The composition or method according to any one of claims 1 to 19 wherein the relative amount of an N-linked high mannose glycan in the Fab region(s) to the total amount of glycosylated Fab in the composition is analysed by hydrophilic-interaction chromatography-ultra high performance liquid chromatography (HILIC-UHPLC) with subsequent fluorescence detection of 2-aminobenzamide labelled glycans.