

BIOMARKERS FOR DIAGNOSING OVARIAN CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to, and the benefit of, U.S. Provisional Patent Application No. 62/968,941, filed January 31, 2020, the entire contents of which are herein incorporated by reference in its entirety for all purposes.

FIELD

[002] The instant disclosure is directed to glycoproteomic biomarkers including, but not limited to, glycans, peptides, and glycopeptides, as well as to methods of using these biomarkers with mass spectroscopy and in clinical applications.

BACKGROUND

[003] Changes in glycosylation have been described in relationship to disease states such as cancer. *See, e.g.,* Dube, D. H.; Bertozzi, C. R. Glycans in Cancer and Inflammation – Potential for Therapeutics and Diagnostics. *Nature Rev. Drug Disc.* 2005, 4, 477-88, the entire contents of which are herein incorporated by reference in its entirety for all purposes. However, clinically relevant, non-invasive assays for diagnosing cancer, such as ovarian cancer, in a patient based on glycosylation changes in a sample from that patient are still needed.

[004] Conventional clinical assays for diagnosing ovarian cancer, for example, include measuring the amount of the protein CA 125 (cancer antigen 125) in a patient's blood by an enzyme-linked immunosorbent assay (ELISA). However, ELISA has limited sensitivity and precision. ELISA, for example, only measures CA 125 at concentrations in the ng/mL range. This narrow measurement range limits the relevance of this assay by failing to measure biomarkers at concentrations substantially above or below this concentration range. Also, the CA 125 ELISA assay is limited with respect to the types of samples which can be assayed. As a consequence of the lack of more precise and sensitive tests, patients who might

otherwise be diagnosed with ovarian cancer are not and thereby fail to receive proper follow-up medical attention.

[005] As an alternative, mass spectroscopy (MS) offers sensitive and precise measurement of cancer-specific biomarkers including glycopeptides. *See*, for example, Ruhaak, L.R., *et al.*, Protein-Specific Differential Glycosylation of Immunoglobulins in Serum of Ovarian Cancer Patients DOI: 10.1021/acs.jproteome.5b01071; *J. Proteome Res.*, 2016, 15, 1002–1010 (2016); also Miyamoto, S., *et al.*, Multiple Reaction Monitoring for the Quantitation of Serum Protein Glycosylation Profiles: Application to Ovarian Cancer, DOI: 10.1021/acs.jproteome.7b00541, *J. Proteome Res.* 2018, 17, 222–233 (2017), the entire contents of which are herein incorporated by reference in its entirety for all purposes. However, using MS to diagnose cancer, generally, or ovarian cancer specifically, has not been demonstrated to date in a clinically relevant manner.

[006] What is needed are new biomarkers and new methods of using MS to diagnose disease states such as cancer using these biomarkers. Set forth herein in the disclosure below are such biomarkers comprising glycans, peptides, and glycopeptides, as well as fragments thereof, and methods of using the biomarkers with MS to diagnose ovarian cancer.

SUMMARY

[007] In another embodiment, set forth herein is a glycopeptide or peptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[008] In another embodiment, set forth herein is a method for detecting one or more MRM transitions, comprising: obtaining a biological sample from a patient; digesting and/or fragmenting a glycopeptide in the sample; and detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76, as described herein.

[009] In another embodiment, set forth herein a method for identifying a classification for a sample, the method comprising: quantifying by mass spectroscopy (MS) one or more glycopeptides in a sample wherein the glycopeptides each, individually in each instance, comprises a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof; and inputting the quantification into a trained model to generate an output probability; determining if the output probability is above or below a threshold for a classification; and identifying a

classification for the sample based on whether the output probability is above or below a threshold for a classification.

[0010] In yet another embodiment, set forth herein is a method for classifying a biological sample, comprising: obtaining a biological sample from a patient; digesting and/or fragmenting glycopeptides in the sample; detecting a MRM transition selected from the group consisting of transitions 1 - 76; and quantifying the glycopeptides; inputting the quantification into a trained model to generate a output probability; determining if the output probability is above or below a threshold for a classification; and classifying the biological sample based on whether the output probability is above or below a threshold for a classification.

[0011] In another embodiment, set forth herein is a method for treating a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; digesting and/or fragmenting one or more glycopeptides in the sample; and detecting and quantifying one or more multiple-reaction-monitoring (MRM) transitions selected from the group consisting of transitions 1 - 76; inputting the quantification into a trained model to generate an output probability; determining if the output probability is above or below a threshold for a classification; and classifying the patient based on whether the output probability is above or below a threshold for a classification, wherein the classification is selected from the group consisting of: (A) a patient in need of a chemotherapeutic agent; (B) a patient in need of an immunotherapeutic agent; (C) a patient in need of hormone therapy; (D) a patient in need of a targeted therapeutic agent; (E) a patient in need of surgery; (F) a patient in need of neoadjuvant therapy; (G) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, before surgery; (H) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, after surgery; (I) or a combination thereof; administering a therapeutically effective amount of a therapeutic agent to the patient: wherein the therapeutic agent is selected from chemotherapy if classification A or I is determined; wherein the therapeutic agent is selected from immunotherapy if classification B or I is determined; wherein the therapeutic agent is selected from hormone therapy if classification C or I is determined; wherein the therapeutic agent is selected from targeted therapy if classification D or I is determined; or wherein the therapeutic agent is selected from neoadjuvant therapy if classification F or I is determined; wherein the therapeutic agent is selected from chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic

agent, neoadjuvant therapy, or a combination thereof if classification G or I is determined; and wherein the therapeutic agent is selected from chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof if classification H or I is determined.

[0012] In another embodiment, set forth herein is a method for training a machine learning algorithm, comprising: providing a first data set of MRM transition signals indicative of a sample comprising a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; providing a second data set of MRM transition signals indicative of a control sample; and comparing the first data set with the second data set using a machine learning algorithm.

[0013] In another embodiment, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect and quantify one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect and quantify one or more MRM transitions selected from transitions 1-76; inputting the quantification of the detected glycopeptides or the MRM transitions into a trained model to generate an output probability, determining if the output probability is above or below a threshold for a classification; identifying a diagnostic classification for the patient based on whether the output probability is above or below a threshold for a classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification. In some examples, the method includes performing mass spectroscopy of the biological sample using MRM-MS with a QQQ.

[0014] In another embodiment, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0015] Figures 1 through 14 illustrate glycan chemical structures, using the Symbol Nomenclature for Glycans (SNFG) system. Each glycan structure is associated with a glycan reference code number.

[0016] Figures 15 and 16 show work flows for detecting transitions 1-76 by mass spectroscopy.

[0017] Figure 17 is a plot of intensity by retention time (RT) obtained by liquid chromatography / mass spectrometry (LC/MS) detection of a biomarker analyte. The top plot shows predicted probabilities from the PB-Net algorithm, and final (RT) start and stop prediction for the integrated peak.

[0018] Figure 18 shows LC retention time analysis.

[0019] Figure 19 is plot of ELISA results for measuring CA 125 protein in benign and malignant ovarian cancer samples, as set forth in Example 3.

[0020] Figure 20 is a plot of probability of having cancer in benign and malignant ovarian cancer samples, as set forth in Example 4.

[0021] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

DETAILED DESCRIPTION

[0022] The following description is presented to enable one of ordinary skill in the art to make and use the invention and to incorporate it in the context of particular applications. Various modifications, as well as a variety of uses in different applications will be readily apparent to those skilled in the art, and the general principles defined herein may be applied to a wide range of embodiments. Thus, the inventions herein are not intended to be limited to the embodiments presented, but are to be accorded their widest scope consistent with the principles and novel features disclosed herein.

[0023] All the features disclosed in this specification, (including any accompanying claims, abstract, and drawings) may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

[0024] Please note, if used, the labels left, right, front, back, top, bottom, forward, reverse, clockwise and counter clockwise have been used for convenience purposes only and are not intended to imply any particular fixed direction. Instead, they are used to reflect relative locations and/or directions between various portions of an object.

I. GENERAL

[0025] The instant disclosure provides methods and compositions for the profiling, detecting, and/or quantifying of glycans in a biological sample. In some examples, glycan and glycopeptide panels are described for diagnosing and screening patients having ovarian cancer. In some examples, glycan and glycopeptide panels are described for diagnosing and screening patients having cancer, an autoimmune disease, or fibrosis.

[0026] Certain techniques for analyzing biological samples using mass spectroscopy are known. *See*, for example, International PCT Patent Application Publication No. WO2019079639A1, filed October 18, 2018 as International Patent Application No. PCT/US2018/56574, and titled IDENTIFICATION AND USE OF BIOLOGICAL PARAMETERS FOR DIAGNOSIS AND TREATMENT MONITORING, the entire contents of which are herein incorporated by reference in its entirety for all purposes. *See*, also, US Patent Application Publication No. US20190101544A1, filed August 31, 2018 as US Patent Application No. 16/120,016, and titled IDENTIFICATION AND USE OF GLYCOPEPTIDES AS BIOMARKERS FOR DIAGNOSIS AND TREATMENT MONITORING, the entire contents of which are herein incorporated by reference in its entirety for all purposes.

II. DEFINITIONS

[0027] As used herein, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0028] As used herein, the phrase “biological sample,” refers to a sample derived from, obtained by, generated from, provided from, take from, or removed from an organism; or from fluid or tissue from the organism. Biological samples include, but are not limited to synovial fluid, whole blood, blood serum, blood plasma, urine, sputum, tissue, saliva, tears, spinal fluid, tissue section(s) obtained by biopsy; cell(s) that are placed in or adapted to tissue culture; sweat, mucous, fecal material, gastric fluid, abdominal fluid, amniotic fluid, cyst fluid, peritoneal fluid, pancreatic juice, breast milk, lung lavage, marrow, gastric acid, bile, semen, pus, aqueous humor, transudate, and the like including derivatives, portions and combinations of the foregoing. In some examples, biological samples include, but are not

limited, to blood and/or plasma. In some examples, biological samples include, but are not limited, to urine or stool. Biological samples include, but are not limited, to saliva. Biological samples include, but are not limited, to tissue dissections and tissue biopsies. Biological samples include, but are not limited, any derivative or fraction of the aforementioned biological samples.

[0029] As used herein, the term “glycan” refers to the carbohydrate residue of a glycoconjugate, such as the carbohydrate portion of a glycopeptide, glycoprotein, glycolipid or proteoglycan. Glycan structures are described by a glycan reference code number, and also illustrated in International PCT Patent Application No. PCT/US2020/016286, filed January 31, 2020, which is herein incorporated by reference in its entirety for all purposes. For example *see* Figures 1 through 14 of PCT Patent Application No. PCT/US2020/016286, filed January 31, 2020, which are herein incorporated by reference in their entirety for all purposes. Glycans are illustrated using the Symbol Nomenclature for Glycans (SNFG) for illustrating glycans. An explanation of this illustration system is available on the internet at www.ncbi.nlm.nih.gov/glycans/snfg.html, the entire contents of which are herein incorporated by reference in its entirety for all purposes. Symbol Nomenclature for Graphical Representation of Glycans as published in *Glycobiology* 25: 1323-1324, 2015, which is available on the internet at doi.org/10.1093/glycob/cwv091. Additional information showing illustrations of the SNFG system are. Within this system, the term, Hex_i: is interpreted as follows: i indicates the number of green circles (mannose) and the number of yellow circles (galactose). The term, HexNAC_j, uses j to indicate the number of blue squares (GlcNAC's). The term Fuc_d, uses d to indicate the number of red triangles (fucose). The term Neu5AC_l, uses l to indicate the number of purple diamonds (sialic acid). The glycan reference codes used herein combine these i, j, d, and l terms to make a composite 4-5 number glycan reference code, *e.g.*, 5300 or 5320. As an example, glycans 3200 and 3210 in Figure 1 both include 3 green circles (mannose), 2 blue squares (GlcNAC's), and no purple diamonds (sialic acid) but differ in that glycan 3210 also includes 1 red triangle (fucose).

[0030] As used herein, the term “glycopeptide,” refers to a peptide having at least one glycan residue bonded thereto. In each embodiment described herein, the glycopeptide may comprise, consist essentially of, or consist of, the amino acid sequence specified by the indicated SEQ ID NO together with one or more glycans, for instance those described herein associated with that SEQ ID NO. For instance, a glycopeptide according to SEQ ID NO: 1, as used herein, can refer to a glycopeptide according to the amino acid sequence of SEQ ID NO: 1 and glycan 5402, wherein the glycan is bonded to residue 1424 of SEQ ID NO: 1. A

glycopeptide comprising SEQ ID NO: 1, as used herein, can refer to a glycopeptide comprising the amino acid sequence of SEQ ID NO: 1 and glycan 5402, wherein the glycan is bonded to residue 1424. A glycopeptide consisting essentially of SEQ ID NO: 1, as used herein, can refer to a glycopeptide consisting essentially of the amino acid sequence of SEQ ID NO: 1 and glycan 5402, wherein the glycan is bonded to residue 1424. A glycopeptide consisting of SEQ ID NO: 1, as used herein, can refer to a glycopeptide consisting of the amino acid sequence of SEQ ID NO: 1 and glycan 5402, wherein the glycan is bonded to residue 1424. Similarly usage applies to SEQ ID NOs: 2-30, with the glycans described in sections below.

[0031] As used herein, the phrase “glycosylated peptides,” refers to a peptide bonded to a glycan.

[0032] As used herein, the phrase “glycopeptide fragment” or “glycosylated peptide fragment” or “glycopeptide” refers to a glycosylated peptide (or glycopeptide) having an amino acid sequence that is the same as part (but not all) of the amino acid sequence of the glycosylated protein from which the glycosylated peptide is obtained, *e.g.*, ion fragmentation within a MRM-MS instrument. MRM refers to multiple-reaction-monitoring. Unless specified otherwise, within the specification, “glycopeptide fragments” or “fragments of a glycopeptide” refer to the fragments produced directly by using a mass spectrometer optionally after the glycoprotein has been digested enzymatically to produce the glycopeptides.

[0033] As used herein, the phrase “glycoprotein” refers to the glycosylated protein from which the glycosylated peptide is obtained.

[0034] As used herein, the phrase “peptide,” is meant to include glycopeptides, and not the glycosylated protein from which the glycosylated peptide is obtained, unless stated otherwise.

[0035] As used herein, the phrase “multiple reaction monitoring mass spectrometry (MRM-MS),” refers to a highly sensitive and selective method for the targeted quantification of glycans and peptides in biological samples. Unlike traditional mass spectrometry, MRM-MS is highly selective (targeted), allowing researchers to fine tune an instrument to specifically look for certain peptides fragments of interest. MRM allows for greater sensitivity, specificity, speed and quantitation of peptides fragments of interest, such as a potential biomarker. MRM-MS involves using one or more of a triple quadrupole (QQQ) mass spectrometer and a quadrupole time-of-flight (qTOF) mass spectrometer.

[0036] As used herein, the phrase “digesting a glycopeptide,” refers to a biological process that employs enzymes to break specific amino acid peptide bonds. For example, digesting a glycopeptide includes contacting a glycopeptide with an digesting enzyme, *e.g.*, trypsin to produce fragments of the glycopeptide. In some examples, a protease enzyme is used to digest a glycopeptide. The term “protease” refers to an enzyme that performs proteolysis or breakdown of large peptides into smaller polypeptides or individual amino acids. Examples of a protease include, but are not limited to, one or more of a serine protease, threonine protease, cysteine protease, aspartate protease, glutamic acid protease, metalloprotease, asparagine peptide lyase, and any combinations of the foregoing.

[0037] As used herein, the phrase “fragmenting a glycopeptide,” refers to the ion fragmentation process which occurs in a MRM-MS instrument. Fragmenting may produce various fragments having the same mass but varying with respect to their charge.

[0038] As used herein, the term “subject,” refers to a mammal. The non-liming examples of a mammal include a human, non-human primate, mouse, rat, dog, cat, horse, or cow, and the like. Mammals other than humans can be advantageously used as subjects that represent animal models of disease, pre-disease, or a pre-disease condition. A subject can be male or female. However, in the context of diagnosing ovarian cancer, the subject is female unless explicitly specified otherwise. A subject can be one who has been previously identified as having a disease or a condition, and optionally has already undergone, or is undergoing, a therapeutic intervention for the disease or condition. Alternatively, a subject can also be one who has not been previously diagnosed as having a disease or a condition. For example, a subject can be one who exhibits one or more risk factors for a disease or a condition, or a subject who does not exhibit disease risk factors, or a subject who is asymptomatic for a disease or a condition. A subject can also be one who is suffering from or at risk of developing a disease or a condition.

[0039] As used herein, the term “patient” refers to a mammalian subject. The mammal can be a human, or an animal including, but not limited to an equine, porcine, canine, feline, ungulate, and primate animal. In one embodiment, the individual is a human. The methods and uses described herein are useful for both medical and veterinary uses. A “patient” is a human subject unless specified to the contrary.

[0040] As used herein, the phrase “multiple-reaction-monitoring (MRM) transition,” refers to the mass to charge (m/z) peaks or signals observed when a glycopeptide, or a fragment thereof, is detected by MRM-MS. The MRM transition is detected as the transition of the precursor and product ion.

[0041] As used herein, the phrase “detecting a multiple-reaction-monitoring (MRM) transition,” refers to the process in which a mass spectrometer analyzes a sample using tandem mass spectrometer ion fragmentation methods and identifies the mass to charge ratio for ion fragments in a sample. The phrase also refers to a MS process in which a MRM-MS transition is detected and then compared to a calculated mass to charge ratio (m/z) of a glycopeptide, or fragment thereof, in order to identify the glycopeptide. The absolute value of these identified mass to charge ratios are referred to as transitions. In the context of the methods set forth herein, the mass to charge ratio transitions are the values indicative of glycan, peptide or glycopeptide ion fragments. For some glycopeptides set forth herein, there is a single transition peak or signal. For some other glycopeptides set forth herein, there is more than one transition peak or signal. In some examples, herein, a single transition may be indicative of two more glycopeptides, if those glycopeptides have identical MRM-MS fragmentation patterns. A transition peak or signal includes, but is not limited to, those transitions set forth herein that are associated with a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from SEQ ID NOs: 1-76, and combinations thereof, according to Tables 1-5, e.g., Table 1, Table 2, Table 3, Table 4, or Table 5, or a combination thereof. Background information on MRM mass spectrometry can be found in *Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation*, 4th Edition, J. Throck Watson, O. David Sparkman, ISBN: 978-0-470-51634-8, November 2007, the entire contents of which are here incorporated by reference in its entirety for all purposes.

[0042] As used herein, the term “reference value” refers to a value obtained from a population of individual(s) whose disease state is known. The reference value may be in n -dimensional feature space and may be defined by a maximum-margin hyperplane. A reference value can be determined for any particular population, subpopulation, or group of individuals according to standard methods well known to those of skill in the art.

[0043] As used herein, the term “population of individuals” means one or more individuals. In one embodiment, the population of individuals consists of one individual. In one embodiment, the population of individuals comprises multiple individuals. As used herein, the term “multiple” means at least 2 (such as at least 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30) individuals. In one embodiment, the population of individuals comprises at least 10 individuals.

[0044] As used herein, the term “treatment” or “treating” means any treatment of a disease or condition in a subject, such as a mammal, including: 1) preventing or protecting

against the disease or condition, that is, causing the clinical symptoms not to develop; 2) inhibiting the disease or condition, that is, arresting or suppressing the development of clinical symptoms; and/or 3) relieving the disease or condition that is, causing the regression of clinical symptoms. Treating may include administering therapeutic agents to a subject in need thereof.

[0045] As used herein, the term “about” indicates and encompasses an indicated value and a range above and below that value. In certain embodiments, the term “about” indicates the designated value $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$. In certain embodiments, the term “about” indicates the designated value $\pm$ one standard deviation of that value.

III. BIOMARKERS

[0046] Set forth herein are biomarkers. These biomarkers are useful for a variety of applications, including, but not limited to, diagnosing diseases and conditions. For example, certain biomarkers set forth herein, or combinations thereof, are useful for diagnosing ovarian cancer. In some other examples, certain biomarkers set forth herein, or combinations thereof, are useful for diagnosing and screening patients having cancer, an autoimmune disease, or fibrosis. In some examples, the biomarkers set forth herein, or combinations thereof, are useful for classifying a patient so that the patient receives the appropriate medical treatment. In some other examples, the biomarkers set forth herein, or combinations thereof, are useful for treating or ameliorating a disease or condition in patient by, for example, identifying a therapeutic agent with which to treat a patient. In some other examples, the biomarkers set forth herein, or combinations thereof, are useful for determining a prognosis of treatment for a patient or a likelihood of success or survivability for a treatment regimen.

[0047] In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting of an amino acid sequence selected from SEQ ID NOs: 1-76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting essentially of an amino acid sequence selected from SEQ ID NOs: 1-76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from SEQ ID NOs: 1-76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a

glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from SEQ ID NOs: 1-76 in the sample. In some examples, as described below, the presence, absolute amount, and/or relative amount of a glycopeptide is determined by analyzing the MS results. In some examples, the MS results are analyzed using machine learning.

[0048] Set forth herein are biomarkers selected from glycans, peptides, glycopeptides, fragments thereof, and combinations thereof. In some examples, the glycopeptide consists of an amino acid sequence selected from SEQ ID NOs: 1-76. In some examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NOs: 1-76.

[0049] In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting of an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting essentially of an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 in the sample. In some examples, a sample from a patient is analyzed by MS and the results are used to determine the presence, absolute amount, and/or relative amount of a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 in the sample. In some examples, as described below, the presence, absolute amount, and/or relative amount of a glycopeptide is determined by analyzing the MS results. In some examples, the MS results are analyzed using machine learning.

[0050] Set forth herein are biomarkers selected from glycans, peptides, glycopeptides, fragments thereof, and combinations thereof. In some examples, the glycopeptide consists of an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

a. O-Glycosylation

[0051] In some examples, the glycopeptides set forth herein include O-glycosylated peptides. These peptides include glycopeptides in which a glycan is bonded to the peptide through an oxygen atom of an amino acid. Typically, the amino acid to which the glycan is bonded is threonine (T) or serine (S). In some examples, the amino acid to which the glycan is bonded is threonine (T). In some examples, the amino acid to which the glycan is bonded is serine (S).

[0052] In certain examples, the O-glycosylated peptides include those peptides from the group selected from Apolipoprotein C-III (APOC3), Alpha-2-HS-glycoprotein (FETUA), and combinations thereof. In certain examples, the O-glycosylated peptide, set forth herein, is an Apolipoprotein C-III (APOC3) peptide. In certain examples, the O-glycosylated peptide, set forth herein, is an Alpha-2-HS-glycoprotein (FETUA).

b. N-Glycosylation

[0053] In some examples, the glycopeptides set forth herein include N-glycosylated peptides. These peptides include glycopeptides in which a glycan is bonded to the peptide through a nitrogen atom of an amino acid. Typically, the amino acid to which the glycan is bonded is asparagine (N) or arginine (R). In some examples, the amino acid to which the glycan is bonded is asparagine (N). In some examples, the amino acid to which the glycan is bonded is arginine (R).

[0054] In certain examples, the N-glycosylated peptides include members selected from the group consisting of Alpha-1-antitrypsin (A1AT), Alpha-1B-glycoprotein (A1BG), Leucine-richAlpha-2-glycoprotein (A2GL), Alpha-2-macroglobulin (A2MG), Alpha-1-antichymotrypsin (AACT), Afamin (AFAM), Alpha-1-acid glycoprotein 1 & 2 (AGP12), Alpha-1-acid glycoprotein 1 (AGP1), Alpha-1-acid glycoprotein 2 (AGP2), Apolipoprotein A-I (APOA1), Apolipoprotein B-100 (APOB), Apolipoprotein D (APOD), Beta-2-glycoprotein-1 (APOH), Apolipoprotein M (APOM), Attractin (ATRN), Calpain-3 (CAN3), Ceruloplasmin (CERU), ComplementFactorH (CFAH), ComplementFactorI (CFAI), Clusterin (CLUS), ComplementC3 (CO3), ComplementC4-A&B (CO4A&CO4B), ComplementcomponentC6 (CO6), ComplementComponentC8AChain (CO8A), Coagulation factor XII (FA12), Haptoglobin (HPT), Histidine-rich Glycoprotein (HRG), Immunoglobulin heavy constant alpha 1&2 (IgA12), Immunoglobulin heavy constant alpha 2 (IgA2), Immunoglobulin heavy constant gamma 2 (IgG2), Immunoglobulin heavy constant mu (IgM), Inter-alpha-trypsin inhibitor heavy chain H1 (ITI1), Plasma Kallikrein (KLKB1),

Kininogen-1 (KNG1), Serum paraoxonase/arylesterase 1 (PON1), Selenoprotein P (SEPP1), Prothrombin (THRB), Serotransferrin (TRFE), Transthyretin (TTR), Protein unc-13HomologA (UN13A), Vitronectin (VTNC), Zinc-alpha-2-glycoprotein (ZA2G), Insulin-like growth factor-II (IGF2), Apolipoprotein C-I (APOC1), Hemopexin (HEMO), Immunoglobulin heavy constant gamma 1 (IgG1), Immunoglobulin J chain (IgJ), and combinations thereof.

c. Peptides and Glycopeptides

[0055] In some examples, set forth herein is a glycopeptide or peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[0056] In some examples, set forth herein is a glycopeptide or peptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[0057] In some examples, set forth herein is a glycopeptide or peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[0058] In some examples, set forth herein is a glycopeptide or peptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[0059] In some examples, set forth herein is a glycopeptide or peptide consisting of an amino acid sequence selected from SEQ ID NO: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[0060] In some examples, set forth herein is a glycopeptide or peptide consisting essentially of an amino acid sequence selected from SEQ ID NO: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[0061] In some examples, set forth herein is a glycopeptide or peptide consisting of an amino acid sequence selected from SEQ ID NO: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[0062] In some examples, set forth herein is a glycopeptide or peptide consisting essentially of an amino acid sequence selected from SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26,

28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[0063] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:1. In some examples, the glycopeptide comprises glycan 5402, wherein the glycan is bonded to residue 271. In some examples, the glycopeptide is A1AT-GP001_271_5402. Herein A1AT refers to Alpha-1-antitrypsin.

[0064] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:2. In some examples, the glycopeptide comprises glycan 5412 at residue 271. In some examples, the glycopeptide is A1AT-GP001_271_5412.

[0065] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:3. In some examples, the glycopeptide comprises glycan 5402 at residue 271. In some examples, the glycopeptide is A1AT-GP001_271MC_5402. Herein, "MC" refers to a missed cleavage of a trypsin digestion. A missed cleavage peptide includes the amino acid sequence selected from SEQ ID NO:3 but also includes additional residues which were not cleaved by way of trypsin digestion.

[0066] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:4. In some examples, the glycopeptide comprises glycans 5421 or 5402, or both, at residue 179. In some examples, the glycopeptide is A1BG-GP002_179_5421/5402. Herein, when two glycans are recited with a forward slash (/) between them, this means, unless specified otherwise explicitly, that the mass spectrometry method is unable to distinguish between these two glycans, *e.g.*, because they share a common mass to charge ratio. Unless specified to the contrary, 5421/5402 means that either glycan 5421 or 5402 is present. The quantification of the amount of glycans 5421/5402 includes a summation of the detected amount of glycan 5421 as well as the detected amount of glycan 5402. Herein A1BG refers to Alpha-1B-glycoprotein.

[0067] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:5. In some examples, the glycopeptide comprises glycan 5402 at residue 1424. In some examples, the glycopeptide is A2MG-GP004_1424_5402. Herein A2MG refers to Alpha-2-macroglobulin.

[0068] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:6. In some examples, the glycopeptide comprises glycan 5411 at residue 1424. In some examples, the glycopeptide is A2MG-GP004_1424_5411.

[0069] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:7. In some examples, the glycopeptide comprises glycan 5401 at residue 55. In some examples, the glycopeptide is A2MG-GP004_55_5401.

[0070] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:8. In some examples, the glycopeptide comprises glycan 5402 at residue 55. In some examples, the glycopeptide is A2MG-GP004_55_5402.

[0071] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:9. In some examples, the glycopeptide comprises glycan 5411 at residue 55. In some examples, the glycopeptide is A2MG-GP004_55_5411.

[0072] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:10. In some examples, the glycopeptide comprises glycan 5200 at residue 869. In some examples, the peptide is A2MG-GP004_869_5200.

[0073] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:11. In some examples, the glycopeptide comprises glycan 869 at residue 5401. In some examples, the glycopeptide is A2MG-GP004_869_5401.

[0074] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:12. In some examples, the glycopeptide comprises glycan 6301 at residue 869. In some examples, the glycopeptide is A2MG-GP004_869_6301.

[0075] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:13. In some examples, the glycopeptide comprises glycan 5402 at residue 33. In some examples, the glycopeptide is AFAM-GP006_33_5402. Herein, AFAM refers to Afamin.

[0076] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:14. In some examples, the glycopeptide comprises glycan 6503 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_6503. Herein AGP12 refers to Alpha-1-acid glycoprotein 1&2.

[0077] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:15. In some examples, the glycopeptide comprises glycan 6513 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_6513.

[0078] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:16. In some examples, the glycopeptide comprises glycan 7601 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_7601.

[0079] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:17. In some examples, the glycopeptide comprises glycan 7602 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_7602.

[0080] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:18. In some examples, the glycopeptide comprises glycan 7603 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_7603.

[0081] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:19. In some examples, the glycopeptide comprises glycan 7613 at residue 72. In some examples, the glycopeptide is AGP12-GP007&008_72MC_7613.

[0082] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:20. In some examples, the glycopeptide comprises glycan 5402 at residue 33. In some examples, the glycopeptide is AGP1-GP007_33_5402.

[0083] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:21. In some examples, the glycopeptide comprises glycans 6503 or 6522, or both, at residue 93. In some examples, the glycopeptide is AGP1-GP007_93_6503/6522. Herein AGP1 refers to Alpha-1-acid glycoprotein 1.

[0084] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:22. In some examples, the glycopeptide comprises glycan 6513 at residue 93. In some examples, the glycopeptide is AGP1-GP007_93_6513.

[0085] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:23. In some examples, the glycopeptide comprises glycans 7603 or 7622, or both, at residue 93. In some examples, the glycopeptide is AGP1-GP007_93_7603/7622.

[0086] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:24. In some examples, the glycopeptide comprises glycan 7613 at residue 93. In some examples, the glycopeptide is AGP1-GP007_93_7613.

[0087] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:25. In some examples, the glycopeptide comprises glycan 1102 at residue 74. In some examples, the glycopeptide is APOC3-GP012_74_1102. Herein APOC3 refers to Apolipoprotein C-III.

[0088] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:26. In some examples, the glycopeptide comprises glycan 1102 at residue 74. In some examples, the glycopeptide is APOC3-GP012_74MC_1102.

[0089] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:27. In some examples, the glycopeptide comprises glycan 5401 at residue 253. In some examples, the glycopeptide is APOH-GP015_253_5401. Herein APOH refers to Beta-2-glycoprotein1.

[0090] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:28. In some examples, the glycopeptide comprises glycan 5412 at residue 138. In some examples, the glycopeptide is CERU-GP023_138_5412. Herein CERU refers to Ceruloplasmin.

[0091] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:29. In some examples, the glycopeptide comprises glycans 5421 or 5402, or both, at residue 138. In some examples, the glycopeptide is CERU-GP023_138_5421/5402.

[0092] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:30. In some examples, the glycopeptide comprises glycans 6503 or 6522, or both, at residue 138. In some examples, the glycopeptide is CERU-GP023_138_6503/6522.

[0093] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:31. In some examples, the glycopeptide comprises glycans 5421 or 5402, or both, at residue 882. In some examples, the glycopeptide is CFAH-GP024_882_5421/5402. Herein CFAH refers to ComplementFactorH.

[0094] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:32. In some examples, the glycopeptide comprises glycan 5200 at residue 85. In some examples, the glycopeptide is CO3-GP028_85_5200. Herein CO3 refers to ComplementC3.

[0095] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:33. In some examples, the glycopeptide comprises glycan 6200 at residue 85. In some examples, the glycopeptide is CO3-GP028_85_6200.

[0096] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:34. In some examples, the glycopeptide comprises glycan 5402 at residue 1328. In some examples, the glycopeptide is

CO4A&CO4B-GP029&030_1328_5402. Herein CO4A&CO4B refers to ComplementC4-A&B.

[0097] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:35. In some examples, the glycopeptide comprises glycans 5421 or 5402, or both, at residue 156. In some examples, the glycopeptide is FETUA-GP036_156_5402/5421. Herein FETUA refers to Alpha-2-HS-glycoprotein.

[0098] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:36. In some examples, the glycopeptide comprises glycan 6513 at residue 176. In some examples, the glycopeptide is FETUA-GP036_176_6513.

[0099] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:37. In some examples, the glycopeptide comprises glycan 1101 at residue 346. In some examples, the glycopeptide is FETUA-GP036_346_1101.

[00100] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:38. In some examples, the glycopeptide comprises glycans 5412 or 5431, or both, at residue 187. In some examples, the glycopeptide is HEMO-GP042_187_5412/5431. Herein HEMO refers to Hemopexin.

[00101] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:39. In some examples, the glycopeptide comprises glycans 5420 or 5401, or both, at residue 453. In some examples, the glycopeptide is HEMO-GP042_453_5420/5401.

[00102] In certain examples, the peptide comprises an amino acid sequence selected from SEQ ID NO:40. In some examples, the glycopeptide comprises glycan 6502 at residue 184. In some examples, the glycopeptide is HPT-GP044_184_6502. Herein HPT refers to Haptoglobin.

[00103] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:41. In some examples, the glycopeptide comprises glycan 10803 at residue 207. In some examples, the glycopeptide is HPT-GP044_207_10803.

[00104] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:42. In some examples, the glycopeptide comprises glycan 10804 at residue 207. In some examples, the glycopeptide is HPT-GP044_207_10804.

[00105] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:43. In some examples, the glycopeptide comprises glycan 11904 at residue 207. In some examples, the glycopeptide is HPT-GP044_207_11904.

[00106] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:44. In some examples, the glycopeptide comprises glycan 11914 at residue 207. In some examples, the glycopeptide is HPT-GP044_207_11914.

[00107] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:45. In some examples, the glycopeptide comprises glycan 11915 at residue 207. In some examples, the glycopeptide is HPT-GP044_207_11915.

[00108] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:46. In some examples, the glycopeptide comprises glycans 5401 or 5420, or both, at residue 241. In some examples, the glycopeptide is HPT-GP044_241_5401/5420.

[00109] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:47. In some examples, the glycopeptide comprises glycan 5412 at residue 241. In some examples, the glycopeptide is HPT-GP044_241_5412.

[00110] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:48. In some examples, the glycopeptide comprises glycan 6501 at residue 241. In some examples, the glycopeptide is HPT-GP044_241_6501.

[00111] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:49. In some examples, the glycopeptide comprises glycan 6502 at residue 241. In some examples, the glycopeptide is HPT-GP044_241_6502.

[00112] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:50. In some examples, the glycopeptide comprises glycan 6511 at residue 241. In some examples, the glycopeptide is HPT-GP044_241_6511.

[00113] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:51. In some examples, the glycopeptide comprises glycan 6513 at residue 241. In some examples, the glycopeptide is HPT-GP044_241_6513.

[00114] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:52. In some examples, the glycopeptide comprises glycan 5501 at residue 144. In some examples, the glycopeptide is IgA12-GP046&047_144_5501. Herein IgA12 refers to Immunoglobulin heavy constant alpha 1&2.

[00115] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:53. In some examples, the glycopeptide comprises

glycan 4510 at residue 205. In some examples, the glycopeptide is IgA2-GP047_205_4510. Herein IgA2 refers to Immunoglobulin heavy constant alpha 2.

[00116] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:54. In some examples, the glycopeptide comprises glycan 5412 at residue 205. In some examples, the glycopeptide is IgA2-GP047_205_5412.

[00117] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:55. In some examples, the glycopeptide comprises glycan 5501 at residue 205. In some examples, the glycopeptide is IgA2-GP047_205_5510.

[00118] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:56. In some examples, the glycopeptide comprises glycan 3410 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_3410. Herein IgG1 refers to Immunoglobulin heavy constant gamma 1.

[00119] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:57. In some examples, the glycopeptide comprises glycan 4400 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_4400.

[00120] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:58. In some examples, the glycopeptide comprises glycan 4510 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_4510.

[00121] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:59. In some examples, the glycopeptide comprises glycan 5400 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_5400.

[00122] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:60. In some examples, the glycopeptide comprises glycan 5410 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_5410.

[00123] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:61. In some examples, the glycopeptide comprises glycan 5411 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_5411.

[00124] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:62. In some examples, the glycopeptide comprises glycan 5510 at residue 297. In some examples, the glycopeptide is IgG1-GP048_297_5510.

[00125] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:63. In some examples, the glycopeptide is IgG1-GP048_297_nonglycosylated.

[00126] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:64. In some examples, the glycopeptide comprises glycan 3510 at residue 297. In some examples, the glycopeptide is IgG2-GP049_297_3510. Herein IgG2 refers to Immunoglobulin heavy constant gamma 2.

[00127] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:65. In some examples, the glycopeptide comprises glycan 4411 at residue 297. In some examples, the glycopeptide is IgG2-GP049_297_4411.

[00128] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:66. In some examples, the glycopeptide comprises glycan 4510 at residue 297. In some examples, the glycopeptide is IgG2-GP049_297_4510.

[00129] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:67. In some examples, the glycopeptide comprises glycan 5401 at residue 71. In some examples, the glycopeptide is IgJ-GP052_71_5401. Herein IgJ refers to Immunoglobulin J chain.

[00130] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:68. In some examples, the glycopeptide comprises glycan 6200 at residue 439. In some examples, the glycopeptide is IgM-GP053_439_6200. Herein IgM refers to Immunoglobulin heavy constant mu.

[00131] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:69. In some examples, the glycopeptide comprises glycan 4311 at residue 46. In some examples, the glycopeptide is IgM-GP053_46_4311.

[00132] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:70. In some examples, the glycopeptide comprises glycan 6503 at residue 205. In some examples, the glycopeptide is KNG1-GP057_205_6503. Herein KNG1 refers to Kininogen-1.

[00133] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:71. In some examples, the glycopeptide comprises glycan 5402 at residue 432. In some examples, the glycopeptide is TRFE-GP064_432_5402. Herein TRFE refers to Serotransferrin.

[00134] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:72. In some examples, the glycopeptide comprises glycan 5401 at residue 630. In some examples, the glycopeptide is TRFE-GP064_630_5401.

[00135] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:73. In some examples, the glycopeptide comprises glycan 6513 at residue 630. In some examples, the glycopeptide is TRFE-GP064_630_6513.

[00136] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:74. In some examples, the glycopeptide comprises glycan 5401 at residue 169. In some examples, the glycopeptide is VTNC-GP067_169_5401. Herein VTNC refers to Vitronectin.

[00137] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:75. In some examples, the glycopeptide comprises glycan 6503 at residue 242. In some examples, the glycopeptide is VTNC-GP067_242_6503.

[00138] In certain examples, the glycopeptide consists essentially of an amino acid sequence selected from SEQ ID NO:76. In some examples, the glycopeptide comprises glycan 5421 at residue 86. In some examples, the glycopeptide is VTNC-GP067_86_5421.

IV. METHODS OF USING BIOMARKERS

A. METHODS FOR DETECTING GLYCOPEPTIDES

[00139] In some embodiments, set forth herein is a method for detecting one or more a multiple-reaction-monitoring (MRM) transition, comprising: obtaining a biological sample from a patient, wherein the biological sample comprises one or more glycopeptides; digesting and/or fragmenting a glycopeptide in the sample; and detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76. In certain examples, the method includes detecting MRM transitions selected from the group consisting of 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, 76, and combinations thereof. In certain examples, the method includes detecting MRM transitions selected from the group consisting of 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof. In certain examples, the method includes detecting MRM transitions selected from the group consisting of 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof. These transitions may include, in various examples, any one or more of the transitions in Tables 1-5. These transitions may include, in various examples, any one or more of the transitions in Tables 1-3. These transitions may include, in various examples, any one or more of the transitions in Table 1. These transitions may include, in various examples, any one or more of the transitions in Table 2. These transitions may include, in various

examples, any one or more of the transitions in Table 3. These transitions may include, in various examples, any one or more of the transitions in Table 4. These transitions may include, in various examples, any one or more of the transitions in Table 5. These transitions may be indicative of glycopeptides.

[00140] In some examples, including any of the foregoing, the methods include fragmenting a glycopeptide in the sample; and detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00141] In some examples, including any of the foregoing, the methods include fragmenting a glycopeptide in the sample; and detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00142] In some examples, including any of the foregoing, the methods include fragmenting a glycopeptide in the sample; and detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[00143] In some examples, set forth herein is a method of detecting one or more glycopeptides, wherein each glycopeptide is individually in each instance selected from a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00144] In some examples, set forth herein is a method of detecting one or more glycopeptides, wherein each glycopeptide is individually in each instance selected from a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00145] In some examples, set forth herein is a method of detecting one or more glycopeptides. In some examples, set forth herein is a method of detecting one or more glycopeptide fragments. In certain examples, the method includes detecting the glycopeptide group to which the glycopeptide, or fragment thereof, belongs. In some of these examples, the glycopeptide group is selected from Alpha-1-antitrypsin (A1AT), Alpha-1B-glycoprotein (A1BG), Leucine-richAlpha-2-glycoprotein (A2GL), Alpha-2-macroglobulin (A2MG), Alpha-1-antichymotrypsin (AACT), Afamin (AFAM), Alpha-1-acid glycoprotein 1 & 2 (AGP12), Alpha-1-acid glycoprotein 1 (AGP1), Alpha-1-acid glycoprotein 2 (AGP2),

Apolipoprotein A-I (APOA1), Apolipoprotein C-III (APOC3), Apolipoprotein B-100 (APOB), Apolipoprotein D (APOD), Beta-2-glycoprotein-1 (APOH), Apolipoprotein M (APOM), Attractin (ATRN), Calpain-3 (CAN3), Ceruloplasmin (CERU), ComplementFactorH (CFAH), ComplementFactorI (CFAI), Clusterin (CLUS), ComplementC3 (CO3), ComplementC4-A&B (CO4A&CO4B), ComplementcomponentC6 (CO6), ComplementComponentC8AChain (CO8A), Coagulation factor XII (FA12), Alpha-2-HS-glycoprotein (FETUA), Haptoglobin (HPT), Histidine-rich Glycoprotein (HRG), Immunoglobulin heavy constant alpha 1&2 (IgA12), Immunoglobulin heavy constant alpha 2 (IgA2), Immunoglobulin heavy constant gamma 2 (IgG2), Immunoglobulin heavy constant mu (IgM), Inter-alpha-trypsin inhibitor heavy chain H1 (ITI1), Plasma Kallikrein (KLKB1), Kininogen-1 (KNG1), Serum paraoxonase/arylesterase 1 (PON1), Selenoprotein P (SEPP1), Prothrombin (THRB), Serotransferrin (TRFE), Transthyretin (TTR), Protein unc-13HomologA (UN13A), Vitronectin (VTNC), Zinc-alpha-2-glycoprotein (ZA2G), Insulin-like growth factor-II (IGF2), Apolipoprotein C-I (APOC1), and combinations thereof.

[00146] In some examples, including any of the foregoing, the method includes detecting a glycopeptide, a glycan on the glycopeptide and the glycosylation site residue where the glycan bonds to the glycopeptide. In certain examples, the method includes detecting a glycan residue. In some examples, the method includes detecting a glycosylation site on a glycopeptide. In some examples, this process is accomplished with mass spectroscopy used in tandem with liquid chromatography.

[00147] In some examples, including any of the foregoing, the method includes obtaining a biological sample from a patient. In some examples, the biological sample is synovial fluid, whole blood, blood serum, blood plasma, urine, sputum, tissue, saliva, tears, spinal fluid, tissue section(s) obtained by biopsy; cell(s) that are placed in or adapted to tissue culture; sweat, mucous, fecal material, gastric fluid, abdominal fluid, amniotic fluid, cyst fluid, peritoneal fluid, pancreatic juice, breast milk, lung lavage, marrow, gastric acid, bile, semen, pus, aqueous humour, transudate, or combinations of the foregoing. In certain examples, the biological sample is selected from the group consisting of blood, plasma, saliva, mucus, urine, stool, tissue, sweat, tears, hair, or a combination thereof. In some of these examples, the biological sample is a blood sample. In some of these examples, the biological sample is a plasma sample. In some of these examples, the biological sample is a saliva sample. In some of these examples, the biological sample is a mucus sample. In some

of these examples, the biological sample is a urine sample. In some of these examples, the biological sample is a stool sample. In some of these examples, the biological sample is a sweat sample. In some of these examples, the biological sample is a tear sample. In some of these examples, the biological sample is a hair sample.

[00148] In some examples, including any of the foregoing, the method also includes digesting and/or fragmenting a glycopeptide in the sample. In certain examples, the method includes digesting a glycopeptide in the sample. In certain examples, the method includes fragmenting a glycopeptide in the sample. In some examples, the digested or fragmented glycopeptide is analyzed using mass spectroscopy. In some examples, the glycopeptide is digested or fragmented in the solution phase using digestive enzymes. In some examples, the glycopeptide is digested or fragmented in the gaseous phase inside a mass spectrometer, or the instrumentation associated with a mass spectrometer. In some examples, the mass spectroscopy results are analyzed using machine learning algorithms. In some examples, the mass spectroscopy results are the quantification of the glycopeptides, glycans, peptides, and fragments thereof. In some examples, this quantification is used as an input in a trained model to generate an output probability. The output probability is a probability of being within a given category or classification, *e.g.*, the classification of having ovarian cancer or the classification of not having ovarian cancer. In some other examples, the output probability is a probability of being within a given category or classification, *e.g.*, the classification of having cancer or the classification of not having cancer. In some other examples, the output probability is a probability of being within a given category or classification, *e.g.*, the classification of having an autoimmune disease or the classification of not having an autoimmune disease. In some other examples, the output probability is a probability of being within a given category or classification, *e.g.*, the classification of having fibrosis or the classification of not having an fibrosis.

[00149] In some examples, including any of the foregoing, the method includes introducing the sample, or a portion thereof, into a mass spectrometer.

[00150] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample after introducing the sample, or a portion thereof, into the mass spectrometer.

[00151] In some examples, including any of the foregoing, the mass spectroscopy is performed using multiple reaction monitoring (MRM) mode. In some examples, the mass spectroscopy is performed using QTOF MS in data-dependent acquisition. In some examples, the mass spectroscopy is performed using or MS-only mode. In some examples, an

immunoassay is used in combination with mass spectroscopy. In some examples, the immunoassay measures CA-125 and HE4.

[00152] In some examples, including any of the foregoing, the method includes digesting a glycopeptide in the sample occurs before introducing the sample, or a portion thereof, into the mass spectrometer.

[00153] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide ion, a peptide ion, a glycan ion, a glycan adduct ion, or a glycan fragment ion.

[00154] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00155] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00156] In some examples, including any of the foregoing, the method includes digesting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00157] In some examples, including any of the foregoing, the method includes digesting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00158] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00159] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.

[00160] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino

acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00161] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00162] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00163] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00164] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[00165] In some examples, including any of the foregoing, the method includes fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[00166] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof. In some

examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1 - 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1 - 76.

[00167] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75.

[00168] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino

acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76.

[00169] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

[00170] In some examples, including any of the foregoing, the method includes performing mass spectroscopy on the biological sample using multiple-reaction-monitoring mass spectroscopy (MRM-MS).

[00171] In some examples, including any of the foregoing, the method includes digesting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof. In certain examples, the biological sample is combined with chemical

reagents. In certain examples, the biological sample is combined with enzymes. In some examples, the enzymes are lipases. In some examples, the enzymes are proteases. In some examples, the enzymes are serine proteases. In some of these examples, the enzyme is selected from the group consisting of trypsin, chymotrypsin, thrombin, elastase, and subtilisin. In some of these examples, the enzyme is trypsin. In some examples, the methods includes contacting at least two proteases with a glycopeptide in a sample. In some examples, the at least two proteases are selected from the group consisting of serine protease, threonine protease, cysteine protease, aspartate protease. In some examples, the at least two proteases are selected from the group consisting of trypsin, chymotrypsin, endoproteinase, Asp-N, Arg-C, Glu-C, Lys-C, pepsin, thermolysin, elastase, papain, proteinase K, subtilisin, clostripain, and carboxypeptidase protease, glutamic acid protease, metalloprotease, and asparagine peptide lyase.

[00172] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1 - 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1 - 76.

[00173] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from

the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75.

[00174] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof.

61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76.

[00175] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide or glycan residue, wherein the glycopeptide consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof. In some examples, the method includes detecting a MRM transition indicative of a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof. In some examples, the method includes detecting more than one MRM transition selected from a combination of members from the group consisting of transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the method includes detecting more than one MRM transition indicative of a combination of glycopeptides having amino acid sequences selected from a combination of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

[00176] In some examples, including any of the foregoing, the method includes performing mass spectroscopy on the biological sample using multiple-reaction-monitoring mass spectroscopy (MRM-MS).

[00177] In some examples, including any of the foregoing, the method includes digesting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid

sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof. In certain examples, the biological sample is contacted with one or more chemical reagents. In certain examples, the biological sample is contacted with one or more enzymes. In some examples, the enzymes are lipases. In some examples, the enzymes are proteases. In some examples, the enzymes are serine proteases. In some of these examples, the enzyme is selected from the group consisting of trypsin, chymotrypsin, thrombin, elastase, and subtilisin. In some of these examples, the enzyme is trypsin. In some examples, the methods includes contacting at least two proteases with a glycopeptide in a sample. In some examples, the at least two proteases are selected from the group consisting of serine protease, threonine protease, cysteine protease, aspartate protease. In some examples, the at least two proteases are selected from the group consisting of trypsin, chymotrypsin, endoproteinase, Asp-N, Arg-C, Glu-C, Lys-C, pepsin, thermolysin, elastase, papain, proteinase K, subtilisin, clostripain, and carboxypeptidase protease, glutamic acid protease, metalloprotease, and asparagine peptide lyase.

[00178] In some examples, including any of the foregoing, the MRM transition is selected from the transitions, or any combinations thereof, in any one of Tables 1, 2 or 3.

[00179] In some examples, including any of the foregoing, the method includes conducting tandem liquid chromatography-mass spectroscopy on the biological sample.

[00180] In some examples, including any of the foregoing, the method includes multiple-reaction-monitoring mass spectroscopy (MRM-MS) mass spectroscopy on the biological sample.

[00181] In some examples, including any of the foregoing, the method includes detecting a MRM transition using a triple quadrupole (QQQ) and/or a quadrupole time-of-flight (qTOF) mass spectrometer. In certain examples, the method includes detecting a MRM transition using a QQQ mass spectrometer. In certain other examples, the method includes detecting using a qTOF mass spectrometer. In some examples, a suitable instrument for use with the instant methods is an Agilent 6495B Triple Quadrupole LC/MS, which can be found at www.agilent.com/en/products/mass-spectrometry/lc-ms-instruments/triple-quadrupole-lc-ms/6495b-triple-quadrupole-lc-ms. In certain other examples, the method includes detecting using a QQQ mass spectrometer. In some examples, a suitable instrument for use with the instant methods is an Agilent 6545 LC/Q-TOF, which can be found at <https://www.agilent.com/en/products/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/quadrupole-time-of-flight-lc-ms/6545-q-tof-lc-ms>.

[00182] In some examples, including any of the foregoing, the method includes detecting more than one MRM transition using a QQQ and/or qTOF mass spectrometer. In certain examples, the method includes detecting more than one MRM transition using a QQQ mass spectrometer. In certain examples, the method includes detecting more than one MRM transition using a qTOF mass spectrometer. In certain examples, the method includes detecting more than one MRM transition using a QQQ mass spectrometer.

[00183] In some examples, including any of the foregoing, the methods herein include quantifying one or more glycomic parameters of the one or more biological samples comprises employing a coupled chromatography procedure. In some examples, these glycomic parameters include the identification of a glycopeptide group, identification of glycans on the glycopeptide, identification of a glycosylation site, identification of part of an amino acid sequence which the glycopeptide includes. In some examples, the coupled chromatography procedure comprises: performing or effectuating a liquid chromatography-mass spectrometry (LC-MS) operation. In some examples, the coupled chromatography procedure comprises: performing or effectuating a multiple reaction monitoring mass spectrometry (MRM-MS) operation. In some examples, the methods herein include a coupled chromatography procedure which comprises: performing or effectuating a liquid chromatography-mass spectrometry (LC-MS) operation; and effectuating a multiple reaction monitoring mass spectrometry (MRM-MS) operation. In some examples, the methods include training a machine learning algorithm using one or more glycomic parameters of the one or more biological samples obtained by one or more of a triple quadrupole (QQQ) mass spectrometry operation and/or a quadrupole time-of-flight (qTOF) mass spectrometry operation. In some examples, the methods include training a machine learning algorithm using one or more glycomic parameters of the one or more biological samples obtained by a triple quadrupole (QQQ) mass spectrometry operation. In some examples, the methods include training a machine learning algorithm using one or more glycomic parameters of the one or more biological samples obtained by a quadrupole time-of-flight (qTOF) mass spectrometry operation. In some examples, the methods include quantifying one or more glycomic parameters of the one or more biological samples comprises employing one or more of a triple quadrupole (QQQ) mass spectrometry operation and a quadrupole time-of-flight (qTOF) mass spectrometry operation. In some examples, machine learning algorithms are used to quantify these glycomic parameters. In some examples, including any of the foregoing, the mass spectroscopy is performed using multiple reaction monitoring (MRM) mode. In some examples, the mass spectroscopy is performed using QTOF MS in data-

dependent acquisition. In some examples, the mass spectroscopy is performed using or MS-only mode. In some examples, an immunoassay (*e.g.*, ELISA) is used in combination with mass spectroscopy. In some examples, the immunoassay measures CA-125 and HE4 proteins.

[00184] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00185] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00186] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof.

[00187] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, and 75 and combinations thereof.

[00188] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00189] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00190] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof.

[00191] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41,

42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, and 76 and combinations thereof.

[00192] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00193] In some examples, including any of the foregoing, the glycopeptide or combination thereof consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00194] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00195] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00196] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00197] In some examples, including any of the foregoing, the method includes digesting and/or fragmenting a glycopeptide in the sample to provide a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76 and combinations thereof.

[00198] In some examples, including any of the foregoing, the method includes detecting one or more MRM transitions indicative of glycans selected from the group consisting of glycan 3200, 3210, 3300, 3310, 3320, 3400, 3410, 3420, 3500, 3510, 3520, 3600, 3610, 3620, 3630, 3700, 3710, 3720, 3730, 3740, 4200, 4210, 4300, 4301, 4310, 4311, 4320, 4400, 4401, 4410, 4411, 4420, 4421, 4430, 4431, 4500, 4501, 4510, 4511, 4520, 4521, 4530, 4531, 4540, 4541, 4600, 4601, 4610, 4611, 4620, 4621, 4630, 4631, 4641, 4650, 4700, 4701, 4710, 4711, 4720, 4730, 5200, 5210, 5300, 5301, 5310, 5311, 5320, 5400, 5401, 5402,

5410, 5411, 5412, 5420, 5421, 5430, 5431, 5432, 5500, 5501, 5502, 5510, 5511, 5512, 5520, 5521, 5522, 5530, 5531, 5541, 5600, 5601, 5602, 5610, 5611, 5612, 5620, 5621, 5631, 5650, 5700, 5701, 5702, 5710, 5711, 5712, 5720, 5721, 5730, 5731, 6200, 6210, 6300, 6301, 6310, 6311, 6320, 6400, 6401, 6402, 6410, 6411, 6412, 6420, 6421, 6432, 6500, 6501, 6502, 6503, 6510, 6511, 6512, 6513, 6520, 6521, 6522, 6530, 6531, 6532, 6540, 6541, 6600, 6601, 6602, 6603, 6610, 6611, 6612, 6613, 6620, 6621, 6622, 6623, 6630, 6631, 6632, 6640, 6641, 6642, 6652, 6700, 6701, 6711, 6721, 6703, 6713, 6710, 6711, 6712, 6713, 6720, 6721, 6730, 6731, 6740, 7200, 7210, 7400, 7401, 7410, 7411, 7412, 7420, 7421, 7430, 7431, 7432, 7500, 7501, 7510, 7511, 7512, 7600, 7601, 7602, 7603, 7604, 7610, 7611, 7612, 7613, 7614, 7620, 7621, 7622, 7623, 7632, 7640, 7700, 7701, 7702, 7703, 7710, 7711, 7712, 7713, 7714, 7720, 7721, 7722, 7730, 7731, 7732, 7740, 7741, 7751, 8200, 9200, 9210, 10200, 11200, 12200, and combinations thereof. Herein, these glycans are illustrated in Figures 1-14.

[00199] In some examples, including any of the foregoing, the method includes quantifying a glycan.

[00200] In some examples, including any of the foregoing, the method includes quantifying a first glycan and quantifying a second glycan; and further comprising comparing the quantification of the first glycan with the quantification of the second glycan.

[00201] In some examples, including any of the foregoing, the method includes associating the detected glycan with a peptide residue site, whence the glycan was bonded.

[00202] In some examples, including any of the foregoing, the method includes generating a glycosylation profile of the sample.

[00203] In some examples, including any of the foregoing, the method includes spatially profiling glycans on a tissue section associated with the sample. In some examples, including any of the foregoing, the method includes spatially profiling glycopeptides on a tissue section associated with the sample. In some examples, the method includes matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) mass spectroscopy in combination with the methods herein.

[00204] In some examples, including any of the foregoing, the method includes quantifying relative abundance of a glycan and/or a peptide.

[00205] In some examples, including any of the foregoing, the method includes normalizing the amount of a glycopeptide by quantifying a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof and comparing that quantification to the amount of another chemical species. In some examples, the method includes normalizing the amount of a

peptide by quantifying a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof, and comparing that quantification to the amount of another glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In some examples, the method includes normalizing the amount of a peptide by quantifying a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof, and comparing that quantification to the amount of another glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00206] In some examples, including any of the foregoing, the method includes normalizing the amount of a glycopeptide by quantifying a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof and comparing that quantification to the amount of another chemical species. In some examples, the method includes normalizing the amount of a peptide by quantifying a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof, and comparing that quantification to the amount of another glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76. In some examples, the method includes normalizing the amount of a peptide by quantifying a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof, and comparing that quantification to the amount of another glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

B. METHODS FOR CLASSIFYING SAMPLES COMPRISING GLYCOPEPTIDES

[00207] In another embodiment, set forth herein a method for identifying a classification for a sample, the method comprising: quantifying by mass spectroscopy (MS) one or more glycopeptides in a sample wherein the glycopeptides each, individually in each instance, comprises a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of, or consisting essentially of, SEQ ID NOs: 1 - 76, and

combinations thereof; and inputting the quantification into a trained model to generate a output probability; determining if the output probability is above or below a threshold for a classification; and identifying a classification for the sample based on whether the output probability is above or below a threshold for a classification.

[00208] In some examples, set forth herein is a method for classifying glycopeptides, comprising: obtaining a biological sample from a patient; digesting and/or fragmenting a glycopeptide in the sample; detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76; and classifying the glycopeptides based on the MRM transitions detected. In some examples, a machine learning algorithm is used to train a model using the analyzed the MRM transitions as inputs. In some examples, a machine learning algorithm is trained using the MRM transitions as a training data set. In some examples, the methods herein include identifying glycopeptides, peptides, and glycans based on their mass spectroscopy relative abundance. In some examples, a machine learning algorithm or algorithms select and/or identify peaks in a mass spectroscopy spectrum.

[00209] In some examples, set forth herein is a method for classifying glycopeptides, comprising: obtaining a biological sample from an individual; digesting and/or fragmenting a glycopeptide in the sample; detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76; and classifying the glycopeptides based on the MRM transitions detected. In some examples, a machine learning algorithm is used to train a model using the analyzed the MRM transitions as inputs. In some examples, a machine learning algorithm is trained using the MRM transitions as a training data set. In some examples, the methods herein include identifying glycopeptides, peptides, and glycans based on their mass spectroscopy relative abundance. In some examples, a machine learning algorithm or algorithms select and/or identify peaks in a mass spectroscopy spectrum.

[00210] In some examples, set forth herein is a method of training a machine learning algorithm using MRM transitions as an input data set. In some examples, set forth herein is a method for identifying a classification for a sample, the method comprising quantifying by mass spectroscopy (MS) a glycopeptide in a sample wherein the glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof; and identifying a classification based on the quantification. In some examples, the quantifying includes determining the presence or absence of a glycopeptide, or combination of glycopeptides, in a sample. In some examples, the quantifying includes determining the relative abundance of a glycopeptide, or combination of glycopeptides, in a sample.

- [00211] In some examples, including any of the foregoing, the sample is a biological sample from a patient having a disease or condition.
- [00212] In some examples, including any of the foregoing, the patient has ovarian cancer.
- [00213] In some examples, including any of the foregoing, the patient has cancer.
- [00214] In some examples, including any of the foregoing, the patient has fibrosis.
- [00215] In some examples, including any of the foregoing, the patient has an autoimmune disease.
- [00216] In some examples, including any of the foregoing, the disease or condition is ovarian cancer.
- [00217] In some examples, including any of the foregoing, the MS is MRM-MS with a QQQ and/or qTOF mass spectrometer.
- [00218] In some examples, including any of the foregoing, the mass spectroscopy is performed using multiple reaction monitoring (MRM) mode. In some examples, the mass spectroscopy is performed using QTOF MS in data-dependent acquisition. In some examples, the mass spectroscopy is performed using or MS-only mode. In some examples, an immunoassay is used in combination with mass spectroscopy. In some examples, the immunoassay measures CA-125 and HE4.
- [00219] In some examples, including any of the foregoing, the machine learning algorithm is selected from the group consisting of a deep learning algorithm, a neural network algorithm, an artificial neural network algorithm, a supervised machine learning algorithm, a linear discriminant analysis algorithm, a quadratic discriminant analysis algorithm, a support vector machine algorithm, a linear basis function kernel support vector algorithm, a radial basis function kernel support vector algorithm, a random forest algorithm, a genetic algorithm, a nearest neighbor algorithm, k-nearest neighbors, a naive Bayes classifier algorithm, a logistic regression algorithm, or a combination thereof. In certain examples, the machine learning algorithm is lasso regression.
- [00220] In some examples, including any of the foregoing, the method includes classifying a sample as within, or embraced by, a disease classification or a disease severity classification.
- [00221] In some examples, including any of the foregoing, the classification is identified with 80 % confidence, 85 % confidence, 90 % confidence, 95 % confidence, 99 % confidence, or 99.9999 % confidence.

[00222] In some examples, including any of the foregoing, the method includes quantifying by MS the glycopeptide in a sample at a first time point; quantifying by MS the glycopeptide in a sample at a second time point; and comparing the quantification at the first time point with the quantification at the second time point.

[00223] In some examples, including any of the foregoing, the method includes quantifying by MS a different glycopeptide in a sample at a third time point; quantifying by MS the different glycopeptide in a sample at a fourth time point; and comparing the quantification at the fourth time point with the quantification at the third time point.

[00224] In some examples, including any of the foregoing, the method includes monitoring the health status of a patient.

[00225] In some examples, including any of the foregoing, monitoring the health status of a patient includes monitoring the onset and progression of disease in a patient with risk factors such as genetic mutations, as well as detecting cancer recurrence.

[00226] In some examples, including any of the foregoing, the method includes quantifying by MS a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00227] In some examples, including any of the foregoing, the method includes quantifying by MS a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00228] In some examples, including any of the foregoing, the method includes quantifying by MS a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

[00229] In some examples, including any of the foregoing, the method includes quantifying by MS a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76.

[00230] In some examples, including any of the foregoing, the method includes quantifying by MS one or more glycans selected from the group consisting of glycan 3200, 3210, 3300, 3310, 3320, 3400, 3410, 3420, 3500, 3510, 3520, 3600, 3610, 3620, 3630, 3700, 3710, 3720, 3730, 3740, 4200, 4210, 4300, 4301, 4310, 4311, 4320, 4400, 4401, 4410, 4411, 4420, 4421, 4430, 4431, 4500, 4501, 4510, 4511, 4520, 4521, 4530, 4531, 4540, 4541, 4600, 4601, 4610, 4611, 4620, 4621, 4630, 4631, 4641, 4650, 4700, 4701, 4710, 4711, 4720, 4730, 5200, 5210, 5300, 5301, 5310, 5311, 5320, 5400, 5401, 5402, 5410, 5411, 5412, 5420, 5421,

5430, 5431, 5432, 5500, 5501, 5502, 5510, 5511, 5512, 5520, 5521, 5522, 5530, 5531, 5541, 5600, 5601, 5602, 5610, 5611, 5612, 5620, 5621, 5631, 5650, 5700, 5701, 5702, 5710, 5711, 5712, 5720, 5721, 5730, 5731, 6200, 6210, 6300, 6301, 6310, 6311, 6320, 6400, 6401, 6402, 6410, 6411, 6412, 6420, 6421, 6432, 6500, 6501, 6502, 6503, 6510, 6511, 6512, 6513, 6520, 6521, 6522, 6530, 6531, 6532, 6540, 6541, 6600, 6601, 6602, 6603, 6610, 6611, 6612, 6613, 6620, 6621, 6622, 6623, 6630, 6631, 6632, 6640, 6641, 6642, 6652, 6700, 6701, 6711, 6721, 6703, 6713, 6710, 6711, 6712, 6713, 6720, 6721, 6730, 6731, 6740, 7200, 7210, 7400, 7401, 7410, 7411, 7412, 7420, 7421, 7430, 7431, 7432, 7500, 7501, 7510, 7511, 7512, 7600, 7601, 7602, 7603, 7604, 7610, 7611, 7612, 7613, 7614, 7620, 7621, 7622, 7623, 7632, 7640, 7700, 7701, 7702, 7703, 7710, 7711, 7712, 7713, 7714, 7720, 7721, 7722, 7730, 7731, 7732, 7740, 7741, 7751, 8200, 9200, 9210, 10200, 11200, 12200, and combinations thereof. Herein, these glycans are illustrated in Figures 1-14.

[00231] In some examples, including any of the foregoing, the method includes diagnosing a patient with a disease or condition based on the quantification.

[00232] In some examples, including any of the foregoing, the method includes diagnosing the patient as having ovarian cancer based on the quantification.

[00233] In some examples, including any of the foregoing, the method includes treating the patient with a therapeutically effective amount of a therapeutic agent selected from the group consisting of a chemotherapeutic, an immunotherapy, a hormone therapy, a targeted therapy, a neoadjuvant therapy, surgery, and combinations thereof.

[00234] In some examples, including any of the foregoing, the method includes diagnosing an individual with a disease or condition based on the quantification.

[00235] In some examples, including any of the foregoing, the method includes diagnosing the individual as having an aging condition.

[00236] In some examples, including any of the foregoing, the method includes treating the individual with a therapeutically effective amount of an anti-aging agent. In some examples, the anti-aging agent is selected from hormone therapy. In some examples, the anti-aging agent is testosterone or a testosterone supplement or derivative. In some examples, the anti-aging agent is estrogen or an estrogen supplement or derivative.

C. METHODS OF TREATMENT

[00237] In some examples, set forth herein is a method for treating a patient having a disease or condition, comprising measuring by mass spectroscopy a glycopeptide in a sample from the patient. In some examples, the patient is a human. In certain examples, the patient is a female. In certain other examples, the patient is a female with ovarian cancer. In certain

examples, the patient is a female with ovarian cancer at Stage 1. In certain examples, the patient is a female with ovarian cancer at Stage 2. In certain examples, the patient is a female with ovarian cancer at Stage 3. In certain examples, the patient is a female with ovarian cancer at Stage 4. In some examples, the female has an age equal or between 10-20 years. In some examples, the female has an age equal or between 20-30 years. In some examples, the female has an age equal or between 30-40 years. In some examples, the female has an age equal or between 40-50 years. In some examples, the female has an age equal or between 50-60 years. In some examples, the female has an age equal or between 60-70 years. In some examples, the female has an age equal or between 70-80 years. In some examples, the female has an age equal or between 80-90 years. In some examples, the female has an age equal or between 90-100 years.

[00238] In another embodiment, set forth herein is a method for treating a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; digesting and/or fragmenting one or more glycopeptides in the sample; and detecting and quantifying one or more multiple-reaction-monitoring (MRM) transitions selected from the group consisting of transitions 1 - 76; inputting the quantification into a trained model to generate an output probability; determining if the output probability is above or below a threshold for a classification; and classifying the patient based on whether the output probability is above or below a threshold for a classification, wherein the classification is selected from the group consisting of: (A) a patient in need of a chemotherapeutic agent; (B) a patient in need of an immunotherapeutic agent; (C) a patient in need of hormone therapy; (D) a patient in need of a targeted therapeutic agent; (E) a patient in need of surgery; (F) a patient in need of neoadjuvant therapy; (G) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, before surgery; (H) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, after surgery; (I) or a combination thereof; administering a therapeutically effective amount of a therapeutic agent to the patient: wherein the therapeutic agent is selected from chemotherapy if classification A or I is determined; wherein the therapeutic agent is selected from immunotherapy if classification B or I is determined; or wherein the therapeutic agent is selected from hormone therapy if classification C or I is determined; or wherein the therapeutic agent is selected from targeted therapy if classification D or I is determined wherein the therapeutic agent is selected from neoadjuvant therapy if classification F or I is determined; wherein the therapeutic agent is selected from

chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof if classification G or I is determined; and wherein the therapeutic agent is selected from chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof if classification H or I is determined.

[00239] In some examples, the machine learning is used to identify MS peaks associated with MRM transitions. In some examples, the MRM transitions are analyzed using machine learning. In some examples, the machine learning is used to train a model based on the quantification of the amount of glycopeptides associated with an MRM transition(s). In some examples, the MRM transitions are analyzed with a trained machine learning algorithm. In some of these examples, the trained machine learning algorithm was trained using MRM transitions observed by analyzing samples from patients known to have ovarian cancer.

[00240] In some examples, the patient is treated with a therapeutic agent selected from targeted therapy. In some examples, the methods herein include administering a therapeutically effective amount of a (poly(ADP)-ribose polymerase) (PARP) inhibitor if combination D is detected. In some examples, the therapeutic agent is selected from Olaparib (Lynparza), Rucaparib (Rubraca), and Niraparib (Zejula).

[00241] In some examples, the patient is an adult with platinum-sensitive relapsed high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer.

[00242] In some examples, the therapeutic agent is administered at 150 mg, 250 mg, 300 mg, 350 mg, and 600 mg doses. In some examples, the therapeutic agent is administered twice daily.

[00243] Chemotherapeutic agents include, but are not limited to, platinum-based drug such as carboplatin (Paraplatin) or cisplatin with a taxane such as paclitaxel (Taxol) or docetaxel (Taxotere). Paraplatin may be administered at 10mg/mL injectable concentrations (in vials of 50, 150, 450, and 600 mg). For advanced ovarian carcinoma a single agent dose of 360 mg/m² IV for 4 weeks may be administered. Paraplatin may be administered in combination = as 300 mg/m² IV (plus cyclophosphamide 600 mg/m² IV) q4Weeks. Taxol may be administered at 175 mg/m² IV over 3 hours q3Weeks (follow with cisplatin). Taxol may be administered at 135 mg/m² IV over 24 hours q3Weeks (follow with cisplatin). Taxol may be administered at 135-175 mg/m² IV over 3 hours q3Weeks.

[00244] Immunotherapeutic agents include, but are not limited to, Zejula (Niraparib). Niraparib may be administered at 300 mg PO qDay.

[00245] Hormone therapeutic agents include, but are not limited to, Luteinizing-hormone-releasing hormone (LHRH) agonists, Tamoxifen, and Aromatase inhibitors.

[00246] Targeted therapeutic agents include, but are not limited to, PARP inhibitors.

[00247] In some examples, including any of the foregoing, the method includes conducting multiple-reaction-monitoring mass spectroscopy (MRM-MS) on the biological sample.

[00248] In some examples, including any of the foregoing, the mass spectroscopy is performed using multiple reaction monitoring (MRM) mode. In some examples, the mass spectroscopy is performed using QTOF MS in data-dependent acquisition. In some examples, the mass spectroscopy is performed using or MS-only mode. In some examples, an immunoassay (*e.g.*, ELISA) is used in combination with mass spectroscopy. In some examples, the immunoassay measures CA-125 and HE4.

[00249] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00250] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00251] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00252] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00253] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00254] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, 76, and combinations thereof.

[00255] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, 76, and combinations thereof.

[00256] In some examples, including any of the foregoing, the method includes quantifying one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 and combinations thereof.

[00257] In some examples, including any of the foregoing, the method includes detecting a multiple-reaction-monitoring (MRM) transition selected from the group consisting of transitions 1 - 76 using a QQQ and/or a qTOF mass spectrometer.

[00258] In some examples, including any of the foregoing, the method includes training a machine learning algorithm to identify a classification based on the quantifying step.

[00259] In some examples, including any of the foregoing, the method includes using a machine learning algorithm to identify a classification based on the quantifying step.

[00260] In some examples, including any of the foregoing, the machine learning algorithm is selected from the group consisting of a deep learning algorithm, a neural network algorithm, an artificial neural network algorithm, a supervised machine learning algorithm, a linear discriminant analysis algorithm, a quadratic discriminant analysis algorithm, a support vector machine algorithm, a linear basis function kernel support vector algorithm, a radial basis function kernel support vector algorithm, a random forest algorithm, a genetic algorithm, a nearest neighbor algorithm, k-nearest neighbors, a naive Bayes classifier algorithm, a logistic regression algorithm, or a combination thereof.

D. METHODS FOR DIAGNOSING PATIENTS

[00261] In some examples, set forth herein is a method for diagnosing a patient having a disease or condition, comprising measuring by mass spectroscopy a glycopeptide in a sample from the patient.

[00262] In another embodiment, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ

and/or qTOF spectrometer to detect and quantify one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect and quantify one or more MRM transitions selected from transitions 1-76; inputting the quantification of the detected glycopeptides or the MRM transitions into a trained model to generate an output probability, determining if the output probability is above or below a threshold for a classification; and identifying a diagnostic classification for the patient based on whether the output probability is above or below a threshold for a classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification.

[00263] In another embodiment, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect and quantify one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect and quantify one or more MRM transitions selected from transitions 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76; inputting the quantification of the detected glycopeptides or the MRM transitions into a trained model to generate an output probability, determining if the output probability is above or below a threshold for a classification; and identifying a diagnostic classification for the patient based on whether the output probability is above or below a threshold for a classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification.

[00264] In another embodiment, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: inputting the quantification of detected glycopeptides or MRM transitions into a trained model to generate an output probability, determining if the output probability is above or below a threshold for a classification; and identifying a diagnostic classification for the patient based on whether the output probability is above or below a threshold for a classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification. In some examples, the method includes obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect and quantify one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76; or to detect and quantify one or more MRM transitions selected from transitions 1-76.

[00265] In some examples, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect one or more glycopeptides consisting or, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect one or more MRM transitions selected from transitions 1-76; analyzing the detected glycopeptides or the MRM transitions to identify a diagnostic classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification.

[00266] In some examples, set forth herein is a method for diagnosing a patient having ovarian cancer; the method comprising: analyzing detected or quantified glycopeptides or MRM transitions to identify a diagnostic classification; and diagnosing the patient as having ovarian cancer based on the diagnostic classification. In some examples, the method includes obtaining a biological sample from the patient; and performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect one or more glycopeptides consisting or, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect one or more MRM transitions selected from transitions 1-76.

[00267] In some examples, set forth herein is a method for diagnosing, monitoring, or classifying aging in an individual; the method comprising: obtaining a biological sample from the patient; performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect one or more glycopeptides consisting or, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect one or more MRM transitions selected from transitions 1-76; analyzing the detected glycopeptides or the MRM transitions to identify a diagnostic classification; and diagnosing, monitoring, or classifying the individual as having an aging classification based on the diagnostic classification.

E. DISEASES AND CONDITIONS

[00268] Set forth herein are biomarkers for diagnosing a variety of diseases and conditions.

[00269] In some examples, the diseases and conditions include cancer. In some examples, the diseases and conditions are not limited to cancer.

[00270] In some examples, the diseases and conditions include fibrosis. In some examples, the diseases and conditions are not limited to fibrosis.

[00271] In some examples, the diseases and conditions include an autoimmune disease. In some examples, the diseases and conditions are not limited to an autoimmune disease.

[00272] In some examples, the diseases and conditions include ovarian cancer. In some examples, the diseases and conditions are not limited to ovarian cancer.

[00273] In some examples, the condition is aging. In some examples, the “patient” described herein is equivalently described as an “individual.” For example, in some methods herein, set forth are biomarkers for monitoring or diagnosing aging or aging conditions in an individual. In some of these examples, the individual is not necessarily a patient who has a medical condition in need of therapy. In some examples, the individual is a male. In some examples, the individual is a female. In some examples, the individual is a male mammal. In some examples, the individual is a female mammal. In some examples, the individual is a male human. In some examples, the individual is a female human.

[00274] In some examples, the individual is 1 year old. In some examples, the individual is 2 years old. In some examples, the individual is 3 years old. In some examples, the individual is 4 years old. In some examples, the individual is 5 years old. In some examples, the individual is 6 years old. In some examples, the individual is 7 years old. In some examples, the individual is 8 years old. In some examples, the individual is 9 years old. In some examples, the individual is 10 years old. In some examples, the individual is 11 years old. In some examples, the individual is 12 years old. In some examples, the individual is 13 years old. In some examples, the individual is 14 years old. In some examples, the individual is 15 years old. In some examples, the individual is 16 years old. In some examples, the individual is 17 years old. In some examples, the individual is 18 years old. In some examples, the individual is 19 years old. In some examples, the individual is 20 years old. In some examples, the individual is 21 years old. In some examples, the individual is 22 years old. In some examples, the individual is 23 years old. In some examples, the individual is 24 years old. In some examples, the individual is 25 years old. In some examples, the individual is 26 years old. In some examples, the individual is 27 years old. In some examples, the individual is 28 years old. In some examples, the individual is 29 years old. In some examples, the individual is 30 years old. In some examples, the individual is 31 years old. In some examples, the individual is 32 years old. In some examples, the individual is 33 years old. In some examples, the individual is 34 years old. In some examples, the individual is 35 years old. In some examples, the individual is 36 years old. In some examples, the individual is 37 years old. In some examples, the individual is 38 years old. In some examples, the individual is 39 years old. In some examples, the individual is 40 years old. In some

[illegible]

V. MACHINE LEARNING

[00275] In some examples, including any of the foregoing, the methods herein include quantifying one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 using mass spectroscopy and/or liquid chromatography. In some examples, the quantification results are used as inputs in a trained model. In some examples, the quantification results are classified or categorized with a diagnostic algorithm based on the absolute amount, relative amount, and/or type of each glycan or glycopeptide quantified in the test sample, wherein the diagnostic algorithm is trained on corresponding values for each marker obtained from a population of individuals having known diseases or conditions. In some examples, the disease or condition is ovarian cancer.

[00276] In some examples, including any of the foregoing, set forth herein is a method for training a machine learning algorithm, comprising: providing a first data set of MRM transition signals indicative of a sample comprising a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; providing a second data set of MRM transition signals indicative of a control sample; and comparing the first data set with the second data set using a machine learning algorithm.

[00277] In some examples, including any of the foregoing, the method herein include using a sample comprising a glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76 is a sample from a patient having ovarian cancer.

[00278] In some examples, including any of the foregoing, the method herein include using a sample comprising a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76 is a sample from a patient having ovarian cancer.

[00279] In some examples, including any of the foregoing, the method herein include using a control sample, wherein the control sample is a sample from a patient not having ovarian cancer.

[00280] In some examples, including any of the foregoing, the method herein include using a sample comprising a glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76, which is a pooled sample from one or more patients having ovarian cancer.

[00281] In some examples, including any of the foregoing, the method herein include using a control sample, which is a pooled sample from one or more patients not having ovarian cancer.

[00282] In some examples, including any of the foregoing, the methods include generating machine learning models trained using mass spectrometry data (*e.g.*, MRM-MS transition signals) from patients having a disease or condition and patients not having a disease or condition. In some examples, the disease or condition is ovarian cancer. In some examples, the methods include optimizing the machine learning models by cross-validation with known standards or other samples. In some examples, the methods include qualifying the performance using the mass spectrometry data to form panels of glycans and glycopeptides with individual sensitivities and specificities. In certain examples, the methods include determining a confidence percent in relation to a diagnosis. In some examples, one to ten glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 may be useful for diagnosing a patient with ovarian cancer with a certain confidence percent. In some examples, ten to fifty glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 may be useful for diagnosing a patient with ovarian cancer with a higher confidence percent.

[00283] In some examples, including any of the foregoing, the methods include performing MRM-MS and/or LC-MS on a biological sample. In some examples, the methods include constructing, by a computing device, theoretical mass spectra data representing a plurality of mass spectra, wherein each of the plurality of mass spectra corresponds to one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In some examples, the methods include comparing, by the computing device, the mass spectra data with the theoretical mass spectra data to generate comparison data indicative of a similarity of each of the plurality of mass spectra to each of the plurality of theoretical target mass spectra associated with a corresponding glycopeptide of the plurality of glycopeptides.

[00284] In some examples, machine learning algorithms are used to determine, by the computing device and based on the MRM-MS data, a distribution of a plurality of characteristic ions in the plurality of mass spectra; and determining, by the computing device and based on the distribution, whether one or more of the plurality of characteristic ions is a glycopeptide ion.

[00285] In some examples, the methods herein include training a diagnostic algorithm. Herein, training the diagnostic algorithm may refer to supervised learning of a diagnostic algorithm on the basis of values for one or more glycopeptides consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. Training the diagnostic algorithm may refer to variable selection in a statistical model on the basis of values for one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. Training a diagnostic algorithm may for example include determining a weighting vector in feature space for each category, or determining a function or function parameters.

[00286] In some examples, including any of the foregoing, the machine learning algorithm is selected from the group consisting of a deep learning algorithm, a neural network algorithm, an artificial neural network algorithm, a supervised machine learning algorithm, a linear discriminant analysis algorithm, a quadratic discriminant analysis algorithm, a support vector machine algorithm, a linear basis function kernel support vector algorithm, a radial basis function kernel support vector algorithm, a random forest algorithm, a genetic algorithm, a nearest neighbor algorithm, k-nearest neighbors, a naive Bayes classifier algorithm, a logistic regression algorithm, or a combination thereof. In certain examples, the machine learning algorithm is lasso regression.

[00287] In certain examples, the machine learning algorithm is LASSO, Ridge Regression, Random Forests, K-nearest Neighbors (KNN), Deep Neural Networks (DNN), and Principal Components Analysis (PCA). In certain examples, DNN's are used to process mass spec data into analysis-ready forms. In some examples, DNN's are used for peak picking from a mass spectra. In some examples, PCA is useful in feature detection.

[00288] In some examples, LASSO is used to provide feature selection.

[00289] In some examples, machine learning algorithms are used to quantify peptides from each protein that are representative of the protein abundance. In some examples, this quantification includes quantifying proteins for which glycosylation is not measured.

[00290] In some examples, glycopeptide sequences are identified by fragmentation in the mass spectrometer and database search using Byonic software.

[00291] In some examples, the methods herein include unsupervised learning to detect features of MRMS-MS data that represent known biological quantities, such as protein function or glycan motifs. In certain examples, these features are used as input for classifying by machine. In some examples, the classification is performed using LASSO, Ridge Regression, or Random Forest nature.

[00292] In some examples, the methods herein include mapping input data (*e.g.*, MRM transition peaks) to a value (*e.g.*, a scale based on 0-100) before processing the value in an algorithm. For example, after a MRM transition is identified and the peak characterized, the methods herein include assessing the MS scans in an *m/z* and retention time window around the peak for a given patient. In some examples, the resulting chromatogram is integrated by a machine learning algorithm that determines the peak start and stop points, and calculates the area bounded by those points and the intensity (height). The resulting integrated value is the abundance, which then feeds into machine learning and statistical analyses training and data sets.

[00293] In some examples, machine learning output, in one instance, is used as machine learning input in another instance. For example, in addition to the PCA being used for a classification process, the DNN data processing feeds into PCA and other analyses. This results in at least three levels of algorithmic processing. Other hierarchical structures are contemplated within the scope of the instant disclosure.

[00294] In some examples, including any of the foregoing, the methods include comparing the amount of each glycan or glycopeptide quantified in the sample to corresponding reference values for each glycan or glycopeptide in a diagnostic algorithm. In some examples, the methods includes a comparative process by which the amount of a glycan or glycopeptide quantified in the sample is compared to a reference value for the same glycan or glycopeptide using a diagnostic algorithm. The comparative process may be part of a classification by a diagnostic algorithm. The comparative process may occur at an abstract level, *e.g.*, in *n*-dimensional feature space or in a higher dimensional space.

[00295] In some examples, the methods herein include classifying a patient's sample based on the amount of each glycan or glycopeptide quantified in the sample with a diagnostic algorithm. In some examples, the methods include using statistical or machine learning classification processes by which the amount of a glycan or glycopeptide quantified in the test sample is used to determine a category of health with a diagnostic algorithm. In some examples, the diagnostic algorithm is a statistical or machine learning classification algorithm.

[00296] In some examples, including any of the foregoing, classification by a diagnostic algorithm may include scoring likelihood of a panel of glycan or glycopeptide values belonging to each possible category, and determining the highest-scoring category. Classification by a diagnostic algorithm may include comparing a panel of marker values to previous observations by means of a distance function. Examples of diagnostic algorithms

suitable for classification include random forests, support vector machines, logistic regression (e.g. multiclass or multinomial logistic regression, and/or algorithms adapted for sparse logistic regression). A wide variety of other diagnostic algorithms that are suitable for classification may be used, as known to a person skilled in the art.

[00297] In some examples, the methods herein include supervised learning of a diagnostic algorithm on the basis of values for each glycan or glycopeptide obtained from a population of individuals having a disease or condition (e.g., ovarian cancer). In some examples, the methods include variable selection in a statistical model on the basis of values for each glycan or glycopeptide obtained from a population of individuals having ovarian cancer. Training a diagnostic algorithm may for example include determining a weighting vector in feature space for each category, or determining a function or function parameters.

[00298] In one embodiment, the reference value is the amount of a glycan or glycopeptide in a sample or samples derived from one individual. Alternatively, the reference value may be derived by pooling data obtained from multiple individuals, and calculating an average (for example, mean or median) amount for a glycan or glycopeptide. Thus, the reference value may reflect the average amount of a glycan or glycopeptide in multiple individuals. Said amounts may be expressed in absolute or relative terms, in the same manner as described herein.

[00299] In some examples, the reference value may be derived from the same sample as the sample that is being tested, thus allowing for an appropriate comparison between the two. For example, if the sample is derived from urine, the reference value is also derived from urine. In some examples, if the sample is a blood sample (e.g. a plasma or a serum sample), then the reference value will also be a blood sample (e.g. a plasma sample or a serum sample, as appropriate). When comparing between the sample and the reference value, the way in which the amounts are expressed is matched between the sample and the reference value. Thus, an absolute amount can be compared with an absolute amount, and a relative amount can be compared with a relative amount. Similarly, the way in which the amounts are expressed for classification with the diagnostic algorithm is matched to the way in which the amounts are expressed for training the diagnostic algorithm.

[00300] When the amounts of the glycan or glycopeptide are determined, the method may comprise comparing the amount of each glycan or glycopeptide to its corresponding reference value. When the cumulative amount of one, some or all the glycan or glycopeptides are determined, the method may comprise comparing the cumulative amount to a corresponding reference value. When the amounts of the glycan or glycopeptides are

combined with each other in a formula to form an index value, the index value can be compared to a corresponding reference index value derived in the same manner.

[00301] The reference values may be obtained either within (*i.e.*, constituting a step of) or external to the (*i.e.*, not constituting a step of) methods described herein. In some examples, the methods include a step of establishing a reference value for the quantity of the markers. In other examples, the reference values are obtained externally to the method described herein and accessed during the comparison step of the invention.

[00302] In some examples, including any of the foregoing, training of a diagnostic algorithm may be obtained either within (*i.e.*, constituting a step of) or external to (*i.e.*, not constituting a step of) the methods set forth herein. In some examples, the methods include a step of training of a diagnostic algorithm. In some examples, the diagnostic algorithm is trained externally to the method herein and accessed during the classification step of the invention. The reference value may be determined by quantifying the amount of a glycan or glycopeptide in a sample obtained from a population of healthy individual(s). The diagnostic algorithm may be trained by quantifying the amount of a glycan or glycopeptide in a sample obtained from a population of healthy individual(s). As used herein, the term “healthy individual” refers to an individual or group of individuals who are in a healthy state, *e.g.*, patients who have not shown any symptoms of the disease, have not been diagnosed with the disease and/or are not likely to develop the disease. Preferably said healthy individual(s) is not on medication affecting the disease and has not been diagnosed with any other disease. The one or more healthy individuals may have a similar sex, age and body mass index (BMI) as compared with the test individual. The reference value may be determined by quantifying the amount of a glycan or glycopeptide in a sample obtained from a population of individual(s) suffering from the disease. The diagnostic algorithm may be trained by quantifying the amount of a marker in a sample obtained from a population of individual(s) suffering from the disease. More preferably such individual(s) may have similar sex, age and body mass index (BMI) as compared with the test individual. The reference value may be obtained from a population of individuals suffering from ovarian cancer. The diagnostic algorithm may be trained by quantifying the amount of a glycan or glycopeptide in a sample obtained from a population of individuals suffering from ovarian cancer. Once the characteristic glycan or glycopeptide profile of ovarian cancer is determined, the profile of markers from a biological sample obtained from an individual may be compared to this reference profile to determine whether the test subject also has ovarian cancer. Once the diagnostic algorithm is trained to classify ovarian cancer, the profile of markers from a

biological sample obtained from an individual may be classified by the diagnostic algorithm to determine whether the test subject is also at that particular stage of ovarian cancer.

VI. Kits

[00303] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00304] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00305] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[00306] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.

[00307] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00308] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.

[00309] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00310] In some examples, including any of the foregoing, set forth herein is a kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00311] In some examples, including any of the foregoing, set forth herein is a kit for diagnosing or monitoring cancer in an individual wherein the glycan or glycopeptide profile of a sample from said individual is determined and the measured profile is compared with a profile of a normal patient or a profile of a patient with a family history of cancer. In some examples, the kit comprises one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In some examples, the kit comprises one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In some examples, the kit comprises one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In some examples, the kit comprises one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In some examples, the kit comprises one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75. In some examples, the kit comprises one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75. In some examples, the kit comprises one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof. In some examples, the kit comprises one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00312] In some examples, including any of the foregoing, set forth herein is a kit comprising the reagents for quantification of the oxidised, nitrated, and/or glycated free adducts derived from glycopeptides.

VII. Clinical Assays

[00313] In some examples, including any of the foregoing, the biomarkers, methods, and/or kits may be used in a clinical setting for diagnosing patients. In some of these examples, the analysis of samples includes the use of internal standards. These standards may include one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. These standards may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In certain examples, these standards may include one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In certain examples, these standards may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In certain examples, these standards may include one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof. In certain examples, these standards may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof. In certain examples, these standards may include one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof. In certain examples, these standards may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00314] In a clinical setting, samples may be prepared (*e.g.*, by digestion) to include one or more glycopeptides consisting of an amino acid sequence selected from the group

consisting of SEQ ID NOs: 1 - 76. In certain examples, these samples may include one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In certain examples, these samples may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof. In certain examples, these samples may include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00315] In a clinical setting, samples may be prepared (*e.g.*, by digestion) to include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76. In certain examples, the samples may be prepared (*e.g.*, by digestion) to include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof. In certain examples, the samples may be prepared (*e.g.*, by digestion) to include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof. In certain examples, the samples may be prepared (*e.g.*, by digestion) to include one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

[00316] In some examples, the amount of a glycan or glycopeptide may be assessed by comparing the amount of one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 to the concentration of another biomarker.

[00317] In some examples, the amount of a glycan or glycopeptide may be assessed by comparing the amount of one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 to the concentration of another biomarker.

[00318] In some examples, the amount of a glycan or glycopeptide may be assessed by comparing the amount of one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 to the amount of one or more glycopeptides consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00319] In some examples, the amount of a glycan or glycopeptide may be assessed by comparing the amount of one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 to the amount of one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00320] In some examples, including any of the foregoing, the kit may include software for computing the normalization of a glycopeptide MRM transition signal.

[00321] In some examples, including any of the foregoing, the kit may include software for quantifying the amount of a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00322] In some examples, including any of the foregoing, the kit may include software for quantifying the relative amount of a glycopeptide consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.

[00323] In some examples, including any of the foregoing, a trained model is stored on a server which is accessed by a clinician performing a method, set forth herein. In some examples, the clinician inputs the quantification of the MRM transition signals from a patient's sample into a trained model which are stored on a server. In some examples, the server is accessed by the internet, wireless communication, or other digital or telecommunication methods.

[00324] In some examples, including any of the foregoing, a trained model is stored on a server which is accessed by a clinician performing a method, set forth herein. In some examples, the clinician inputs the quantification of the glycopeptide or glycopeptides consisting of, or consisting essentially of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 from a patient's sample into a trained model which are stored on a server. In some examples, the server is accessed by the internet, wireless communication, or other digital or telecommunication methods.

[00325] In some examples, including any of the foregoing, MRM transition signals 1-76 are stored on a server which is accessed by a clinician performing a method, set forth

herein. In some examples, the clinician compares the MRM transition signals from a patient's sample to the MRM transition signals 1-76 which are stored on a server. In some examples, the server is accessed by the internet, wireless communication, or other digital or telecommunication methods.

[00326] In some examples, including any of the foregoing, a machine learning algorithm, which has been trained using the MRM transition signals 1-76, described herein, is stored on a server which is accessed by a clinician performing a method, set forth herein. In some examples, the machine learning algorithm, accessed remotely on a server, analyzes the MRM transition signals from a patient's sample. In some examples, the server is accessed by the internet, wireless communication, or other digital or telecommunication methods.

VIII. EXAMPLES

[00327] Chemicals and Reagents. Glycoprotein standards purified from human serum/plasma were purchased from Sigma-Aldrich (St. Louis, MO). Sequencing grade trypsin was purchased from Promega (Madison, WI). Dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Sigma-Aldrich (St. Louis, MO). Human serum was purchased from Sigma-Aldrich (St. Louis, MO).

[00328] Sample Preparation. Serum samples and glycoprotein standards were reduced, alkylated and then digested with trypsin in a water bath at 37 °C for 18 hours.

[00329] LC-MS/MS Analysis. For quantitative analysis, tryptic digested serum samples were injected into an high performance liquid chromatography (HPLC) system coupled to triple quadrupole (QqQ) mass spectrometer. The separation was conducted on a reverse phase column. Solvents A and B used in the binary gradient were composed of mixtures of water, acetonitrile and formic acid. Typical positive ionization source parameters were utilized after source tuning with vendor supplied standards. The following ranges were evaluated: source spray voltage between 3-5 kV, temperature 250-350 °C, and nitrogen sheath gas flow rate 20-40 psi. The scan mode of instrument used was dMRM.

[00330] For the glycoproteomic analysis, enriched serum glycopeptides were analyzed with a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass spectrometer or an Agilent 6495B Triple Quadrupole LC/MS.

[00331] MRM Mass Spectroscopy settings, sample preparation, and reagents are set forth in Li, *et al.*, Site-Specific Glycosylation Quantification of 50 serum Glycoproteins Enhanced by Predictive Glycopeptidomics for Improved Disease Biomarker Discovery, *Anal. Chem.* 2019, 91, 5433-5445; DOI: 10.1021/acs.analchem.9b00776, the entire contents of which are herein incorporated by reference in its entirety for all purposes.

Example 1 – Identifying Glycopeptide Biomarkers

[00332] This Example refers to Figures 15 and 17-18.

[00333] As shown in Figure 15, in step 1, samples from patients having ovarian cancer and samples from patients not having ovarian cancer were provided. In step 2, the samples were digested using protease enzymes to form glycopeptide fragments. In step 3, the glycopeptide fragments were introduced into a tandem LC-MS/MS instrument to analyze the retention time and MRM-MS transition signals associated with the aforementioned samples. In step 4, glycopeptides and glycan biomarkers were identified. Machine learning algorithms selected MRM-MS transition signals from a series of MS spectra and associated those signals with the calculated mass of certain glycopeptide fragments. See Figures 17-18 for retention times analysis for biomarker signals identified by machine learning algorithms.

[00334] In step 5, the glycopeptides identified in samples from patients having ovarian cancer were compared using machine learning algorithms, including lasso regression, with the glycopeptides identified in samples from patients not having ovarian cancer. This comparison included a comparison of the types, absolute amounts, and relative amounts of glycopeptides. From this comparison, normalization of peptides, and relative abundance of glycopeptides was calculated. See Figure 18 for output results of this comparison.

Example 2 – Identifying Glycopeptide Biomarkers

[00335] This Example refers to Figure 16.

[00336] As shown in Figure 16, in step 1, samples from patients are provided. In step 2, the samples were digested using protease enzymes to form glycopeptide fragments. In step 3, the glycopeptide fragments were introduced into a tandem LC-MS/MS instrument to analyze the retention time and MRM-MS transition signals associated with the sample. In step 4, the glycopeptides were identified using machine learning algorithms which select MRM-MS transition signals and associate those signals with the calculated mass of certain glycopeptide fragments. In step 5, the data is normalized. In step 6, machine learning is used to analyzed the normalized data to identify biomarkers indicative of a patient having ovarian cancer.

IX. TABLES**Table 1. Transition Numbers for Glycopeptides from Glycopeptide Groups.**

Transition No.	Compound Group	Compound Name
1	GP001-P01009 Alpha-1-antitrypsin A1AT	A1AT-GP001_271_5402

Transition No.	Compound Group	Compound Name
2	GP001-P01009 Alpha-1-antitrypsin A1AT	A1AT-GP001_271_5412
3	GP001-P01009 Alpha-1-antitrypsin A1AT	A1AT-GP001_271MC_5402
4	GP002-P04217 Alpha-1B-glycoprotein A1BG	A1BG-GP002_179_5421/5402
5	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_1424_5402
6	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_1424_5411
7	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_55_5401
8	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_55_5402
9	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_55_5411
10	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_869_5200
11	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_869_5401
12	GP004-P01023 Alpha-2-macroglobulin A2MG	A2MG-GP004_869_6301
13	GP006-P43652 Afamin AFAM	AFAM-GP006_33_5402
14	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_6503
15	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_6513
16	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_7601
17	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_7602
18	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_7603
19	GP007&008-P02763&P19652 Alpha-1-acid glycoprotein 1&2 AGP12	AGP12-GP007&008_72MC_7613
20	GP007-P02763 Alpha-1-acid glycoprotein 1 AGP1	AGP1-GP007_33_5402
21	GP007-P02763 Alpha-1-acid glycoprotein 1 AGP1	AGP1-GP007_93_6503/6522
22	GP007-P02763 Alpha-1-acid glycoprotein 1 AGP1	AGP1-GP007_93_6513
23	GP007-P02763 Alpha-1-acid glycoprotein 1 AGP1	AGP1-GP007_93_7603/7622
24	GP007-P02763 Alpha-1-acid glycoprotein 1 AGP1	AGP1-GP007_93_7613
25	GP012-P02656 Apolipoprotein C-III APOC3	APOC3-GP012_74_1102
26	GP012-P02656 Apolipoprotein C-III APOC3	APOC3-GP012_74MC_1102

Transition No.	Compound Group	Compound Name
27	GP015-P02749 Beta-2-glycoprotein1 APOH	APOH-GP015_253_5401
28	GP023-P00450 Ceruloplasmin CERU	CERU-GP023_138_5412
29	GP023-P00450 Ceruloplasmin CERU	CERU-GP023_138_5421/5402
30	GP023-P00450 Ceruloplasmin CERU	CERU-GP023_138_6503/6522
31	GP024-P08603 ComplementFactorH CFAH	CFAH-GP024_882_5421/5402
32	GP028-P01024 ComplementC3 CO3	CO3-GP028_85_5200
33	GP028-P01024 ComplementC3 CO3	CO3-GP028_85_6200
34	GP029&030-P0C0L4&P0C0L5 ComplementC4-A&B CO4A&CO4B	CO4A&CO4B-GP029&030_1328_5402
35	GP036-P02765 Alpha-2-HS-glycoprotein FETUA	FETUA-GP036_156_5402/5421
36	GP036-P02765 Alpha-2-HS-glycoprotein FETUA	FETUA-GP036_176_6513
37	GP036-P02765 Alpha-2-HS-glycoprotein FETUA	FETUA-GP036_346_1101
38	GP042-P02790 Hemopexin HEMO	HEMO-GP042_187_5412/5431
39	GP042-P02790 Hemopexin HEMO	HEMO-GP042_453_5420/5401
40	GP044-P00738 Haptoglobin HPT	HPT-GP044_184_6502
41	GP044-P00738 Haptoglobin HPT	HPT-GP044_207_10803
42	GP044-P00738 Haptoglobin HPT	HPT-GP044_207_10804
43	GP044-P00738 Haptoglobin HPT	HPT-GP044_207_11904
44	GP044-P00738 Haptoglobin HPT	HPT-GP044_207_11914
45	GP044-P00738 Haptoglobin HPT	HPT-GP044_207_11915
46	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_5401/5420
47	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_5412
48	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_6501
49	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_6502
50	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_6511
51	GP044-P00738 Haptoglobin HPT	HPT-GP044_241_6513
52	GP046&047-P01876&P01877 Immunoglobulin heavy constant alpha 1&2 IgA12	IgA12-GP046&047_144_5501
53	GP047-P01877 Immunoglobulin heavy constant alpha 2 IgA2	IgA2-GP047_205_4510
54	GP047-P01877 Immunoglobulin heavy constant alpha 2 IgA2	IgA2-GP047_205_5412
55	GP047-P01877 Immunoglobulin heavy constant alpha 2 IgA2	IgA2-GP047_205_5510
56	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_3410
57	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_4400
58	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_4510

Transition No.	Compound Group	Compound Name
59	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_5400
60	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_5410
61	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_5411
62	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_5510
63	GP048-P01857 Immunoglobulin heavy constant gamma 1 IgG1	IgG1-GP048_297_nonglycosylated
64	GP049-P01859 Immunoglobulin heavy constant gamma 2 IgG2	IgG2-GP049_297_3510
65	GP049-P01859 Immunoglobulin heavy constant gamma 2 IgG2	IgG2-GP049_297_4411
66	GP049-P01859 Immunoglobulin heavy constant gamma 2 IgG2	IgG2-GP049_297_4510
67	GP052-P01591 Immunoglobulin J chain Ig-J	IgJ-GP052_71_5401
68	GP053-P01871 Immunoglobulin heavy constant mu IgM	IgM-GP053_439_6200
69	GP053-P01871 Immunoglobulin heavy constant mu IgM	IgM-GP053_46_4311
70	GP057-P01042 Kininogen-1 KNG1	KNG1-GP057_205_6503
71	GP064-P02787 Serotransferrin TRFE	TRFE-GP064_432_5402
72	GP064-P02787 Serotransferrin TRFE	TRFE-GP064_630_5401
73	GP064-P02787 Serotransferrin TRFE	TRFE-GP064_630_6513
74	GP067-P04004 Vitronectin VTNC	VTNC-GP067_169_5401
75	GP067-P04004 Vitronectin VTNC	VTNC-GP067_242_6503
76	GP067-P04004 Vitronectin VTNC	VTNC-GP067_86_5421

Table 2. Transition Numbers with Precursor Ion and Product Ion (m/z)

Transition No.	Precursor Ion	Product Ion
1	991.2	366.1
2	1027.7	366.1
3	1149.9	366.1
4	1209.5	366.1
5	1093.2	366.1
6	1057	366.1
7	1079	366.1
8	1151.7	366.1
9	1115.4	366.1
10	1158.8	1206.9
11	1066.7	366.1
12	1322.3	366.1
13	1134.1	366.1

Transition No.	Precursor Ion	Product Ion
14	1152.7	366.1
15	1181.9	366.1
16	1109.1	366.1
17	1167.3	366.1
18	1225.5	366.1
19	1254.7	366.1
20	1196.5	366.1
21	1195.3	366.1
22	1231.8	274.1
23	1286.6	366.1
24	1323.1	366.1
25	1028.8	274.1
26	1109.8	274.1
27	1055.8	366.1
28	1062.2	366.1
29	1367.2	366.1
30	1189.5	366.1
31	1057.7	366.1
32	1157.9	204.1
33	1211.9	366.1
34	1103.8	366.1
35	995.4	366.1
36	1343.8	366.1
37	891.8	274.1
38	1253.2	366.1
39	1217.9	366.1
40	1051	366.1
41	1116.4	366.1
42	1174.6	366.1
43	1247.7	366.1
44	1276.9	366.1
45	1335.1	366.1
46	1237.3	366.1
47	1383	366.1
48	1019.5	366.1
49	1092.3	366.1
50	1055.7	366.1
51	1201.5	366.1
52	1017.3	366.1
53	923.5	366.1
54	1103.8	366.1
55	977.8	366.1
56	879	204.1
57	884.4	204.1
58	1000.7	204.1
59	938.4	366.1
60	987.1	366.1

Transition No.	Precursor Ion	Product Ion
61	1084.1	366.1
62	1054.7	366.1
63	595.3	640.3
64	935.8	204.1
65	1019.4	204.1
66	989.9	204.1
67	1048.1	366.1
68	1248.5	1284.7
69	993.8	366.1
70	1069.9	366.1
71	921.4	366.1
72	1108.4	366.1
73	1105.6	366.1
74	942.4	366.1
75	1409.1	366.1
76	1148	366.1

MS1 and MS2 resolution was 1 unit.

Table 3. Transition Numbers with Retention Time, Δ Retention Time, Fragmentor and Collision Energy

Transition No.	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy
1	29.8	3	380	24
2	29.8	3	380	25
3	42.5	4	380	28
4	24	2	380	36
5	43.7	3	380	22
6	43.1	3	380	22
7	39.8	3	380	22
8	41.1	3	380	23
9	39.3	3	380	22
10	25.3	2	380	23
11	26	2	380	22
12	26.2	2	380	24
13	9.3	1	380	30
14	38.2	3	380	28
15	38.2	3	380	29
16	37.3	3	380	30
17	36.5	3	380	29
18	38	3	380	31
19	37.8	3	380	31
20	29	2	380	30
21	17.6	1.2	380	30
22	17.6	1.2	380	31
23	17.5	1.2	380	32
24	17.5	1.2	380	33

Transition No.	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy
25	30.2	2	380	25
26	29.3	2	380	27
27	14.4	2	380	33
28	13	2	380	33
29	13.2	2	380	40
30	13.5	2	380	36
31	12.4	1.2	380	33
32	19.6	1.2	380	30
33	19.5	1.2	380	30
34	13.6	1.2	380	34
35	20.1	1.2	380	24
36	22	1.2	380	34
37	17.1	2	380	21
38	16.2	1.2	380	37
39	21.4	1.2	380	37
40	24.2	1.4	380	26
41	10.8	1.2	380	27
42	11	1.2	380	29
43	10.7	1.2	380	31
44	10.9	1.4	380	32
45	10.9	1.4	380	34
46	20.7	1.4	380	31
47	21.6	1.4	380	35
48	20.6	1.2	380	25
49	21.5	1.3	380	27
50	20.6	1.2	380	30
51	22.2	1.2	380	30
52	38.1	2	380	25
53	10.2	1.2	380	22
54	11.3	1.2	380	27
55	10.2	1.2	380	24
56	5.6	1	380	21
57	5.6	1	380	21
58	5.8	1.2	380	24
59	5.5	1	380	22
60	5.5	1	380	24
61	6	1	380	27
62	5.7	1	380	26
63	15.6	1.2	380	13
64	10.7	1.2	380	22
65	11.3	1	380	25
66	10.6	1	380	24
67	12.4	1	380	26
68	22.2	1.2	380	25
69	4.3	1.2	380	24
70	13.3	2	380	33

Transition No.	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy
71	19.7	1.2	380	22
72	22.5	1.2	380	27
73	24.2	1.2	380	27
74	18.4	1.2	380	30
75	29.3	3	380	41
76	14.5	2	380	35

Cell accelerator voltage was 5.

Table 4. Glycan Residue Compound Numbers, Molecular Mass, and Glycan Fragment mass-to-charge (m/z) (+2) & (m/z) (+3) ratios

Composition	mass	m/z (+2)	m/z (+3)
3200	910.327	456.1708	304.449633
3210	1056.386	529.2003	353.135967
3300	1113.407	557.7108	372.142967
3310	1259.465	630.7398	420.828967
3320	1405.523	703.7688	469.514967
3400	1316.487	659.2508	439.8363
3410	1462.544	732.2793	488.521967
3420	1608.602	805.3083	537.207967
3500	1519.566	760.7903	507.5293
3510	1665.624	833.8193	556.2153
3520	1811.682	906.8483	604.9013
3600	1722.645	862.3298	575.2223
3610	1868.703	935.3588	623.9083
3620	2014.761	1008.3878	672.5943
3630	2160.89	1081.4523	721.303967
3700	1925.724642	963.869621	642.915514
3710	2071.782551	1036.898576	691.601484
3720	2217.84046	1109.92753	740.287453
3730	2363.898369	1182.956485	788.973423
3740	2509.956277	1255.985439	837.659392
4200	1072.380603	537.1976015	358.467501
4210	1218.438512	610.226556	407.153471
4300	1275.459976	638.737288	426.160625
4301	1566.555392	784.284996	523.192431
4310	1421.517884	711.766242	474.846595
4311	1712.613301	857.3139505	571.8784
4320	1567.575793	784.7951965	523.532564
4400	1478.539348	740.276974	493.853749
4401	1769.634765	885.8246825	590.885555
4410	1624.597257	813.3059285	542.539719
4411	1915.692673	958.8536365	639.571524
4420	1770.655166	886.334883	591.225689
4421	2061.750582	1031.882591	688.257494
4430	1916.713074	959.363837	639.911658

Composition	mass	m/z (+2)	m/z (+3)
4431	2207.808491	1104.911546	736.943464
4500	1681.618721	841.8166605	561.546874
4501		1.0073	1.0073
4510	1972.714137	987.3643685	658.578679
4511	2118.772046	1060.393323	707.264649
4520	1973.734538	987.874569	658.918813
4521	2264.829955	1133.422278	755.950618
4530	2119.792447	1060.903524	707.604782
4531	2410.887864	1206.451232	804.636588
4540	2265.850356	1133.932478	756.290752
4541	2556.945772	1279.480186	853.322557
4600	1884.698093	943.3563465	629.239998
4601	2175.79351	1088.904055	726.271803
4610	2030.756002	1016.385301	677.925967
4611	2321.851418	1161.933009	774.957773
4620	2176.813911	1089.414256	726.611937
4621	2467.909327	1234.961964	823.643742
4630	2322.87182	1162.44321	775.297907
4631	2613.967236	1307.990918	872.329712
4641	2760.025145	1381.019873	921.015682
4650	2614.987637	1308.501119	872.669846
4700	2087.777466	1044.896033	696.933122
4701	2378.872882	1190.443741	793.964927
4710	2233.835374	1117.924987	745.619091
4711	2524.930791	1263.472696	842.650897
4720	2379.893283	1190.953942	794.305061
4730	2525.951192	1263.982896	842.991031
5200	1234.433426	618.224013	412.485109
5210	1380.491335	691.2529675	461.171078
5300	1437.512799	719.7636995	480.178233
5301	1728.608215	865.3114075	577.210038
5310	1583.570708	792.792654	528.864203
5311	1874.666124	938.340362	625.896008
5320	1729.628617	865.8216085	577.550172
5400	1640.592171	821.3033855	547.871357
5401	1931.687588	966.851094	644.903163
5402	2222.783005	1112.398803	741.934968
5410	1786.65008	894.33234	596.557327
5411	2077.745497	1039.880049	693.589132
5412	2368.840913	1185.427757	790.620938
5420	1932.707989	967.3612945	645.243296
5421	2223.803406	1112.909003	742.275102
5430	2078.765898	1040.390249	693.929266
5431	2369.861314	1185.937957	790.961071
5432	2660.956731	1331.485666	887.992877
5500	1843.671544	922.843072	615.564481
5501	2134.766961	1068.390781	712.596287

Composition	mass	m/z (+2)	m/z (+3)
5502	2425.862377	1213.938489	809.628092
5510	1989.729453	995.8720265	664.250451
5511	2280.824869	1141.419735	761.282256
5512	2571.920286	1286.967443	858.314062
5520	2135.787362	1068.900981	712.936421
5521	2426.882778	1214.448689	809.968226
5522	2717.978195	1359.996398	907.000032
5530	2281.84527	1141.929935	761.62239
5531	2572.940687	1287.477644	858.654196
5541	2718.998596	1360.506598	907.340165
5600	2046.750917	1024.382759	683.257606
5601	2337.846333	1169.930467	780.289411
5602	2628.94175	1315.478175	877.321217
5610	2192.808825	1097.411713	731.943575
5611	2483.904242	1242.959421	828.975381
5612	2774.999658	1388.507129	926.007186
5620	2338.866734	1170.440667	780.629545
5621	2629.962151	1315.988376	877.66135
5631	2776.020059	1389.01733	926.34732
5650	2777.040461	1389.527531	926.687454
5700	2249.830289	1125.922445	750.95073
5701	2540.925706	1271.470153	847.982535
5702	2832.021122	1417.017861	945.014341
5710	2395.888198	1198.951399	799.636699
5711	2686.983614	1344.499107	896.668505
5712	2978.079031	1490.046816	993.70031
5720	2541.946107	1271.980354	848.322669
5721	2833.041523	1417.528062	945.354474
5730	2688.004016	1345.009308	897.008639
5731	2979.099432	1490.557016	994.040444
6200	1396.48625	699.250425	466.502717
6210	1542.544159	772.2793795	515.188686
6300	1599.565622	800.790111	534.195841
6301	1890.661039	946.3378195	631.227646
6310	1745.623531	873.8190655	582.88181
6311	2036.718948	1019.366774	679.913616
6320	1891.68144	946.84802	631.56778
6400	1802.644995	902.3297975	601.888965
6401	2093.740411	1047.877506	698.92077
6402	2384.835828	1193.425214	795.952576
6410	1948.702904	975.358752	650.574935
6411	2239.79832	1120.90646	747.60674
6412	2530.893737	1266.454169	844.638546
6420	2094.760813	1048.387707	699.260904
6421	2385.856229	1193.935415	796.29271
6432	2823.009554	1412.512077	942.010485
6500	2005.724367	1003.869484	669.582089

Composition	mass	m/z (+2)	m/z (+3)
6501	2296.819784	1149.417192	766.613895
6502	2587.9152	1294.9649	863.6457
6503	2879.010617	1440.512609	960.677506
6510	2151.782276	1076.898438	718.268059
6511	2442.877693	1222.446147	815.299864
6512	2733.973109	1367.993855	912.33167
6513	3025.068526	1513.541563	1009.36348
6520	2297.840185	1149.927393	766.954028
6521	2588.935602	1295.475101	863.985834
6522	2880.031018	1441.022809	961.017639
6530	2443.898094	1222.956347	815.639998
6531	2734.99351	1368.504055	912.671803
6532	3026.088927	1514.051764	1009.70361
6540	2589.956003	1295.985302	864.325968
6541	2881.051419	1441.53301	961.357773
6600	2208.80374	1105.40917	737.275213
6601	2499.899157	1250.956879	834.307019
6602	2790.994573	1396.504587	931.338824
6603	3082.08999	1542.052295	1028.37063
6610	2354.861649	1178.438125	785.961183
6611	2645.957065	1323.985833	882.992988
6612	2937.052482	1469.533541	980.024794
6613	3228.147898	1615.081249	1077.0566
6620	2500.919558	1251.467079	834.647153
6621	2792.014974	1397.014787	931.678958
6622	3083.110391	1542.562496	1028.71076
6623	3374.205807	1688.110204	1125.74257
6630	2646.977466	1324.496033	883.333122
6631	2938.072883	1470.043742	980.364928
6632	3229.168299	1615.59145	1077.39673
6640	2793.035375	1397.524988	932.019092
6641	3084.130792	1543.072696	1029.0509
6642	3375.226208	1688.620404	1126.0827
6652	3521.284117	1761.649359	1174.76867
6700	2411.883113	1206.948857	804.968338
6701	2702.978529	1352.496565	902.000143
6703	3285.169362	1643.591981	1096.06375
6710	2557.941021	1279.977811	853.654307
6711	2849.036438	1425.525519	950.686113
6711	2849.036438	1425.525519	950.686113
6712	3140.131854	1571.073227	1047.71792
6713	3431.227271	1716.620936	1144.74972
6713	3431.227271	1716.620936	1144.74972
6720	2703.99893	1353.006765	902.340277
6721	2995.094347	1498.554474	999.372082
6721	2995.094347	1498.554474	999.372082
6730	2850.056839	1426.03572	951.026246

Composition	mass	m/z (+2)	m/z (+3)
6731	3141.152255	1571.583428	1048.05805
6740	2996.114748	1499.064674	999.712216
7200	1558.539073	780.2768365	520.520324
7210	1704.596982	853.305791	569.206294
7400	1964.697818	983.356209	655.906573
7401	2255.793235	1128.903918	752.938378
7410	2110.755727	1056.385164	704.592542
7411	2401.851144	1201.932872	801.624348
7412	2692.94656	1347.48058	898.656153
7420	2256.813636	1129.414118	753.278512
7421	2547.909052	1274.961826	850.310317
7430	2402.871545	1202.443073	801.964482
7431	2693.966961	1347.990781	898.996287
7432	2985.062378	1493.538489	996.028093
7500	2167.777191	1084.895896	723.599697
7501	2458.872607	1230.443604	820.631502
7510	2313.8351	1157.92485	772.285667
7511	2604.930516	1303.472558	869.317472
7512	2896.025933	1449.020267	966.349278
7600	2370.856563	1186.435582	791.292821
7601	2661.95198	1331.98329	888.324627
7602	2953.047396	1477.530998	985.356432
7603	3244.142813	1623.078707	1082.38824
7604	3535.23823	1768.626415	1179.42004
7610	2516.914472	1259.464536	839.978791
7611	2808.009889	1405.012245	937.010596
7612	3099.105305	1550.559953	1034.0424
7613	3390.200722	1696.107661	1131.07421
7614	3681.296138	1841.655369	1228.10601
7620	2662.972381	1332.493491	888.66476
7621	2954.067798	1478.041199	985.696566
7622	3245.163214	1623.588907	1082.72837
7623	3536.258631	1769.136616	1179.76018
7632	3391.221123	1696.617862	1131.41434
7640	2955.088199	1478.5514	986.0367
7700	2573.935936	1287.975268	858.985945
7701	2865.031352	1433.522976	956.017751
7702	3156.126769	1579.070685	1053.04956
7703	3447.222186	1724.618393	1150.08136
7710	2719.993845	1361.004223	907.671915
7711	3011.089261	1506.551931	1004.70372
7712	3302.184678	1652.099639	1101.73553
7713	3593.280094	1797.647347	1198.76733
7714	3884.375511	1943.195056	1295.79914
7720	2866.051754	1434.033177	956.357885
7721	3157.14717	1579.580885	1053.38969
7722	3448.242587	1725.128594	1150.4215

Composition	mass	m/z (+2)	m/z (+3)
7730	3012.109662	1507.062131	1005.04385
7731	3303.205079	1652.60984	1102.07566
7732	3594.300495	1798.157548	1199.10747
7740	3158.167571	1580.091086	1053.72982
7741	3449.262988	1725.638794	1150.76163
7751	3595.320897	1798.667749	1199.4476
8200	1720.591897	861.3032485	574.537932
9200	1882.64472	942.32966	628.55554
9210	2028.702629	1015.358615	677.24151
10200	2044.697544	1023.356072	682.573148
11200	2206.750367	1104.382484	736.590756
12200	2368.80319	1185.408895	790.608363

Table 5. Glycan Residue Compound Numbers, Molecular Mass, and Classification

Compound	Glycan Mass	Glycan Composition	Class
3200	910.328	GlcNAc ₂ Man ₃	HM
3200			
3210	1056.386	GlcNAc ₂ Man ₃ Fuc ₁	HM-F
3210			
3300	1113.407	Hex ₃ HexNAc ₃	C
3300			
3310	1259.465	Hex ₃ HexNAc ₃ Fuc ₁	C-F
3310			
3320	1405.523	Hex ₃ HexNAc ₃ Fuc ₂	C-F
3400	1316.487	Hex ₃ HexNAc ₄	C
3410	1462.544	Hex ₃ HexNAc ₄ Fuc ₁	C-F
3410			
3420	1608.602	Hex ₃ HexNAc ₄ Fuc ₂	C-F
3500	1519.566	Hex ₃ HexNAc ₅	C
3510	1665.624	Hex ₃ HexNAc ₅ Fuc ₁	C-F
3520	1811.682	Hex ₃ HexNAc ₅ Fuc ₂	C-F
3600	1722.645	Hex ₃ HexNAc ₆	C
3610	1868.703	Hex ₃ HexNAc ₆ Fuc ₁	C-F
3620	2014.761	Hex ₃ HexNAc ₆ Fuc ₂	C-F
3630	2160.819	Hex ₃ HexNAc ₆ Fuc ₃	C-F
3700	1925.725	Hex ₃ HexNAc ₇	C
3710	2071.783	Hex ₃ HexNAc ₇ Fuc ₁	C-F
3720	2217.841	Hex ₃ HexNAc ₇ Fuc ₂	C-F
3720	2217.841	Hex ₃ HexNAc ₇ Fuc ₂	C-F
3730	2363.898	Hex ₃ HexNAc ₇ Fuc ₃	C-F
3740	2509.956	Hex ₃ HexNAc ₇ Fuc ₄	C-F
4200	1072.381	GlcNAc ₂ Man ₄	HM
4200			
4210	1218.438	GlcNAc ₂ Man ₄ Fuc ₁	HM-F

Compound	Glycan Mass	Glycan Composition	Class
4210			
4300	1275.460	Hex ₄ HexNAc ₃	C/H
4300			
4301	1566.555	Hex ₄ HexNAc ₃ Neu5Ac ₁	C-S
4301	1566.555	Hex ₄ HexNAc ₃ Neu5Ac ₁	C-S
4301			
4310	1421.518	Hex ₄ HexNAc ₃ Fuc ₁	C/H-F
4310	1566.555	Hex ₄ HexNAc ₃ Neu5Ac ₁	C-S
4310			
4311	1712.613	Hex ₄ HexNAc ₃ Fuc ₁ Neu5Ac ₁	C-FS
4311			
4320			
4400	1478.539	Hex ₄ HexNAc ₄	C/H
4400			
4401	1769.635	Hex ₄ HexNAc ₄ Neu5Ac ₁	C-S
4410	1624.597	Hex ₄ HexNAc ₄ Fuc ₁	C/H-F
4410			
4411	1915.693	Hex ₄ HexNAc ₄ Fuc ₁ Neu5Ac ₁	C-FS
4411			
4420	1770.655	Hex ₄ HexNAc ₄ Fuc ₂	C/H-F
4420			
4421	2061.751	Hex ₄ HexNAc ₄ Fuc ₂ Neu5Ac ₁	C-FS
4430	1916.713	Hex ₄ HexNAc ₄ Fuc ₃	C/H-F
4431	2207.808	Hex ₄ HexNAc ₄ Fuc ₃ Neu5Ac ₁	C-FS
4431	2207.808	Hex ₄ HexNAc ₄ Fuc ₃ Neu5Ac ₁	C-FS
4531	2410.888	Hex ₄ HexNAc ₅ Fuc ₃ Neu5Ac ₁	C-FS
4541	2556.946	Hex ₄ HexNAc ₅ Fuc ₄ Neu5Ac ₁	C-FS
4600	1884.698	Hex ₄ HexNAc ₆	C
4601	2175.794	Hex ₄ HexNAc ₆ Neu5Ac ₁	C-S
4610	2030.756	Hex ₄ HexNAc ₆ Fuc ₁	C-F
4611	2321.851	Hex ₄ HexNAc ₆ Fuc ₁ Neu5Ac ₁	C-FS
4620	2176.814	Hex ₄ HexNAc ₆ Fuc ₂	C-F
4621	2467.909	Hex ₄ HexNAc ₆ Fuc ₂ Neu5Ac ₁	C-FS
4630	2322.872	Hex ₄ HexNAc ₆ Fuc ₃	C-F
4641	2760.025	Hex ₄ HexNAc ₆ Fuc ₄ Neu5Ac ₁	C-FS
4650	2614.988	Hex ₄ HexNAc ₆ Fuc ₅	C-F
4700	2087.778	Hex ₄ HexNAc ₇	C
4701	2378.873	Hex ₄ HexNAc ₇ Neu5Ac ₁	C-S
4710	2233.835	Hex ₄ HexNAc ₇ Fuc ₁	C-F
4711	2524.931	Hex ₄ HexNAc ₇ Fuc ₁ Neu5Ac ₁	C-FS
4720	2379.893	Hex ₄ HexNAc ₇ Fuc ₂	C-F
4730	2525.951	Hex ₄ HexNAc ₇ Fuc ₃	C-F
5200			
5200			
5210	1380.491	GlcNAc ₂ Man ₅ Fuc ₁	HM-F
5300	1437.513	Hex ₅ HexNAc ₃	H
5300			

Compound	Glycan Mass	Glycan Composition	Class
5301	1728.608	Hex ₅ HexNAc ₃ Neu5Ac ₁	H-S
5301			
5310	1583.571	Hex ₅ HexNAc ₃ Fuc ₁	H-F
5310			
5311	1874.666	Hex ₅ HexNAc ₃ Fuc ₁ Neu5Ac ₁	H-FS
5311			
5320	1729.629	Hex ₅ HexNAc ₃ Fuc ₂	H-F
5320			
5400			
5401			
5401			
5402			
5410			
5411		Hex ₅ HexNAc ₄ Fuc ₁ Neu5Ac ₁	C-FS
5411			
5412			
5420			
5421			
5430			
5431	2369.861	Hex ₅ HexNAc ₄ Fuc ₃ Neu5Ac ₁	C/H-FS
5432	2660.957	Hex ₅ HexNAc ₄ Fuc ₃ Neu5Ac ₂	C-FS
5432	2660.957	Hex ₅ HexNAc ₄ Fuc ₃ Neu5Ac ₂	C-FS
5531	2572.941	Hex ₅ HexNAc ₅ Fuc ₃ Neu5Ac ₁	C/H-FS
5541	2718.999	Hex ₅ HexNAc ₅ Fuc ₄ Neu5Ac ₁	C-FS
5631	2776.020	Hex ₅ HexNAc ₆ Fuc ₃ Neu5Ac ₁	C-FS
5650	2777.040	Hex ₅ HexNAc ₆ Fuc ₅	C-F
5700	2249.830	Hex ₅ HexNAc ₇	C
5701	2540.926	Hex ₅ HexNAc ₇ Neu5Ac ₁	C-S
5702	2832.021	Hex ₅ HexNAc ₇ Neu5Ac ₂	C-S
5710	2395.888	Hex ₅ HexNAc ₇ Fuc ₁	C-F
5711	2686.984	Hex ₅ HexNAc ₇ Fuc ₁ Neu5Ac ₁	C-FS
5712	2978.079	Hex ₅ HexNAc ₇ Fuc ₁ Neu5Ac ₂	C-FS
5720	2541.946	Hex ₅ HexNAc ₇ Fuc ₂	C-F
5721	2833.042	Hex ₅ HexNAc ₇ Fuc ₂ Neu5Ac ₁	C-FS
5730	2688.004	Hex ₅ HexNAc ₇ Fuc ₃	C-F
5730	2688.004	Hex ₅ HexNAc ₇ Fuc ₃	C-F
5731	2979.099	Hex ₅ HexNAc ₇ Fuc ₃ Neu5Ac ₁	C-FS
6200			
6200			
6210	1542.544	GlcNAc ₂ Man ₆ Fuc ₁	HM-F
6300	1599.566	Hex ₆ HexNAc ₃	H
6300			
6301	1890.661	Hex ₆ HexNAc ₃ Neu5Ac ₁	H-S
6301			
6310	1745.623	Hex ₆ HexNAc ₃ Fuc ₁	H-F
6310			
6311	2036.719	Hex ₆ HexNAc ₃ Fuc ₁ Neu5Ac ₁	H-FS

Compound	Glycan Mass	Glycan Composition	Class
6311	2036.719	Hex ₆ HexNAc ₃ Fuc ₁ Neu5Ac ₁	H-FS
6311			
6320	1891.681	Hex ₆ HexNAc ₃ Fuc ₂	H-F
6400	1802.645	Hex ₆ HexNAc ₄	H
6401	2093.740	Hex ₆ HexNAc ₄ Neu5Ac ₁	H-S
6401			
6402	2384.836	Hex ₆ HexNAc ₄ Neu5Ac ₂	H-S
6410	1948.703	Hex ₆ HexNAc ₄ Fuc ₁	H-F
6410			
6411	2239.798	Hex ₆ HexNAc ₄ Fuc ₁ Neu5Ac ₁	H-FS
6421	2385.856	Hex ₆ HexNAc ₄ Fuc ₂ Neu5Ac ₁	H-FS
6432	2823.009	Hex ₆ HexNAc ₄ Fuc ₃ Neu5Ac ₂	H-FS
6500	2005.724	Hex ₆ HexNAc ₅	C/H
6500			
6501	2296.820	Hex ₆ HexNAc ₅ Neu5Ac ₁	C/H-S
6501			
6502	2587.915	Hex ₆ HexNAc ₅ Neu5Ac ₂	C/H-S
6503	2879.011	Hex ₆ HexNAc ₅ Neu5Ac ₃	C-S
6510	2151.782	Hex ₆ HexNAc ₅ Fuc ₁	C/H-F
6510			
6511	2442.878	Hex ₆ HexNAc ₅ Fuc ₁ Neu5Ac ₁	C/H-FS
6511	2442.878	Hex ₆ HexNAc ₅ Fuc ₁ Neu5Ac ₁	C/H-FS
6511			
6512	2733.973	Hex ₆ HexNAc ₅ Fuc ₁ Neu5Ac ₂	C/H-FS
6513	3025.068	Hex ₆ HexNAc ₅ Fuc ₁ Neu5Ac ₃	C-FS
6520			
6521	2588.936	Hex ₆ HexNAc ₅ Fuc ₂ Neu5Ac ₁	C/H-FS
6522	2880.031	Hex ₆ HexNAc ₅ Fuc ₂ Neu5Ac ₂	C/H-FS
6530	2443.898	Hex ₆ HexNAc ₅ Fuc ₃	C/H-F
6530	2879.011	Hex ₆ HexNAc ₅ Neu5Ac ₃	C-S
6531	2734.993	Hex ₆ HexNAc ₅ Fuc ₃ Neu5Ac ₁	C/H-FS
6532	3026.089	Hex ₆ HexNAc ₅ Fuc ₃ Neu5Ac ₂	C/H-FS
6603	3082.090	Hex ₆ HexNAc ₆ Neu5Ac ₃	C-S
6623	3374.206	Hex ₆ HexNAc ₆ Fuc ₂ Neu5Ac ₃	C-FS
6630	3082.090	Hex ₆ HexNAc ₆ Neu5Ac ₃	C-S
6631	2938.073	Hex ₆ HexNAc ₆ Fuc ₃ Neu5Ac ₁	C-FS
6632	3229.168	Hex ₆ HexNAc ₆ Fuc ₃ Neu5Ac ₂	C-FS
6641	3084.131	Hex ₆ HexNAc ₆ Fuc ₄ Neu5Ac ₁	C-FS
6642	3375.226	Hex ₆ HexNAc ₆ Fuc ₄ Neu5Ac ₂	C-FS
6652	3521.284	Hex ₆ HexNAc ₆ Fuc ₅ Neu5Ac ₂	C-FS
6713	3431.227	Hex ₆ HexNAc ₇ Fuc ₁ Neu5Ac ₃	C-FS
6731	3141.152	Hex ₆ HexNAc ₇ Fuc ₃ Neu5Ac ₁	C-FS
6740	2996.115	Hex ₆ HexNAc ₇ Fuc ₄	C-F
7200	1558.539	GlcNAc ₂ Man ₇	HM
7200			
7200			
7210	1704.597	GlcNAc ₂ Man ₇ Fuc ₁	HM-F

Compound	Glycan Mass	Glycan Composition	Class
7400	1964.698	Hex ₇ HexNAc ₄	H
7400			
7401	2255.793	Hex ₇ HexNAc ₄ Neu5Ac ₁	H-S
7410	2110.756	Hex ₇ HexNAc ₄ Fuc ₁	H-F
7411	2401.851	Hex ₇ HexNAc ₄ Fuc ₁ Neu5Ac ₁	H-FS
7412	2692.946	Hex ₇ HexNAc ₄ Fuc ₁ Neu5Ac ₂	H-FS
7420	2256.814	Hex ₇ HexNAc ₄ Fuc ₂	H-F
7421	2547.909	Hex ₇ HexNAc ₄ Fuc ₂ Neu5Ac ₁	H-FS
7430	2402.871	Hex ₇ HexNAc ₄ Fuc ₃	H-F
7431	2693.967	Hex ₇ HexNAc ₄ Fuc ₃ Neu5Ac ₁	H-FS
7432	2985.062	Hex ₇ HexNAc ₄ Fuc ₃ Neu5Ac ₂	H-FS
7500	2167.777	Hex ₇ HexNAc ₅	H
7500	2167.777	Hex ₇ HexNAc ₅	H
7511	2604.930	Hex ₇ HexNAc ₅ Fuc ₁ Neu5Ac ₁	H-FS
7512	2896.026	Hex ₇ HexNAc ₅ Fuc ₁ Neu5Ac ₂	H-FS
7601	2661.952	Hex ₇ HexNAc ₆ Neu5Ac ₁	C-S
7602	2953.047	Hex ₇ HexNAc ₆ Neu5Ac ₂	C-S
7610	2516.914	Hex ₇ HexNAc ₆ Fuc ₁	C-F
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7611	2808.010	Hex ₇ HexNAc ₆ Fuc ₁ Neu5Ac ₁	C-FS
7611			
7612	3099.105	Hex ₇ HexNAc ₆ Fuc ₁ Neu5Ac ₂	C-FS
7613	3390.201	Hex ₇ HexNAc ₆ Fuc ₁ Neu5Ac ₃	C-FS
7620	2662.972	Hex ₇ HexNAc ₆ Fuc ₂	C-F
7621	2954.068	Hex ₇ HexNAc ₆ Fuc ₂ Neu5Ac ₁	C-FS
7640	2955.088	Hex ₇ HexNAc ₆ Fuc ₄	C-F
7713	3593.280	Hex ₇ HexNAc ₇ Fuc ₁ Neu5Ac ₃	C-FS
7731	3303.205	Hex ₇ HexNAc ₇ Fuc ₃ Neu5Ac ₁	C-FS
7740	3158.168	Hex ₇ HexNAc ₇ Fuc ₄	C-F
7741	3449.263	Hex ₇ HexNAc ₇ Fuc ₄ Neu5Ac ₁	C-FS
8200	1720.592	GlcNAc ₂ Man ₈	HM
8200		GlcNAc ₂ Man ₈	
8200			
9200	1882.645	GlcNAc ₂ Man ₉	HM
9200		GlcNAc ₂ Man ₉	
9200			
9210	2028.702	GlcNAc ₂ Man ₉ Fuc ₁	HM-F
9210	2028.702	GlcNAc ₂ Man ₉ Fuc ₁	HM-F
10200	2044.697	GlcNAc ₂ Man ₁₀	HM
10200			
11200			

Example 3 – CA 125 ELISA

[00337] This Example refers to Figure 20.

[00338] An protein CA 125 (cancer antigen 125) enzyme-linked immunosorbent assay (ELISA) was performed on patient samples. The patient pool consisted of the first n=41 enrolled women from InterVenn's prospective trial to detect ovarian cancer malignancy (VOCAL). There were n=12 women with borderline or malignant cancer, as well as n=29 women with benign pelvic masses, representing the exact patient population that would be targeted in the clinical setting. Models were trained on previously purchased retrospective samples from commercial biobanks, and applied blind to the VOCAL trial participants.

[00339] The results of the ELISA assay are shown in Figure 20.

[00340] At a Cutoff = 35; the ELISA assay was observed to diagnose malignant ovarian cancer at the following levels of accuracy, sensitivity and specificity:

Accuracy = 56% Sensitivity = 86% Specificity = 48%

[00341] The samples had a positive predictive value at 20% Prevalence = 29%

[00342] The samples had a negative predictive value at 20% Prevalence = 93%

[00343] There are approximately 22,000 new cases of ovarian cancer in the United States every year, which stem from approximately 110,000 pelvic masses (at 20% prevalence). As observed the CA-125 test would correctly identify 18,920 of the malignant cancers and 42,240 of the benign cancers. There would be 45,760 false positives and 3080 false negatives.

Example 4 – Glycoproteomic Trained Model Test

[00344] This Example refers to Figure 21.

[00345] A model trained using SEQ ID NOs.: 1-76 was to identify the probability that a given patient sample had ovarian cancer.

[00346] The patient pool consisted of the first n=41 enrolled women from InterVenn's prospective trial to detect ovarian cancer malignancy (VOCAL). There were n=12 women with borderline or malignant cancer, as well as n=29 women with benign pelvic masses, representing the exact patient population that would be targeted in the clinical setting. Models were trained on previously purchased retrospective samples from commercial biobanks, and applied blind to the VOCAL trial participants.

[00347] The results are shown in Figure 21.

[00348] At a Cutoff = 0.54; the model was observed to diagnose malignant ovarian cancer at the following levels of accuracy, sensitivity and specificity:

Accuracy = 92% Sensitivity = 86% Specificity = 93%

[00349] The samples had a positive predictive value at 20% Prevalence = 75.0%.

[00350] The samples had a negative predictive value at 20% Prevalence = 96%.

[00351] There are approximately 22,000 new cases of ovarian cancer in the United States every year, which stem from approximately 110,000 pelvic masses (at 20% prevalence).

[00352] The glycoproteomic test set forth in this Example 4 would correctly identify 18,920 of the malignant cancers and 81,840 of the benign cancers. There would be 6,160 false positives and 3,080 false negatives.

[00353] Compared with CA-125, and in the United States alone, this would amount to 39,600 less incorrect cancer diagnoses per year. These additional correctly-diagnosed patients would all be triaged to the appropriate surgery and surgeon, where they would not have been with the CA-125 test. This results in significantly less stress on patients, as well as on the gynecologic oncologists required to perform surgeries on predicted malignancies.

[00354] The embodiments and examples described above are intended to be merely illustrative and non-limiting. Those skilled in the art will recognize or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials and procedures. All such equivalents are considered to be within the scope and are encompassed by the appended claims.

XI. SEQUENCE LISTING

SEQUENCE LISTING

<110> DANIEL SERIE
 VENN BIOSCIENCES CORPORATION

<120> BIOMARKERS FOR DIAGNOSING OVARIAN CANCER

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<160> 76

<170> PatentIn version 3.5

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20 25

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<211> 9

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<400> 57

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<223> Synthetic: IgG1-GP048_297_4510

<400> 58

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<210> 62

<211> 9

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<223> Synthetic: IgG1-GP048_297_5510

<400> 62

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1 5

<210> 63

<211> 9

<212> PRT

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<223> Synthetic: IgG1-GP048_297_nonglycosylated

<400> 63

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1 5

<210> 64

<211> 9

<212> PRT

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<223> Synthetic: IgG2-GP049_297_3510

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<211> 9

<212> PRT

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<223> Synthetic: IgG2-GP049_297_4411

<400> 65

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<210> 66

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<223> Synthetic: IgG2-GP049_297_4510

<400> 66

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<210> 67

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<223> Synthetic: IgJ-GP052_71_5401

<400> 67

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<210> 69

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Phe Cys Leu Phe Arg
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<400> 73

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 1 5 10 15

Phe Cys Leu Phe Arg
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<400> 74

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<210> 75
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<220>
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<400> 75

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 1 5 10 15

Leu Ala Leu Pro Ala His Ser Tyr Ser Gly Arg
 20 25

<210> 76

<211> 23

<212> PRT

<213> Artificial Sequence

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<223> Synthetic: VTNC-GP067_86_5421

<400> 76

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1 5 10 15

Asp Leu Gln Ala Gln Ser Lys
20

What is claimed is:

1. A method of detecting one or more multiple-reaction-monitoring (MRM) transitions, comprising:
obtaining, or having obtained, a biological sample from a patient, wherein the biological sample comprises one or more glycans or glycopeptides;
digesting and/or fragmenting a glycopeptide in the sample; and
detecting a MRM transition selected from the group consisting of transitions 1 - 76.
2. The method of claim 1, wherein the fragmenting a glycopeptide in the sample occurs after introducing the sample, or a portion thereof, into the mass spectrometer.
3. The method of any one of claims 1 or 2, wherein the fragmenting a glycopeptide in the sample produces a glycopeptide ion, a peptide ion, a glycan ion, a glycan adduct ion, or a glycan fragment ion.
4. The method of any one of claims 1-3, wherein the digesting and/or fragmenting a glycopeptide in the sample produces a peptide or glycopeptide:
consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof;
consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof;
consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof; or
consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.
5. The method of any one of claims 1-4, wherein the digesting a glycopeptide in the sample produces a peptide or glycopeptide consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.
6. The method of any one of claims 1-4, wherein the fragmenting a glycopeptide in the sample produces a peptide or glycopeptide:

- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof;
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof;
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof; or
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.
7. The method of any one of claims 1-6, wherein the MRM transition is selected from the transitions, or any combinations thereof, in any one of Tables 1-5.
8. The method of any one of claims 1-7, further comprising conducting tandem liquid chromatography-mass spectroscopy on the biological sample.
9. The method of any one of claims 1-8, wherein detecting a MRM transition selected from the group consisting of transitions 1 - 76 comprises conducting multiple-reaction-monitoring mass spectroscopy (MRM-MS) mass spectroscopy on the biological sample.
10. The method of any one of claims 1-3 and 7-9, wherein the one or more glycopeptides comprises a peptide or glycopeptide:
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof;
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof;
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof; or
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35,

36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59,
60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

11. The method of any one of claims 1-10, comprising detecting one or more MRM transitions indicative of one or more glycans selected from the group consisting of glycan 3200, 3210, 3300, 3310, 3320, 3400, 3410, 3420, 3500, 3510, 3520, 3600, 3610, 3620, 3630, 3700, 3710, 3720, 3730, 3740, 4200, 4210, 4300, 4301, 4310, 4311, 4320, 4400, 4401, 4410, 4411, 4420, 4421, 4430, 4431, 4500, 4501, 4510, 4511, 4520, 4521, 4530, 4531, 4540, 4541, 4600, 4601, 4610, 4611, 4620, 4621, 4630, 4631, 4641, 4650, 4700, 4701, 4710, 4711, 4720, 4730, 5200, 5210, 5300, 5301, 5310, 5311, 5320, 5400, 5401, 5402, 5410, 5411, 5412, 5420, 5421, 5430, 5431, 5432, 5500, 5501, 5502, 5510, 5511, 5512, 5520, 5521, 5522, 5530, 5531, 5541, 5600, 5601, 5602, 5610, 5611, 5612, 5620, 5621, 5631, 5650, 5700, 5701, 5702, 5710, 5711, 5712, 5720, 5721, 5730, 5731, 6200, 6210, 6300, 6301, 6310, 6311, 6320, 6400, 6401, 6402, 6410, 6411, 6412, 6420, 6421, 6432, 6500, 6501, 6502, 6503, 6510, 6511, 6512, 6513, 6520, 6521, 6522, 6530, 6531, 6532, 6540, 6541, 6600, 6601, 6602, 6603, 6610, 6611, 6612, 6613, 6620, 6621, 6622, 6623, 6630, 6631, 6632, 6640, 6641, 6642, 6652, 6700, 6701, 6711, 6721, 6703, 6713, 6710, 6711, 6712, 6713, 6720, 6721, 6730, 6731, 6740, 7200, 7210, 7400, 7401, 7410, 7411, 7412, 7420, 7421, 7430, 7431, 7432, 7500, 7501, 7510, 7511, 7512, 7600, 7601, 7602, 7603, 7604, 7610, 7611, 7612, 7613, 7614, 7620, 7621, 7622, 7623, 7632, 7640, 7700, 7701, 7702, 7703, 7710, 7711, 7712, 7713, 7714, 7720, 7721, 7722, 7730, 7731, 7732, 7740, 7741, 7751, 8200, 9200, 9210, 10200, 11200, 12200, and combinations thereof.
12. The method of claim 11, further comprising quantifying a first glycan and quantifying a second glycan; and further comprising comparing the quantification of the first glycan with the quantification of the second glycan.
13. The method of claim 11 or 12, further comprising associating the detected glycan with a peptide residue site, whence the glycan was bonded.
14. The method of claim 13, further comprising quantifying relative abundance of a glycan and/or a peptide.
15. The method of any one of claims 1-14, comprising normalizing the amount of glycopeptide based on the amount of peptide or glycopeptide consisting essentially of an amino acid having a SEQ ID. No: 1-76.
16. A method for identifying a classification for a sample, the method comprising

- quantifying by mass spectroscopy (MS) one or more glycopeptides in a sample
wherein the glycopeptides each, individually in each instance, comprises a
glycopeptide consisting essentially of an amino acid sequence selected from
the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof; and
inputting the quantification into a trained model to generate a output probability;
determining if the output probability is above or below a threshold for a classification;
and
identifying a classification for the sample based on whether the output probability is
above or below a threshold for a classification.
17. The method of claim 16, wherein the glycopeptides each, individually in each
instance, comprises a glycopeptide:
consisting essentially of an amino acid sequence selected from the group
consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51,
56, 57, 58, 62, 74, and 76, and combinations thereof;
consisting essentially of an amino acid sequence selected from the group
consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19,
20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74,
75, and combinations thereof; or
consisting essentially of an amino acid sequence selected from the group
consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35,
36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59,
60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.
18. The method of claim 16, wherein the sample is a biological sample from a patient or
individual having a disease or condition.
19. The method of claim 18, wherein the patient has cancer, an autoimmune disease, or
fibrosis.
20. The method of claim 18, wherein the patient has ovarian cancer.
21. The method of claim 18, wherein the individual has an aging condition.
22. The method of claim 18, wherein the disease or condition is ovarian cancer.
23. The method of claim any one of claims 16-22, wherein the trained model was trained
used a machine learning algorithm selected from the group consisting of a deep
learning algorithm, a neural network algorithm, an artificial neural network algorithm,
a supervised machine learning algorithm, a linear discriminant analysis algorithm, a

- quadratic discriminant analysis algorithm, a support vector machine algorithm, a linear basis function kernel support vector algorithm, a radial basis function kernel support vector algorithm, a random forest algorithm, a genetic algorithm, a nearest neighbor algorithm, k-nearest neighbors, a naive Bayes classifier algorithm, a logistic regression algorithm, or a combination thereof.
24. The method of claim any one of claims 16-23, wherein the classification is a disease classification or a disease severity classification.
25. The method of claim 24, wherein the classification is identified with greater than 80 % confidence, greater than 85 % confidence, greater than 90 % confidence, greater than 95 % confidence, greater than 99 % confidence, or greater than 99.9999 % confidence.
26. The method of claim any one of claims 13-25, further comprising:
quantifying by MS a first glycopeptide in a sample at a first time point;
quantifying by MS a second glycopeptide in a sample at a second time point; and
comparing the quantification at the first time point with the quantification at the second time point.
27. The method of claim 26, further comprising:
quantifying by MS a third glycopeptide in a sample at a third time point;
quantifying by MS a fourth glycopeptide in a sample at a fourth time point; and
comparing the quantification at the fourth time point with the quantification at the third time point.
28. The method of any one of claims 16-27, further comprising monitoring the health status of a patient.
29. The method of any one of claims 16-28, further comprising quantifying by MS a glycopeptide from whence the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76 was fragmented.
30. The method of any one of claims 16-29, further comprising diagnosing a patient with a disease or condition based on the classification.
31. The method of claim 30, further comprising diagnosing the patient as having ovarian cancer based on the classification.
32. The method of any one of claims 16-31, further comprising treating the patient with a therapeutically effective amount of a therapeutic agent selected from the group

consisting of a chemotherapeutic, an immunotherapy, a hormone therapy, a targeted therapy, and combinations thereof.

33. A method for treating a patient having ovarian cancer; the method comprising:
- obtaining, or having obtained, a biological sample from the patient;
 - digesting and/or fragmenting, or having digested or having fragmented, one or more glycopeptides in the sample; and
 - detecting and quantifying one or more multiple-reaction-monitoring (MRM) transitions selected from the group consisting of transitions 1 - 76;
 - inputting the quantification into a trained model to generate an output probability;
 - determining if the output probability is above or below a threshold for a classification; and
 - classifying the patient based on whether the output probability is above or below a threshold for a classification, wherein the classification is selected from the group consisting of:
 - (A) a patient in need of a chemotherapeutic agent;
 - (B) a patient in need of an immunotherapeutic agent;
 - (C) a patient in need of hormone therapy;
 - (D) a patient in need of a targeted therapeutic agent;
 - (E) a patient in need of surgery;
 - (F) a patient in need of neoadjuvant therapy;
 - (G) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, before surgery;
 - (H) a patient in need of chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof, after surgery;
 - (I) or a combination thereof;
 - administering a therapeutically effective amount of a therapeutic agent to the patient:
 - wherein the therapeutic agent is selected from chemotherapy if classification A or I is determined;
 - wherein the therapeutic agent is selected from immunotherapy if classification B or I is determined; or

- wherein the therapeutic agent is selected from hormone therapy if classification C or I is determined; or
- wherein the therapeutic agent is selected from targeted therapy if classification D or I is determined
- wherein the therapeutic agent is selected from neoadjuvant therapy if classification F or I is determined;
- wherein the therapeutic agent is selected from chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof if classification G or I is determined; and
- wherein the therapeutic agent is selected from chemotherapeutic agent, immunotherapeutic agent, hormone therapy, targeted therapeutic agent, neoadjuvant therapy, or a combination thereof if classification H or I is determined.
34. The method of claim 33, comprising conducting multiple-reaction-monitoring mass spectroscopy (MRM-MS) on the biological sample.
35. The method of claim 46 or 47, comprising quantifying one or more glycopeptides:
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof;
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof; or
- consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.
36. The method of any one of claims 33-35, wherein the analyzing the transitions comprises selecting peaks and/or quantifying detected glycopeptide fragments with a machine learning algorithm.
37. A method for diagnosing a patient having ovarian cancer; the method comprising:
- obtaining, or having obtained, a biological sample from the patient;

- performing mass spectroscopy of the biological sample using MRM-MS with a QQQ and/or qTOF spectrometer to detect and quantify one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-76; or to detect one or more MRM transitions selected from transitions 1-76;
- inputting the quantification of the detected glycopeptides or the MRM transitions into a trained model to generate an output probability,
- determining if the output probability is above or below a threshold for a classification;
- and
- identifying a diagnostic classification for the patient based on whether the output probability is above or below a threshold for a classification; and
- diagnosing the patient as having ovarian cancer based on the diagnostic classification.
38. The method of claim 37, wherein the analyzing the detected glycopeptides comprises using a machine learning algorithm.
39. A glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76, and combinations thereof.
40. A glycopeptide consisting essentially an amino acid sequence selected from the group consisting essentially of SEQ ID NOs: 1 - 76, and combinations thereof.
41. A glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.
42. A glycopeptide consisting essentially an amino acid sequence selected from the group consisting essentially of SEQ ID NOs: 1, 6, 7, 8, 10, 15, 21, 22, 23, 42, 47, 48, 51, 56, 57, 58, 62, 74, and 76, and combinations thereof.
43. A glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.
44. A glycopeptide consisting essentially an amino acid sequence selected from the group consisting essentially of SEQ ID NOs: 1, 3, 4, 5, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 30, 32, 34, 43, 45, 51, 54, 55, 65, 68, 71, 73, 74, 75, and combinations thereof.
45. A glycopeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40,

41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

46. A glycopeptide consisting essentially an amino acid sequence selected from the group consisting essentially of SEQ ID NOs: 2, 6, 7, 9, 10, 15, 21, 26, 28, 29, 31, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 72, 76, and combinations thereof.

47. A kit comprising a glycopeptide standard, a buffer, and one or more glycopeptides consisting essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 - 76.