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(54) Title: METHODS RELATED TO TRASTUZUMAB

FIG. 1

Trastuzumab HC sequence (SEQ ID NO:1):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSV
KGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK

(57) Abstract: The present invention relates to the characterization and production of trastuzumab.

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METHODS RELATED TO TRASTUZUMAB

This disclosure provides compositions and methods related to trastuzumab.

This application claims the benefit of U.S. Provisional Application No. 61/654539, filed June 1, 2012; and U.S. Provisional Application 61/783042, filed March 14, 2013.

BACKGROUND OF THE INVENTION

Trastuzumab (HERCEPTIN®) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd = 5nM) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2 (also known as neu). The antibody is an IgG_1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

Trastuzumab is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing gentamicin. Gentamicin is not detectable in the final product. HERCEPTIN® is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. The nominal content of each HERCEPTIN® vial is 440 mg Trastuzumab, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, 400 mg α,α-trehalose dihydrate, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.1% benzyl alcohol as a preservative, yields a multi-dose solution containing 2 1 mg/mL Trastuzumab, at a pH of approximately 6.

Trastuzumab is a HER2/neu receptor antagonist presently indicated for the treatment of HER2 overexpressing breast cancer and for the treatment of HER2-overexpressing metastatic gastric or gastroeophageal junction adenocarcinoma. (*See* HERCEPTIN® Product Label dated October 29, 2010, Genentech, Inc.)

SUMMARY OF THE INVENTION

The present disclosure provides, in part, methods for evaluating, identifying, and/or producing (e.g., manufacturing) trastuzumab. In some instances, methods herein allow highly resolved evaluation of trastuzumab useful for, *inter alia*, manufacturing trastuzumab, characterizing trastuzumab, identifying and/or confirming trastuzumab, monitoring the structure of trastuzumab, comparing trastuzumab preparations made over time or made under different conditions, and/or controlling the structure of trastuzumab.

In certain aspects, the disclosure provides methods of evaluating a glycoprotein preparation (e.g., such as a glycoprotein drug substance or drug product preparation). Such methods can include evaluating the glycoprotein preparation for the presence, absence, level and/or ratio of one or more (e.g., two or more when working with ratios) trastuzumab-specific parameters (i.e., acquiring information (e.g., value(s)) pertaining to the trastuzumab-specific parameters). Such methods can also optionally include providing, e.g., acquiring, a determination of whether the presence, absence, level and/or ratio of one or more trastuzumab-specific parameters evaluated meets a reference criteria for the one or more trastuzumab-specific parameters, which determination includes, for example, comparing the presence, absence, level and/or ratio of one or more trastuzumab -specific parameters evaluated with the reference criteria and/or confirming that the presence, absence, level or ratio of one or more trastuzumab -specific parameters evaluated has a defined (e.g., predefined) relationship with the reference criteria. In some instances, the one or more (e.g., two or more when working with ratios) trastuzumab -specific parameters evaluated include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22) parameters disclosed in Table 1.

In certain other aspects, the disclosure provides methods of manufacturing trastuzumab drug product, such methods include a first step of providing (e.g., producing or expressing (e.g., in small scale or large scale cell culture) or manufacturing) or obtaining (e.g., receiving and/or purchasing from a third party (including a contractually related third party or a non-contractually-related (e.g., an independent) third party) a test glycoprotein preparation (e.g., a sample of a test glycoprotein preparation), a second step of acquiring (e.g., detecting, measuring, receiving, or obtaining, as discussed subsequently herein) at least one value (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22) for an trastuzumab parameter listed in

Table 1 for the test glycoprotein preparation, and a third step of processing at least a portion of the test glycoprotein preparation (e.g., processing a portion of a manufacturing lot, batch, or run, an entire manufacturing lot, batch, or run, or multiple manufacturing lots, batches, or runs) as trastuzumab drug product (e.g., in a form or packaging intended for marketing or administration as described subsequently herein) if the at least one value for the test glycoprotein preparation meets a reference criterion shown in Table 1 for the parameter, thereby manufacturing trastuzumab drug product. In some instances, the second step of such methods includes acquiring values for any combination of two or more trastuzumab parameters listed in Table 1, and the third step of such methods includes processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the values for the any combination of two or more trastuzumab parameters for the test glycoprotein preparation meet the corresponding reference criterion shown in Table 1 for the parameters. In some instances, the any combination of two or more trastuzumab parameters can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of the trastuzumab parameters listed in Table 1 and/or any two or more of parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1. In some instances, the second step of such methods includes acquiring a value for a plurality of trastuzumab parameters listed in Table 1, and the third step of such methods includes processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the value for the plurality for the test glycoprotein preparation meets the corresponding reference criterion shown in Table 1 for the parameters. In some instances, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of the trastuzumab parameters listed in Table 1 and/or parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1. In some instances, the second step of such methods includes acquiring a value for at least one value of trastuzumab parameters listed in Table 1, and the third step of such methods includes processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if at least one of the at least one value for the plurality for the test glycoprotein preparation meets the corresponding reference criterion shown in Table 1 for the parameter.

In some instances, the test glycoprotein preparation obtained or produced in the first step of such methods includes a recombinant antibody composition having a first amino acid sequence with at

least 85% identity to SEQ ID NO:1 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:1) and a second amino acid sequence with at least 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2). In some instances, the recombinant antibody composition includes a first amino acid sequence with 100% identity to SEQ ID NO:1 and a second amino acid sequence with 100% identity to SEQ ID NO:2. In either instance, the first and second amino acid sequence combine when expressed to form the recombinant antibody in which the first sequence is the antibody heavy chain and the second sequence is the antibody light chain.

In some instances, evaluation methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more trastuzumab-specific parameters and, optionally, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature.

In some instances, evaluation methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more of the trastuzumab parameters disclosed in Table 1, and, optionally, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature. For example, for a given glycoprotein preparation, methods can include: evaluating HM7 and obtaining a value therefor, and, optionally, determining whether the value conforms to the reference criterion for HM7 provided in Table 1, wherein, in this example, the reference criterion for HM7 is a trastuzumab signature. In this instance, the value for HM7 would conform to the trastuzumab signature if it is less than 0.05%.

In another aspect, the disclosure provides methods of identifying a test glycoprotein preparation (e.g., such as a glycoprotein drug substance or drug product preparation) as trastuzumab. In some instances, identification methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more trastuzumab-specific parameters, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming

that the information has a defined (e.g., predefined) relationship with the trastuzumab signature, and identifying the glycoprotein preparation as trastuzumab if the information meets the trastuzumab signature.

In some instances, identification methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more of the 'trastuzumab parameters' disclosed in Table 1, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature, and identifying the glycoprotein preparation as trastuzumab if the acquired information meets the trastuzumab signature. For example, for a given glycoprotein preparation, methods can include: evaluating HM7 and obtaining a value therefor, determining whether the value conforms to the reference criterion for HM7 provided in Table 1, and identifying the glycoprotein preparation as trastuzumab if the information conforms, wherein, in this example, the reference criterion for HM7 is a trastuzumab signature. In this instance, the value for HM7 would conform to the trastuzumab signature if it is less than 0.05%.

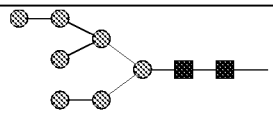
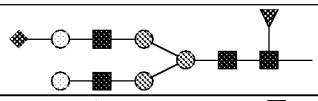
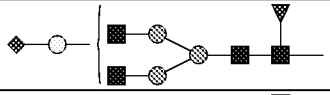
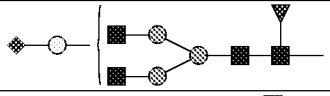
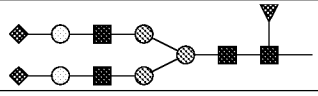
In a further aspect, the disclosure provides methods of producing (e.g., manufacturing) trastuzumab (e.g., trastuzumab drug product). In some instances, production methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more trastuzumab-specific parameters, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature, and processing the glycoprotein preparation (e.g., as trastuzumab drug product) if the information meets the trastuzumab signature, thereby producing trastuzumab (e.g., trastuzumab drug product).

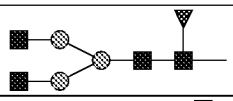
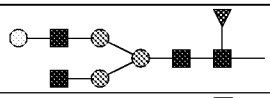
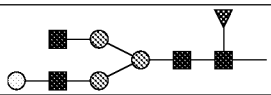
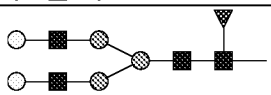
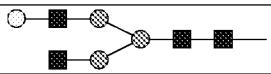
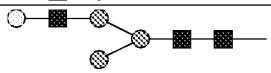
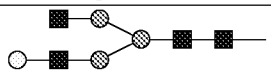
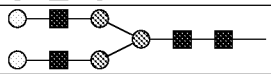
In some instances, production methods include, for a glycoprotein preparation, evaluating information (e.g., value(s)) pertaining to one or more trastuzumab parameters disclosed in Table 1, providing, e.g., acquiring, a determination of whether the information meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature,

and processing the glycoprotein preparation (e.g., as trastuzumab drug product) if the information meets the trastuzumab signature, thereby producing trastuzumab (e.g., trastuzumab drug product). For example, for a given glycoprotein preparation, production methods can include: evaluating a value for HM7 for the glycoprotein preparation, comparing the value with the reference criterion for HM7 provided in Table 1, determining whether the value obtained meets with the reference value for HM7, and processing the glycoprotein preparation as trastuzumab drug product if the value obtained meets the reference criterion for HM7, wherein, in this example, the reference criterion for HM7 is a trastuzumab signature. In this instance, the value for HM7 would conform to the reference criterion for HM7 if it is less than 0.05%. In some instances, these methods can further include packaging, labeling, and/or shipping the trastuzumab drug product, e.g., as discussed in further detail herein.

As used herein, a *trastuzumab signature* comprises a plurality of reference criteria or rules for a plurality of parameters that define trastuzumab. In some instances, a trastuzumab signature can be a pharmaceutical specification, a commercial product release specification, a product acceptance criterion, a pharmacopeial standard, or a product labeling description. In some instances, the trastuzumab signature comprises a plurality of reference criteria or rules for a plurality of parameters shown in Table 1:

Table 1: Reference Criteria for Trastuzumab Parameters

Parameter #	Parameter Category	Parameter Mannose Fucose GlcNAc Galactose Sialic Acid	Reference Criterion (rule)
1	HM7		<0.05%*
2	Sialylated		>0.80%*
3	Sialylated		>0.20%*&
4	Sialylated		>0.10%*&
5	Sialylated		>0.10%*

6	Complex G0F		<55.00%*
7	Complex G1F		>25.00%*
8	Complex G1F		>8.00%*
9	Complex G2F		>3.50%*
10	Complex G1		>1.0%*
11	Complex		>0.15%*
12	Complex G1		>0.70%*
13	Complex G2		>0.03%*
14	C-terminal-lysine	Amount of lysine present at the C-terminus of the heavy chain	<5.00%$
15	HC-pyroglu	Pyroglutamate (pyroglu) at the N-terminus of the heavy chain	<10.00%#
16	LC-pyroglu	Pyroglutamate at the N-terminus of the light chain	<3.00%#
17	HC-M256-Sulfo	Post-translational modification of the M256 residue (Kabat et al. numbering) of the heavy chain – residue is oxidized to form methionine sulfoxide	<4.00%#
18	LC135	Amount of free cysteine (*e.g.* not paired in disulfides) at cysteine 135 in the light chain	>4.00%^
19	HC148	Amount of free cysteine (*e.g.* not paired in disulfides) at cysteine 148 in the heavy chain	>25.00%^
20	HC204	Amount of free cysteine (*e.g.* not paired in disulfides) at cysteine 204 in the heavy chain	>10.00%^
21	HC371	Amount of free cysteine (*i.e.* not paired in disulfides) at cysteine 371 in the heavy chain	>40.00%^
22	HC429	Amount of free cysteine (*i.e.* not paired in disulfides) at cysteine 429 in the heavy chain	>20.00%^

*For any given parameter, percent refers to the number of moles of PNGase F-released glycan X relative to total moles of PNGase F-released glycan detected as disclosed in Table 2, wherein X represents the parameter of interest (e.g., parameter(s) 1-13).

For any given parameter, percent refers to the level of modified peptide Y relative to the sum of the levels of modified peptide Y and unmodified peptide Y, detected as disclosed in Table 2, wherein Y represents the parameter of interest (e.g., parameter(s) 15-17).

&For related parameters with the same listed structure, the two isomers are assigned in order of their retention time from a reverse-phase C18 column.

$For C-terminal-lysine, percent refers to the level of C-terminal-lysine-containing peptide relative to the sum of the levels of C-terminal-lysine-containing and C-terminal-lysine-free peptides detected as disclosed in Table 2.

^For free cysteine, percent refers to the level of non-disulfide-linked peptide relative to the sum of the levels of non-disulfide-linked and disulfide-linked peptides, detected as disclosed in Table 2.

While the present disclosure provides exemplary units and methods for the evaluation, identification, and production methods disclosed herein (*see*, e.g., Tables 1 and 2), a person of ordinary skill in the art will appreciate that performance of the evaluation, identification, and production methods herein is not limited to use of those units and/or methods. For example, trastuzumab signatures described herein are generally described, for each parameter, as a value for a glycan or structure relative to total glycan on a mass/mass basis (*see*, e.g., Table 1). A person of skill in the art understands that although the use of other metrics or units (e.g., mole percent vs. weight percent) to measure a described parameter might give rise to different absolute values than those described herein, e.g., in Table 1, a test glycoprotein preparation meets a disclosed trastuzumab reference criterion or signature even if other units or metrics are used, as long as the test glycoprotein preparation meets the herein disclosed reference criterion or signature when the herein disclosed units and metrics are used, e.g., allowing for the sensitivity (e.g., analytical variability) of the method being used to measure the value.

Trastuzumab parameters shown in Table 1 are parameters that, alone, in any combination, or together, distinguish trastuzumab from non-trastuzumab glycoprotein (*see* below). In some instances, a trastuzumab parameter is part of the glycoprotein, e.g., connected with the rest of the glycoprotein by a covalent bond, i.e., an *intrinsic parameter*. *Intrinsic parameters* include the presence, absence, level, ratio (with another entity), or distribution of a physical moiety, e.g., a moiety arising from or associated with a post-translational event. Exemplary parameters include the presence (or absence), abundance, absolute or relative amount, ratio (with another entity), or distribution of a glycan, a linkage, a glycoform, or post-translationally added components of the preparation. In some instances, a parameter is not part of the glycoprotein but is present in the preparation with the glycoprotein (i.e., in a glycoprotein preparation), i.e., an *extrinsic, parameter*. Exemplary parameters of this type include the presence (or absence), abundance, ratio (with another entity), or distribution of, e.g., impurities, e.g., host cell proteins, residue from purification processes, viral impurities, and enclosure components.

In some instances, a trastuzumab signature comprises reference criteria or rules for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or substantially all, parameters shown in Table 1. In some instances, a trastuzumab signature comprises reference criteria or rules for two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22) of trastuzumab parameter(s) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22. In some instances, a trastuzumab signature comprises predetermined reference criteria or rules for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 parameters shown in Table 1. In some instances, a trastuzumab signature comprises reference criteria or rules for one or more, including any combination or all, of parameter number(s) 2, 7, 9, 21, and/or 22.

In some instances, methods (i.e., evaluation, identification, and production methods) can further include, e.g., one or more of: providing or obtaining a glycoprotein preparation (e.g., such as a glycoprotein drug substance or a precursor thereof); memorializing confirmation or identification of the glycoprotein preparation as trastuzumab using a recordable medium (e.g., on paper or in a computer readable medium, e.g., in a Certificate of Testing, Certificate of Analysis, Material Safety Data Sheet (MSDS), batch record, or Certificate of Analysis (CofA)); informing a party or entity (e.g., a contractual or manufacturing partner, a care giver or other end-user, a regulatory entity, e.g., the FDA or other U.S., European, Japanese, Chinese or other governmental agency, or another entity, e.g., a compendial entity (e.g., U.S. Pharmacopoeia (USP)) or insurance company) that a glycoprotein preparation is trastuzumab; selecting the glycoprotein preparation for further processing (e.g., processing (e.g., formulating) the glycoprotein preparation as a drug product (e.g., a pharmaceutical product) if the glycoprotein preparation is identified as trastuzumab; reprocessing or disposing of the glycoprotein preparation if the glycoprotein preparation is not identified as trastuzumab.

In some instances, methods (i.e., evaluation, identification, and production methods) include taking action (e.g., physical action) in response to the methods disclosed herein. For example, the glycoprotein preparation is classified, selected, accepted or discarded, released or withheld, processed into a drug product, shipped, moved to a different location, formulated, labeled, packaged, released into commerce, or sold or offered for sale, depending on whether the preselected relationship is met.

In some instances, *processing* may include formulating, packaging (e.g., in a syringe or vial), labeling, or shipping at least a portion of the glycoprotein preparation. In some instances, processing includes formulating, packaging (e.g., in a syringe or vial), and labeling at least a portion of the glycoprotein as trastuzumab drug product. Processing can include directing and/or contracting another party to process as described herein.

Definitions

As used herein, a *glycoprotein* refers to amino acid sequences that include one or more oligosaccharide chains (e.g., glycans) covalently attached thereto. Exemplary amino acid sequences include peptides, polypeptides and proteins. Exemplary glycoproteins include glycosylated antibodies and antibody-like molecules (e.g., Fc fusion proteins). Exemplary antibodies include monoclonal antibodies and/or fragments thereof, polyclonal antibodies and/or fragments thereof, and Fc domain containing fusion proteins (e.g., fusion proteins containing the Fc region of IgG1, or a glycosylated portion thereof). A *glycoprotein preparation* is a composition or mixture that includes at least one glycoprotein.

A glycoprotein preparation (e.g., such as a glycoprotein drug substance or a precursor thereof) included herein is or includes a glycoprotein (e.g., an antibody) that has a first amino acid sequence with at least 85% identity to SEQ ID NO:1 and a second amino acid sequence with at least 85% identity to SEQ ID NO:2. In some instances, the first and/or second amino acid sequence(s) have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:1 and/or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:2.

In some instances, a glycoprotein preparation (e.g., such as a glycoprotein drug substance or a precursor thereof) can be a sample from a proposed or test batch of trastuzumab drug substance or drug product. As used herein, a *batch* of a glycoprotein preparation refers to a single production run of the glycoprotein. Evaluation of different batches thus means evaluation of different production runs or batches. As used herein *sample(s)* refer to separately procured samples. For example, evaluation of separate samples could mean evaluation of different commercially available containers or vials of the same batch or from different batches.

As used herein, *trastuzumab* is the generic, compendial, nonproprietary, or official FDA name for the product marketed as HERCEPTIN® by Genentech/Roche Group and a product that is interchangeable with or equivalent to the product marketed as HERCEPTIN®.

As used herein, *evaluating*, e.g., in the evaluation/evaluating, identifying, and/or producing aspects disclosed herein means reviewing, considering, determining, assessing, analyzing, measuring, and/or detecting the presence, absence, level, and/or ratio of one or more trastuzumab-specific parameters in a glycoprotein preparation to provide information pertaining to the one or more trastuzumab-specific parameters. In some instances, evaluating can include performing a process that involves a physical change in a sample or another substance, e.g., a starting material. Exemplary changes include making a physical entity from two or more starting materials, shearing or fragmenting a substance, separating or purifying a substance, combining two or more separate entities into a mixture, performing a chemical reaction that includes breaking or forming a covalent or non-covalent bond. *Evaluating* can include performing an analytical process which includes a physical change in a substance, e.g., a sample, analyte, or reagent (sometimes referred to herein as "physical analysis"), performing an analytical method, e.g., a method which includes one or more of the following: separating or purifying a substance, e.g., an analyte, or a fragment or other derivative thereof, from another substance; combining an analyte, or fragment or other derivative thereof, with another substance, e.g., a buffer, solvent, or reactant; or changing the structure of an analyte, or a fragment or other derivative thereof, e.g., by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the analyte; or by changing the structure of a reagent, or a fragment or other derivative thereof, e.g., by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the reagent. In some instances, evaluating a glycoprotein preparation includes detecting the presence, absence, level or ratio of one or more (e.g., two or more when working with ratios) disclosed in Table 1 using methods disclosed in Table 2.

Information (e.g., value(s)) pertaining to a *trastuzumab-specific parameter* or a *trastuzumab parameter* means information, regardless of form, that describes the presence, absence, abundance, absolute or relative amount, ratio (with another entity), or distribution of a moiety associated with the glycoprotein preparation and/or trastuzumab. Information is evaluated in a glycoprotein preparation as disclosed herein. Information is also conveyed in a trastuzumab

signature. Information can be qualitative, e.g., present, absent, intermediate, or quantitative, e.g., a numerical value such as a single number, or a range, for a parameter. In some instances, information is from a single sample or batch or a plurality of samples or batches. In some instances, information can be a range or average (or other measure of central tendency), e.g., based on the values from any X samples or batches, e.g., wherein at least of the samples or batches is being evaluated for commercial release, wherein X is equal to, at least, or no more than, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In some instances, information can be, for example: a statistical function, e.g., an average, of a number of values; a function of another value, e.g., of the presence, distribution or amount of a second entity present in the sample, e.g., an internal standard; a statistical function, e.g., an average, of a number of values; a function of another value, e.g., of the presence, distribution or amount of a second entity present in the sample, e.g., an internal standard; a value, e.g., a qualitative value, e.g., present, absent, "below limit of detection", "within normal limits" or intermediate. In some instances, information can be a quantitative value, e.g., a numerical value such as a single number, a range of values, a "no less than x amount" value, a "no more than x amount" value. In some instances, information can be abundance. Abundance can be expressed in relative terms, e.g., abundance can be expressed in terms of the abundance of a structure in relation to another component in the preparation. E.g., abundance can be expressed as: the abundance of a structure (or a first group of structures) in Table 1 relative to the amount of protein; the abundance of a structure (or a first group of structures) in Table 1 relative to the abundance of a second structure (or second group of structures) in Table 1. Abundance, e.g., abundance of a first structure relative to another structure, can be with regard to the preparation as a whole, a single molecule, or a selected site on the protein backbone. E.g., the parameter can be the relative proportion of a first structure from Table 1 and a second structure from Table 1 at a selected site and the value can be expressed as, e.g., a proportion, ratio or percentage. Information can be expressed in any useful term or unit, e.g., in terms of weight/weight, number/number, number/weight, and weight/number. In many cases, the reference criterion is defined by a range of values.

As used herein, *acquire* or *acquiring* (e.g., *acquiring information*) means obtaining possession of a physical entity, or a value, e.g., a numerical value, by "directly acquiring" or "indirectly acquiring" the physical entity or value. *Directly acquiring* means performing a process (e.g., performing an assay or test on a sample or "analyzing a sample" as that term is defined herein) to

obtain the physical entity or value. *Indirectly acquiring* refers to receiving the physical entity or value from another party or source (e.g., a third party laboratory that directly acquired the physical entity or value). *Directly acquiring* a physical entity includes performing a process, e.g., analyzing a sample, that includes a physical change in a physical substance, e.g., a starting material. Exemplary changes include making a physical entity from two or more starting materials, shearing or fragmenting a substance, separating or purifying a substance, combining two or more separate entities into a mixture, performing a chemical reaction that includes breaking or forming a covalent or non-covalent bond. *Directly acquiring* a value includes performing a process that includes a physical change in a sample or another substance, e.g., performing an analytical process which includes a physical change in a substance, e.g., a sample, analyte, or reagent (sometimes referred to herein as "physical analysis"), performing an analytical method, e.g., a method which includes one or more of the following: separating or purifying a substance, e.g., an analyte, or a fragment or other derivative thereof, from another substance; combining an analyte, or fragment or other derivative thereof, with another substance, e.g., a buffer, solvent, or reactant; or changing the structure of an analyte, or a fragment or other derivative thereof, e.g., by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the analyte; or by changing the structure of a reagent, or a fragment or other derivative thereof, e.g., by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the reagent. Exemplary analytical methods are shown in Table 2.

All literature and similar material cited in this application, including, but not limited to, patents, patent applications, articles, books, treatises, and web pages, regardless of the format of such literature and similar materials, are expressly incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described in any way.

These, and other aspects of the invention, are described in more detail below and in the claims.

DESCRIPTION OF THE DRAWINGS

FIG. 1 | Amino acid sequence of heavy chain of trastuzumab (SEQ ID NO: 1).

FIG. 2 | Amino acid sequence of light chain of trastuzumab (SEQ ID NO:2).

DETAILED DESCRIPTION

Detailed, high resolution, structural information about HERCEPTIN® (e.g., related to the presence of signature glycan species or quantitative analyses ascribing site-specificity for backbone modifications) is useful to be able to make and test products that qualify as trastuzumab, e.g., that are interchangeable versions of HERCEPTIN®. Such information is also useful in monitoring product changes and controlling structural drift that may occur as a result of manufacturing changes. The art supports, however, that information necessary to be able to make and test products that qualify as trastuzumab, e.g., that are interchangeable versions of HERCEPTIN®, or any other branded biologic, is unavailable (*see*, e.g., Nowicki, "Basic Facts about Biosimilars," Kidney Blood Press. Res., 30:267-272 (2007); Hincal "An Introduction To Safety Issues In Biosimilars/Follow-On Biopharmaceuticals", J. Med. CBR Def., 7:1-18, (2009); Roger, "Biosimilars: current status and future directions," Expert Opin. Biol. Ther., 10(7):1011-1018 (2010); Schellekens et al., Nat. Biotechnol. 28:28-31 (2010); Sekhon et al., Biosimilars, 1:1-11 (2011)). One exemplary report states that "[t]he size and complexity of … therapeutic proteins make the production of an exact replica almost impossible; therefore, there are no true generic forms of these proteins… Verification of the similarity of biosimilars to innovator medicines remains a key challenge" (Hincal, *supra*). This disclosure provides, in part, methods and compositions sufficient to make and test products that qualify as trastuzumab, e.g., that are interchangeable versions of HERCEPTIN®.

Glycoprotein preparations can be obtained from any source. In some instances, providing or obtaining a glycoprotein preparation (e.g., such as a glycoprotein drug substance or a precursor thereof), e.g., that is or includes a glycoprotein, can include providing a host cell, e.g., a mammalian host cell (e.g., a CHO cell) that is genetically engineered to express a glycoprotein having an amino acid sequence at least 85% identical to SEQ ID NO:1 and an amino acid sequence at least 85% identical to SEQ ID NO:2 (e.g., a genetically engineered cell); culturing the host cell under conditions suitable to express the glycoprotein (e.g., mRNA and/or protein); and, optionally, purifying the expressed glycoproteins, e.g., in the form of a recombinant

antibody) from the cultured cell, thereby producing a glycoprotein preparation. In some instances, the host cell is genetically engineered to express a glycoprotein having an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:1 and an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:2, wherein the expressed amino acid sequences form a recombinant antibody composition.

As used herein *percent (%) sequence identity* with respect to a sequence is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. (E.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In one embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, e.g., at least 40%, e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. In some instances a product will include amino acid variants, e.g., species that differ at terminal residues, e.g., at one or two terminal residues. In instances of such cases the sequence identity which is compared is the identity between the primary amino acid sequences of the most abundant active species in each of the products being compared. In some instances sequence identity refers to the amino acid sequence encoded by a nucleic acid that can be used to make the product.

In some instances, a trastuzumab signature disclosed herein can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of the trastuzumab parameters (e.g., the

reference criterion therefor) shown in Table 1 (e.g., including any combination of 2 or more (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22) of parameter numbers 1-22 shown in Table 1).

In some instances, a trastuzumab signature disclosed herein can include, other structures or characteristics (whether intrinsic or extrinsic) of trastuzumab, e.g., that distinguish trastuzumab from non- trastuzumab glycoprotein (see application entitled Methods of Evaluating and Making Biologics, filed on June 1, 2012, as USSN 61/654,467, for exemplary structures or characteristics). Examples of structures or characteristics include: the amount of GalNAc in the preparation (e.g., relative to total glycans of the preparation); the amount of truncated core glycans; the amount of aglycosylated glycans; the amount of each species of high mannose glycans; the amount of sialylated glycans or particular species of sialylated glycans; the ratio of monosialylated:diasylated glycans, the amount of diacetylated sialic acids (NeuXAc2), the amount of one or more of: NeuGc; NeuAc; Neu5,7,Ac2; Neu5Gc,9Ac; Neu5,8Ac2; Neu5,9Ac2; Neu4,5Ac2. Examples of parameters related to the glycan linkage composition of a glycoprotein preparation can be: the presence or amount of one or more of terminal fucose; terminal mannose; terminal galactose; 2 linked mannose; 3.6 linked mannose; terminal GlcNAc; terminal GalNAc; 4 linked GlcNAc; 4,6 linked GlcNAc. A parameter may also be the ratio of one of these to another or to another property. Examples of parameters related to the glycoform composition of a glycoprotein preparation include: the absence or presence of one or more specific glycoforms (e.g., one or more glycoforms described in Table 1); the amount or abundance of a specific glycoform in the preparation relative to total glycoforms (e.g., in a w/w basis); the ratio of one particular glycoform to another. Examples of parameters related to post-translational modification in the preparation include: the absence or presence of one or more specific post-translational modification; the abundance or distribution of one or more specific post-translational modification.

In some instances, the present disclosure includes determining whether information evaluated for a glycoprotein preparation meets a trastuzumab signature, e.g., by comparing the information with the trastuzumab signature and/or confirming that the information has a defined (e.g., predefined) relationship with the trastuzumab signature.

In some instances, methods disclosed herein can be used to confirm the identity and/or quality of trastuzumab preparations. For example, methods can include assessing preparations (e.g., samples, lots, and/or batches) of a test glycoprotein to confirm whether the test glycoprotein qualifies as trastuzumab, and, optionally, qualifying the test protein as trastuzumab if qualifying criteria (e.g. predefined qualifying criteria) are met; thereby evaluating, identifying, and/or producing (e.g., manufacturing) trastuzumab.

Methods of the disclosure have a variety of applications and include, e.g., quality control at different stages of manufacture, analysis of trastuzumab preparations prior to or after completion of manufacture (e.g., prior to or after distribution to a fill/finish environment or facility), prior to or after release into commerce (e.g., before distribution to a pharmacy, a caregiver, a patient, or other end-user). Thus, the preparation can be any preparation that potentially comprises trastuzumab. In an embodiment the trastuzumab preparation is a drug substance (an active pharmaceutical ingredient or "API") or a drug product (an API formulated for use in a subject such as a human patient). In an embodiment the preparation is from a stage of manufacture or use that is prior to release to care givers or other end-users; prior to packaging into individual dosage forms, such as syringes, pens, vials, or multi-dose vials; prior to determination that the batch can be commercially released, prior to production of a Certificate of Testing, Material Safety Data Sheet (MSDS) or Certificate of Analysis (CofA) of the preparation. In an embodiment the glycoprotein preparation from an intermediate step in production, e.g., it is after secretion of the glycoprotein from a cell but prior to purification of drug substance.

Evaluations from methods of the invention are useful for guiding, controlling or implementing a number of activities or steps in the process of making, distributing, and monitoring and providing for the safe and efficacious use of trastuzumab. Thus, in an embodiment, e.g., responsive to the evaluation, e.g., depending on whether a criterion is met, a decision or step is taken. The method can further comprise one or both of the decision to take the step and/or carrying out the step

itself. E.g., the step can comprise one in which the preparation (or another preparation for which the preparation is representative) is: classified; selected; accepted or discarded; released or processed into a drug product; rendered unusable for commercial release, e.g., by labeling it, sequestering it, or destroying it; passed on to a subsequent step in manufacture; reprocessed (e.g., the preparation may undergo a repetition of a previous process step or subjected to a corrective process); formulated, e.g., into drug substance or drug product; combined with another component, e.g., an excipient, buffer or diluent; disposed into a container; divided into smaller aliquots, e.g., unit doses, or multi-dose containers; combined with another preparation of trastuzumab; packaged; shipped; moved to a different location; combined with another element to form a kit; combined, e.g., placed into a package with a delivery device, diluent, or package insert; released into commerce; sold or offered for sale; delivered to a care giver or other end-user; or administered to a subject. E.g., based on the result of the determination or whether one or more subject entities is present, or upon comparison to a reference standard, the batch from which the preparation is taken can be processed, e.g., as just described.

Methods described herein may include making a decision: (a) as to whether a preparation may be formulated into drug substance or drug product; (b) as to whether a preparation may be reprocessed (e.g., the preparation may undergo a repetition of a previous process step); or (c) that the preparation is not suitable for formulation into drug substance or drug product. In instances the method comprises: formulating as referred to in step (a), reprocessing as referred to in step (b), or rendering the preparation unusable for commercial release, e.g., by labeling it or destroying it, as referred to in step (c).

Parameter Evaluation

The amino acid sequence of the heavy chain of trastuzumab (HERCEPTIN®) is disclosed herein as SEQ ID NO:1. The amino acid sequence of the light chain of trastuzumab (HERCEPTIN®) is disclosed herein as SEQ ID NO:2.

Parameters disclosed herein can be analyzed by any available suitable method. In some instances, glycan structure and composition as described herein are analyzed, for example, by one or more, enzymatic, chromatographic, mass spectrometry (MS), chromatographic followed by MS, electrophoretic methods, electrophoretic methods followed by MS, nuclear magnetic

resonance (NMR) methods, and combinations thereof. Exemplary enzymatic methods include contacting a glycoprotein preparation with one or more enzymes under conditions and for a time sufficient to release one or more glycans (e.g., one or more exposed glycans). In some instances, the one or more enzymes includes PNGase F. Exemplary chromatographic methods include, but are not limited to, Strong Anion Exchange chromatography using Pulsed Amperometric Detection (SAX-PAD), liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), thin layer chromatography (TLC), amide column chromatography, and combinations thereof. Exemplary mass spectrometry (MS) include, but are not limited to, tandem MS, LC-MS, LC-MS/MS, matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS), Fourier transform mass spectrometry (FTMS), ion mobility separation with mass spectrometry (IMS-MS), electron transfer dissociation (ETD-MS), and combinations thereof. Exemplary electrophoretic methods include, but are not limited to, capillary electrophoresis (CE), CE-MS, gel electrophoresis, agarose gel electrophoresis, acrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using antibodies that recognize specific glycan structures, and combinations thereof. Exemplary nuclear magnetic resonance (NMR) include, but are not limited to, one-dimensional NMR (1D-NMR), two-dimensional NMR (2D-NMR), correlation spectroscopy magnetic-angle spinning NMR (COSY-NMR), total correlated spectroscopy NMR (TOCSY-NMR), heteronuclear single-quantum coherence NMR (HSQC-NMR), heteronuclear multiple quantum coherence (HMQC-NMR), rotational nuclear overhauser effect spectroscopy NMR (ROESY-NMR), nuclear overhauser effect spectroscopy (NOESY-NMR), and combinations thereof.

In some instances, techniques described herein may be combined with one or more other technologies for the detection, analysis, and or isolation of glycans or glycoproteins. For example, in certain instances, glycans are analyzed in accordance with the present disclosure using one or more available methods (to give but a few examples, see Anumula, *Anal. Biochem.* 350(1):1, 2006; Klein et al., *Anal. Biochem.,* 179:162, 1989; and/or Townsend, R.R. Carbohydrate Analysis" High Performance Liquid Chromatography and Capillary Electrophoresis., Ed. Z. El Rassi, pp 181-209, 1995, each of which is incorporated herein by reference in its entirety). For example, in some instances, glycans are characterized using one or

more of chromatographic methods, electrophoretic methods, nuclear magnetic resonance methods, and combinations thereof.

In some instances, methods for evaluating one or more trastuzumab-specific parameters, e.g., in a glycoprotein preparation, e.g., one or more of trastuzumab parameters disclosed in Table 1 in a glycoprotein preparation are known in the art and/or are disclosed in Table 2:

Table 2

Method(s)	Relevant literature	Parameter
C18 UPLC Mass Spec.*	Chen and Flynn, Anal. Biochem., 370:147-161 (2007) Chen and Flynn, J. Am. Soc. Mass Spectrom., 20:1821-1833 (2009)	Glycan(s) (e.g., N-linked glycan, exposed N-linked glycan, glycan detection, glycan identification, and characterization; site specific glycation; glycoform detection (e.g., parameters 1-13); percent glycosylation; and/or aglycoosyl)
Peptide LC-MS (reducing/non-reducing)	Dick et al., Biotechnol. Bioeng., 100:1132-1143 (2008) Yan et al., J. Chrom. A., 1164:153-161 (2007) Chelius et al., Anal. Chem., 78:2370-2376 (2006) Miller et al., J. Pharm. Sci., 100:2543-2550 (2011)	C-terminal lysine (e.g., parameter 14)
LC-MS (reducing/non-reducing/alkylated)	Dick et al., Biotechnol. Bioeng., 100:1132-1143 (2008) Goetze et al., Glycobiol., 21:949-959 (2011)	
Weak cation exchange (WCX) chromatography	Dick et al., Biotechnol. Bioeng., 100:1132-1143 (2008)	
LC-MS (reducing/non-reducing/alkylated)	Dick et al., Biotechnol. Bioeng., 100:1132-1143 (2008) Goetze et al., Glycobiol., 21:949-959 (2011)	N-terminal pyroglu (e.g., parameters 15-16)
PeptideLC-MS (reducing/non-reducing)	Yan et al., J. Chrom. A., 1164:153-161 (2007) Chelius et al., Anal. Chem.,	

	78:2370-2376 (2006) Miller et al., J. Pharm. Sci., 100:2543-2550 (2011)	
Peptide LC-MS (reducing/non-reducing)	Yan et al., J. Chrom. A., 1164:153-161 (2007); Xie et al., mAbs, 2:379-394 (2010)	Methionine oxidation (e.g., parameter 17)
Peptide LC-MS (reducing/non-reducing)	Wang et al., Anal. Chem., 83:3133-3140 (2011); Chumsae et al., Anal. Chem., 81:6449-6457 (2009)	Free cysteine (e.g., parameters 18-22)

Literature shown in Table 2 are hereby incorporated by reference in their entirety or, in the alternative, to the extent that they pertain to one or more of the methods disclosed in Table 2.

EXAMPLES

Example 1: Characterization of Trastuzumab

HERCEPTIN® sample was analyzed to determine the amino acid sequences of the heavy and light chains of the trastuzumab antibody. The sequence of the heavy chain is shown as SEQ ID NO:1 and the sequence of the light chain is shown as SEQ ID NO:2.

Characterization of HERCEPTIN® was performed by orthogonal methods. 3 lots Measurements made included use of glycan profiling, glycoform analysis, post-translational modification analysis, and analysis of other intrinsic and extrinsic structures or features. Of 113 HERCEPTIN®/trastuzumab structures or features that were measured or determined, 22 were determined to be trastuzumab parameters, i.e., parameters of trastuzumab that distinguish trastuzumab from non-trastuzumab antibody products. These 22 trastuzumab parameters and values are listed in Table 3 for an illustrative sample of trastuzumab.

Table 3: Acquired Values for Each Parameter

Parameter #	Parameter Category[1]	Value[2]
1	HM7	0.01
2	Sialylated	0.93
3	Sialylated	0.27
4	Sialylated	0.19
5	Sialylated	0.27
6	Complex G0F	36.06
7	Complex G1F	32.82

8	Complex G1F	8.96
9	Complex G2F	8.51
10	Complex G1	1.7
11	Complex	0.18
12	Complex G1	0.85
13	Complex G2	0.27
14	C-terminal-lysine	1.8
15	HC-pyroglu	2.3
16	LC-pyroglu	0
17	HC-M256-Sulfo	2.4
18	LC135	5.4
19	HC148	32.7
20	HC204	12.5
21	HC371	46.7
22	HC429	24.9

[1]Detailed descriptions of the structures/features of each parameter are provided in Table 1.
[2]See Table 1 for unit information.

The information (values) shown for each trastuzumab parameter in Table 3 were used to formulate a reference criterion or rule for each trastuzumab parameter (shown in Table 1).

Example 2: Qualification of Glycoprotein Preparations

The reference criterion or rules described in Table 1 were used to determine whether samples qualify as trastuzumab.

Sample A was analyzed and values were obtained for each of the trastuzumab parameters in Table 1. The values of these parameters in sample A are presented in Table 4 below. In

addition, values obtained for sample A were compared to the reference criteria for trastuzumab as shown in Table 4:

Table 4: Acquired Values of Sample A Compared with Reference Values

Parameter #	Parameter Category[1]	Sample A Value	Reference Criterion[2]	Comparison of "A" Values and reference criterion
1	HM7	1.18	<0.05%	
2	Sialylated	0.35	>0.80%	
3	Sialylated	0.04	>0.20%	
4	Sialylated	0.05	>0.10%	
5	Sialylated	0.01	>0.10%	
6	Complex G0F	45.64	<40.00%	
7	Complex G1F	22.83	>25.00%	
8	Complex G1F	5.9	>8.00%	
9	Complex G2F	3.47	>7.50%	
10	Complex G1	0.84	>1.0%	
11	Complex	0.15	>0.15%	
12	Complex G1	0.38	>0.70%	
13	Complex G2	0.07	>0.10%	
14	C-terminal-lysine	45.20	<5.00%	
15	HC-pyroglu	100.00	<10.00%	
16	LC-pyroglu	70.00	<3.00%	
17	HC-M256-Sulfo	5.50	<4.00%	

18	LC135	2.00	>4.00%	
19	HC148	13.40	>25.00%	
20	HC204	4.10	>10.00%	
21	HC371	28.60	>40.00%	
22	HC429	19.40	>20.00%	

[1]Detailed descriptions of the structures/features of each parameter are provided in Table 1.
[2]See Table 1 for unit information.
✓ Illustrates that a value meets the reference criterion/rule.

Data plotted in Table 4 confirms that sample A is not trastuzumab, according to the methods herein. Sample A does not meet any of the reference criteria for trastuzumab. Based on these data, sample A does not meet a trastuzumab signature that comprises all 22 parameters and, thus, does not qualify as trastuzumab.

A control HERCEPTIN® sample was also analyzed and values were obtained for each of the trastuzumab parameters in Table 1. The values of these parameters in the control are presented in Table 5 below. In addition, values obtained for the control were compared to the reference criteria for trastuzumab as shown in Table 5:

Table 5:

Parameter #	**Parameter Category[1]**	**Control HERCEPTIN sample value**	**Reference Criterion[2]**	**Comparison of "A" Values and reference criterion**
1	HM7	0.01	<0.05%	✓
2	Sialylated	0.93	>0.80%	✓
3	Sialylated	0.27	>0.20%	✓
4	Sialylated	0.19	>0.10%	✓
5	Sialylated	0.27	>0.10%	✓
6	Complex G0F	36.06	<40.00%	✓
7	Complex G1F	32.82	>25.00%	✓

8	Complex G1F	8.96	>8.00%	✓
9	Complex G2F	8.51	>7.50%	✓
10	Complex G1	1.7	>1.0%	✓
11	Complex	0.18	>0.15%	✓
12	Complex G1	0.85	>0.70%	✓
13	Complex G2	0.27	>0.10%	✓
14	C-terminal-lysine	1.8	<5.00%	✓
15	HC-pyroglu	2.3	<10.00%	✓
16	LC-pyroglu	0	<3.00%	✓
17	HC-M256-Sulfo	2.4	<4.00%	✓
18	LC135	5.4	>4.00%	✓
19	HC148	32.7	>25.00%	✓
20	HC204	12.5	>10.00%	✓
21	HC371	46.7	>40.00%	✓
22	HC429	24.9	>20.00%	✓

[1]Detailed descriptions of the structures/features of each parameter are provided in Table 1.

[2]See Table 1 for unit information.

✓ Illustrates that a value meets the reference criterion/rule.

As shown in Table 5, the control HERCEPTIN® sample meets all listed reference criteria signatures for trastuzumab. Accordingly, the control HERCEPTIN® sample does meet a trastuzumab signature that includes all 22 parameters and, thus, qualifies as trastuzumab.

While the methods have been described in conjunction with various instances and examples, it is not intended that the methods be limited to such instances or examples. On the contrary, the

methods encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

WHAT IS CLAIMED IS:

1. A method of manufacturing an trastuzumab drug product, comprising:

providing or obtaining a test glycoprotein preparation;

acquiring at least one value for an trastuzumab parameter listed in Table 1 for the test glycoprotein preparation; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the at least one value for the test glycoprotein preparation meets a reference criterion shown in Table 1 for the parameter,

thereby manufacturing an trastuzumab drug product.

2. The method of claim 1, comprising:

acquiring values for any combination of two or more trastuzumab parameters listed in Table 1; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the values for the any combination of two or more trastuzumab parameters for the test glycoprotein preparation meet the corresponding reference criterion shown in Table 1 for the parameters.

3. The method of claim 2, wherein the any combination of two or more trastuzumab parameters comprises:

2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 of the trastuzumab parameters listed in Table 1; or

any two or more of parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1.

4. The method of claim 1, comprising:

acquiring a value for a plurality of trastuzumab parameters listed in Table 1; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the value for the plurality for the test glycoprotein preparation meets the corresponding reference criterion shown in Table 1 for the parameters.

5. The method of claim 4, wherein the plurality comprises:

2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 of the trastuzumab parameters listed in Table 1; and/or

parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1.

6. The method of claim 1, wherein the test glycoprotein preparation comprises a recombinant antibody composition having a first amino acid sequence with at least 85% identity to SEQ ID NO:1 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:1) and a second amino acid sequence with at least 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2).

7. The method of claim 6, wherein the test glycoprotein preparation comprises a recombinant antibody composition having a first amino acid sequence with 100% identity to SEQ ID NO:1 and a second amino acid sequence with 100% identity to SEQ ID NO:2.

8. The methods of any one of claims 6 or 7, wherein the first and second amino acid sequences form a recombinant antibody.

9. A method of manufacturing an trastuzumab drug product, comprising:

providing or obtaining a test glycoprotein preparation;

acquiring a value for each parameter listed in Table 1 for the test glycoprotein preparation; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the value for each parameter listed in Table 1 for the test glycoprotein preparation meets the reference criterion shown in Table 1,

thereby manufacturing an trastuzumab drug product.

10. The method of claim 9, wherein the test glycoprotein preparation comprises a recombinant antibody composition having a first amino acid sequence with at least 85% identity

to SEQ ID NO:1 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:1) and a second amino acid sequence with at least 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2).

11. The method of claim 8, wherein the test glycoprotein preparation comprises a recombinant antibody composition having a first amino acid sequence with 100% identity to SEQ ID NO:1 and a second amino acid sequence with 100% identity to SEQ ID NO:2.

12. The methods of any one of claims 10 or 11, wherein the first and second amino acid sequences form a recombinant antibody.

13. A method of manufacturing an trastuzumab drug product, comprising:

providing a host cell that is genetically engineered to express a first amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:1 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:1) and a second amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2), wherein the expressed amino acid sequences form a recombinant antibody composition,

culturing the host cell under conditions whereby the cell expresses the first and second amino acid sequences, wherein the expressed first and second amino acid sequences form recombinant antibodies,

harvesting the recombinant antibodies from the host cell culture to produce an antibody preparation,

acquiring a value for each parameter listed in Table 1 for the antibody preparation; and

processing at least a portion of the antibody preparation into trastuzumab drug product if the value for each parameter listed in Table 1 for the antibody preparation meets the reference criterion shown in Table 1,

thereby manufacturing an trastuzumab drug product.

14. A method of manufacturing an trastuzumab drug product, comprising:

providing a host cell that is genetically engineered to express a first amino acid sequence having the sequence of SEQ ID NO:1 and a second amino acid sequence having the sequence of

SEQ ID NO:2, wherein the expressed amino acid sequences form a recombinant antibody composition,

culturing the host cell under conditions whereby the cell expresses the first and second amino acid sequences, wherein the expressed first and second amino acid sequences form recombinant antibodies,

harvesting the recombinant antibodies from the host cell culture to produce an antibody preparation,

acquiring at least one value for an trastuzumab parameter listed in Table 1 for the antibody preparation; and

processing or directing the processing of at least a portion of the antibody preparation as trastuzumab drug product if the at least one value for the antibody preparation meets a reference criterion shown in Table 1,

thereby manufacturing an trastuzumab drug product.

15. The method of claim 14, comprising:

acquiring values for any combination of two or more trastuzumab parameters listed in Table 1; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the values for the any combination of two or more trastuzumab parameters for the test glycoprotein preparation meet the corresponding reference criterion shown in Table 1 for the parameters.

16. The method of claim 15, wherein the any combination of two or more trastuzumab parameters comprises:

2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 of the trastuzumab parameters listed in Table 1; or

any two or more of parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1.

17. The method of claim 14, comprising:

acquiring a value for a plurality of trastuzumab parameters listed in Table 1; and

processing at least a portion of the test glycoprotein preparation as trastuzumab drug product if the value for the plurality for the test glycoprotein preparation meets the corresponding reference criterion shown in Table 1 for the parameters.

18. The method of claim 17, wherein the plurality comprises: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 of the trastuzumab parameters listed in Table 1; and/or

any two or more of parameter numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and/or 22 shown in Table 1.

19. The method of any of claims 1, 2, 4, 8, 11, 12, 13, or 15, further comprising:

after the step of acquiring the value(s) and before the step of processing, obtaining a plurality of assessments made by comparing the value(s) with a corresponding reference criterion shown in Table 1.

20. The any one of claims 1-19, wherein at least one value is directly obtained by performing an analytical test on the test antibody or glycoprotein preparation.

21. The method of claim 20, wherein the value is directly obtained using a method provided in Table 2.

22. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein the processing step comprises combining the test antibody preparation with an excipient or buffer.

23. The method of any of claims 1, 2, 4, 9, 13, 14, 15, 17 or 22, wherein the processing step comprises one or more of: formulating the test protein preparation; processing the test protein preparation into a drug product; combining the test protein preparation with a second component, e.g., an excipient or buffer; changing the concentration of the test protein in the preparation; lyophilizing the test protein preparation; combining a first and second aliquot of the test protein to provide a third, larger, aliquot; dividing the test protein preparation into smaller aliquots; disposing the test protein preparation into a container, e.g., a gas or liquid tight

container; packaging the test protein preparation; associating a container comprising the test protein preparation with a label (e.g., labeling); shipping or moving the test protein preparation to a different location.

24. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein the processed drug product or antibody is approved under Section 351(k) of the Public Health Service (PHS) Act.

25. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein the processed drug product or antibody is not approved under biologics license application (BLA) under Section 351(a) of the Public Health Service (PHS) Act.

26. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein the value for the test glycoprotein preparation comprises an average (e.g., mean) of a range of values for the parameter for multiple (e.g., 2, 3, 4, 5, 10, 15, 20, or more) batches or samples of the target protein.

27. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein one or more, including all, of the reference criterion shown in Table 1 is/are a specification for commercial release of a drug product under Section 351(k) of the Public Health Service (PHS) Act.

28. The method of any of claims 1, 2, 4, 9, 13, 14, 15, or 17, wherein the value is acquired for one, two, or more samples or batches.

FIG. 1

Trastuzumab HC sequence (SEQ ID NO:1):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSV
KGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK

FIG. 2

Trastuzumab LC sequence (SEQ ID NO:2):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSG
SRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC