

Fc-galactosylation modulates antibody-dependent cellular cytotoxicity of therapeutic antibodies.

Molecular immunology 2016; 73:69-75).

[0005] Different factors influence the glycan structure and thus the ultimate glycosylated form (glycoform) of the protein (glycoprotein). For example, the cell line expressing the antibody, the cell culture medium, the feed medium composition, and the timing of the feeds during cell culture can impact the production of glycoforms of the protein. While research groups have suggested many ways to influence the levels of particular glycoforms of an antibody, there still is a need in the biopharmaceutical industry for simple and efficient methods to predict the level of effector function a particular antibody composition will exhibit based on the given glycoform profile for that antibody composition. Additionally, there is a need in the art for methods of determining the levels of particular glycans, e.g., afucosylated glycans, high mannose glycans, that will achieve a desired level effector function.

SUMMARY

[0006] In one aspect, the present disclosure provides a method of determining product quality of an antibody composition, wherein the ADCC activity level of the antibody composition is a criterion upon which product quality of the antibody composition is based, said method comprising i. determining the total afucosylated (TAF) glycan content of a sample of an antibody composition, ii. determining the product quality of the antibody composition as acceptable and/or achieving the ADCC activity level criterion when the TAF glycan content determined in (i) is within a target range, wherein the target range of TAF glycan content is based on (1) a target range of ADCC activity levels for a reference antibody and (2) a first model which correlates ADCC activity level of the antibody composition to the TAF glycan content of the antibody composition, wherein the ADCC predicted by the first model is about 95% to about 105% of the ADCC predicted by a second model, wherein the second model correlates the ADCC activity level of the antibody composition to the high mannose (HM) glycan content and the afucosylated (AF) glycan content of the antibody composition.

[0006a] In another aspect, the present disclosure provides a method of monitoring product quality of an antibody composition, comprising determining product quality of an antibody composition in accordance with a method as described herein, with a first sample obtained at a first timepoint and with a second sample taken at a second timepoint which is different from the first timepoint.

[0006b] In yet another aspect, the present disclosure provides a method of producing an antibody composition, comprising determining the product quality of the antibody composition, wherein product quality of the antibody composition is determined in accordance with a method as described herein, wherein the sample is a sample of in-process material, wherein, when the TAF glycan content determined in (i) is not within the target range, the method further comprises (iii) modifying one or more conditions of the cell culture to obtain a modified cell culture and (iv) determining the TAF glycan content of a sample of the antibody composition obtained from the modified cell culture, optionally, repeating steps (ii) and (iii) until the TAF glycan content is within the target range.

[0006c] The present disclosure provides methods of determining product quality of an antibody composition, wherein the ADCC activity level of the antibody composition is a criterion upon which product quality of the antibody composition is based. The method in various aspectexamples determines the product quality in terms of the ADCC activity level criterion. In exemplary embodimentexamples, the method comprises (i) determining the total afucosylated (TAF) glycan content of a sample of an antibody composition; and (ii) determining the product quality as acceptable and/or achieving the ADCC activity level criterion when the TAF glycan content determined in (i) is within a target range. In exemplary aspectexamples, the target range of TAF glycan content is based on (1) a target range of ADCC activity levels for a reference antibody and (2) a first model which correlates ADCC activity level of the antibody composition to TAF glycan content of the antibody composition. In exemplary aspectexamples, the ADCC predicted by the first model is about 95% to about 105% of the ADCC predicted by a second model, wherein the second model correlates the ADCC activity level of the antibody composition to the HM glycan content of the antibody composition and the AF glycan content of the antibody composition. As used herein, the term “predicted” in the context of ADCC activity level(s) refers to a calculated ADCC activity level, wherein the ADCC activity level is calculated according to a model, e.g., a first model, a second model. Advantageously, the ADCC predicted by the first model is statistically significantly similar to the ADCC predicted by the second model. For example, the ADCC activity level predicted by the first model is about 95% to about 105% of the ADCC activity level predicted by the second model.

Optionally, the ADCC activity level predicted by the first model is about 95%, about 96%, about 97%, about 98%, about 99%, about 100%, about 101%, about 102%, about 103%, about 104%, or about 105% of the ADCC activity level predicted by the second model. The ADCC activity level predicted by the first model is, in various instances, about 100% of the ADCC predicted by the second model. In certain [aspectexamples](#), there is a one-to-one correspondence between the ADCC predicted by the first model and the ADCC predicted by the second model. In various instances, the first model and/or the second model is/are statistically significant. For instance, the p-value of the first model is less than 0.0001 and/or the p-value of the second model is less than 0.0001. Optionally, each of the first model and the second model has a p-value which is less than 0.0001. In exemplary [aspectexamples](#), the ADCC activity level predicted by the first model is $\sim 12Q * \%TAF$, wherein Q is the number of antibody binding sites on the antigen to which the antibody binds and $\%TAF$ is the TAF glycan content of the antibody composition. In exemplary instances, the target range of TAF glycan content is m to n , wherein m is $[ADCC_{min} / 12Q]$, wherein $ADCC_{min}$ is the minimum of the target range of ADCC activity level for a reference antibody, and n is $[ADCC_{max} / 12Q]$, wherein $ADCC_{max}$ is the maximum of the target range of ADCC activity level for the reference antibody. In various instances, Q is 2. In various instances, the ADCC activity level predicted by the first model is $\sim 24 * \%TAF$. In various instances, the target range of TAF glycan content is m to n wherein m is $[ADCC_{min} / 24]$ and n is $[ADCC_{max} / 24]$. In various instances, the ADCC activity level predicted by the second model is $\sim 27 * \%HM + \sim 22 * \%AF$, wherein $\%AF$ is the AF glycan content of the antibody composition and $\%HM$ is the HM glycan content of the antibody composition. In various instances, Q is 1. In various [aspectexamples](#), the ADCC activity level predicted by the first model is $\sim 12 * \%TAF$. In various instances, the target range of TAF glycan content is m to n wherein m is $[ADCC_{min} / 12]$ and n is $[ADCC_{max} / 12]$. In various instances, the ADCC activity level predicted by the second model is $\sim 14.8 * \%HM + \sim 12.8 * \%AF$. Suitable alternative first models and second models are described herein. In exemplary instances, the first model is any of one of the models (e.g., equations) described herein which correlate ADCC and TAF glycan content, including but not limited to, Equations 1, 3, 5, and 7 and Equation A. In exemplary instances, the second model is any of one of the models (e.g., equations) described herein which correlate ADCC and HM glycan content and AF glycan content, including but not limited to, Equations 2, 4, 6, and 8 and Equation B. For example, in various [aspectexamples](#), the target range for TAF glycan content is m° to n° , wherein m° is defined as $[(ADCC_{min} - y) / x]$, wherein $ADCC_{min}$ is the minimum of the target range of ADCC activity level, and n° is defined as $[(ADCC_{max} - y) / x]$, wherein $ADCC_{max}$ is the maximum of the target range of ADCC activity level. Optionally, x is about 20.4 to about 27.7 and y is about -11.4 to about 16.7. Alternatively, x is about 9.7 to about 15.2 and y is about -15.6 to

about 34.2. In various [aspectexample](#)s, the target range for TAF glycan content is m' to n' , wherein m' is $[\text{ADCC}_{\min} / x']$, wherein $\text{ADCC}_{\min}$ is the minimum of the target range of ADCC activity level, and n' is $[\text{ADCC}_{\max} / x']$, wherein $\text{ADCC}_{\max}$ is the maximum of the target range of ADCC activity level. Optionally, x' is about 24.1 to about 25.4. Alternatively, x' is about 13.0 to about 13.95. In various instances, the ADCC activity level of the antibody composition is about $13.5\% \pm 0.5\%$ for every 1% TAF present in the antibody composition, optionally, wherein the antibody of the antibody composition binds to an antigen comprising only one antibody binding site. In various [aspectexample](#)s, the ADCC activity level of the antibody composition is about $24.74\% \pm 0.625\%$ for every 1% TAF present in the antibody composition, optionally, wherein the antibody of the antibody composition binds to an antigen comprising only two antibody binding sites. In exemplary [aspectexample](#)s, the ADCC activity level of the antibody composition is about $12\% \pm 1.5\% * Q$ for every 1% TAF present in the antibody composition, Q is the number of antibody binding sites present on the antigen. In exemplary instances, the reference antibody is infliximab. In exemplary [aspectexample](#)s, the reference antibody is rituximab. In exemplary [aspectexample](#)s, the method is a quality control (QC) assay. In exemplary [aspectexample](#)s, the method is an in-process QC assay. In various [aspectexample](#)s, the sample is a sample of in-process material. In various instances, the TAF glycan content is determined pre-harvest or post-harvest. In exemplary instances, the TAF glycan content is determined after a chromatography step. Optionally, the chromatography step comprises a capture chromatography, intermediate chromatography, and/or polish chromatography. In some [aspectexample](#)s, the TAF glycan content is determined after a virus inactivation and neutralization, virus filtration, or a buffer exchange. The method in various instances is a lot release assay. The sample in some [aspectexample](#)s is a sample of a manufacturing lot. In various [aspectexample](#)s, the method further comprises selecting the antibody composition for downstream processing, when the TAF glycan content determined in (i) is within a target range. When the TAF glycan content determined in (i) is not within the target range, one or more conditions of the cell culture are modified to obtain a modified cell culture, in various [aspectexample](#)s. The method in some [aspectexample](#)s, further comprises determining the TAF glycan content of a sample of the antibody composition obtained after one or more conditions of the cell culture are modified. In various [aspectexample](#)s, when the TAF glycan content determined in (i) is not within the target range, the method further comprises (iii) modifying one or more conditions of the cell culture to obtain a modified cell culture and (iv) determining the TAF glycan content of a sample of the antibody composition obtained from the modified cell culture. In exemplary [aspectexample](#)s, when the TAF glycan content determined in (i) is not within the target range, the method further comprises (iii) and (iv) until the TAF glycan content determined in (iv) is within the target range. In exemplary instances, an assay which directly measures ADCC activity of the antibody composition is

carried out on the antibody composition only when the TAF glycan content determined in (i) is not within the target range, e.g., outside the target range. Assays which directly measure ADCC activity include for example a cell-based assay that measures the release of a detectable reagent upon lysis of antigen-expressing cells comprising the detectable agent by effector cells that are bound to antibody binding both antigen-expressing and effector cells. In exemplary instances, an assay which directly measures ADCC activity of the antibody composition is not carried out on the antibody composition. In various [aspectexamples](#), determining the TAF glycan content is the only step required to determine the product quality with regard to the ADCC activity level criterion. Without being bound to theory, the statistically significant correlations of the first model and the second model allow for TAF glycan content to indicate ADCC activity level such that assays that directly measure ADCC activity level are not needed. Accordingly, direct measurement of the ADCC activity level of the antibody composition is not needed and thus not carried out in various [aspectexamples](#) of the presently disclosed methods.

[0007] The present disclosure also provides methods of monitoring product quality of an antibody composition, wherein the ADCC activity level of the antibody composition is a criterion upon which product quality of the antibody composition is based. In exemplary [embodimentexamples](#), the method comprises determining product quality of an antibody composition in accordance with a method of the present disclosures, with a first sample obtained at a first timepoint and with a second sample taken at a second timepoint which is different from the first timepoint. In various instances, each of the first sample and second sample is a sample of in-process material. In various [aspectexamples](#), the first sample is a sample of in-process material and the second sample is a sample of a manufacturing lot. Optionally, the first sample is a sample obtained before one or more conditions of the cell culture are modified and the second sample is a sample obtained after the one or more conditions of the cell culture are modified. In exemplary instances, the TAF glycan content is determined for each of the first sample and second sample. Product quality of the antibody composition depends on whether the TAF glycan content is within a target range. In exemplary [aspectexamples](#), the target range of TAF glycan content is based on (1) a target range of ADCC activity levels for a reference antibody and (2) a first model which correlates ADCC activity level of the antibody composition to TAF glycan content of the antibody composition. In exemplary [aspectexamples](#), the ADCC predicted by the first model is about 95% to about 105% of the ADCC predicted by a second model, wherein the second model correlates the ADCC activity level of the antibody composition to the HM glycan content of the antibody composition and the AF glycan content of the antibody composition.

[0008] The present disclosure provides methods of producing an antibody composition. In exemplary [embodimentexample](#), the method comprises determining product quality of the antibody composition wherein product quality of the antibody composition is determined in accordance with a method of the present disclosures. Optionally, the method comprises determining the TAF glycan content of a sample of an antibody composition and the sample is a sample of in-process material. In various instances, the method comprises determining the product quality of the antibody composition as acceptable and/or achieving the ADCC activity level criterion when the TAF glycan content determined in (i) is within a target range, as defined herein. In exemplary [aspectexample](#), the target range of TAF glycan content is based on (1) a target range of ADCC activity levels for a reference antibody and (2) a first model which correlates ADCC activity level of the antibody composition to TAF glycan content of the antibody composition. In exemplary [aspectexample](#), the ADCC predicted by the first model is about 95% to about 105% of the ADCC predicted by a second model, wherein the second model correlates the ADCC activity level of the antibody composition to the HM glycan content of the antibody composition and the AF glycan content of the antibody composition. In various [aspectexamples](#), when the TAF glycan content determined in (i) is not within the target range, the method further comprises (iii) modifying one or more conditions of the cell culture to obtain a modified cell culture and (iv) determining the TAF glycan content of a sample of the antibody composition obtained from the modified cell culture, optionally, repeating steps (iii) and (iv) until the TAF glycan content is within the target range. In various instances, the sample is a sample of a cell culture comprising cells expressing an antibody of the antibody composition. In various instances, one or more conditions of the cell culture are modified to modify the TAF glycan content. In various [aspectexamples](#), the TAF glycan content of the antibody composition is achieved by modifying the AF glycan content. In exemplary [aspectexamples](#), one or more conditions of the cell culture are modified to modify the AF glycan content of the antibody composition. In exemplary [aspectexample](#), the one or more conditions primarily modify the AF glycan content. In various instances, the one or more conditions modify the AF glycan content and does not modify the HM glycan content. In exemplary [aspectexample](#), the method comprises the TAF glycan content of the antibody composition is achieved by modifying the HM glycan content. Optionally, one or more conditions of the cell culture are modified to modify the HM glycan content of the antibody composition. In some instances, the one or more conditions primarily modify the HM glycan content. In some [aspectexamples](#), the one or more conditions modify the HM glycan content and does not modify the AF glycan content. In various instances, the method comprises repeating the modifying of the afucosylated (AF) glycan content and/or repeating the modifying of the high mannose (HM) glycan, until the TAF glycan content is within a target range.

[0009] In exemplary [embodimentexamples](#), the method of producing an antibody composition comprises (i) determining the TAF glycan content of a sample of an antibody composition; and (ii) selecting the antibody composition for downstream processing based on the TAF glycan content determined in (i). In various [aspectexamples](#), the sample is taken from a cell culture comprising cells expressing an antibody of the antibody composition. In various instances, the method further comprises modifying the TAF glycan content of the antibody composition and determining the modified TAF glycan content. Optionally, one or more conditions of the cell culture are modified in order to modify the TAF glycan content. In exemplary [aspectexamples](#), the method comprises repeating the modifying until the TAF glycan content is within a target range. In exemplary instances, the target range is based on a target range of ADCC activity level for the antibody. Without being bound to theory, the TAF glycan content correlates with the ADCC activity level of the antibody composition such that the ADCC activity level of an antibody composition may be predicted based on the TAF glycan content of the antibody composition. The ADCC activity level of the antibody composition may be a criteria worth considering when deciding whether the antibody composition should be selected for downstream processing. Therefore, in various [aspectexamples](#), the method comprises (i) determining the TAF glycan content of a sample of an antibody composition; (ii) determining the ADCC activity level of the antibody composition based on the TAF glycan content determined in (i), and, optionally, (iii) selecting the antibody composition for downstream processing when the ADCC level of the antibody composition determined in (ii) is within a target range of ADCC activity level. In various [aspectexamples](#), the target range of ADCC activity level is known for the antibody of the antibody composition. The antibody of the antibody composition, in various [aspectexamples](#), is a biosimilar of a reference antibody. In various instances, a target range of TAF glycan content is based or determined (e.g., calculated) based on the target range of ADCC activity level which is known. Accordingly, in exemplary [aspectexamples](#), the method comprises (i) determining the TAF glycan content of a sample of an antibody composition; and (ii) selecting the antibody composition for downstream processing when the TAF glycan content determined in (i) is within a target range. When the method further comprises modifying the TAF glycan content of the antibody composition, the method in various instances comprises modifying the afucosylated (AF) glycan content to modify the TAF glycan content. Optionally, one or more conditions of the cell culture are modified to modify the AF glycan content of the antibody composition, which, in turn, modifies the TAF glycan content. Alternatively or additionally, when the method further comprises modifying the TAF glycan content of the antibody composition, the method in various instances comprises modifying the high mannose (HM) glycan content to modify the TAF glycan content. Optionally, one or more conditions of the cell culture are modified to modify the HF glycan

content of the antibody composition, which, in turn, modifies the TAF glycan content. In exemplary [aspectexamples](#), the one or more conditions primarily modify the AF glycan content. In exemplary instances, the one or more conditions primarily modify the HM glycan content. In exemplary [aspectexamples](#), the one or more conditions modify the AF glycan content and not the HM glycan content. In exemplary instances, the one or more conditions modify the HM glycan content and not the AF glycan content. The method optionally comprises repeating the modifying of the afucosylated (AF) glycan content and/or repeating the modifying of the high mannose (HM) glycan, until the TAF glycan content is within a target range. In exemplary [aspectexamples](#), the antibody of the antibody composition is an IgG, optionally, an IgG₁. In various [aspectexamples](#), the target range for TAF glycan content is m to n , wherein m is $[(ADCC_{min} - y) / x]$, wherein $ADCC_{min}$ is the minimum of the target range of ADCC activity level, and n is $[(ADCC_{max} - y) / x]$, wherein $ADCC_{max}$ is the maximum of the target range of ADCC activity level. Optionally, x is about 20.4 to about 27.7 and y is about -11.4 to about 16.7. Alternatively, x is about 9.7 to about 15.2 and y is about -15.6 to about 34.2. In various [aspectexamples](#), the target range for TAF glycan content is m' to n' , wherein m' is $[ADCC_{min} / x']$, wherein $ADCC_{min}$ is the minimum of the target range of ADCC activity level, and n' is $[ADCC_{max} / x']$, wherein $ADCC_{max}$ is the maximum of the target range of ADCC activity level. Optionally, x' is about 24.1 to about 25.4. Alternatively, x' is about 13.0 to about 13.95. In various instances, the ADCC activity level of the antibody composition is about $13.5\% \pm 0.5\%$ for every 1% TAF present in the antibody composition, optionally, wherein the antibody of the antibody composition binds to an antigen comprising only one antibody binding site. In various [aspectexamples](#), the ADCC activity level of the antibody composition is about $24.74\% \pm 0.625\%$ for every 1% TAF present in the antibody composition, optionally, wherein the antibody of the antibody composition binds to an antigen comprising only two antibody binding sites. In exemplary [aspectexamples](#), the ADCC activity level of the antibody composition is about $12\% \pm 1.5\% * Q$ for every 1% TAF present in the antibody composition, Q is the number of antibody binding sites present on the antigen. In exemplary instances, Q is 1 and optionally the antibody is infliximab or a biosimilar thereof. Optionally, Q is 2 and optionally the antibody is rituximab or a biosimilar thereof.

[0010] In exemplary [embodimentexamples](#), the method of producing an antibody composition comprises (i) determining the % total afucosylated (TAF) glycans of an antibody composition; (ii) calculating a % antibody dependent cellular cytotoxicity (ADCC) of the antibody composition based on the % TAF using Equation A:

$$Y = 2.6 + 24.1 * X$$

[Equation A],

wherein **Y** is the % ADCC and **X** is the % TAF glycans determined in step (i),

[0011] and (iii) selecting the antibody composition for one or more downstream processing steps when **Y** is within a target % ADCC range.

[0012] The present disclosure also provides a method of producing an antibody composition, wherein, the method comprises (i) determining the % high mannose glycans and the % afucosylated glycans of an antibody composition; (ii) calculating a % antibody dependent cellular cytotoxicity (ADCC) of the antibody composition based on the % high mannose glycans and the % afucosylated glycans using Equation B:

$$Y = (0.24 + 27*HM + 22.1*AF)$$

[Equation B],

wherein **Y** is the % ADCC, **HM** is the % high mannose glycans determined in step (i), and **AF** is the % afucosylated glycans determined in step (i),

[0013] and (iii) selecting the antibody composition for one or more downstream processing steps when **Y** is within a target % ADCC range.

[0014] The present disclosure additionally provides methods of producing an antibody composition with a target % ADCC. In exemplary embodiment examples, the method comprises (i) calculating a target % total afucosylated (TAF) glycans for the target % ADCC using Equation A:

$$Y = 2.6 + 24.1*X$$

[Equation A],

wherein **Y** is the target % ADCC and **X** is the target % TAF glycans,

[0015] and (ii) maintaining glycosylation-competent cells in a cell culture to produce an antibody composition with the target % TAF glycans, **X**.

[0016] The present disclosure further provides methods of producing an antibody composition with a target % ADCC, wherein the method comprises (i) calculating a target % afucosylated glycans and a target % high mannose glycans for the target % ADCC using Equation B:

$$Y = (0.24 + 27*HM + 22.1*AF)$$

[Equation B],

wherein **Y** is the target % ADCC, **HM** is the target % high mannose glycans and **AF** is the target % afucosylated glycans

and (ii) maintaining glycosylation-competent cells in a cell culture to produce an antibody composition with the target % high mannose glycans and the target % afucosylated glycans.

[0017] In exemplary [aspectexamples](#) of the presently disclosed methods, the target % ADCC is within a target % ADCC range. Optionally, the target % ADCC range is greater than or about 40 and less than or about 170. In various [aspectexamples](#), the target % ADCC range is greater than or about 44 and less than or about 165. In various instances, the target % ADCC range is greater than or about 60 and less than or about 130. In exemplary [aspectexamples](#), the target % ADCC range is $Y \pm 20$, e.g., $Y \pm 17$ or $Y \pm 18$.

[0018] Further provided are methods of producing an antibody composition with a % ADCC, Y , which is optionally greater than or about 40 and less than or about 170, said method comprising (i) determining the % total afucoylated (TAF) glycans, X , of the antibody composition, and (ii) selecting the antibody composition for one or more downstream processing steps, when X is equivalent to $(Y-2.6)/24.1$. In exemplary [aspectexamples](#), X is greater than or about 1.55% and less than or about 6.95%. In various [aspectexamples](#), Y is greater than or about 44% and less than or about 165%, and optionally, wherein X is about 1.72% to about 6.74%.

[0019] The present disclosure provides method of producing an antibody composition with a % ADCC, Y , said method comprising (i) determining the % total afucoylated (TAF) glycans, X , of the antibody composition, and (ii) selecting the antibody composition for one or more downstream processing steps, when the X is equivalent to $(Y-2.6)/24.1$, optionally, wherein X is greater than or about $X-0.4$ and less than or about $X+0.4$, and wherein the % ADCC is greater than about $Y-17$ and less than or about $Y+17$. Also provided is a method of producing an antibody composition with a % ADCC, said method comprising (i) determining the % afucosylated glycans and the % high mannose glycans of the antibody composition, and (ii) selecting the antibody composition for one or more downstream processing steps, when **AF** and **HM** are related to Y according to Equation B

$$Y = (0.24 + 27*HM + 22.1*AF)$$

[Equation B],

wherein Y is the % ADCC, **HM** is the % high mannose glycans determined in step (i), and **AF** is the afucosylated glycans determined in step (i). In exemplary [aspectexamples](#), Y is greater than or about 40 and less than or about 175, optionally, about 41 to about 171, wherein **AF** is about 1 to about 4 and wherein **HM** is about 40 to about 175. Optionally, Y is about 30 to about 185, optionally, about 32 to about 180, wherein **HM** is about 1 to about 4 and wherein **AF** is about 30 to about 185. In exemplary instances, the % ADCC of the antibody composition is within a range defined by Y . Optionally, the % ADCC of the

antibody composition is within a range of $Y \pm 18$. In exemplary [aspectexamples](#), **AF** is about 1 to about 4. Optionally, the % high mannose glycans is a value within a range defined by **HM**, optionally, wherein the range is $HM \pm 1$. In various instances, **HM** is about 1 to about 4. Optionally, the % afucosylated glycans is a value within a range defined by **AF** optionally, wherein the range is $AF \pm 1$.

[0020] In exemplary [embodimentexamples](#), the presently disclosed methods of producing an antibody composition comprises modifying total afucosylated (TAF) glycan content of an antibody composition produced by cells of a cell culture. In various instances, one or more conditions of the cell culture are modified to modify the TAF glycan content. In various [aspectexamples](#), the method comprises determining the modified TAF glycan content. Optionally, the modifying is repeated until the determined TAF glycan content is in a target range of TAF. Without being bound to a particular theory, the TAF glycan content may be modified by changing the afucosylated (AF) glycan content or the high mannose (HM) content, or a combination thereof, since each impacts the TAF glycan content. Accordingly, the methods advantageously allow for multiple ways to achieve the target range of TAF glycan content. For example, one or more conditions of the cell culture are modified to modify the AF glycan content in order to modify the TAF glycan content. Alternatively, one or more conditions of the cell culture are modified to modify the HM glycan content in order to modify the TAF glycan content. In various instances, one or more conditions of the cell culture are modified to modify the AF glycan content and the HM glycan content in order to modify the TAF glycan content. Therefore, the present disclosure further provides methods of modifying total afucosylated (TAF) glycan content of an antibody composition produced by cells of a cell culture. In exemplary [embodimentexamples](#), the method comprises modifying the AF glycan content. In exemplary [embodimentexamples](#), the method comprises modifying the HM glycan content. In various [aspectexamples](#), the method comprises (i) determining the afucosylated (AF) glycan content and the high mannose (HM) glycan content of a sample of an antibody composition; (ii) determining a target range of AF glycan content based on a target range of ADCC activity level of an antibody of the antibody composition, assuming the HM glycan content is constant; and (iii) selecting the antibody composition for downstream processing when the AF glycan content is in the target range of AF glycan content. In various instances, the method comprises (i) determining the afucosylated (AF) glycan content and the high mannose (HM) glycan content of a sample of an antibody composition; (ii) determining a target range of HM glycan content based on a target range of ADCC activity level of an antibody of the antibody composition, assuming the AF glycan content is constant; and (iii) selecting the antibody composition for downstream processing when the HM glycan content is in the target range of AF glycan content. In various instances, the method comprises (i) determining the AF glycan content and the HM

glycan content of a sample of the antibody composition and (ii) determining a target range of AF glycan content based on the HM glycan content determined in (i), and (iii) modifying the AF glycan content until it is within the target range of AF glycan content, wherein the HM glycan content is unmodified. Alternatively, the method comprises (i) determining the AF glycan content and the HM glycan content of a sample of the antibody composition and (ii) determining a target range of HM glycan content based on the AF glycan content determined in (i), and (iii) modifying the HM glycan content until it is within the target range of HM glycan content, wherein the AF glycan content is unmodified. In exemplary [aspectexample](#)s, the model which correlates ADCC activity level of the antibody composition to the TAF glycan content of the antibody composition predicts essentially the same ADCC activity level predicted by the model which correlates ADCC to HM and AF glycan content.

[0021] In various [aspectexamples](#) of the presently disclosed methods, the % TAF glycans is determined by calculating the sum of the % high mannose glycans and the % afucosylated glycans. In various instances, the % high mannose glycans and the % afucosylated glycans are determined by hydrophilic interaction chromatography. Optionally, the % high mannose glycans and the % afucosylated glycans are determined by the method described in Example 1. In various [aspectexamples](#), the % ADCC is determined by a quantitative cell-based assay which measures the ability of the antibodies of the antibody composition to mediate cell cytotoxicity in a dose-dependent manner in cells expressing the antigen of the antibodies and engaging Fc-gammaRIIIA receptors on effector cells through the Fc domain of the antibodies. In various instances, the % ADCC is determined by the assay described in Example 2. In exemplary [aspectexamples](#), the determining step is carried out after a harvest step. Optionally, the determining step is carried out after a chromatography step. In various [aspectexamples](#), the chromatography step is a Protein A chromatography step. In various instances of the presently disclosed methods, the one or more downstream processing steps comprise(s): a dilution step, a filling step, a filtration step, a formulation step, a chromatography step, a viral filtration step, a viral inactivation step, or a combination thereof. Optionally, the chromatography step is an ion exchange chromatography step, optionally, a cation exchange chromatography step or an anion exchange chromatography step.

[0022] In various [aspectexamples](#) of the present disclosure, each antibody of the antibody composition is an IgG, optionally, each antibody of the antibody composition is an IgG₁. In exemplary instances, each antibody of the antibody composition binds to a tumor-associated antigen. In exemplary [aspectexamples](#), the tumor-associated antigen comprises the amino acid sequence of SEQ ID NO. 3. In exemplary [aspectexamples](#), each antibody of the antibody composition is an anti-CD20 antibody. In various instances, each

antibody of the antibody composition comprises: (i) a light chain (LC) CDR1 comprising an amino acid sequence of SEQ ID NO: 4 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 4 or a variant amino acid sequence of SEQ ID NO: 4 with 1 or 2 amino acid substitutions, (ii) a LC CDR2 comprising an amino acid sequence of SEQ ID NO: 5 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 5 or a variant amino acid sequence of SEQ ID NO: 5 with 1 or 2 amino acid substitutions, (iii) a LC CDR3 comprising an amino acid sequence of SEQ ID NO: 6 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 6 or a variant amino acid sequence of SEQ ID NO: 6 with 1 or 2 amino acid substitutions, (iv) a heavy chain (HC) CDR1 comprising an amino acid sequence of SEQ ID NO: 7 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 7 or a variant amino acid sequence of SEQ ID NO: 7 with 1 or 2 amino acid substitutions; (v) a HC CDR2 comprising an amino acid sequence of SEQ ID NO: 8 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 8 or a variant amino acid sequence of SEQ ID NO: 8 with 1 or 2 amino acid substitutions; and/or (vi) a HC CDR3 comprising an amino acid sequence of SEQ ID NO: 9 or an amino acid sequence which is at least 90% identical to SEQ ID NO: 9 or a variant amino acid sequence of SEQ ID NO: 9 with 1 or 2 amino acid substitutions.

[0023] In exemplary [aspectexamples](#), each antibody of the antibody composition comprises a LC variable region comprising an amino acid sequence of SEQ ID NO: 10, an amino acid sequence which is at least 90% identical to SEQ ID NO: 10, or a variant amino acid sequence of SEQ ID NO: 10 with 1 to 10 amino acid substitutions. Optionally, each antibody of the antibody composition comprises a HC variable region comprising an amino acid sequence of SEQ ID NO: 11, an amino acid sequence which is at least 90% identical to SEQ ID NO: 11, or a variant amino acid sequence of SEQ ID NO: 11 with 1 to 10 amino acid substitutions. In exemplary [aspectexamples](#), each antibody of the antibody composition comprises a light chain comprising an amino acid sequence of SEQ ID NO: 12, an amino acid sequence which is at least 90% identical to SEQ ID NO: 12, or a variant amino acid sequence of SEQ ID NO: 12 with 1 to 10 amino acid substitutions. In exemplary instances, each antibody of the antibody composition comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 13, an amino acid sequence which is at least 90% identical to SEQ ID NO: 13, or a variant amino acid sequence of SEQ ID NO: 13 with 1 to 10 amino acid substitutions.

[0024] In exemplary [aspectexamples](#), the tumor-associated antigen comprises the amino acid sequence of SEQ ID NO. 14. In exemplary [aspectexamples](#), each antibody of the antibody composition is an anti-TNF α antibody, optionally, infliximab or a biosimilar thereof. In exemplary [aspectexamples](#), each antibody of the antibody

composition comprises a LC variable region comprising an amino acid sequence of SEQ ID NO: 15, an amino acid sequence which is at least 90% identical to SEQ ID NO: 15, or a variant amino acid sequence of SEQ ID NO: 15 with 1 to 10 amino acid substitutions. Optionally, each antibody of the antibody composition comprises a HC variable region comprising an amino acid sequence of SEQ ID NO: 16, an amino acid sequence which is at least 90% identical to SEQ ID NO: 16, or a variant amino acid sequence of SEQ ID NO: 16 with 1 to 10 amino acid substitutions.

[0025] The present disclosure further provides methods of producing an antibody composition within a target % ADCC range said method comprises: (i) measuring the % ADCC of a series of samples comprising varying glycoforms of an antibody, (ii) determining the % total afucosylated (TAF) glycans for each sample of the series, (iii) determining a linear equation of a best fit line of a graph which plots for each sample of the series the % ADCC as measured in step (i) as a function of the % TAF glycans as determined in step (ii), (iv) determining the % TAF for an antibody composition and then calculating a % ADCC using the linear equation of step (iii), and (v) selecting the antibody composition for one or more downstream processing steps when the % ADCC calculated in step (iv) is within a target % ADCC range.

[0026] A method of producing an antibody composition within a target range of TAF glycan content is provided wherein said method comprises: (i) measuring the ADCC activity level of a series of samples comprising varying glycoforms of an antibody, (ii) determining the TAF glycan content for each sample of the series, (iii) creating a model which correlates the ADCC activity level to the TAF glycan content, (iv) determining the ADCC activity level for an antibody composition and then calculating a TAF glycan content using the model or determining the TAF glycan content for the antibody composition and calculating the ADCC activity level using the model, and (v) selecting the antibody composition for one or more downstream processing steps when the TAF glycan content calculated in step (iv) is within a target range of TAF glycan content or when the ADCC activity level calculated in step (iv) is within a target range of ADCC activity level.

[0027] A method of producing an antibody composition within a target % TAF range is provided wherein said method comprises: (i) measuring the % ADCC of a series of samples comprising varying glycoforms of an antibody, (ii) determining the % total afucosylated (TAF) glycans for each sample of the series, (iii) determining a linear equation of a best fit line of a graph which plots for each sample of the series the % ADCC as measured in step (i) as a function of the % TAF glycans as determined in step (ii), (iv) determining a linear equation of a best fit line of a graph which plots for each sample of the series the % ADCC as measured in step (i) as a function of the % TAF glycans as determined in step (ii), (v)

determining the % ADCC for an antibody composition and then calculating a % TAF using the linear equation of step (iii), and (iv) selecting the antibody composition for one or more downstream processing steps when the % TAF calculated in step (iv) is within a target % TAF range. Also provided is a method of producing an antibody composition within a target % TAF range wherein the method comprises the following steps: (i) generating a linear equation of a best fit graph by plotting the % ADCC and %TAF glycans of a series of at least 5 reference antibody compositions produced under cell culture conditions, each reference antibody composition having the same amino acid sequence as the antibody composition, (ii) selecting a target %TAF glycan range based on the linear equation generated in step (i) and desired %ADCC activity; (iii) culturing the antibody composition under cell culture conditions; (iv) purifying the antibody composition, (v) sampling the antibody composition to determine the %TAF and (vi) determining whether the %TAF of the antibody composition is within the target %TAF range of step (ii). In exemplary ~~aspect~~examples, the method further comprises selecting the antibody composition for one or more downstream processing steps when the % TAF calculated in step (v) is within the target %TAF range.

[0028] Also provided is a method of determining % antibody dependent cellular cytotoxicity (ADCC) of an antibody composition, said method comprising: (i) determining the % total afucosylated (TAF) glycans of an antibody composition; and (ii) calculating the % ADCC of the antibody composition based on the % TAF using Equation A

$$Y = 2.6 + 24.1 * X$$

[Equation A],

wherein **Y** is the % ADCC and **X** is the % TAF glycans determined in step (i),

[0029] Additionally, a method of determining % antibody dependent cellular cytotoxicity (ADCC) of an antibody composition, is provided, said method comprising (i) determining the % high mannose glycans and the % afucosylated glycans of an antibody composition, and (ii) calculating the % ADCC of the antibody composition based on the % high mannose glycans and the % afucosylated glycans using Equation B:

$$Y = (0.24 + 27 * HM + 22.1 * AF)$$

[Equation B],

wherein **Y** is the % ADCC, **HM** is the % high mannose glycans determined in step (i), and **AF** is the % afucosylated glycans determined in step (i).