

As such, there remains a need for alternative methods of reducing HCPs in the purification process of therapeutic or diagnostic proteins. Such alternative methods reduce HCPs preferably without affecting product stability, yield, or cost to ultimately maintain product quality and is amenable to large scale manufacturing and ensuring patient safety.

Summary

In one aspect of the present disclosure, there is provided a pharmaceutical composition comprising an antibody that binds to human N3pGlu A β (anti-N3pGlu A β antibody), wherein the anti-N3pGlu A β antibody was prepared by a process comprising purifying the anti-N3pGlu antibody from a mammalian host cell, and wherein the total content of host cell proteins (HCPs) in the composition is less than about 100 ppm (as measured by LCMS) and the composition comprises one of, combinations of, or all of the following host cell proteins: protein S100-A6, protein S100-A11, phospholipase B-like 2 protein, lysosomal protective protein, ubiquitin-40S ribosomal protein S27a, kallikrein-11, serine protease HTRA1 isoform X1, complement C1r subcomponent, actin, aortic smooth muscle isoform X1, heat shock cognate 71 kDa protein, and peroxiredoxin-1.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising an antibody that binds to human N3pGlu A β (anti-N3pGlu A β antibody), wherein the anti-N3pGlu A β antibody was prepared by a process comprising purifying the anti-N3pGlu antibody from a mammalian host cell, and wherein the total content of host cell proteins (HCPs) in the composition is less than about 10 ppm (as measured by LCMS) and the composition comprises one of, combinations of, or all of the following host cell proteins: polyubiquitin, lysosomal protective protein, glutathione S-transferase Y1, 40S ribosomal protein S28, thioredoxin isoform X1, basement membrane-specific heparan sulfate proteoglycan core protein isoform X1, tubulointerstitial nephritis antigen-like protein, actin – partial cytoplasmic 2 isoform X2, galectin-1, peroxiredoxin-1, and cornifin alpha.

Description

Accordingly, the present invention addresses one or more of the above problems by providing alternative methods of reducing HCPs in the preparation of therapeutic or diagnostic antibodies or antigen-binding fragments thereof. The methods of the present

invention provide reproducible methods that are highly effective in removing HCPs, whilst preserving antibody stability, reducing aggregation, maintaining product yield and have a potential to lower immunogenicity risk. Such methods can effectively remove HCPs without requiring increased antibody preparation volume. Surprisingly, the

5 methods of the present invention achieved HCP counts well below the industry acceptable standards of < 100 ppm. Surprisingly, other embodiments of the present invention achieved HCP counts of < 50 ppm whilst preserving protein stability, reducing aggregation, and maintaining product yield. More surprisingly, other embodiments of the present invention achieved HCP counts of < 20 ppm, < 10 ppm, < 5 ppm, < 1 ppm, or ~ 0

10 ppm, whilst preserving protein stability, reducing aggregation, and maintaining product yield. Furthermore, embodiments of the present invention provide methods of HCP removal that are applicable to a broad range of molecules. Other embodiments of the present invention enable the elimination of additional purification steps, resulting in a reduction in batch processing time, and decreased manufacturing costs. The disclosed

15 methods may be performed in order to produce antibody compositions having reduced host cell content, wherein the host cell content of the antibody compositions is less < 100 ppm, < 50 ppm, < 10 ppm, < 5 ppm, < 1 ppm, or ~ 0 ppm.

Accordingly, there is provided methods of reducing host cell protein content in an anti-N3pGlu A β antibody ("an anti-N3pG antibody") preparation. In some embodiments,

20 the anti-N3pG antibody is recombinantly produced in a mammalian host cell, such as a Chinese hamster ovary cell host cell.

Accordingly, in particular embodiments, provided is a method of reducing host cell protein content in a protein preparation comprising an anti-N3pG antibody

Claims

1. A pharmaceutical composition comprising an antibody that binds to human N3pGlu A β (anti-N3pGlu A β antibody), wherein the anti-N3pGlu A β antibody was prepared by a process comprising purifying the anti-N3pGlu antibody from a mammalian host cell, and wherein the total content of host cell proteins (HCPs) in the composition is less than about 100 ppm (as measured by LCMS) and the composition comprises one of, combinations of, or all of the following host cell proteins: protein S100-A6, protein S100-A11, phospholipase B-like 2 protein, lysosomal protective protein, ubiquitin-40S ribosomal protein S27a, kallikrein-11, serine protease HTRA1 isoform X1, complement C1r subcomponent, actin, aortic smooth muscle isoform X1, heat shock cognate 71 kDa protein, and peroxiredoxin-1.
2. A pharmaceutical composition according to claim 1, wherein the mammalian cell is a CHO cell.
3. A pharmaceutical composition according to claim 1 or claim 2, wherein the anti-N3pGlu A β antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a bispecific antibody, or an antibody fragment.
4. A pharmaceutical composition according to claim 3, wherein the anti-N3pGlu A β antibody is an IgG1 antibody.
5. A pharmaceutical composition according to claim 1, wherein the anti-N3pGlu A β antibody comprises a heavy chain (HC) and a light chain (LC), wherein the light chain comprises a light chain variable region (LCVR) and the heavy chain comprises a heavy chain variable region (HCVR), wherein the LCVR comprises amino acid sequences LCDR1, LCDR2, and LCDR3, and the HCVR comprises amino acid sequences HCDR1, HCDR2, and HCDR3, wherein LCDR1 is KSSQSLLYSRGKTYLN (SEQ ID NO:17), LCDR2 is AVSKLDS (SEQ ID NO:18), LCDR3 is VQGTHYPFT (SEQ ID NO:19), HCDR1 is GYDFTRYIN (SEQ ID NO:20), HCDR2 is WINPGSGNTKYNEKFKG (SEQ ID NO:21), and HCDR3 is EGITVY (SEQ ID NO:22).
6. A pharmaceutical composition according to claim 1, wherein the LC of the anti-N3pGlu A β antibody comprises a LCVR and the HC of the anti-N3pGlu A β antibody

comprises a HCVR, wherein the LCVR is

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSRGKTYLNWLLQKPGQSPQLLIY
AVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHYPFTFGQG
TKLEIK (SEQ ID NO:13) and the HCVR is
QVQLVQSGAEVKKPGSSVKVSKKASGYDFTRYINWVRQAPGQGLEWMGW
INPGSGNTKYNEKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCAREGITV
YWGGQTTVTVSS (SEQ ID NO: 14).

7. A pharmaceutical composition according claim 1, wherein the LC of the anti-N3pGlu A β antibody is

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSRGKTYLNWLLQKPGQSPQLLIY
AVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHYPFTFGQG
TKLEIKRTVAAPS VFIFPPSDEQLKSGTASVCLLN NFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC (SEQ ID NO: 15) and the HC of the anti-N3pGlu A β antibody is
QVQLVQSGAEVKKPGSSVKVSKKASGYDFTRYINWVRQAPGQGLEWMGW
INPGSGNTKYNEKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCAREGITV
YWGGQTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 16).

8. A pharmaceutical composition according to claim 1, wherein the anti-N3pGlu A β antibody is donanemab.
9. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm of protein S100-A6 (as measured by LCMS).
10. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm of protein S100-A11 (as measured by LCMS).

11. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 10 ppm of phospholipase B-like 2 protein (as measured by LCMS).
12. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm of lysosomal protective protein (as measured by LCMS).
13. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5ppm of ubiquitin-40S ribosomal protein S27a (as measured by LCMS).
14. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm of kallikrein-11 (as measured by LCMS).
15. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm serine protease HTRA1 isoform X1 (as measured by LCMS).
16. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm complement C1r subcomponent (as measured by LCMS).
17. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm actin (as measured by LCMS).
18. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm aortic smooth muscle isoform X1 (as measured by LCMS).
19. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm heat shock cognate 71 kDa protein (as measured by LCMS).
20. A pharmaceutical composition according to claim 1, wherein the composition comprises less than about 5 ppm peroxiredoxin-1 (as measured by LCMS).
21. A pharmaceutical composition comprising an antibody that binds to human N3pGlu A β (anti-N3pGlu A β antibody), wherein the anti-N3pGlu A β antibody was prepared by a process comprising purifying the anti-N3pGlu antibody from a mammalian host cell, and wherein the total content of host cell proteins (HCPs) in the composition is

less than about 10 ppm (as measured by LCMS) and the composition comprises one of, combinations of, or all of the following host cell proteins: polyubiquitin, lysosomal protective protein, glutathione S-transferase Y1, 40S ribosomal protein S28, thioredoxin isoform X1, basement membrane-specific heparan sulfate proteoglycan core protein isoform X1, tubulointerstitial nephritis antigen-like protein, actin – partial cytoplasmic 2 isoform X2, galectin-1, peroxiredoxin-1, and cornifin alpha.

22. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of polyubiquitin (as measured by LCMS).
23. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of lysosomal protective protein (as measured by LCMS).
24. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of glutathione S-transferase Y1 (as measured by LCMS).
25. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of 40S ribosomal protein S28 (as measured by LCMS).
26. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of thioredoxin isoform X1 (as measured by LCMS).
27. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of basement membrane-specific heparan sulfate proteoglycan core protein isoform X1 (as measured by LCMS).
28. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of tubulointerstitial nephritis antigen-like protein (as measured by LCMS).
29. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of actin – partial cytoplasmic 2 isoform X2 (as measured by LCMS).
30. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of galectin-1 (as measured by LCMS).

31. A pharmaceutical composition according to claim 21, wherein the composition comprises less than about 1 ppm of peroxiredoxin-1 (as measured by LCMS).
32. A pharmaceutical composition according to claim 21, wherein the mammalian cell is a CHO cell.
33. A pharmaceutical composition according to claim 21, wherein the anti-N3pGlu A β antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a bispecific antibody, or an antibody fragment.
34. A pharmaceutical composition according to claim 33, wherein the anti-N3pGlu A β antibody is an IgG1 antibody.
35. The pharmaceutical composition according to claim 21, wherein the anti-N3pGlu A β antibody comprises a heavy chain (HC) and a light chain (LC), wherein the light chain comprises a light chain variable region (LCVR) and the heavy chain comprises a heavy chain variable region (HCVR), wherein the LCVR comprises amino acid sequences LCDR1, LCDR2, and LCDR3, and the HCVR comprises amino acid sequences HCDR1, HCDR2, and HCDR3, wherein LCDR1 is RASQSLGNWLA (SEQ ID NO: 27), LCDR2 is YQASTLES (SEQ ID NO: 28). LCDR3 is QHYKGSFWT (SEQ ID NO: 29), HCDR1 is AASGFTFSSYPMS (SEQ ID NO: 30), HCDR2 is AISGSGGSTYYADSVKG (SEQ ID NO: 31), and HCDR3 is AREGGSGSYNGFDY (SEQ ID NO: 32).
36. The pharmaceutical composition of claim 21, wherein the LC of the anti-N3pGlu A β antibody comprises a LCVR and the HC of the anti-N3pGlu A β antibody comprises a HCVR, wherein the LCVR is
DIQMTQSPSTLSASVGDRVTITCRASQSLGNWLAWYQQKPGKAPKLLIYQASTLE
SGVPSRFSGSGSGTEFTLTISLQPDDFATYYCQHYKGSFWTFGQGGTKVEIK (SEQ
ID NO:23) and the HCVR is
EVQLLES GGGLVQPGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAISGS
GGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREGGSGSYN
GFDYWGGQGLVTVSS (SEQ ID NO: 24).
37. The pharmaceutical composition of claim 21, wherein the LC of the anti-N3pGlu A β antibody is
DIQMTQSPSTLSASVGDRVTITCRASQSLGNWLAWYQQKPGKAPKLLIYQASTLE
SGVPSRFSGSGSGTEFTLTISLQPDDFATYYCQHYKGSFWTFGQGGTKVEIKRTVA

APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO: 25) and the HC of the anti-N3pGlu A β antibody is
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAISGS
GGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREGGSGSYYN
GFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO: 26).