analytical chemistry

Article

pubs.acs.org/ac

Multiplexed Surrogate Analysis of Glycotransferase Activity in Whole Biospecimens

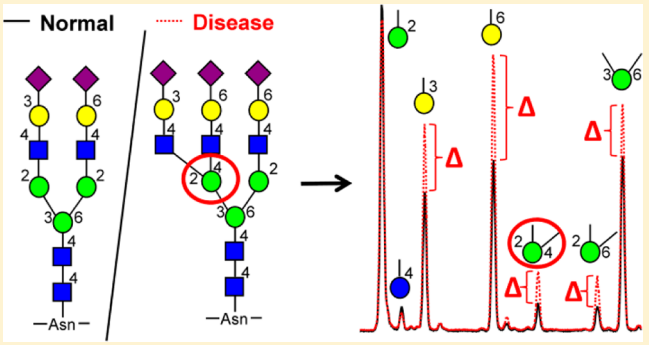
Chad R. Borges,[†,*] Douglas S. Rehder,[†] and Paolo Boffetta[‡]

[†]Molecular Biomarkers Unit, The Biodesign Institute at Arizona State University, Tempe, Arizona 85287, United States

[‡]Institute for Translational Epidemiology and Tisch Cancer Institute, Mount Sinai School of Medicine, New York, New York 10029, United States

Supporting Information

ABSTRACT: Dysregulated glycotransferase enzymes in cancer cells produce aberrant glycans—some of which can help facilitate metastases. Within a cell, individual glycotransferases promiscuously help to construct dozens of unique glycan structures, making it difficult to comprehensively track their activity in biospecimens—especially where they are absent or inactive. Here, we describe an approach to deconstruct glycans in whole biospecimens then analytically pool together resulting monosaccharide-and-linkage-specific degradation products ("glycan nodes") that directly represent the activities of specific glycotransferases. To implement this concept, a reproducible, relative quantitation-based glycan methylation analysis methodology was developed that simultaneously captures information from N-, O-, and lipid linked glycans and is compatible with whole biofluids and homogenized tissues; in total, over 30 different glycan nodes are detectable per gas chromatography–mass spectrometry (GC-MS) run. Numerous nonliver organ cancers are known to induce the production of abnormally glycosylated serum proteins. Thus, following analytical validation, in blood plasma, the technique was applied to a group of 59 lung cancer patient plasma samples and age/gender/smoking-status-matched non-neoplastic controls from the Lung Cancer in Central and Eastern Europe (CEE) study to gauge the clinical utility of the approach toward the detection of lung cancer. Ten smoking-independent glycan node ratios were found that detect lung cancer with individual receiver operating characteristic (ROC) c-statistics ranging from 0.76 to 0.88. Two glycan nodes provided novel evidence for altered ST6Gal-I and GnT-IV glycotransferase activities in lung cancer patients. In summary, a conceptually novel approach to the analysis of glycans in unfractionated human biospecimens has been developed that, upon clinical validation for specific applications, may provide diagnostic and/or predictive information in glycan-altering diseases.



Glycans are complex, heterogeneous biological sugar polymers generally found attached to proteins or lipids and displayed on cell and macromolecule surfaces. The construction and display of abnormal glycan structures is an established hallmark of nearly every known type of tumor cell and appears to facilitate their ability to metastasize.[1] In addition, there are numerous types of cancer, including ovarian,[2,3] prostate,[4,5] pancreatic,[6,7] liver,[8,9] multiple myeloma,[10] breast,[11,12] lung,[13,14] gastric,[15,16] thyroid,[17] and colorectal cancer,[18] as well as other inflammation-related diseases[19,20] that are able to induce aberrant glycosylation of abundant blood plasma proteins.

Glycans are created in the endoplasmic reticulum and golgi apparatus organelles by enzymes known as glycotransferases (GTs). Aberrant GT expression and/or activity is generally the immediate upstream cause of irregular glycan production.[1] Unfortunately, however, the ability to directly track the activity of one or more GTs in human biospecimens is technically difficult and/or generally precluded in common clinical samples where GTs tend to lose activity ex vivo or are simply absent.

The natural complexity and structural heterogeneity of glycans comes in part from the fact that GTs build at glycan polymer branch-points and chain link sites in a non-template-driven, first-come-first-build manner—i.e., there are no biologically embedded templates or instruction sets that drive glycan construction in a precise, well-defined manner (such as is the case with DNA and proteins). Yet, amidst this seemingly chaotic process, individual GTs generally exhibit strict donor, acceptor, and linkage specificity,[21] allowing for a moderate degree of consistency in routine glycan production.

When viewed across all protein and lipid substrates, the altered expression of a single GT can result in the production of a complex, heterogeneous mixture of *n* number of unique, abnormal whole-glycan structures rather than a uniformly increased expression of a single whole-glycan structure (see Figure 1). These heterogeneous mixtures of whole-glycan

Received: December 13, 2012
Accepted: January 31, 2013
Published: January 31, 2013

   dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927–2936

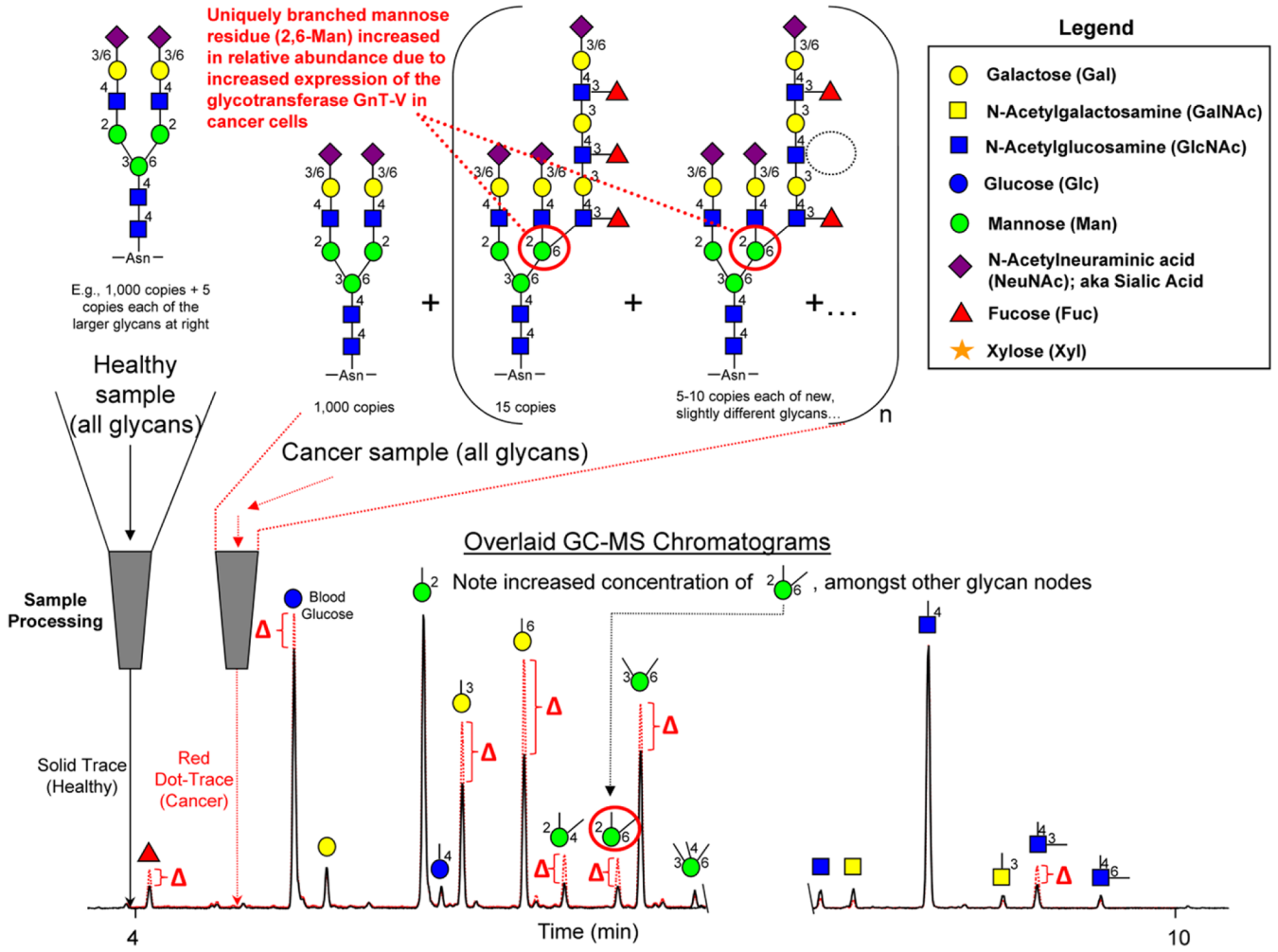


Figure 1. Conceptual overview of the analytical concept: An upregulated glycotransferase (GT) (e.g., GnT-V) causes an increase in the quantity of a specific, uniquely linked glycan monosaccharide residue (a 2,6-linked mannose "node" in this example)—which, through the subsequent action of other GTs, can lead to formation of a mixture of heterogeneous whole-glycan structures at low copy number each—all of which can be difficult to detect and quantify in routine fashion. Analytically pooling together the "glycan nodes" from among all the aberrant glycan structures provides a more direct surrogate measurement of GnT-V activity than any single intact glycan. Simultaneous measurement of N-, O-, and lipid linked "glycan nodes" in whole biospecimens as described here represents a conceptually novel means by which to detect and monitor glycan-affective diseases such as cancer. Actual extracted ion chromatograms from 10-μL blood plasma samples are shown. Numbers adjacent to monosaccharide residues in glycan structures indicate the position at which the higher residue is linked to the lower residue. If no linkage positions are indicated in the chromatogram annotation, the residue is either in the terminal position or free in solution (e.g., glucose). All residues except sialic acid link downward via their 1-position; sialic acid links downward via its 2-position. The split in the chromatogram indicates the change in extracted ion chromatograms: m/z 117 + 129 for hexose residues and m/z 116 + 158 for *N*-acetylhexosamine (HexNAc) residues.

structures are difficult to fully characterize routinely—so existing cancer markers and novel candidate biomarkers that are based on intact glycan structure are generally based on one or a few particular aberrant glycan structures (out of n)—or perhaps a set of very closely related aberrant glycan structures that result in a unique antibody or lectin epitope.

With this background in mind, we developed the idea that monosaccharide-and-linkage-specific glycan polymer chain links and branch points ("glycan nodes", as we refer to them), if broken down and quantified from the pool of all glycan structures in a biological sample, may, in numerous cases, serve as direct, 1:1 molecular surrogates of aberrant GT activity—a complementary contrast to traditional glycomics approaches that focus on the analysis of whole, intact glycans that represent $1/n$:1 molecular surrogates of GT activity (Figure 1).

Below, we describe the development and technical characteristics of a clinical sample-compatible protocol by which we have implemented this analytical concept. In the context of lung cancer, we provide an initial assessment of its utility as a methodology for routine measurement of novel glycan-based cancer markers.

EXPERIMENTAL SECTION

Materials. Heavy, stable-isotope-labeled D-glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D7, 97%–98%) was obtained from Cambridge Isotope Laboratories. *N*-acetyl-D-[UL-$^{13}C_6$]glucosamine and L-[UL-$^{13}C_6$]fucose were obtained from Omicron Biochemicals, Inc. 6′-Sialyl-*N*-acetyllactosamine and *N*-acetyllactosamine were purchased from Carbosynth (U.K.). Additional monosaccharide and glycan polymer standards for verification of partially methylated alditol acetate (PMAA) identities via gas chromatography–mass spectroscopy (GC-MS) were obtained from Carbosynth, Sigma–Aldrich, V-Laboratories (which is a U.S. subsidiary of Dextra U.K.), and The Scripps Research

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927–2936

Institute/Consortium for Functional Glycomics. Prepurified proteins were obtained from EMD Millipore (Human Serum Amyloid P), Sigma–Aldrich (Bovine Ribonuclease B), and Athens Research & Technology (Human Vitamin D Binding Protein); prepurified neutral glycosphingolipids were obtained from Enzo Life Sciences. Sodium hydroxide beads (20–40 mesh) were purchased from Sigma–Aldrich. Spin columns (0.9 mL) equipped with plugs and polyethylene frits were purchased from the Pierce division of ThermoFisher Scientific (Cat. No. 69705). GC-MS autosampler vials and Teflon-lined pierceable caps were also obtained from ThermoFisher Scientific. GC consumables were acquired from Agilent; MS consumables were obtained from Waters. All other solvents and chemicals were of the highest purity available and obtained from either ThermoFisher Scientific or Sigma–Aldrich.

Samples. A group of 59 blood plasma samples from lung cancer patients and age/gender/smoking-status matched controls that were enrolled in the Lung Cancer in Central and Eastern Europe (CEE) study were a gift from the International Agency for Research on Cancer Biobank in Lyon, France. Additional serum samples from nominally healthy individuals, lung cancer patients, and colorectal cancer patients were purchased from ProMedDx (Norton, MA). Serum samples from prostate cancer patients were purchased from the Cooperative Human Tissue Network (Vanderbilt, TN). Plasma samples from patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), Type 2 diabetes (T2D), and T2D with cardiovascular disease (CVD), the latter of which is defined as a history of heart attack or stroke and/or the presence of microalbuminuria or macroalbuminuria, were provided through an ongoing NIH-sponsored collaboration with Dr. Craig Stump and Dr. Hussein Yassine, endocrinologists at the University of Arizona. (Dr. Yassine is now at the University of Southern California.) Other biospecimens were purchased from Bioreclamation (Hicksville, NY), including a 300-mL plasma sample from an individual donor, which was analyzed in every batch as a quality control sample.

Permethylation and Semipurification of Whole Biofluid Glycans. Permethylation and subsequent cleanup procedures were adapted from the protocol of Goetz et al.,[22] which was designed to permethylate and release O-linked glycans from preisolated glycoproteins. Nine microliters (9 μL) of a whole biofluid sample (e.g., blood plasma, serum, seminal fluid, homogenized tissue, etc.) was added to a 1.5-mL polypropylene test tube. To this was added 1 μL of an internal tracer stock solution containing 10 mM each of D-glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D7, 97%–98%), *N*-acetyl-D-[UL-$^{13}C_6$]glucosamine, and L-[UL-$^{13}C_6$]fucose. (As explained below, these internal standards are useful for qualitative verification of proper sample processing, but for purposes of relative quantification, the glycan nodes within a sample are best-normalized to themselves.) To the 10-μL sample-plus-internal-standard mixture was added 270 μL of dimethylsulfoxide (DMSO) and 105 μL of iodomethane. This solution was mixed thoroughly and placed onto a plugged 1-mL spin column containing ∼0.7 g sodium hydroxide (NaOH) beads that had been preconditioned with acetonitrile, followed by two rinses with DMSO. Samples were allowed to stand for 10–12 min with occasional stirring. Samples were then unplugged and spun in a microcentrifuge for 30 s at 4000 rpm (800 g) to retrieve the glycan-containing liquid. Samples were then transferred to a silanized 13 × 100 glass test tube. Three hundred microliters (300 μL) of acetonitrile was then added to the spin column, to wash off all of the permethylated glycan. Spin columns were then centrifuged at 10 000 rpm (5000*g*) for 30 s to collect the acetonitrile that was pooled with the rest of the sample. To the liquid sample was added 3.5 mL of 0.5 M NaCl, followed by 1.2 mL of chloroform. Liquid/liquid extraction was performed 3 times, saving the chloroform layers, which were dried under a gentle stream of nitrogen.

Glycan Methylation Analysis. The following procedure was adapted from Heiss et al.:[23] trifluoroacetic acid (TFA) hydrolysis, reduction of sugar aldehydes, acetylation of nascent hydroxyl groups, and then final cleanup.

Trifluoroacetic Acid (TFA) Hydrolysis. Three hundred twenty five microliters (325 μL) of 2 M TFA was added to each sample, which was then capped tightly and heated at 121 °C for 2 h. TFA was then removed by heat-assisted evaporation under a gentle stream of nitrogen.

Reduction of Sugar Aldehydes. A fresh 10 mg/mL solution of sodium borohydride in freshly prepared 1 M ammonium hydroxide was prepared and added (475 μL) to each test tube, mixed thoroughly, and allowed to react for 1 h at room temperature. Residual borate was removed by adding five drops of methanol to each sample, drying under nitrogen, then adding 125 μL of 9:1 (v/v) methanol (MeOH):acetic acid and drying again under nitrogen. Samples were then dried for ∼30 min in a vacuum desiccator before proceeding.

Acetylation of Nascent Hydroxyl Groups. Two hundred and fifty microliters (250 μL) of freshly made water-saturated acetic anhydride (16:234 (v/v) water:acetic anhydride) was added to each sample, which was mixed thoroughly to dissolve as much of the sample residue as possible. Next, 230 μL of concentrated TFA was added to each sample, which was then mixed, capped, and incubated at 50 °C for 10 min.

Final Cleanup. Two milliliters (2 mL) of dichloromethane was added to each sample, along with 2 mL of water. Liquid/liquid extraction carried out twice with water. The final organic layer was then dried in a silanized autosampler vial under nitrogen and reconstituted in eight drops of acetone, mixed, capped, and placed on the GC-MS autosampler rack.

Overall sample throughput is limited by the time required for sample preparation. One analyst can reasonably process ∼60–75 samples per week. We anticipate that automated sample processing robotics will be able to significantly reduce this bottleneck in throughput.

Gas Chromatography–Mass Spectrometry. GC/MS was carried out on an Agilent Model A7890 gas chromatograph (equipped with a CTC PAL autosampler) coupled to a Waters GCT (time-of-flight) mass spectrometer.

One microliter (1 μL) was injected in split mode onto an Agilent split-mode liner (Cat. No. 5183-4647) containing a small plug of silanized glass wool, maintained at 280 °C. All injections were made in duplicate: once at a split ratio of 50 and once at a split ratio of 75. Using helium as the carrier gas (0.8 mL/min, constant flow mode), samples were analyzed via chromatography over a 30-m DB-5 ms GC column. The oven was initially held at 165 °C for 0.5 min, followed by ramping at 10 °C/min to 265 °C then immediately ramping at 30 °C/min to 325 °C and holding for 3 min (15.5 min of total run time). The transfer line was maintained at 250 °C. Sample components eluting from the GC column were subjected to electron ionization (70 eV, 250 °C) and analyzed from m/z 40 to m/z 800 with a "scan cycle" time of 0.2 s. The mass

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927–2936

spectrometer was tuned and calibrated (to within 10 ppm mass accuracy) daily, using perfluorotributylamine.

Data Analysis. Initial identification of PMAAs was made through the analysis of glycan standards and verified through comparison with the online electron ionization mass spectral library of PMAAs at the University of Georgia's Complex Carbohydrate Research Center: http://www.ccrc.uga.edu/databases/index.php#.

The topmost abundant and/or diagnostic fragment ions for each glycan node in blood plasma/serum were summed (using a 0.15 Da extracted ion chromatogram mass window) for quantification (see Table S1 in the Supporting Information). Quantification was carried out by integration of summed extracted ion chromatogram peak areas in automated fashion, using QuanLynx software. Integrated peaks were manually verified then exported to a spreadsheet for further calculation.

All statistical analyses, including the generation of receiver operating characteristic (ROC) curves, were carried out using XLSTAT Version 2012.3.01. All *t*-tests for significant differences between group means were two-sided and pre-evaluated for variance scedasticity. No weighting was employed during analysis of variance (ANOVA) or ROC calculations.

RESULTS AND DISCUSSION

Strategy and Initial Development. For decades now, glycan methylation analysis (Figure 2) has been employed to collect monosaccharide-specific linkage information from preisolated glycans. Given the fact that numerous monosaccharide-specific linkage patterns (i.e., glycan nodes) are created by one or just a few GTs (see Table S2 in the Supporting Information), our goal was to enable glycan methylation analysis as a biomarker development tool by adapting it for routine use with common human biological specimens—minimizing or eliminating sample prefractionation steps.

Solid-phase sodium hydroxide-based permethylation procedures were first developed by Cicanu and co-workers[24−26] and were later refined into online and spin-column based approaches by Kang et al.[27,28] A spin-column based procedure reported in 2009 by Goetz, Novotny, and Mechref[22] discussed the specific chemical release of O-glycans from intact proteins and, for us, represented a promising front-end preparatory step. With little modification, we found that this method could not only release O-linked protein glycans but also, when coupled with a TFA-based methylation analysis protocol,[23] resulted in the release and detection of partially methylated alditol acetates (PMAAs) from N-linked protein glycans as well as glycolipids (Figure 3). As expected, all forms of hexose (plus xylose) and *N*-acetylhexosamine (HexNAc) residues were detectable. Sulfated, hexuronic, and sialic acid residues were only detected *indirectly*, vis-à-vis their linkage positions. Reducing-end monosaccharides in N-linked glycans appeared in the final analysis, unaffected by their unique N-atom linkage. This was evidenced by the routine detection of the PMAA corresponding to 4,6-linked GlcNAc (4,6-GlcNAc) in blood plasma samples (see Figure 1). Based on the database research conducted to create Table S2 in the Supporting Information, there is only one GT capable of producing this glycan node—α-(1,6)-fucosyltransferase—which exclusively catalyzes the addition of a fucose residue to the 6-position of the reducing-end 4-linked GlcNAc residue in N-glycans.

The suitability of this approach for direct application with 10-μL volumes of whole biofluids and homogenized tissue samples

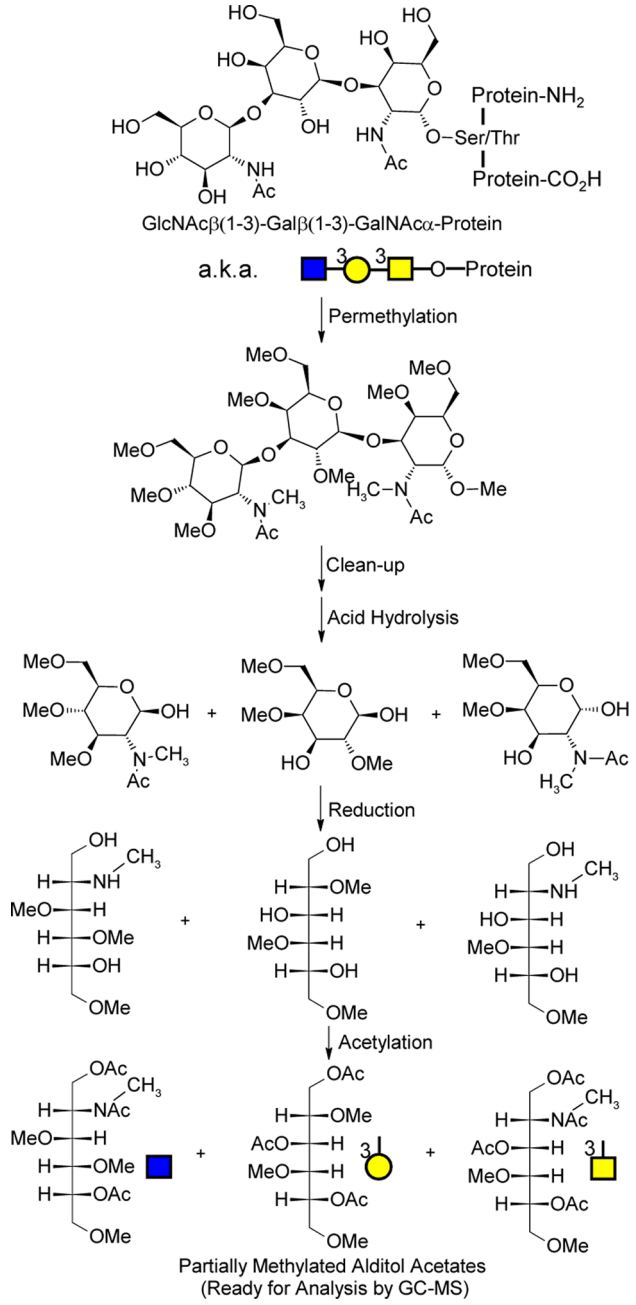


Figure 2. Molecular overview of the global glycan methylation analysis procedure. An O-linked glycan is illustrated; these are released during the permethylation process, which has been adapted from Goetz.[22] Following permethylation and hydrolysis, monosaccharides are reduced and nascent hydroxyl groups "marked" by acetylation. The unique pattern of methylation and acetylation in the final partially methylated alditol acetates (PMAAs) corresponds to the unique "glycan node" in the original intact polymer and provides the molecular basis for separation and quantification by GC-MS. N-linked and glycolipid glycans are released as linkage-marked monosaccharides during acid hydrolysis.

was assessed and, in every biomatrix tested to date, proved qualtitatively compatible (see Figures 1 and 4). These biomatrix compatibility findings opened up the technique to a wide variety of potential clinical applications. In practical terms, however, blood plasma represented the most readily available biomatrix for assessing the potential clinical utility of the technology.

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927−2936

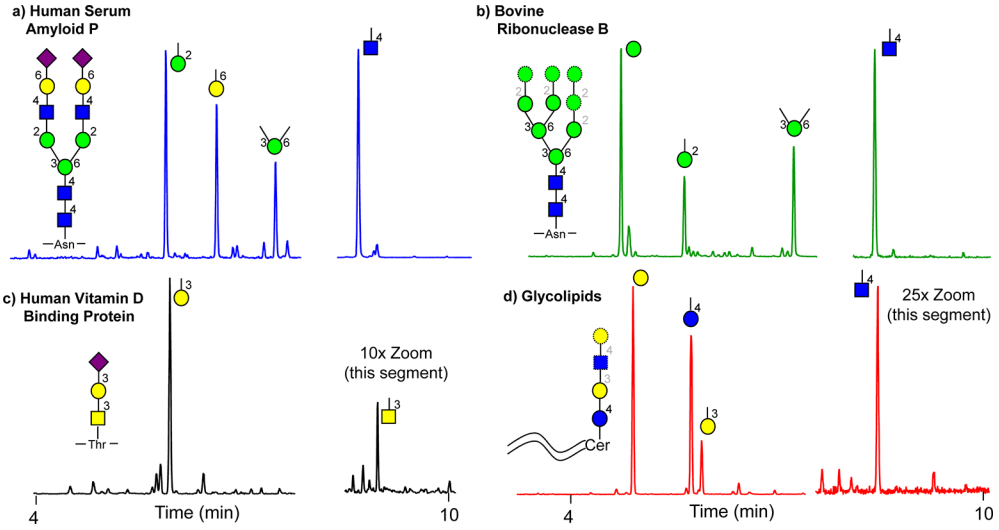


Figure 3. Evidence that N-linked and glycolipid glycans were captured by the cleanup protocol and subsequently subjected to methylation analysis. This occurred despite the fact that they were not released during permethylation, like O-linked glycans:[22] (a) prepurified Human Serum Amyloid P (contains only one complex-type N-linked glycan[46,47]), (b) prepurified Bovine Ribonuclease B (RNase B, contains only one high mannose N-linked glycan[48,49]), (c) prepurified Human Vitamin D Binding Protein (DBP, containing a NeuNAc2−3Gal1−3GalNAc O-linked glycan and no N-linked glycans[50,51]), and (d) prepurified neutral glycosphingolipids from human granulocytes (largely characterized by their lactose (Gal1−4Glc)-base-, 3-Gal-, and 4-GlcNAc-containing structures). The extracted-ion chromatograms and symbol legend in this figure are the same as those in Figure 1. Dotted borders around monosaccharides and greyed out linkage numbers indicate potential heterogeneity in the glycan structure across different protein or lipid molecules.

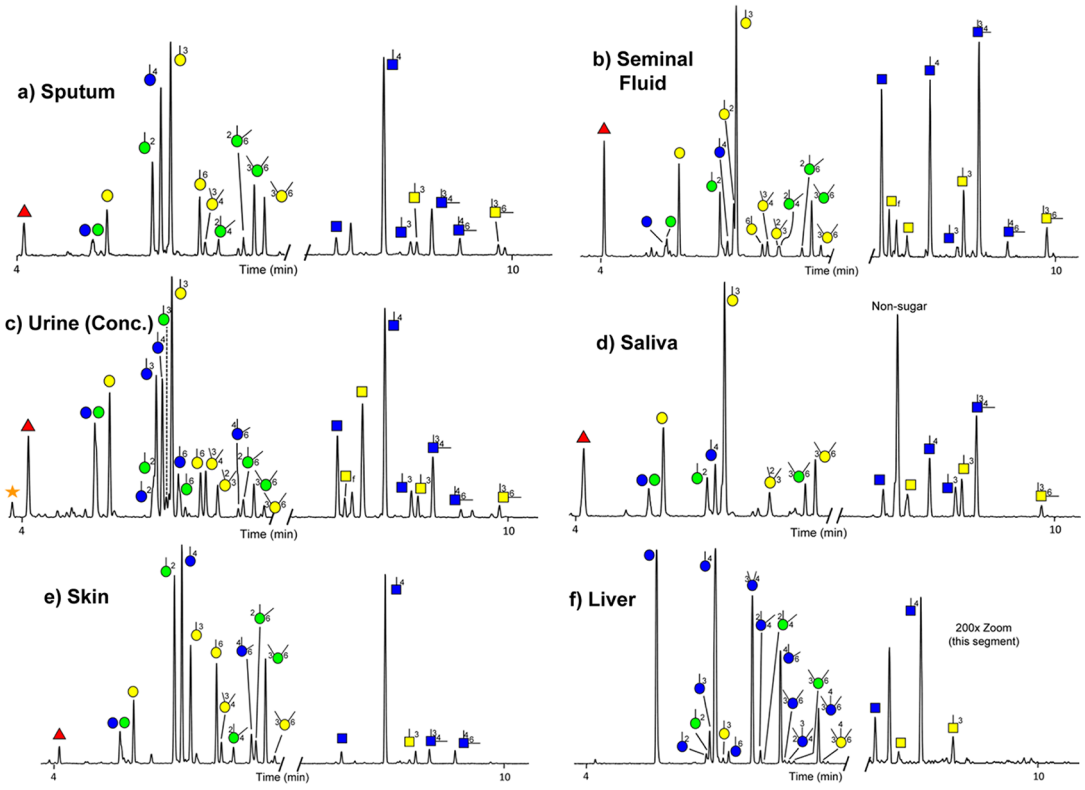


Figure 4. Illustrative results from biomatrix compatibility studies. The analytical technique may be applied to 10-μL volumes of any whole biofluid or homogenized tissue. Qualitatively diverse results are obtained for (a) sputum (homogenized), (b) seminal fluid (without sperm), (c) urine (concentrated ∼10× prior to analysis), (d) saliva, (e) skin harvested from an abrasion wound, and (f) liver (bovine). The figure legend is the same as that provided in Figure 1. The symbol "f" next to terminal t-GalNAc in the urine sample indicates a furanose (5-membered) ring structure, which likely arises from the presence of some structurally interchangeable free GalNAc in the sample. Glycan nodes derived from glycogen dominate the liver sample.

Evaluation in Lung Cancer. Following an initial evaluation of reproducibility in blood plasma (described below), we applied the new analytical technology to a cross-sectional pilot study of 59 archived blood plasma samples from patients

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927−2936

enrolled in the Lung Cancer in Central and Eastern Europe (CEE) study.[29] Summary information on gender, age, and smoking history is shown in Table 1. Additional detailed information on the patients enrolled in this study can be found online (see Tables S3 and S4 in the Supporting Information). In most cases, samples from lung cancer patients were taken within a few days of initial diagnosis. Controls in this study were matched to the lung cancer patients by age, gender, and smoking status and were enrolled upon visiting participating clinics for non-neoplastic conditions unrelated to tobacco smoking.

Table 1. Summary Clinical Information on Gender, Age, and Smoking Status for the 59 Samples Analyzed from the Lung Cancer in Central and Eastern Europe (CEE) Study

	gender	age[a]	tobacco pack years[b]
controls	15 male, 14 female	63.1 ± 7.6	17.3 ± 15.2
lung cancer cases	16 male, 14 female	60 ± 10.7	27.0 ± 20.7

[a]Student's *t*-test *p*-value for controls versus cases = 0.21. [b]Student's *t* test *p*-value for controls vs cases = 0.046.

Randomized samples were analyzed blind in six separate batches. Despite the addition of heavy, stable-isotope labeled monosaccharides to each sample as internal standards, we found that, generally, the ratios of endogenous glycan nodes to each other (GNRs) tended to provide greater analytical precision than the ratios of individual glycan nodes to stable-isotope labeled internal standards (iGNs) (see Table 2 and Table S5 in the Supporting Information, which are described in additional detail below). In the CEE group of samples, 8 iGNs and 29 GNRs were found to be significantly different ($p < 0.005$) in the lung cancer cases, versus controls. The top 2 performing GNRs had ROC c-statistics in the range of 0.8−0.9 (see Figure 5). The top 12 performing GNRs had ROC c-statistics of >0.75, which was better than any single iGN (see Table 2 and Table S5 in the Supporting Information).

To evaluate if these GNRs might be mere indicators of smoking status, the ROC curve analysis was repeated for smokers only and on the basis of smokers (including current and former) versus nonsmokers (i.e., never-smokers/no smoking history), regardless of cancer status (see Table 2). ROC curve analysis for smokers-only demonstrated negligible differences, compared to when nonsmokers were included in the analysis (Table 2). ROC curve analysis for smokers versus nonsmokers (regardless of cancer status) demonstrated an across-the-board loss of diagnostic power for 10 of the top 12 GNRs (see Table 2)—indicating that these markers are linked to the presence of cancer and not smoking history. Interestingly, the two GNR ROC c-statistics that remained the same or increased in this comparison had the same common denominator (3-GalNAc). The biological relationship of this glycan node to smoking, if any, is not yet clear.

Analytical Validation in Blood Plasma. Analytical validation of the approach in blood plasma was undertaken with the goals of determining reproducibility (intraday and interday precision), sample stability, consistency of results in

Table 2. Analytical Reproducibility and Clinical Performance Characteristics of the 12 Top-Performing Blood Plasma-Based Glycan Node Ratios (GNRs) in Lung Cancer[a]

			ROC AUC ± SE			ROC AUC ± SE
glycan node ratio, GNR	intra-assay precision (% CV)	inter-assay precision (% CV)	cancer (n = 28) vs noncancer (n = 29)	smokers only: cancer (n = 25) vs noncancer (n = 23)	trend in cancers (increased (I) or decreased (D))	smokers (n = 48) vs nonsmokers (n = 9)[b]
t-Gal/6-Gal	2.34	3.76	0.878 ± 0.051[c]	0.897 ± 0.048[c]	D	0.681 ± 0.087[d]
t-Gal/3,6-Man	4.35	5.69	0.869 ± 0.048[c]	0.868 ± 0.051[c]	D	NS
2,4-Man/3-GalNAc	7.76	11.97	0.793 ± 0.055	0.775 ± 0.062	I	0.804 ± 0.057
t-Gal/2,4-Man	7.06	7.38	0.79 ± 0.06	0.789 ± 0.063	D	NS
2,4-Man/3,4,6-Man	8.59	11.13	0.786 ± 0.06	0.775 ± 0.066	I	NS
2-Man/2,4-Man	6.08	8.93	0.78 ± 0.058	0.781 ± 0.062	D	NS
6-Gal/3-GalNAc	5.29	9.56	0.777 ± 0.058	0.778 ± 0.064	I	0.88 ± 0.043
2,4-Man/t-GlcNAc	4.47	5.12	0.772 ± 0.058	0.776 ± 0.062	I	NS
6-Gal/3,4,6-Man	4.61	7.15	0.772 ± 0.06	0.767 ± 0.065	I	NS
2,6-Man/3,4,6-Man	5.24	5.59	0.766 ± 0.063	0.749 ± 0.069	I	NS
3,6-Man/3,4,6-Man	3.35	4.89	0.764 ± 0.061	0.752 ± 0.066	I	NS
t-Gal/2,6-Man	5.9	9.82	0.76 ± 0.064	0.767 ± 0.067	D	NS

[a]Reproducibility was assessed through the analysis of six samples per batch on three separate days. Diagnostic capacity was maintained in the analysis of smokers only and, with the exception of two node ratios that share a common denominator (3-GalNAc), there was a loss of diagnostic capacity for smokers vs. non-smokers. [b]Regardless of cancer status. NS = not statistically significant from an ROC AUC of 0.5. [c]Including two lung cancer-group outliers that lie on the *opposite side* of the control distribution and are *completely separate* from it gives ROC c-statistics (AUCs) of 0.82 ± 0.062 and 0.81 ± 0.060 for the top two node ratios, respectively (0.83 ± 0.063 and 0.80 ± 0.064 for the smokers only group). [d]The difference between the "cancer vs. noncancer" group is 0.197 ± 0.10 (statistically significant at the 2σ-level).

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927−2936

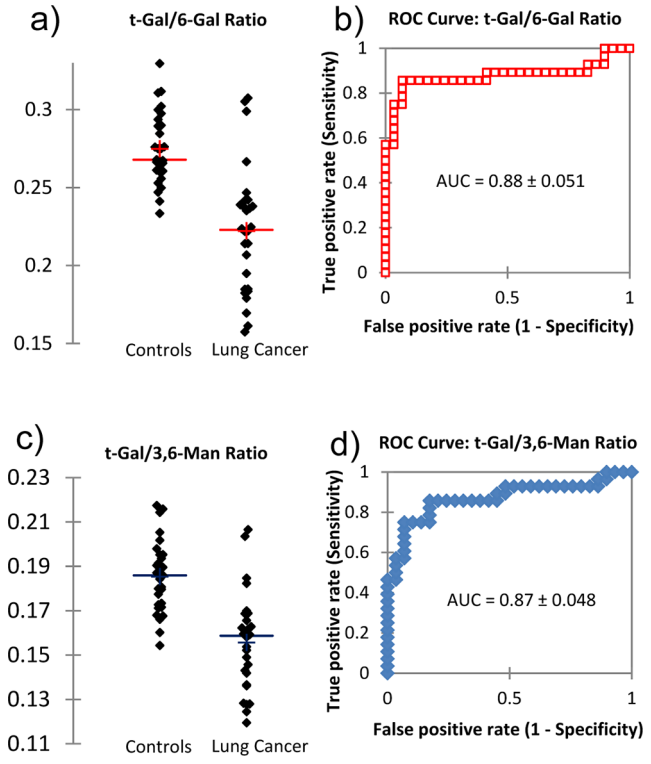


Figure 5. Clinical performance of the top blood plasma-based glycan node ratios (GNRs) in distinguishing newly diagnosed lung cancer patients (n = 28) from age/gender/smoking status-matched controls (n = 29): (a) univariate distribution of the t-Gal/6-Gal GNR, (b) ROC curve for t-Gal/6-Gal, (c) univariate distribution of the t-Gal/3,6-Man GNR, and (d) ROC curve for t-Gal/3,6-Gal. For both of these GNRs, the same two samples from squamous cell carcinoma patients produced two outliers on the opposite side of the control distribution. Since they were completely separate from the control distribution, they were excluded from the ROC curve analysis. Table 1 summarizes the clinical performance characteristics for the top 12 diagnostic GNRs in lung cancer.

serum and four different types of plasma, autosampler stability, and analytical sensitivity and linearity of response. Because of space constraints and the need to avoid the presentation of copious amounts of superfluous data, the analytical validation parameters described below are largely contextualized to the top 12 performing GNRs in lung cancer (see Table 2).

Precision/Reproducibility. Despite the addition of heavy, stable-isotope labeled monosaccharides to each plasma sample as internal standards, we found that, based on the analysis of six aliquots of the same plasma sample per day on three different days, the ratios of individual glycan nodes to stable-isotope labeled internal standards (iGNs) were not highly reproducible (see Table S5 in the Supporting Information) and that the ratios of endogenous glycan nodes to each other (GNRs) tended to provide greater analytical precision. Since ∼20 iGNs were routinely detected in plasma samples, this meant that over 200 GNRs were available for assessment of reproducibility. Of these GNRs, over 80 had both intra-assay and inter-assay reproducibility of <15%. The analytical precision of the top 12 performing GNRs in lung cancer are reported in Table 2.

Sample Stability. GNR stability in blood plasma samples was assessed by creating 12 aliquots of a single sample then placing 6 back into the −80 °C freezer and leaving the remaining 6 at room temperature overnight. None of the top 6, but 2 of the top 12 lung cancer GNRs demonstrated statistically significant difference between the batches (see Table S6 in the Supporting Information). However, these apparent differences were subtle and may have been due to abnormally tight intra-assay precision as the overall differences between the batches were <12%.

Effect of Blood Collection Type. Matched sets of blood serum, 3.8% sodium citrate, Na_2EDTA, K_2EDTA, and K_3EDTA plasma samples were collected from 22 healthy volunteers. Glycan nodes were then analyzed in the resulting 110 samples. Analysis of the results for the top 12 lung cancer GNRs by Repeated Measures ANOVA followed by the Ryan−Einot−Gabriel−Welsch (REGW) multiple comparison test demonstrated no significant differences between the blood collection types (see Table S7 in the Supporting Information).

Autosampler Stability. Autosampler stability was assessed over the time span required to inject 48 samples (∼14.5 h), which was the largest batch size employed during the analysis of samples reported in this paper. Four data points from the same sample were acquired three times—at the beginning, middle, and end of this time period. Passing stability was designated to be within 10% of the average of the first two data points. Autosampler stability passed for the top 12 lung cancer GNRs, except the three with HexNAc nodes as the denominator (see Figure S1 in the Supporting Information)—which were of minimal interest since 2 of these 3 appear to be more diagnostic of smoking rather than lung cancer and the third is not in the top 6 GNRs (see Table 2).

Analytical Sensitivity and Linearity of Response. IUPAC defines analytical sensitivity as the ability of an analytical procedure to produce a change in signal for a defined change in analyte quantity.[30] They add that, in most cases, this parameter can be observed as the slope of a calibration curve. The fact that strong, statistically significant differences were detected in numerous iGNs and GNRs between the CEE lung cancer cases and controls (see Table 2 and Table S5 in the Supporting Information) suggested that analytical sensitivity was more than adequate to impart the technique with potential clinical applicability—but the expense and limited availability of glycan polymer standards precluded a formal assessment of the analytical sensitivity for all 12 of the top lung cancer GNRs. However, the instrument response ratio of t-Gal/6-Gal versus the actual molar ratio of t-Gal/6-Gal was assessed through the use of *N*-acetyllactosamine (Gal1−4GalNAc) and 6-sialyl-*N*-acetyllactosamine (NeuNAc2−6Gal1−4GalNAc) standards. These were mixed together in ratios spanning the physiologically observed range in blood plasma, such that overall signal intensities approximated those from blood plasma samples. Following analysis on two different days, the resulting data were employed to construct a standard curve (see Figure S2 in the Supporting Information). The standard curve was linear (R^2 = 0.993) and had a slope of 1.31, indicating a change of greater than 1:1 in instrument response per change in actual glycan node molar ratio.

Other Analytical Validation Considerations. The goal of this study was to evaluate *changes* in the relative abundance of readily detectable glycan nodes as potential clinical markers; this is a distinct and separate goal from quantifying low-abundance glycan nodes. As such, unlike most conventional assays, *raw sensitivity* (i.e., limits of detection and limits of quantification) was not a parameter of significant concern because, for each biomatrix, a particular set of glycan nodes was present in every sample at readily detectable levels. For

dx.doi.org/10.1021/ac3035579 | *Anal. Chem.* 2013, 85, 2927−2936

example, although not visible with the particular extracted-ion chromatograms from plasma shown in Figure 1, there were over 18 individual glycan nodes with signal/noise (S/N) ratios of >10 in every plasma sample of hundreds of individual samples tested to date. Thus, in the absence of a specific need to detect low-abundance glycan node(s), raw detection limits were of little practical importance or value, relative to analytical sensitivity. In the future, if raw detection limits are required for a particular application, they will need to be investigated on a biomatrix-specific basis.

Since this is a technique for the relative quantification of the constituent components of heterogeneous biological polymers, *accuracy* cannot be defined by any single molecular standard. But this does not rule out clinical utility or applicability: To achieve these things without a definition of absolute accuracy, good reproducibility/precision of iGN/GNR measurement will be critical (as documented above), but it will eventually have to be coupled with a mechanism to facilitate interlaboratory transferability—for example, through establishment of a "gold standard" sample that can be shared across laboratories or through instrument-specific calibration with predefined standard curves (such as that in Figure S2 in the Supporting Information). Since the latter will have to be based on particular chemical standards, they still will not be able to define accuracy in an absolute sense and, at best, will only ever be considered to provide results that are *approximately* accurate according to strict definition; however, this is essentially irrelevant when it comes to practical application.

Disease Specificity. To further evaluate the specificity of the top-performing blood plasma GNRs for lung cancer, two additional sets of samples were analyzed for comparison. The first set consisted of biobank-purchased serum samples from 80 healthy individuals, an additional 16 lung cancer patients, 10 colorectal cancer and 59 prostate cancer patients. The second set consisted of a cross section of patients from a University of Arizona diabetes study who had undergone an oral glucose tolerance test and ranged from healthy (normal glucose tolerance, NGT, n = 18), to pre-diabetic (impaired glucose tolerance, IGT, n = 12), to stark Type 2 diabetes (T2D, n = 32), to T2D with cardiovascular complications (T2D w/CVD, n = 26). Analysis of the top 6 lung cancer GNRs (Table 2) in these samples by ANOVA, followed by the REGW multiple comparison test, demonstrated a general grouping of lung cancer and colorectal cancer separate from the other sample sets, with T2D aligning more closely with lung and colorectal cancer than did prostate cancer (see Figure S3 in the Supporting Information). These cross-disease comparisons suggested that the GNR markers in blood were not just fluctuating with inflammation and possessed at least a limited degree of specificity for certain types of cancer.

Comparison of GNRs in biobank-purchased serum samples from cancer patients with those from nominally healthy individuals revealed general consistency of GNR behavior in the two lung cancer groups (see Table 2 and Table S8 in the Supporting Information). In addition, there was partial GNR profile overlap between lung and colorectal cancer but not prostate cancer (see Table 2 and Tables 8−10 in the Supporting Information). Based on increases in ROC c-statistics, GNRs were better at distinguishing lung cancer patients from fully healthy individuals than from well-match controls (Table 2 and Table S8 in the Supporting Information), which is an unsurprising but useful observation to note when it comes to the design of biomarker studies. GNRs in serum did not appear to be particularly diagnostic in prostate cancer (see Table S10 in the Supporting Information).

Biological Implications. Glycan nodes observed via this technique are necessarily derived from the most abundant glycan source in the sample under consideration. In blood plasma, roughly half of the glycoproteins are immunoglobulins/complement protein and the other half are liver glycoproteins. Unless near-milligram-per-milliliter plasma concentrations are reached, cancer glycoprotein shedding is unlikely to contribute more than an unobservable fraction to the total plasma glycoprotein content. This means that cancer-induced alterations to the humoral immune system and/or the liver are most likely responsible for the alterations in plasma glycan nodes observed here. Although the mechanisms behind such phenomena (e.g., those discovered by Narisada et al.[31] and Kitazume et al.[32]) are varied and the phenomena themselves are only partially understood, they are by no means unknown,[2−18] even in non-neoplastic diseases.[19,20]

Glycan source notwithstanding, aberrant activity of at least two different GTs in lung and colorectal cancer patients was detected (see Table 2 and Tables S5 and S8−S10 in the Supporting Information). A table of GTs responsible for producing the glycan nodes observed in humans is provided online (see Table S2 in the Supporting Information):

(1) ST6Gal-I: Six (6) of the top 12-performing GNRs in lung cancer (see Table 2) and 5 of the top 7 GNRs in colorectal cancer (Table S9 in the Supporting Information) involved t-Gal and/or 6-Gal. When their behavior was considered in cancer cases, compared to controls, these GNRs showed fluctuations that were consistent with minor changes in t-Gal (p = 0.04) and significant increases in 6-Gal ($p = 8.6 \times 10^{-4}$; ROC c-statistic = 0.737 ± 0.064 (see Table S5 in the Supporting Information)). Together, these data indicated increased β-galactoside:α-2,6-sialyltransferase (ST6Gal-I) enzyme activity—a phenomenon for which there is evidence in cancer cells from numerous other carcinomas including those of the colon/rectum,[33,34] breast,[35] brain (non-neuroectodermal epithelial-like tumors),[36] cervix,[37] and liver (transgenic mouse model of hepatocellular carcinoma),[38] as well as in choriocarcinoma (cell lines)[39] and acute myeloid leukemia.[40]

(2) GnT-IV: Five (5) of the top 12 lung cancer GNRs (see Table 2) and 2 of the top 7 colorectal cancer GNRs (see Table S9 in the Supporting Information) provided evidence for elevated quantities of 2,4-Man. In addition, the 2,4-Man iGN in CEE lung cancer patients was significantly higher than in the controls ($p = 3.4 \times 10^{-4}$; ROC c-statistic = 0.747 ± 0.063 (see Table S5 in the Supporting Information)). Increased 2,4-Man is mediated through increased UDP-*N*-acetylglucosamine:α-1,3-D-mannosidase β1,4-*N*-acetylglucosaminyltransferase IV (GnT-IV) activity. Generally speaking, this enzyme has been documented as overactive during oncogenesis and differentiation.[41] Evidence for its overexpression has been found in cancer cells from colorectal carcinoma,[42] choriocarcinoma,[43] hepatocellular carcinoma,[44] and pancreatic cancer (GnT-IVb form).[45]

To our knowledge this is the first report of altered activity for either ST6Gal-I or GnT-IV in lung cancer patients; in our opinion, the fact that these changes may not be derived directly from tumor cells themselves makes the findings intriguing,

particularly with regard to potential therapeutic implications embedded in the underlying mechanism(s). Based on the CEE group of samples, a total of 12 iGNs were significantly increased in lung cancer patient plasma, providing evidence for activity changes in numerous other GTs as well, including fucosyltransferases (vis-à-vis, increased t-Fuc and 3,4-GlcNAc) and GnT-V (vis-à-vis, increased 2,6-Man) (see Tables S2 and S5 in the Supporting Information).

Four of the seven increasing GNRs in lung cancer (Table 2) contained 3,4,6-Man as their denominator, providing suggestive evidence for decreased β1,4-*N*-acetylglucosaminyltransferase III (GnT-III) activity, which is the GT responsible for adding "bisecting GlcNAc" to N-linked glycans. However, the change in the 3,4,6-Man iGN was not statistically significant. Notably, the 3,6-Man iGN increased in CEE lung cancer patients relative to controls ($p = 1.4 \times 10^{-3}$; see Table S5 in the Supporting Information)—suggesting an overall increase in N-glycans and a relative inability of GnT-III to keep pace.

CONCLUSIONS

There is an urgent need for blood-borne markers of risk and progression in lung cancer, as well as other types of cancer (and glycan-affective disorders) to which this analytical approach will likely be applicable. Based on current knowledge of human GTs, the glycan nodes 6-Gal, 2,4-Man, 2,6-Man, and 3,4,6-Man represent 1:1 (or nearly 1:1) molecular surrogates for ST6Gal-I, GnT-IV, GnT-V, and GnT-III, respectively—and there are other glycan nodes that hold this same relationship with their respective GTs (Table S2 in the Supporting Information). Additional glycan nodes, such as 3-Gal, may represent the activity of multiple GTs but they are still potentially modulated by the aberrant expression of just one of the GTs that leads to their existence.

By condensing and pooling together, into a single analytical signal, the inherent molecular heterogeneity introduced by aberrantly expressed GTs (Figure 1)—and doing so for multiple GTs simultaneously from 10 μL of whole, unprocessed biofluid without the use of enzyme or antibody reagents—this analytical approach represents a promising means by which to access glycans as disease markers. Its utility is expected to improve further, once it can be applied to hundreds of well-characterized patient samples for which outcome information is available, then coupled to multivariate modeling algorithms to create disease-specific prognostic biosignatures. Finally, we note that cancer-induced aberrant glycans in plasma/serum may be diluted by significant quantities of normal glycans; as such, going forward, we feel that application of this technology to biofluids or tissues obtained directly from putatively cancerous organs (e.g., as demonstrated in Figure 4) may represent a more powerful use of this technology to address specific medical needs for better cancer markers.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel.: 480-727-9928. Fax: 480-727-9464. E-mail: chad.borges@asu.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by a Flinn Foundation Award and, in part, by NIH Grant Nos. R01DK082542 and R24DK083948, as well as Grant No. GM62116 to the Consortium for Functional Glycomics. We thank Dr. Mark Holl and Dr. Mia Hashibe for helpful discussions. We also thank the national PIs of the CEE study for allowing the use of the samples: Drs. D. Zaridze, Moscow, Russia; J. Lissowska, Warsaw, Poland; N. Szeszenia-Dabrowska, Lodz, Poland; E. Fabianova, Banska-Bystrica, Slovakia; P. Rudnai, Budapest, Hungary; D. Mates, Bucharest, Romania; V. Bencko, Prague, Czech Republic; L. Foretova, Brno, Czech Republic; V. Janout, Olomouc, Czech Republic.

REFERENCES

(1) Varki, A.; Kannagi, R.; Toole, B. P. In *Essentials of Glycobiology*, 2nd Edition; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2009; Chapter 44.

(2) Gercel-Taylor, C.; Bazzett, L. B.; Taylor, D. D. *Gynecol. Oncol.* **2001**, *81*, 71−76 (DOI: 10.1006/gyno.2000.6102).

(3) An, H. J.; Miyamoto, S.; Lancaster, K. S.; Kirmiz, C.; Li, B.; Lam, K. S.; Leiserowitz, G. S.; Lebrilla, C. B. *J. Proteome Res.* **2006**, *5*, 1626−1635 (DOI: 10.1021/pr060010k).

(4) Kanoh, Y.; Mashiko, T.; Danbara, M.; Takayama, Y.; Ohtani, S.; Egawa, S.; Baba, S.; Akahoshi, T. *Anticancer Res.* **2004**, *24*, 3135−3139.

(5) Kyselova, Z.; Mechref, Y.; Al Bataineh, M. M.; Dobrolecki, L. E.; Hickey, R. J.; Vinson, J.; Sweeney, C. J.; Novotny, M. V. *J. Proteome Res.* **2007**, *6*, 1822−1832 (DOI: 10.1021/Pr060664t).

(6) Okuyama, N.; Ide, Y.; Nakano, M.; Nakagawa, T.; Yamanaka, K.; Moriwaki, K.; Murata, K.; Ohigashi, H.; Yokoyama, S.; Eguchi, H.; Ishikawa, O.; Ito, T.; Kato, M.; Kasahara, A.; Kawano, S.; Gu, J.; Taniguchi, N.; Miyoshi, E. *Int. J. Cancer* **2006**, *118*, 2803−2808 (DOI: 10.1002/ijc.21728).

(7) Zhao, J.; Patwa, T. H.; Qiu, W.; Shedden, K.; Hinderer, R.; Misek, D. E.; Anderson, M. A.; Simeone, D. M.; Lubman, D. M. *J. Proteome Res.* **2007**, *6*, 1864−1874 (DOI: 10.1021/pr070062p).

(8) Comunale, M. A.; Lowman, M.; Long, R. E.; Krakover, J.; Philip, R.; Seeholzer, S.; Evans, A. A.; Hann, H. W.; Block, T. M.; Mehta, A. S. *J. Proteome Res.* **2006**, *5*, 308−315 (DOI: 10.1021/pr050328x).

(9) Goldman, R.; Ressom, H. W.; Varghese, R. S.; Goldman, L.; Bascug, G.; Loffredo, C. A.; Abdel-Hamid, M.; Gouda, I.; Ezzat, S.; Kyselova, Z.; Mechref, Y.; Novotny, M. V. *Clin. Cancer Res.* **2009**, *15*, 1808−1813 (DOI: 10.1158/1078-0432.Ccr-07-5261).

(10) Aurer, I.; Lauc, G.; Dumic, J.; Rendic, D.; Matisic, D.; Milos, M.; Heffer-Lauc, M.; Flogel, M.; Labar, B. *Coll. Antropol.* **2007**, *31*, 247−251.

(11) Abd Hamid, U. M.; Royle, L.; Saldova, R.; Radcliffe, C. M.; Harvey, D. J.; Storr, S. J.; Pardo, M.; Antrobus, R.; Chapman, C. J.; Zitzmann, N.; Robertson, J. F.; Dwek, R. A.; Rudd, P. M. *Glycobiology* **2008**, *18*, 1105−1118 (DOI: 10.1093/glycob/cwn095).

(12) Kyselova, Z.; Mechref, Y.; Kang, P.; Goetz, J. A.; Dobrolecki, L. E.; Sledge, G. W.; Schnaper, L.; Hickey, R. J.; Malkas, L. H.; Novotny, M. V. *Clin. Chem.* **2008**, *54*, 1166−1175 (DOI: 10.1373/clinchem.2007.087148).

(13) Hongsachart, P.; Huang-Liu, R.; Sinchaikul, S.; Pan, F. M.; Phutrakul, S.; Chuang, Y. M.; Yu, C. J.; Chen, S. T. *Electrophoresis* **2009**, *30*, 1206−1220 (DOI: 10.1002/elps.200800405).

(14) Arnold, J. N.; Saldova, R.; Galligan, M. C.; Murphy, T. B.; Mimura-Kimura, Y.; Telford, J. E.; Godwin, A. K.; Rudd, P. M. *J. Proteome Res.* **2011**, *10*, 1755−1764 (DOI: 10.1021/pr101034t).

(15) Bones, J.; Mittermayr, S.; O'Donoghue, N.; Guttman, A.; Rudd, P. M. *Anal. Chem.* **2010**, *82*, 10208−10215 (DOI: 10.1021/ac102860w).
(16) Kodar, K.; Stadlmann, J.; Klaamas, K.; Sergeyev, B.; Kurtenkov, O. *Glycoconjugate J.* **2012**, *29*, 57−66 (DOI: 10.1007/s10719-011-9364-z).
(17) Chen, G.; Wang, Y.; Qiu, L.; Qin, X.; Liu, H.; Wang, X.; Wang, Y.; Song, G.; Li, F.; Guo, Y.; Li, F.; Guo, S.; Li, Z. *J. Proteomics* **2012**, *75*, 2824−2834 (DOI: 10.1016/j.jprot.2012.02.001).
(18) Takeda, Y.; Shinzaki, S.; Okudo, K.; Moriwaki, K.; Murata, K.; Miyoshi, E. *Cancer* **2012**, *118*, 3036−3043 (DOI: 10.1002/cncr.26490).
(19) Parekh, R. B.; Dwek, R. A.; Sutton, B. J.; Fernandes, D. L.; Leung, A.; Stanworth, D.; Rademacher, T. W.; Mizuochi, T.; Taniguchi, T.; Matsuta, K.; et al. *Nature* **1985**, *316*, 452−457.
(20) Mehta, A. S.; Long, R. E.; Comunale, M. A.; Wang, M.; Rodemich, L.; Krakover, J.; Philip, R.; Marrero, J. A.; Dwek, R. A.; Block, T. M. *J. Virol.* **2008**, *82*, 1259−1270 (DOI: 10.1128/JVI.01600-07).
(21) Rini, J.; Esko, J.; Varki, A. In *Essentials of Glycobiology*, 2nd Edition; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2009; Chapter 5.
(22) Goetz, J. A.; Novotny, M. V.; Mechref, Y. *Anal. Chem.* **2009**, *81*, 9546−9552.
(23) Heiss, C.; Klutts, J. S.; Wang, Z.; Doering, T. L.; Azadi, P. *Carbohyd. Res.* **2009**, *344*, 915−920 (DOI: 10.1016/j.carres.2009.03.003).
(24) Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209−217.
(25) Ciucanu, I.; Costello, C. E. *J. Am. Chem. Soc.* **2003**, *125*, 16213−16219 (DOI: 10.1021/Ja035660t).
(26) Ciucanu, I.; Caprita, R. *Anal. Chim. Acta* **2007**, *585*, 81−85.
(27) Kang, P.; Mechref, Y.; Klouckova, I.; Novotny, M. V. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3421−3428 (DOI: 10.1002/Rcm.2210).
(28) Kang, P.; Mechref, Y.; Novotny, M. V. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 721−734 (DOI: 10.1002/Rcm.3395).
(29) Brennan, P.; Crispo, A.; Zaridze, D.; Szeszenia-Dabrowska, N.; Rudnai, P.; Lissowska, J.; Fabianova, E.; Mates, D.; Bencko, V.; Foretova, L.; Janout, V.; Fletcher, T.; Boffetta, P. *Am. J. Epidemiol.* **2006**, *164*, 1233−1241 (DOI: 10.1093/aje/kwj340).
(30) McNaught, A. D.; Wilkinson, A. *IUPAC. Compendium of Chemical Terminology*, 2nd Edition (the "Gold Book"); Blackwell Scientific Publications: Oxford, U.K., 1997. (XML on-line corrected version: http://goldbook.iupac.org (2006). Created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. DOI: 10.1351/goldbook; Version: 2.3.2. Last update: Aug. 19, 2012.)
(31) Narisada, M.; Kawamoto, S.; Kuwamoto, K.; Moriwaki, K.; Nakagawa, T.; Matsumoto, H.; Asahi, M.; Koyama, N.; Miyoshi, E. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 792−796 (DOI: 10.1016/j.bbrc.2008.10.061).
(32) Kitazume, S.; Oka, R.; Ogawa, K.; Futakawa, S.; Hagiwara, Y.; Takikawa, H.; Kato, M.; Kasahara, A.; Miyoshi, E.; Taniguchi, N.; Hashimoto, Y. *Glycobiology* **2009**, *19*, 479−487 (DOI: 10.1093/glycob/cwp003).
(33) Vierbuchen, M. J.; Fruechtnicht, W.; Brackrock, S.; Krause, K. T.; Zienkiewicz, T. J. *Cancer* **1995**, *76*, 727−735.
(34) Dall'Olio, F.; Malagolini, N.; di Stefano, G.; Minni, F.; Marrano, D.; Serafini-Cessi, F. *Int. J. Cancer* **1989**, *44*, 434−439.
(35) Recchi, M. A.; Hebbar, M.; Hornez, L.; Harduin-Lepers, A.; Peyrat, J. P.; Delannoy, P. *Cancer Res.* **1998**, *58*, 4066−4070.
(36) Kaneko, Y.; Yamamoto, H.; Kersey, D. S.; Colley, K. J.; Leestma, J. E.; Moskal, J. R. *Acta Neuropathol.* **1996**, *91*, 284−292.
(37) Wang, P. H.; Li, Y. F.; Juang, C. M.; Lee, Y. R.; Chao, H. T.; Tsai, Y. C.; Yuan, C. C. *Gynecol. Oncol.* **2001**, *83*, 121−127 (DOI: 10.1006/gyno.2001.6358).
(38) Pousset, D.; Piller, V.; Bureaud, N.; Monsigny, M.; Piller, F. *Cancer Res.* **1997**, *57*, 4249−4256.
(39) Fukushima, K.; Hara-Kuge, S.; Seko, A.; Ikehara, Y.; Yamashita, K. *Cancer Res.* **1998**, *58*, 4301−4306.
(40) Skacel, P. O.; Edwards, A. J.; Harrison, C. T.; Watkins, W. M. *Blood* **1991**, *78*, 1452−1460.
(41) Taniguchi, N.; Korekane, H. *BMB Rep.* **2011**, *44*, 772−781.
(42) D'Arrigo, A.; Belluco, C.; Ambrosi, A.; Digito, M.; Esposito, G.; Bertola, A.; Fabris, M.; Nofrate, V.; Mammano, E.; Leon, A.; Nitti, D.; Lise, M. *Int. J. Cancer* **2005**, *115*, 256−262 (DOI: 10.1002/ijc.20883).
(43) Endo, T.; Nishimura, R.; Kawano, T.; Mochizuki, M.; Kobata, A. *Cancer Res.* **1987**, *47*, 5242−5245.
(44) Yamashita, K.; Totani, K.; Iwaki, Y.; Takamisawa, I.; Tateishi, N.; Higashi, T.; Sakamoto, Y.; Kobata, A. *J. Biochem.* **1989**, *105*, 728−735.
(45) Ide, Y.; Miyoshi, E.; Nakagawa, T.; Gu, J.; Tanemura, M.; Nishida, T.; Ito, T.; Yamamoto, H.; Kozutsumi, Y.; Taniguchi, N. *Biochem. Biophys. Res. Commun.* **2006**, *341*, 478−482 (DOI: 10.1016/j.bbrc.2005.12.208).
(46) Tennent, G. A.; Pepys, M. B. *Biochem. Soc. Trans.* **1994**, *22*, 74−79.
(47) Kiernan, U. A.; Nedelkov, D.; Tubbs, K. A.; Niederkofler, E. E.; Nelson, R. W. *Proteomics* **2004**, *4*, 1825−1829.
(48) Uniprot Consortium. *Nucleic Acids Res.* **2012**, *40*, D71−D75 (DOI: 10.1093/Nar/Gkr981).
(49) Prien, J. M.; Ashline, D. J.; Lapadula, A. J.; Zhang, H.; Reinhold, V. N. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 539−556 (DOI: 10.1016/j.jasms.2008.11.012).
(50) Borges, C. R.; Jarvis, J. W.; Oran, P. E.; Nelson, R. W. *J. Proteome Res.* **2008**, *7*, 4143−4153.
(51) Borges, C. R.; Jarvis, J. W.; Oran, P. E.; Rogers, S. P.; Nelson, R. W. *J. Biomol. Technol.* **2008**, *19*, 167−176.

SUPPORTING INFORMATION

Multiplexed Surrogate Analysis of Glycotransferase Activity in Whole Biospecimens

Chad R. Borges[1,*], Douglas S. Rehder[1], and Paolo Boffetta[2]

[1]Molecular Biomarkers Unit, The Biodesign Institute at Arizona State University, Tempe, AZ 85287

[2]Institute for Translational Epidemiology and Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY 10029

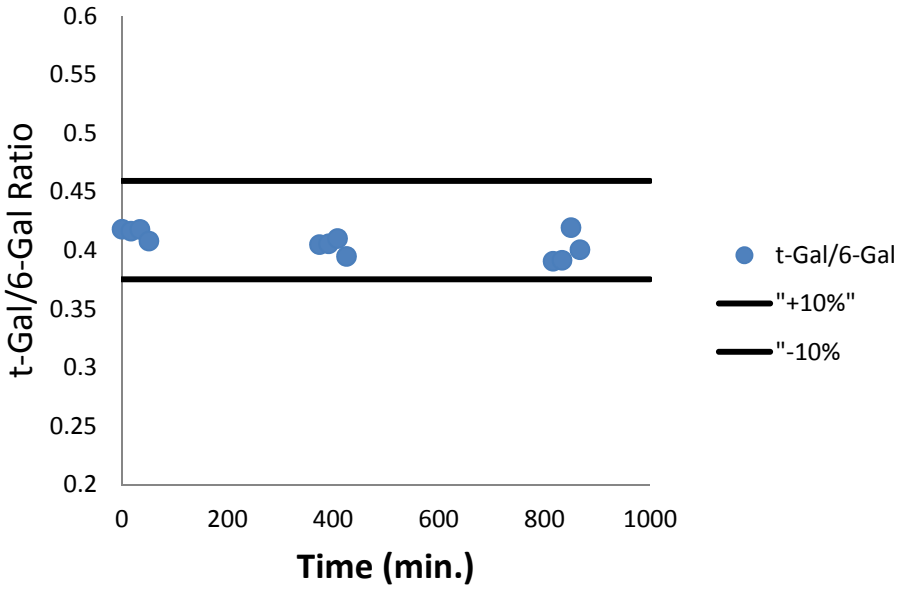
*Author to whom correspondence should be addressed: Chad R. Borges, Molecular Biomarkers Unit, The Biodesign Institute at Arizona State University, P.O. Box 876601, Tempe, AZ 85287. Tel 480-727-9929, fax 480-727-9464; email: chad.borges@asu.edu

Contents: Materials in Supporting Information provide additional details on analytical validation (Figures S1-S3; Tables S5-S7), methodology (Table S1), background information (Tables S2-S4), and clinical verification / disease specificity (Figure S4; Tables S8-S10).

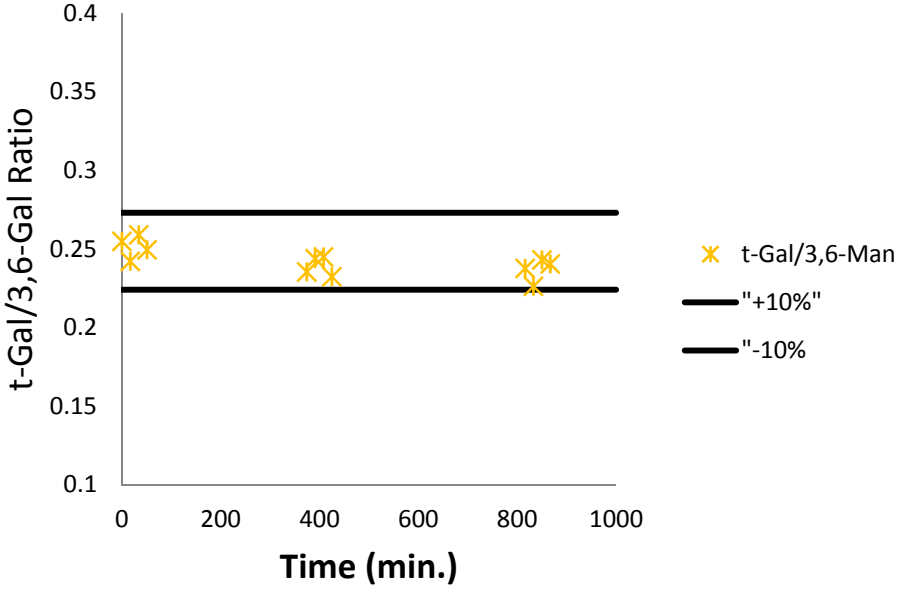
Abbreviations: Glycotransferase enzymes (GTs), individual glycan node (iGN), glycan node ratio (GNR), gas chromatograph-mass spectrometry (GC-MS), Lung Cancer in Central and Eastern Europe Study (CEE), receiver operating characteristic (ROC), normal glucose tolerance (NGT), impaired glucose tolerance (IGT), type 2 diabetes (T2D), cardiovascular disease (CVD)

Supporting Information Figure S1: Autosampler stability for the top 12 performing glycan node ratios (GNRs) over the time span required to inject 48 samples—the largest batch size employed during analysis of the samples reported in this paper. Passing stability was designated to be within 10% of the average of the first two data points.

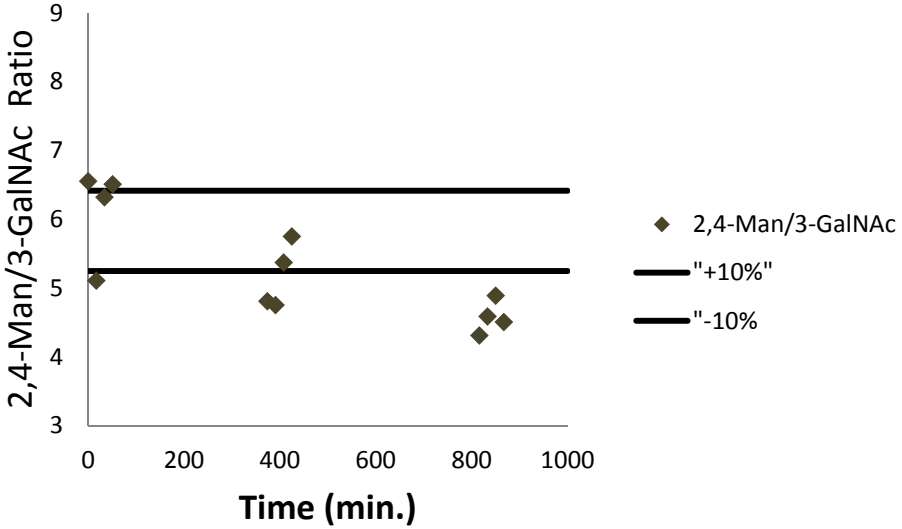
t-Gal/6-Gal Autosampler Stability



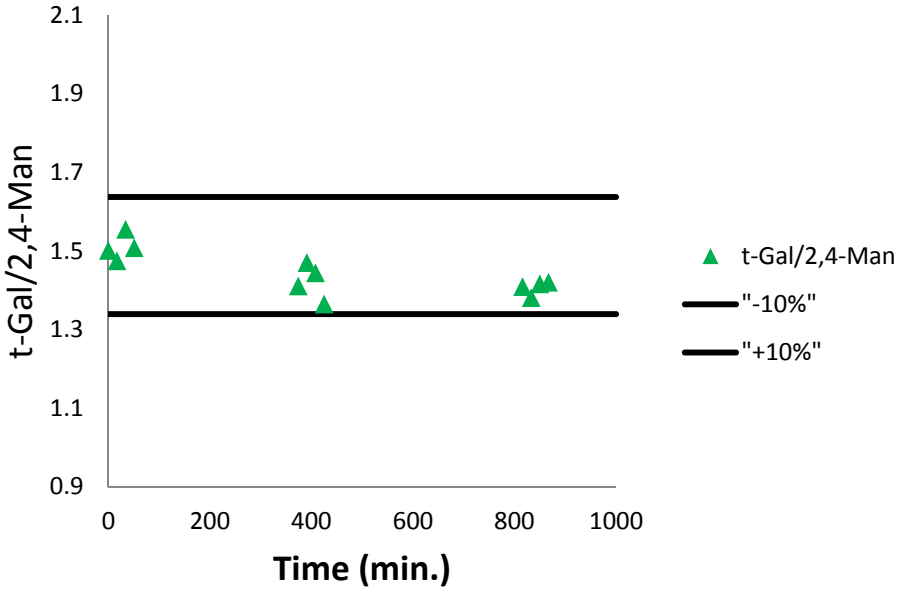
t-Gal/3,6-Gal Autosampler Stability



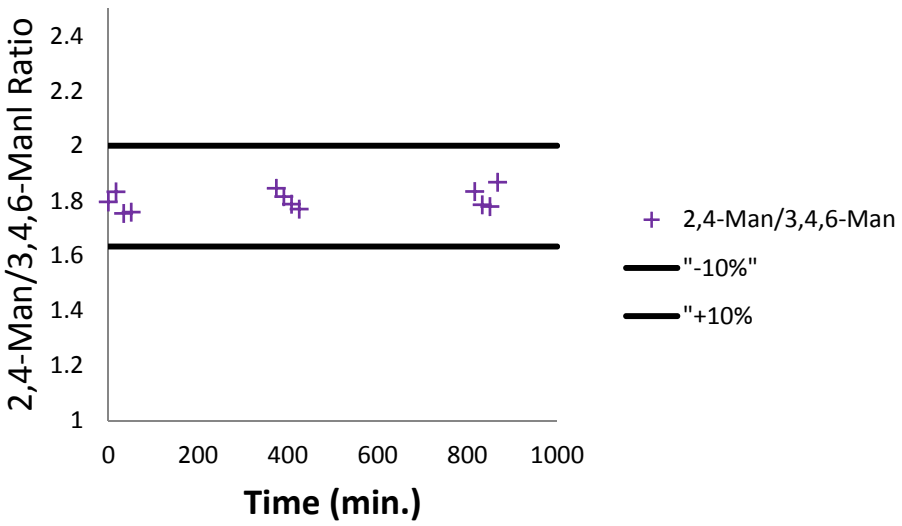
2,4-Man/3-GalNAc Autosampler Stability - FAIL



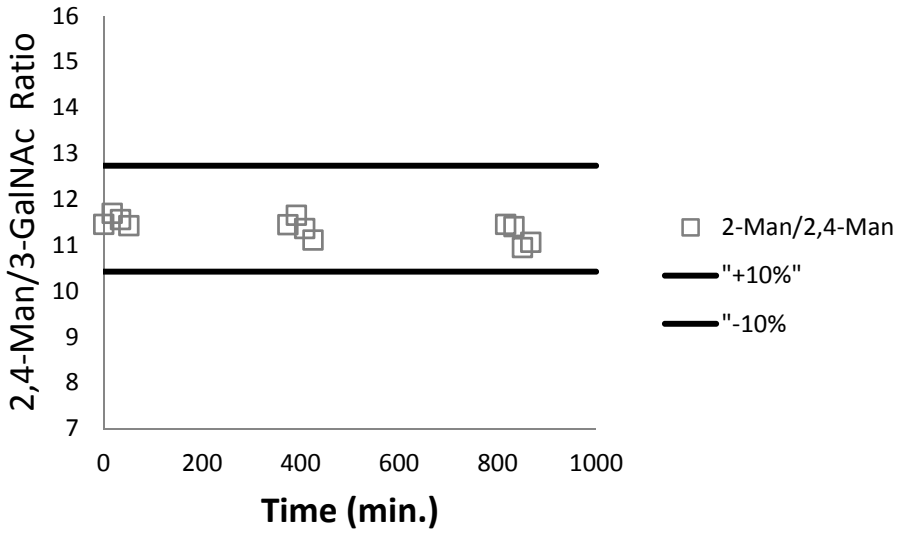
t-Gal/2,4-Man Autosampler Stability



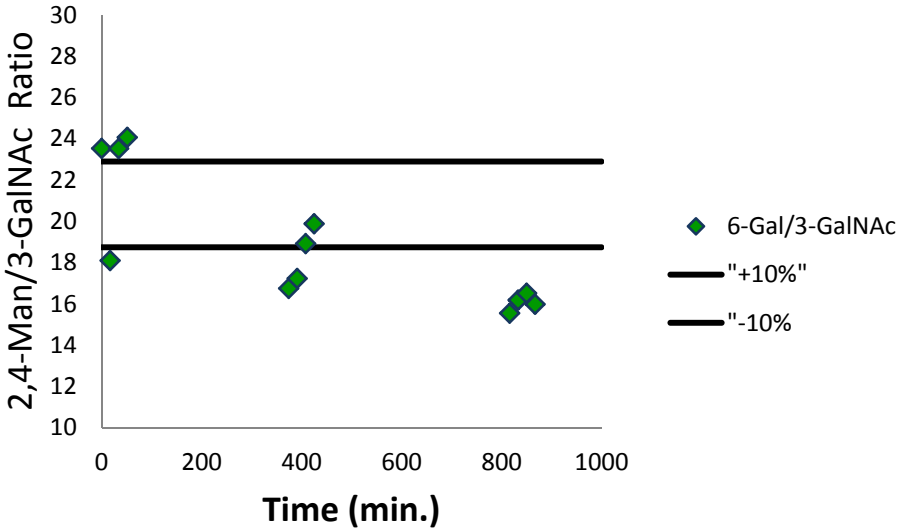
2,4-Man/3,4,6-Man Autosampler Stability



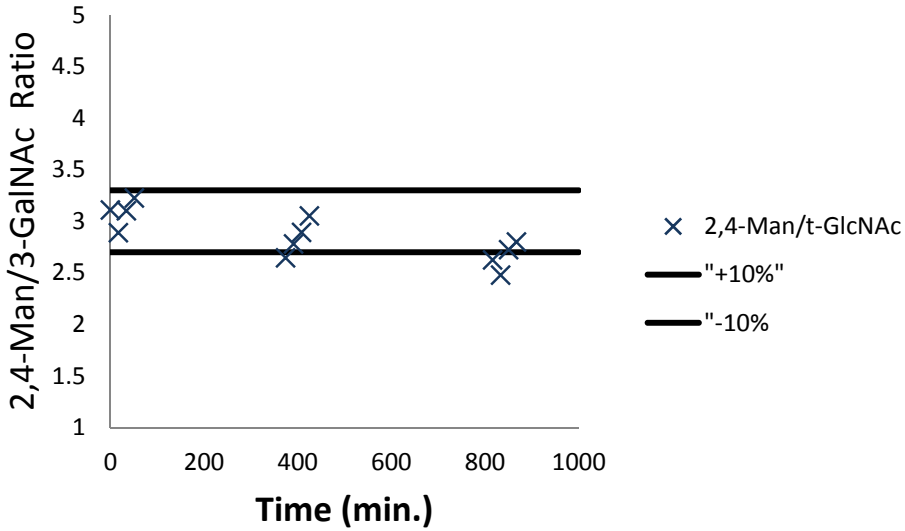
2-Man/2,4-Man Autosampler Stability



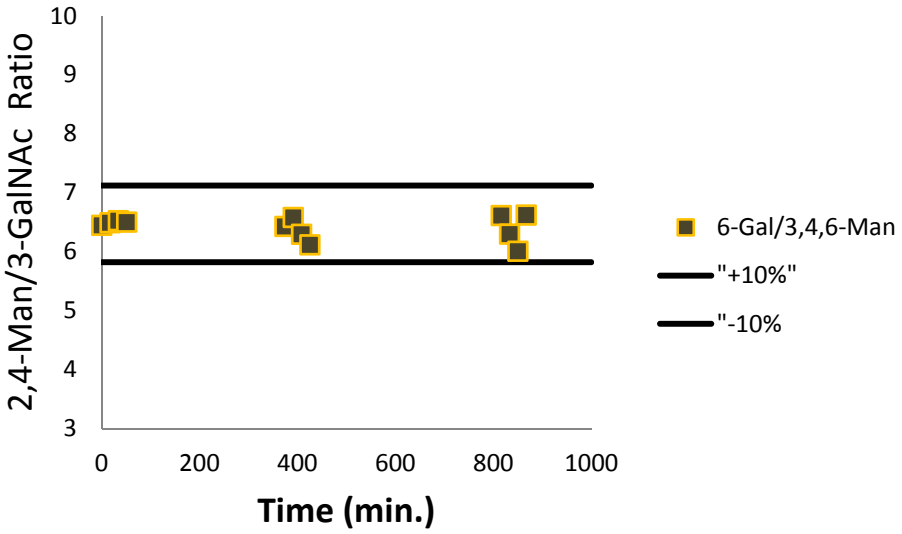
6-Gal/3-GalNAc Autosampler Stability - FAIL



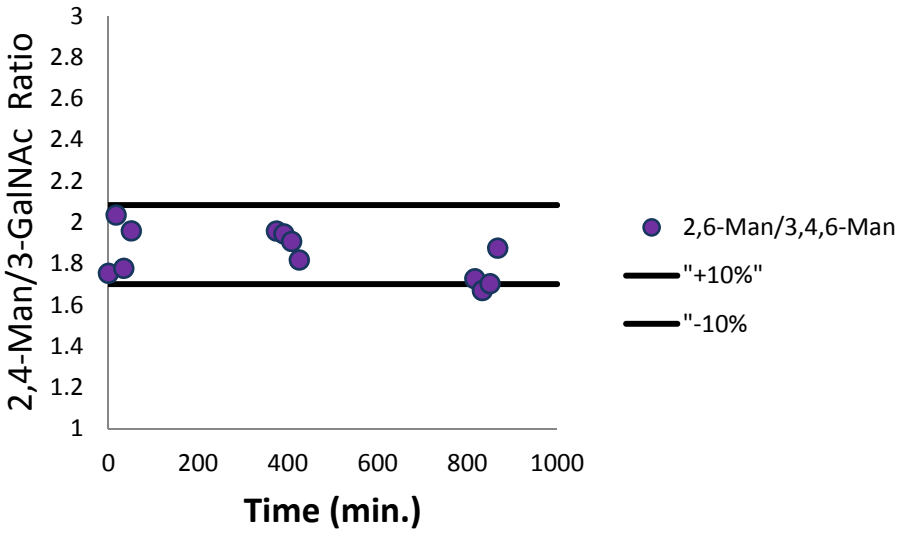
2,4-Man/t-GlcNAc Autosampler Stability - FAIL



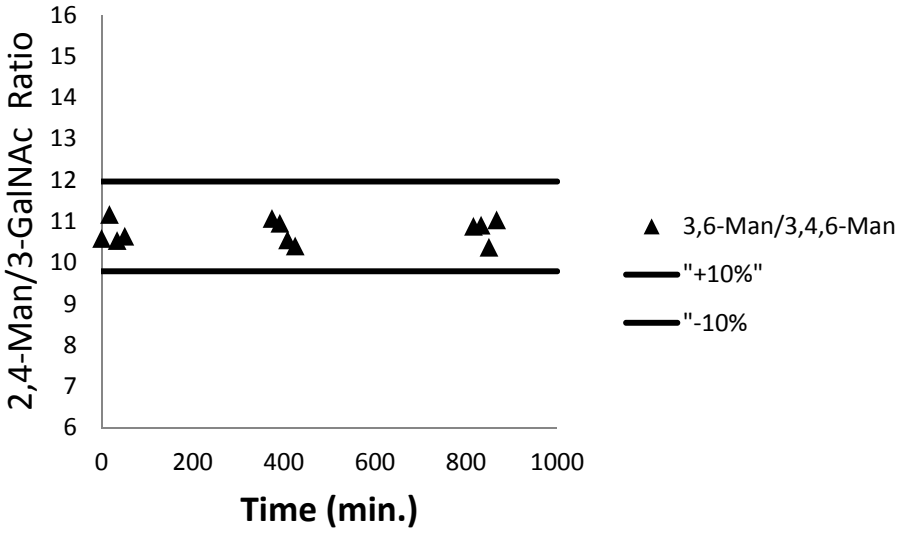
6-Gal/3,4,6-Man Autosampler Stability



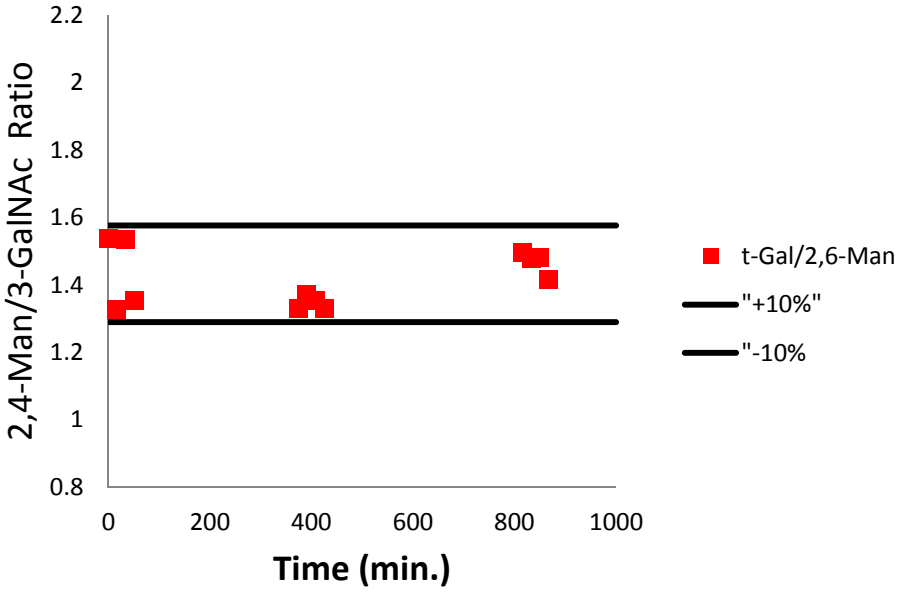
2,6-Man/3,4,6-Man Autosampler Stability

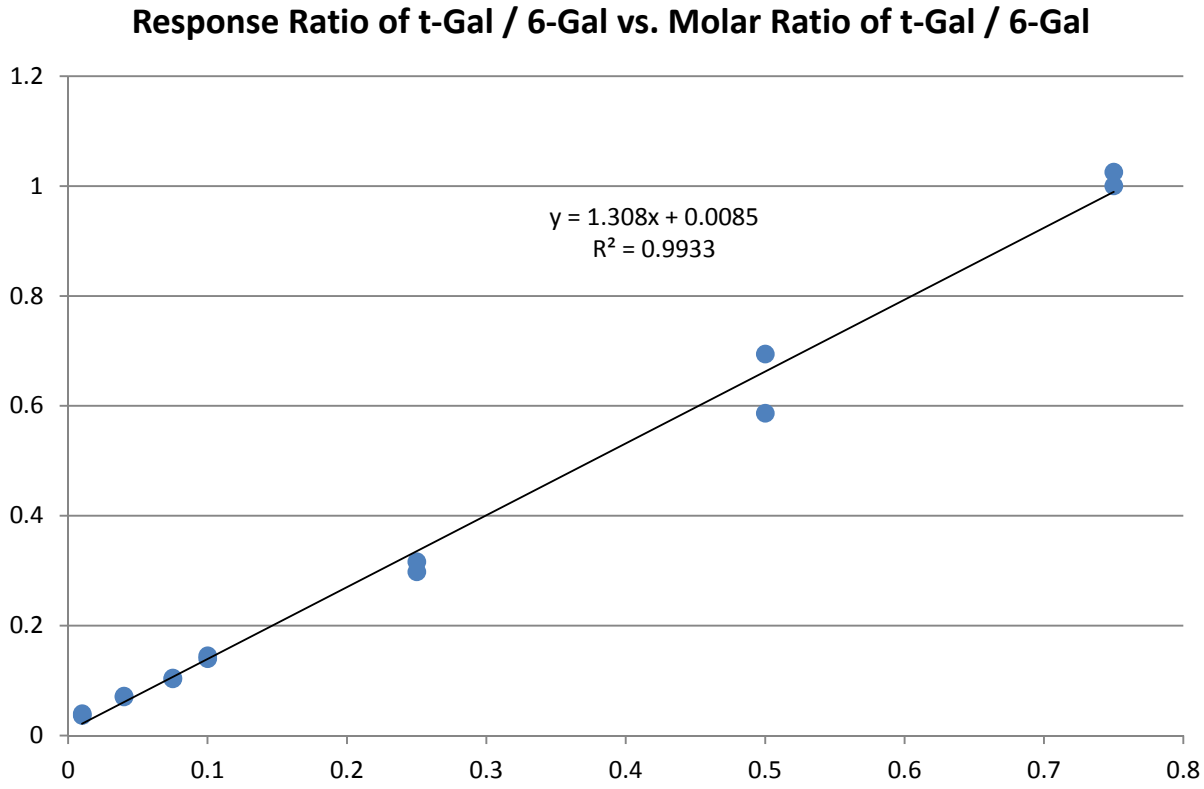


3,6-Man/3,4,6-Man Autosampler Stability

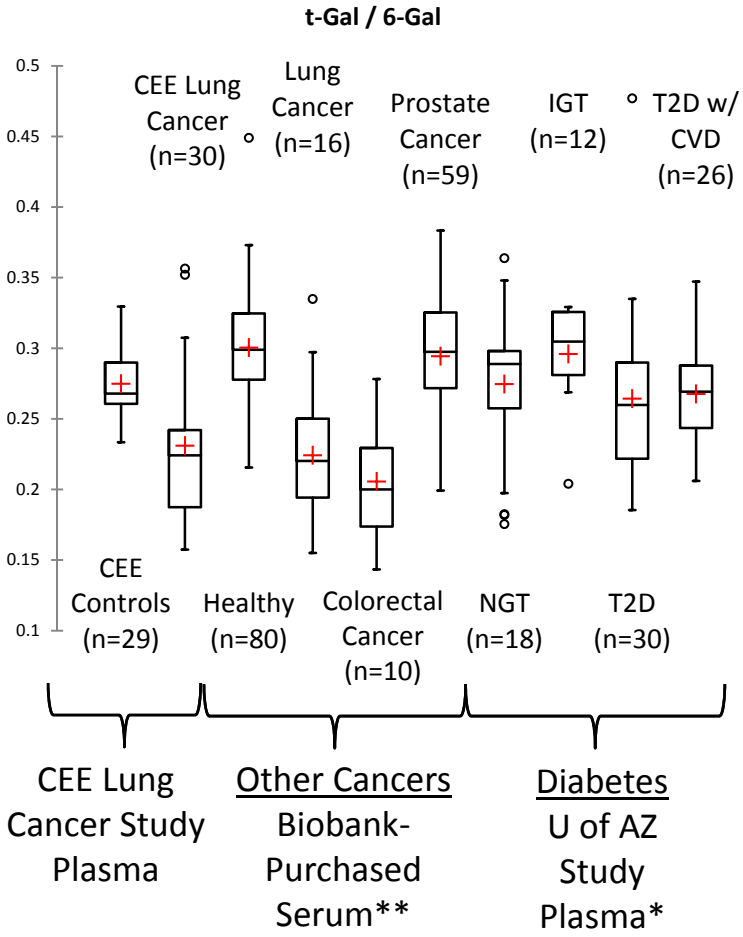


t-Gal/2,6-Man Autosampler Stability



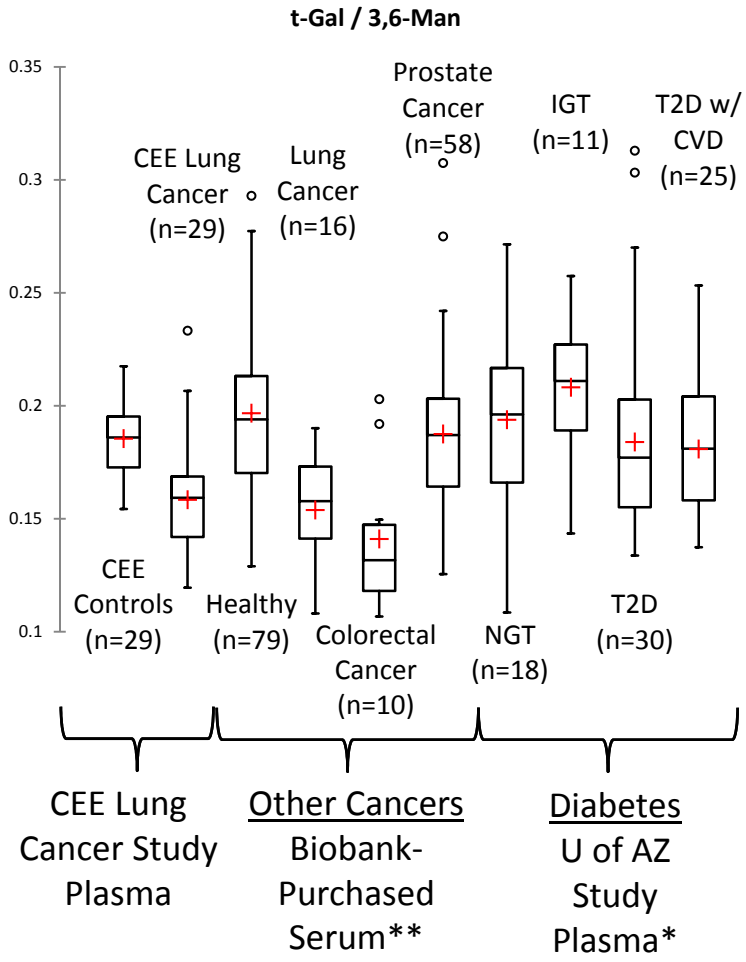


Supporting Information Figure S2: Instrument response ratio of t-Gal/6-Gal vs. actual molar ratio of t-Gal/6-Gal. Standards of N-acetyllactosamine (Gal1-4GalNAc) and 6-Sialyl-N-acetyllactosamine (NeuNAc2-6Gal1-4GalNAc) were employed to construct this curve. Overall signal intensities approximated those from blood plasma samples. Data points represent two standard curves run on two separate days. Extracted ion chromatograms for calculating instrument response ratio are found in Supporting Information Table S1.

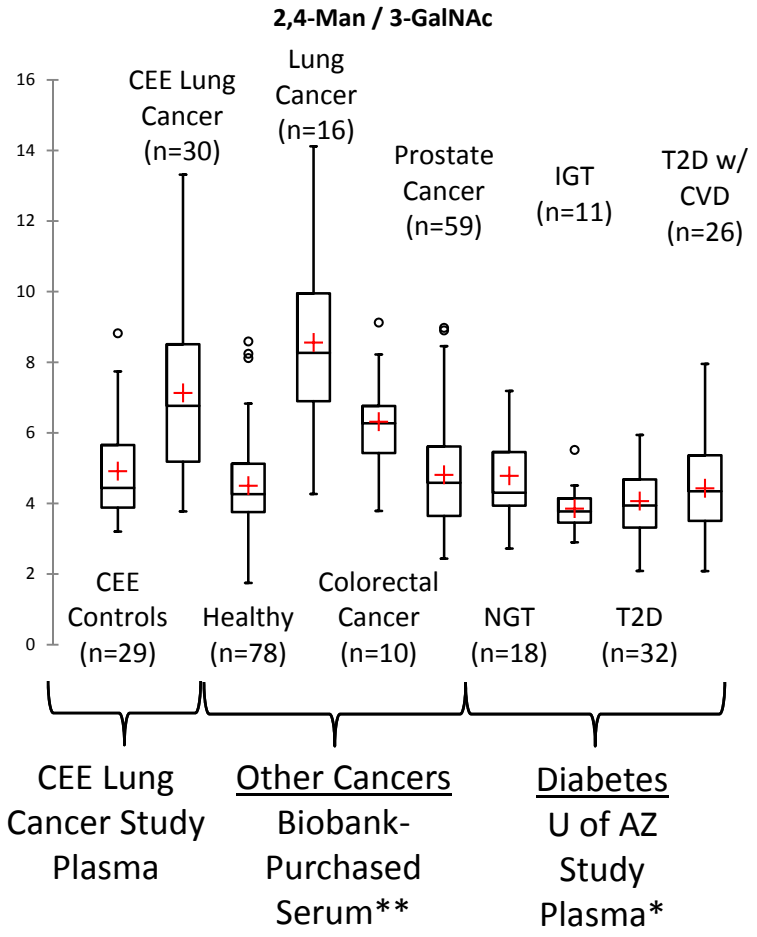


Category	LS means	Groups		
Biobank Healthy	0.300	A		
IGT	0.296	A		
Biobank Prostate Cancer	0.294	A		
CEE Controls	0.275	A	B	
NGT	0.275	A	B	
T2D w/ CVD	0.268		B	
T2D	0.264		B	
CEE Lung Cancer	0.231			C
Biobank Lung Cancer	0.224			C
Biobank Colorectal Cancer	0.206			C

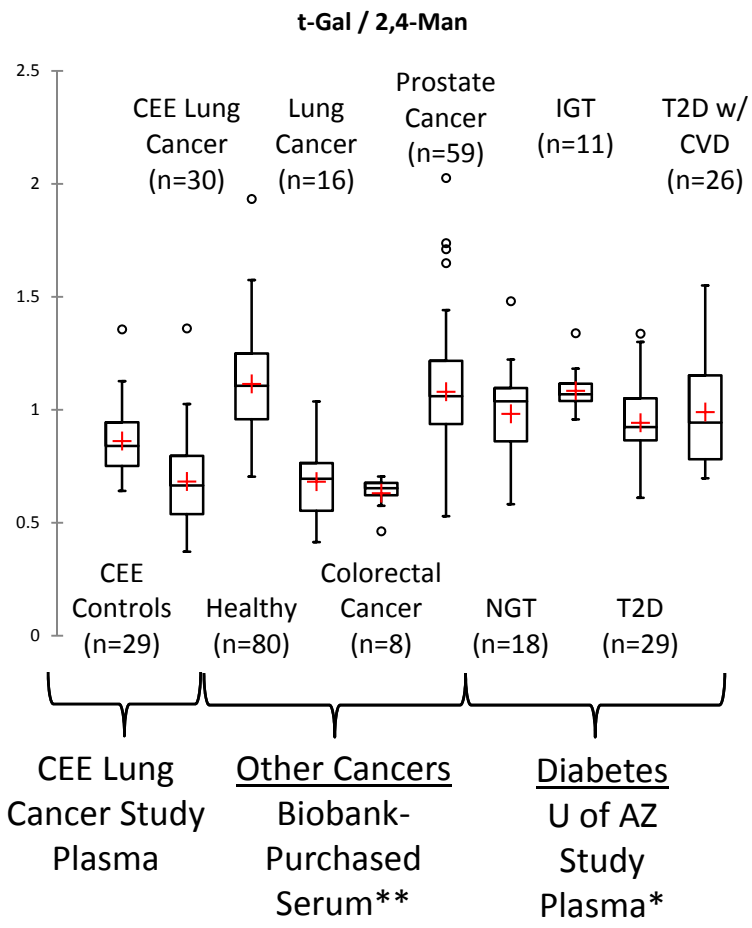
Supporting Information Figures S3: Cancer and chronic disease specificity cross-check for the top 6 performing GNRs in the CEE lung cancer cohort. Statistically significant differences between groups (calculated by ANOVA followed by the Ryan-Einot-Gabriel-Welsch multiple comparison test) are indicated in the adjoining tables at right: Lack of overlap in assigned letters indicates statistical significance; any overlap in an assigned letter indicates lack of a statistically significance difference. As reflected in the n-values, between two and seven extreme outliers from various groups were removed per box-plot / ANOVA comparison. *Diabetes Category Abbreviations: NGT = Normal Glucose Tolerance (Healthy), IGT = Impaired Glucose Tolerance (Pre-T2D), T2D = Type 2 Diabetes, T2D w/ CVD = Type 2 Diabetes with cardiovascular complications. **As described in the manuscript, a pre-analytics study of matched collections of serum and 4 types of plasma show no significant differences in these GNRs.



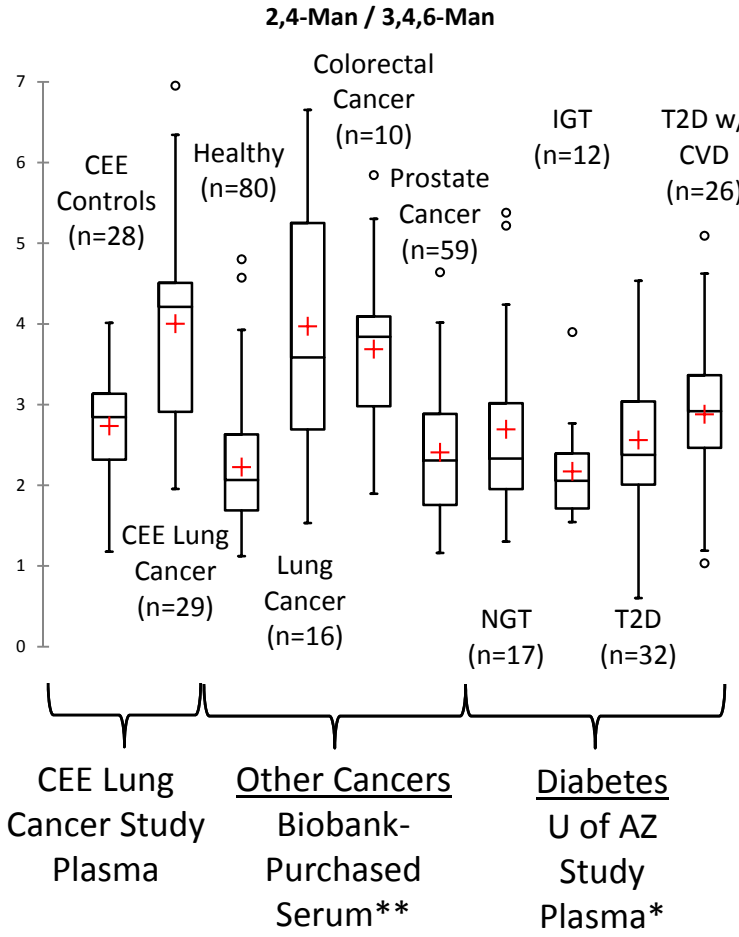
Category	LS means	Groups		
IGT	0.208	A		
Biobank Healthy	0.197	A		
NGT	0.194	A		
Biobank Prostate Cancer	0.187	A		
CEE Controls	0.185	A		
T2D	0.184	A		
T2D w/ CVD	0.181	A	B	
CEE Lung Cancer	0.158		B	C
Biobank Lung Cancer	0.154		B	C
Biobank Colorectal Cancer	0.141			C



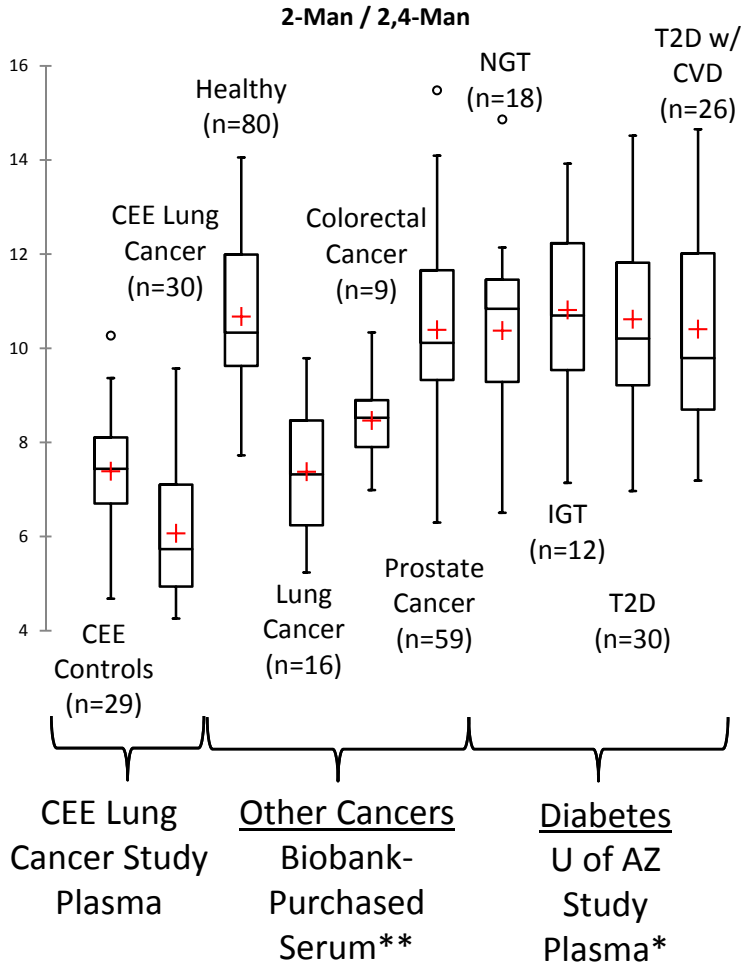
Category	LS means	Groups			
Biobank Lung Cancer	8.560	A			
CEE Lung Cancer	7.129		B		
Biobank Colorectal Cancer	6.313		B	C	
CEE Controls	4.912			C	D
Biobank Prostate Cancer	4.808			C	D
NGT	4.785			C	D
Biobank Healthy	4.500				D
T2D w/ CVD	4.428				D
T2D	4.067				D
IGT	3.851				D



Category	LS means	Groups		
Biobank Healthy	1.114	A		
IGT	1.084	A		
Biobank Prostate Cancer	1.080	A		
T2D w/ CVD	0.989	A	B	
NGT	0.982	A	B	
T2D	0.942		B	
CEE Controls	0.861		B	
CEE Lung Cancer	0.682			C
Biobank Lung Cancer	0.681			C
Biobank Colorectal Cancer	0.631			C



Category	LS means	Groups		
CEE Lung Cancer	4.002	A		
Biobank Lung Cancer	3.970	A		
Biobank Colorectal Cancer	3.687	A		
T2D w/ CVD	2.879		B	
CEE Controls	2.734		B	C
NGT	2.694		B	C
T2D	2.560		B	C
Biobank Prostate Cancer	2.407		B	C
Biobank Healthy	2.225			C
IGT	2.172			C



Category	LS means	Groups		
IGT	10.814	A		
Biobank Healthy	10.673	A		
T2D	10.616	A		
T2D w/ CVD	10.406	A		
Biobank Prostate Cancer	10.391	A		
NGT	10.375	A		
Biobank Colorectal Cancer	8.464		B	
CEE Controls	7.387		B	
Biobank Lung Cancer	7.375		B	C
CEE Lung Cancer	6.069			C

Supporting Information Table S1: Blood plasma/serum data processing parameters. The indicated extracted ion chromatograms (XICs) are summed and automatically integrated by QuanLynx software, manually verified and exported to a spreadsheet.

PMAA Analyte[a]	Summed Ion XIC[b]	Retention Time (min.)
Heavy Fucose	119.06+134.08	4.13
t-Fuc	117.05+131.05	4.13
Heavy Glucose	135.05+153.1+168.1	4.93
t-Glc &/or t-Man	117.05+145.1	4.98
t-Gal	117.05+145.1	5.15
2-Man	129.05+189.1	5.72
4-Gal/4-Man	113.05+117.05+233.1	5.77
4-Glc	113.05+117.05+233.1	5.83
3-Man	117.05	5.87
2-Gal	189.1	5.89
3-Gal	117.05+161.1+233.1	5.95
6-Glc &/or 6-Man	117.05+129.05	6.03
6-Gal	117.05+161.1+233.1	6.31
3,4-Gal	117.05	6.38
2,3-Gal	161.08+261.09	6.53
2,4-Man	129.05+189.1+233.1	6.55
4,6-Glc	117.08+129.08+261.09	6.78
2,6-Man	129.05+189.1	6.86
3,6-Man	117.05+129.05+189.1+233.1	7.00
3,6-Gal	117.05+129.05+189.1+233.1	7.13
3,4,6-Man	117.05+139.05+333.12	7.31
Heavy GlcNAc	118.08+160.09	7.71
t-GlcNAc	116.08+158.08	7.71
t-GalNAc	116.08+158.08	8.04
4-GlcNAc	158.08	8.34
3-GlcNAc	116.08+158.08	8.69
3-GalNAc	116.08+158.08	8.79
6-GlcNAc	116.08+158.08	8.84
3,4-GlcNAc	116.08+158.08	8.99
4-GalNAc	116.08+158.08	9.08
6-GalNAc	116.08+158.08	9.15
4,6-GlcNAc	116.08+158.08	9.37
3,6-GalNAc	116.08+158.08	9.89

[a] PMAA = Partially Methylated Alditol Acetate. "t-" indicates a terminal residue and "n-", "n,n-", or "n,n,n-" indicate linkage positions of the residue in the original glycan polymer. Monosaccharide abbreviations are provided in Fig. 1.

[b] XIC = Extracted Ion Chromatogram. A mass window of ± 0.15 Da is taken around the indicated *m/z*

Undetected Nodes				Individual Nodes (Sort based on these columns)																																		
t-NeuNAc	8-Sial	4-NeuNAc	4-GlcA	t-Xyl	4-Xyl	t-Fuc	3-Fuc	t-Glc	t-Man	t-Gal	2-Man	4-Gal/4-Man	4-Glc	3-Man	2-Gal	3-Gal	6-Glc/6-Man	6-Gal	3,4-Gal	2,3-Gal	2,4-Man	4,6-Glc	2,6-Man	3,6-Man	3,6-Gal	3,4,6-Man	6-Inositol	t-GlcNAc	t-GalNAc	4-GlcNAc	3-GlcNAc	3-GalNAc	6-GlcNAc	3,4-GlcNAc	4-GalNAc	6-GalNAc	4,6-GlcNAc	3,6-GalNAc
						t-Fuc																												3,4-GlcNAc				
						t-Fuc																												3,4-GlcNAc				
						t-Fuc																												3,4-GlcNAc				
						t-Fuc																												3,4-GlcNAc				
						t-Fuc																												3,4-GlcNAc				
						t-Fuc																															4,6-GlcNAc	
																										3,4,6-Man		t-GlcNAc										
																					2,4-Man							t-GlcNAc										
																					2,4-Man							t-GlcNAc										
																					2,4-Man							t-GlcNAc										
t-NeuNAc																		6-Gal																				
t-NeuNAc																		6-Gal																				
t-NeuNAc																		6-Gal																				
																		6-Gal							3,6-Gal			t-GlcNAc										3,6-GalNAc
													4-Glc																									
													4-Glc																									
										t-Gal			4-Glc																	4-GlcNAc								
										t-Gal			4-Glc																	4-GlcNAc								
													4-Glc																									
													4-Glc																									
										t-Gal			4-Glc																									
						t-Fuc									2-Gal																							
						t-Fuc									2-Gal																							
						t-Fuc																																
						t-Fuc																																
t-NeuNAc																																				6-GalNAc		
t-NeuNAc																																				6-GalNAc		
t-NeuNAc																3-Gal																						
t-NeuNAc																3-Gal																						
t-NeuNAc																3-Gal																						
t-NeuNAc																																						3,6-GalNAc
t-NeuNAc																																						3,6-GalNAc
t-NeuNAc	8-Sial																																					
t-NeuNAc																3-Gal																						
t-NeuNAc																3-Gal																						
				t-Xyl																																		
				t-Xyl																																		
																						4,6-Glc																
										t-Gal										2,3-Gal									t-GalNAc									
										t-Gal																				4-GlcNAc								
										t-Gal																				4-GlcNAc					4-GalNAc			
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
																													t-GalNAc									
										t-Gal																												
										t-Gal																												
										t-Gal																												
										t-Gal																						3-GalNAc						
								t-Glc							2-Gal																							
																3-Gal													t-GalNAc									
								t-Glc																														
										t-Gal																					3-GlcNAc							
																													t-GalNAc			3-GalNAc						
																			3,4-Gal										t-GalNAc									
											2-Man																	t-GlcNAc										
																												t-GlcNAc										3,6-GalNAc
																												t-GlcNAc										3,6-GalNAc
									t-Man																													
									t-Man																													
										t-Gal																						3-GalNAc						
																								3,6-Man														
					4-Xyl					t-Gal																												
										t-Gal						3-Gal																						
																3-Gal																						
																3-Gal																						
																3-Gal																						
																														4-GlcNAc								
											2-Man																	t-GlcNAc										
																3-Gal												t-GlcNAc										
																												t-GlcNAc				3-GalNAc						
																												t-GlcNAc										3,6-GalNAc
																3-Gal												t-GlcNAc										
																									3,6-Gal			t-GlcNAc										
																									3,6-Gal			t-GlcNAc										
																									3,6-Gal			t-GlcNAc										
																							2,6-Man					t-GlcNAc										
																							2,6-Man					t-GlcNAc										
																3-Gal												t-GlcNAc										
		4-NeuNAc																											t-GalNAc									
			4-GlcA																										t-GalNAc									
			4-GlcA																										t-GalNAc									
			4-GlcA																										t-GalNAc			3-GalNAc						
			4-GlcA																										t-GalNAc			3-GalNAc						
			4-GlcA																										t-GalNAc			3-GalNAc						
										t-Gal						3-Gal																						
																											6-Inositol	t-GlcNAc										
																											6-Inositol	t-GlcNAc										
																											6-Inositol	t-GlcNAc										
																											6-Inositol	t-GlcNAc										
																											6-Inositol	t-GlcNAc										
																3-Gal												t-GlcNAc										
			4-GlcA																											3-GlcNAc							
			4-GlcA																											3-GlcNAc							
			4-GlcA																											3-GlcNAc							
							3-Fuc																					t-GlcNAc										
							3-Fuc																					t-GlcNAc										
							3-Fuc																					t-GlcNAc										
			4-GlcA																									t-GlcNAc										
			4-GlcA																									t-GlcNAc										
			4-GlcA																											4-GlcNAc								
			4-GlcA																											4-GlcNAc								
			4-GlcA																									t-GlcNAc										
																																3-GalNAc						
										t-Gal		4-Gal																										
																													t-GalNAc	4-GlcNAc								
																													t-GalNAc	4-GlcNAc								
																												t-GlcNAc										
																												t-GlcNAc										
																												t-GlcNAc										

Supporting Information Table S2: Glycotransferases (GTs) responsible for producing human glycan nodes (GNs). As illustrated by the additional worksheets, this Table may be sorted by a GN of interest to obtain a succinct list of GTs responsible for creating that GN. Greyed-out GNs indicate that the GN is usually eliminated later through the action of a downstream-acting GT. In general, only enzymes that employ monosaccharide donors and catalyze potentially final steps in the construction of physiologically observed GNs are listed. This table was constructed by cross-referencing all human glycotransferase enzyme activities (EC numbers) listed on the BRENDA Database website (http://www.brenda-enzymes.info/ectree2/browse.php) with the UniProt DataBase (http://www.uniprot.org). In many cases, multiple human GTs are responsible for a single enzyme activity (EC number). Common names listed are often one of many. Sialic acid (NeuNAc) and glucuronic acid-based nodes (tan colored) are not detected by the methodology employed. 3-Man, 6-GlcNAc, and 6-Glc/6-Man GNs (present at very low quantites in plasma) for which no GTs are listed as responsible may be created by catabolic (debranching) processes.

Summary of Glycan Node(s) Produced	EC Number	Uniprot-Recommended Enzyme Name	Common Name	Gene Name	Evidence Level	Reaction Catalyzed	Function & Notes
t-Fuc, 3,4-GlcNAc	2.4.1.65	Galactoside 3(4)-L-fucosyltransferase	FucT-III	**FUT3**	Evidence at transcript level	GDP-beta-L-fucose + beta-D-gala	May catalyze alpha-1,3 and alpha-1,4 glycosidic linkages involved in the expression of Vim-2, Lewis A, Lewis B, sialyl Lewis X and Lewis X/SSEA-1 antigens. May be involved in blood group Lewis determination; Lewis-positive (Le) individuals have an active enzyme while Lewis-negative (Le) individuals have an inactive enzyme. Also acts on the corresp
t-Fuc, 3,4-GlcNAc	2.4.1.65	Alpha-(1,3)-fucosyltransferase	FucT-V	**FUT5**	Evidence at transcript level	GDP-beta-L-fucose + beta-D-gala	May catalyze alpha-1,3 glycosidic linkages involved in the expression of VIM-2, Lewis X/SSEA-1 and sialyl Lewis X antigens.
t-Fuc, 3,4-GlcNAc	2.4.1.65	Alpha-(1,3)-fucosyltransferase	FucT-VI	**FUT6**	Evidence at protein level	GDP-beta-L-fucose + beta-D-gala	Enzyme involved in the biosynthesis of the E-Selectin ligand, sialyl-Lewis X. Catalyzes the transfer of fucose from GDP-beta-fucose to alpha-2,3 sialylated substrates
t-Fuc, 3,4-GlcNAc	2.4.1.152	Alpha-(1,3)-fucosyltransferase 11	FucT-XI	**FUT11**	Evidence at protein level		Probable fucosyltransferase
t-Fuc, 3,4-GlcNAc	2.4.1.214	glycoprotein 3-alpha-L-fucosyltransferase				GDP-beta-L-fucose + N4-{N-acety	EC Number Not in Uniprot DB
t-Fuc, 4,6-GlcNAc	2.4.1.68	Alpha-(1,6)-fucosyltransferase	Fucosyltransferase 8	**FUT8**	Evidence at protein level	GDP-beta-L-fucose + N(4)-(N-ace	MAJOR for 4,6-GlcNAc: Catalyzes the addition of fucose in alpha 1-6 linkage to the first GlcNAc residue, next to the peptide chains in N-glycans
t-GlcNAc, 3,4,6-Man	2.4.1.144	Beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransfe	GNT-III	**MGAT3**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	MAJOR N-glycans (Bisecting GlcNAc): It is involved in the regulation of the biosynthesis and biological function of glycoprotein oligosaccharides. Catalyzes the addition of N-acetylglucosamine in beta 1-4 linkage to the beta-linked mannose of the trimannosyl core of N-linked sugar chains. It is one of the most important enzymes involved in the re
t-GlcNAc, 2,4-Man	2.4.1.145	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltrans	GnT-IVa	**MGAT4A**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + 3-	MAJOR: N-glycans: Glycosyltransferase that participates in the transfer of N-acetylglucosamine (GlcNAc) to the core mannose residues of N-linked glycans. Catalyzes the formation of the GlcNAcbeta1-4 branch on the GlcNAcbeta1-2Manalpha1-3 arm of the core structure of N-linked glycans. Essential for the production of tri- and tetra-antennar
t-GlcNAc, 2,4-Man	2.4.1.145	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltrans	GnT-IVb	**MGAT4B**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 3-	Not likely main contibutor: Glycosyltransferase that participates in the transfer of N-acetylglucosamine (GlcNAc) to the core mannose residues of N-linked glycans. Catalyzes the formation of the GlcNAcbeta1-4 branch on the GlcNAcbeta1-2Manalpha1-3 arm of the core structure of N-linked glycans. Essential for the production of tri- and tetra-a
t-GlcNAc, 2,4-Man	2.4.1.145	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltrans	GnT-IVc	**MGAT4C**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + 3-	"Evidence for existence at the transcript level": Glycosyltransferase that participates in the transfer of N-acetylglucosamine (GlcNAc) to the core mannose residues of N-linked glycans. Catalyzes the formation of the GlcNAcbeta1-4 branch on the GlcNAcbeta1-2Manalpha1-3 arm of the core structure of N-linked glycans. Essential for the producti
6-Gal	2.4.99.1	Beta-galactoside alpha-2,6-sialyltransferase 1	ST6Gal I	**ST6GAL1**	Evidence at protein level	CMP-N-acetylneuraminate + beta	MAJOR; Transfers sialic acid from the donor of substrate CMP-sialic acid to galactose containing acceptor substrates.
6-Gal	2.4.99.1	Beta-galactoside alpha-2,6-sialyltransferase 2	ST6Gal II	**ST6GAL2**	Evidence at protein level	CMP-N-acetylneuraminate + beta	MINOR (weak or no activities toward glycoproteins and glycolipids)Transfers sialic acid from the donor of substrate CMP-sialic acid to galactose containing acceptor substrates. Has alpha-2,6-sialyltransferase activity toward oligosaccharides that have the Gal-beta-1,4-GlcNAc sequence at the non-reducing end of their carbohydrate groups, but it
6-Gal	2.4.99.11	lactosylceramide alpha-2,6-N-sialyltransferase	GM3 synthase			CMP-N-acetylneuraminate + beta	EC Number Not in Uniprot DB; Glycolipid-specific
t-GlcNAc, 3,6-GalNAc, & 6-Gal, 3,6-Gal	2.4.1.102 & 2.4.1.150	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucos	C2/4GnT	**GCNT3**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	"Evidence at transcript level": Glycosyltransferase that can synthesize all known mucin beta 6 N-acetylglucosaminides. Mediates core 2 and core 4 O-glycan branching, 2 important steps in mucin-type biosynthesis. Has also I-branching enzyme activity by converting linear into branched poly-N-acetyllactosaminoglycans, leading to introduce the b
4-Glc	2.4.1.11	Glycogen [starch] synthase, muscle		**GYS1**	Evidence at protein level	UDP-glucose ((1->4)-alpha-D-gluc	Node from this enzyme likely not present in biofluids;Transfers the glycosyl residue from UDP-Glc to the non-reducing end of alpha-1,4-glucan
4-Glc	2.4.1.11	Glycogen [starch] synthase, liver		**GYS2**	Evidence at protein level	UDP-glucose ((1->4)-alpha-D-gluc	Transfers the glycosyl residue from UDP-Glc to the non-reducing end of alpha-1,4-glucan
4-GlcNAc	2.4.1.22 & 2.4.1.38 & 2.4.1.90 & 2.4.1.n	Beta-1,4-galactosyltransferase 1	b4Gal-T1	**B4GALT1**	Evidence at protein level	UDP-alpha-D-galactose + D-gluco	MINOR in glycans; The Golgi complex form catalyzes the production of lactose in the lactating mammary gland and could also be responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids. The cell surface form functions as a recognition molecule during a va
t-Gal, 4-Glc (as lactose), 4-GlcNAc	2.4.1.22 & 2.4.1.38 & 2.4.1.90 & 2.4.1.n	Beta-1,4-galactosyltransferase 2	b4Gal-T2	**B4GALT2**	Evidence at protein level	UDP-alpha-D-galactose + D-gluco	Responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids. Can produce lactose
4-Glc (Glycogen starter)	2.4.1.186	Glycogenin-1	GN-1	**GYG1**	Evidence at protein level	UDP-alpha-D-glucose + glycogeni	GLYCOGEN Synthesis: Self-glucosylates, via an inter-subunit mechanism, to form an oligosaccharide primer that serves as substrate for glycogen synthase
4-Glc (Glycogen starter)	2.4.1.186	Glycogenin-2	GN-2	**GYG2**	Evidence at protein level	UDP-alpha-D-glucose + glycogeni	GLYCOGEN Synthesis: Self-glucosylates, via an inter-subunit mechanism, to form an oligosaccharide primer that serves as substrate for glycogen synthase
t-Gal, 4-Glc	2.4.1.274	Beta-1,4-galactosyltransferase 6	b4Gal-T6	**B4GALT6**	Evidence at protein level	UDP-galactose + glucosylceramid	MAJOR: Required for the biosynthesis of glycosphingolipids
t-Fuc, 2-Gal	2.4.1.69	Galactoside 2-alpha-L-fucosyltransferase 1	Fucosyltransferase 1	**FUT1**	Evidence at transcript level	GDP-beta-L-fucose + beta-D-gala	GLYCOLIPIDS; Creates a soluble precursor oligosaccharide FuC-alpha ((1,2)Galbeta-) called the H antigen which is an essential substrate for the final step in the soluble A and B antigen synthesis pathway. H and Se enzymes fucosylate the same acceptor substrates but exhibit different Km values
t-Fuc, 2-Gal	2.4.1.69	Galactoside 2-alpha-L-fucosyltransferase 2	Fucosyltransferase 2	**FUT2**	Evidence at transcript level	GDP-beta-L-fucose + beta-D-gala	GLYCOLIPIDS: Creates a soluble precursor oligosaccharide FuC-alpha ((1,2)Galbeta-) called the H antigen which is an essential substrate for the final step in the soluble A and B antigen synthesis pathway. H and Se enzymes fucosylate the same acceptor substrates but exhibit different Km values
t-Fuc	2.4.1.221	GDP-fucose protein O-fucosyltransferase 1	O-FucT-1	**POFUT1**	Evidence at protein level	Transfers an alpha-L-fucosyl resid	Catalyzes the reaction that attaches fucose through an O-glycosidic linkage to a conserved serine or threonine residue in EGF domains. Plays a crucial role in Notch signaling
t-Fuc	2.4.1.221	GDP-fucose protein O-fucosyltransferase 2	O-FucT-2	**POFUT2**	Evidence at protein level	Transfers an alpha-L-fucosyl resid	Catalyzes the reaction that attaches fucose through an O-glycosidic linkage to a conserved serine or threonine residue in thrombospondin type 1 repeats
6-GalNAc	2.4.99.3	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	ST6GalNAc I	**ST6GALNAC1**	Evidence at protein level	CMP-N-acetylneuraminate + glycano-(1->3)-(N-acetyl-alpha-D-galactosaminyl)-glycoprotein = CMP + glycano-((2->6)-alpha-N-acetylneuraminyl)-(N-acetyl-D-galactosaminyl)-glycoprotein	
6-GalNAc	2.4.99.3(?)	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	ST6GalNAc II	**ST6GALNAC2**	Evidence at protein level	CMP-N-acetylneuraminate + glyc	Aberrant O-galactosylation of IgA1 molecules plays a role in the development and progression of IgA nephropathy (IgAN). Genetic interactions of C1GALT1 and ST6GALNAC2 variants influence IgA1 O-glycosylation, disease predisposition, and disease severity, and may contribute to the polygenic nature of IgAN.
3-Gal	2.4.99.4	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltrans	ST3Gal I	**ST3GAL1**	Evidence at transcript level	CMP-N-acetylneuraminate + beta	CORE 1: It may be responsible for the synthesis of the sequence NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc- found on sugar chains O-linked to Thr or Ser and also as a terminal sequence on certain gangliosides. SIAT4A and SIAT4B sialylate the same acceptor substrates but exhibit different Km values
3-Gal	2.4.99.4	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltrans	ST3Gal II	**ST3GAL2**	Evidence at transcript level	CMP-N-acetylneuraminate + beta	CORE 1: It may be responsible for the synthesis of the sequence NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc- found in terminal carbohydrate groups of certain glycoproteins, oligosaccharides and glycolipids. SIAT4A and SIAT4B sialylate the same acceptor substrates but exhibit different Km values.
3-Gal	2.4.99.6	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltran	ST3Gal III	**ST3GAL3**	Evidence at protein level	CMP-N-acetylneuraminate + beta	MAJOR: Catalyzes the formation of the NeuAc-alpha-2,3-Gal-beta-1,4-GlcNAc-, NeuAc-alpha-2,3-Gal-beta-1,3-GlcNAc- or NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc- sequences found in terminal carbohydrate groups of glycoproteins and glycolipids. The highest activity is toward Gal-beta-1,3-GlcNAc and the lowest toward Gal-beta-1,3-GalNAc
3,6-GalNAc	2.4.99.7	Alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3-N-acetyl-galacto	ST6GalNAc IV	**ST6GALNAC4**	Evidence at protein level	CMP-N-acetylneuraminate + N-ac	Involved in the biosynthesis of ganglioside GD1A from GM1B. Transfers CMP-NeuAc with an alpha-2,6-linkage to GalNAc residue on NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc of glycoproteins and glycolipids. Prefers glycoproteins to glycolipids
3,6-GalNAc	2.4.99.?	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	ST6GalNAc III	**ST6GALNAC3**	Evidence at transcript level	CMP-N-acetylneuraminate + alph	Involved in the biosynthesis of ganglioside GD1A from GM1B. Transfers CMP-NeuAc with an alpha-2,6-linkage to GalNAc residue on NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc of glycoproteins and glycolipids. ST6GalNAcIII prefers glycolipids to glycoproteins
8-Sial	2.4.99.8	Alpha-N-acetylneuraminide alpha-2,8-sialyltransferase	ST8SiaI	**ST8SIA1**	Evidence at protein level	CMP-N-acetylneuraminate + alph	Involved in the production of gangliosides GD3 and GT3 from GM3; gangliosides are a subfamily of complex glycosphinglolipds that contain one or more residues of sialic acid
3-Gal	2.4.99.9	Lactosylceramide alpha-2,3-sialyltransferase	ST3GalV	**ST3GAL5**	Evidence at protein level	CMP-N-acetylneuraminate + beta	Catalyzes the formation of ganglioside GM3 (alpha-N-acetylneuraminyl-2,3-beta-D-galactosyl-1, 4-beta-D-glucosylceramide).
3-Gal	2.4.99.10	neolactotetraosylceramide alpha-2,3-sialyltransferase	SAT-3			CMP-N-acetylneuraminate + beta	GLYCOLIPIDS: EC Number Not in Uniprot DB
t-Xyl	2.4.2.26	Xylosyltransferase 1	XylT-I	**XYLT1**	Evidence at protein level	Transfers a beta-D-xylosyl residu	Catalyzes the first step in biosynthesis of glycosaminoglycan. Transfers D-xylose from UDP-D-xylose to specific serine residues of the core protein. Initial enzyme in the biosynthesis of chondroitin sulfate and dermatan sulfate proteoglycans in fibroblasts and chondrocytes
t-Xyl	2.4.2.26	Xylosyltransferase 2	XylT-II	**XYLT2**	Evidence at transcript level	Enzyme Activity not Demonstrate	Probably catalyzes the first step in biosynthesis of glycosaminoglycan. Transfers D-xylose from UDP-D-xylose to specific serine residues of the core protein. Initial enzyme in the biosynthesis of chondroitin sulfate and dermatan sulfate proteoglycans in fibroblasts and chondrocytes
4,6-Glc	2.4.1.18	1,4-alpha-glucan-branching enzyme	Glycogen-branching enzyme	**GBE1**	Evidence at protein level	Transfers a segment of a (1->4)-a	Required for sufficient glycogen accumulation. The alpha 1-6 branches of glycogen play an important role in increasing the solubility of the molecule and, consequently, in reducing the osmotic pressure within cells
2,3-Gal	2.4.1.37 & 2.4.1.40	Histo-blood group ABO system transferase	A transferase & B transferase	**ABO**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	This protein is the basis of the ABO blood group system--based on different mutations in this gene. The histo-blood group ABO involves three carbohydrate antigens: A, B, and H. A, B, and AB individuals express a glycosyltransferase activity that converts the H antigen to the A antigen (by addition of UDP-GalNAc) or to the B antigen (by addition
t-Gal, 4-GlcNAc	2.4.1.38 & 2.4.1.90 & 2.4.1.n	Beta-1,4-galactosyltransferase 3	b4Gal-T3	**B4GALT3**	Evidence at protein level	UDP-alpha-D-galactose + N-acety	Responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids
t-Gal, 4-GlcNAc, 4-GalNAc	2.4.1.90 & 2.4.1.275	Beta-1,4-galactosyltransferase 4	b4Gal-T4	**B4GALT4**	Evidence at protein level	UDP-alpha-D-galactose + N-acety	Responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 1	GalNAc-T1	**GALNT1**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has a broad spectrum of substrates for peptides such as EA2, Muc5AC, Muc1a, Muc1b and Muc7
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 2	GalNAc-T2	**GALNT2**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has a broad spectrum of substrates for peptides such as EA2, Muc5AC, Muc1a, Muc1b. Probably involved in O-linked glycosylation of the immunoglobulin A1 (IgA1) h
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase-like protein 2	GalNAc-T-like protein 2	**GALNTL2**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Although it displays a much weaker activity toward all substrates tested compared to GALNT2, it is able to transfer up to seven GalNAc residues to the Muc5AC pepti
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 3	GalNAc-T3	**GALNT3**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has activity toward HIV envelope glycoprotein gp120, EA2, Muc2 and Muc5. Probably glycosylates fibronectin in vivo. Glycosylates FGF23. Plays a central role in phos
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 4	GalNAc-T4	**GALNT4**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has a highest activity toward Muc7, EA2 and Muc2, with a lowest activity than GALNT2. Glycosylates 'Thr-57' of SELPLG
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 5	GalNAc-T5	**GALNT5**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has activity toward EA2 peptide substrate, but has a weak activity toward Muc2 or Muc1b substrates
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 6	GalNAc-T6	**GALNT6**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. May participate in synthesis of oncofetal fibronectin. Has activity toward Muc1a, Muc2, EA2 and fibronectin peptides
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 9	GalNAc-T9	**GALNT9**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Does not glycosylate apomucin or SDC3
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 10	GalNAc-T10	**GALNT10**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has activity toward Muc5Ac and EA2 peptide substrates.
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 11	GalNAc-T11	**GALNT11**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Displays the same enzyme activity toward Muc1, Muc4.1, and EA2 than GALNT1. Does not appear to be involved in glycosylation of erythropoietin
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 12	GalNAc-T12	**GALNT12**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has activity toward non-glycosylated peptides such as Muc5AC, Muc1a and EA2, and no detectable activity with Muc2 and Muc7. Displays enzymatic activity toward t
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 13	GalNAc-T13	**GALNT13**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has a much stronger activity than GALNT1 to transfer GalNAc to mucin peptides, such as Muc5Ac and Muc7. Able to glycosylate SDC3. May be responsible for the syn
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase 14	GalNAc-T14	**GALNT14**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Displays activity toward mucin-derived peptide substrates such as Muc2, Muc5AC, Muc7, and Muc13 (-58). May be involved in O-glycosylation in kidney
t-GalNAc	2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase-like 6	GalNAc-T17	**GALNTL6 (GALNT17)**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor
t-GalNAc	2.4.1.41	Putative polypeptide N-acetylgalactosaminyltransferase-like protein	GalNAc-T-like protein 1	**GALNTL1**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	May catalyze the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor
t-GalNAc	2.4.1.41	Putative polypeptide N-acetylgalactosaminyltransferase-like protein	GalNAc-T-like protein 3	**WBSCR17 (GALNTL3)**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	May catalyze the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor
t-GalNAc	2.4.1.41	Putative polypeptide N-acetylgalactosaminyltransferase-like protein	GalNAc-T-like protein 4	**GALNTL4**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	May catalyze the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor
t-GalNAc	2.4.1.41	Probable polypeptide N-acetylgalactosaminyltransferase 8	GalNAc-T8	**GALNT8**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Probably catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor
t-Gal	2.4.1.45	2-hydroxyacylsphingosine 1-beta-galactosyltransferase	Ceramide UDP-galactosyltransfera	**UGT8**	Evidence at transcript level	UDP-alpha-D-galactose + 2-(2-hy	NERVOUS System Mainly: Catalyzes the transfer of galactose to ceramide, a key enzymatic step in the biosynthesis of galactocerebrosides, which are abundant sphingolipids of the myelin membrane of the central nervous system and peripheral nervous system
t-Gal	2.4.1.50	Procollagen galactosyltransferase 1	Hydroxylysine galactosyltransfera	**GLT25D1**	Evidence at protein level	UDP-alpha-D-galactose + 5-hydro	COLLAGEN: Has a beta-galactosyltransferase activity; transfers beta-galactose to hydroxylysine residues of collagen
t-Gal	2.4.1.50	Procollagen galactosyltransferase 2	Hydroxylysine galactosyltransfera	**GLT25D2**	Evidence at protein level	UDP-alpha-D-galactose + 5-hydro	COLLAGEN: Has a beta-galactosyltransferase activity; transfers beta-galactose to hydroxylysine residues on collagen
t-Gal, 3-GalNAc	2.4.1.62	Beta-1,3-galactosyltransferase 4	b3Gal-T4	**B3GALT4**	Evidence at transcript level	UDP-alpha-D-galactose + N-acety	GANGLIOSIDES: Involved in GM1/GD1B/GA1 ganglioside biosynthesis
t-Glc, 2-Gal	2.4.1.66	procollagen glucosyltransferase				UDP-glucose + (2S,5R)-5-O-(beta-	EC Number Not in Uniprot DB; COLLAGEN
t-GalNAc, 3-Gal	2.4.1.79	UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1	b3Gal-T3	**B3GALNT1**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	GLYCOLIPIDS: Transfers N-acetylgalactosamine onto globotriaosylceramide
t-Glc	2.4.1.80	Ceramide glucosyltransferase	Glucosylceramide synthase	**UGCG**	Evidence at protein level	UDP-glucose + N-acylsphingosine	GLYCOLIPIDS: Catalyzes the first glycosylation step in glycosphingolipid biosynthesis, the transfer of glucose to ceramide. May also serve as a "flippase"
t-Gal, 3-GlcNAc	2.4.1.86	glucosaminylgalactosylglucosylceramide beta-galactosyltransferase				UDP-galactose + N-acetyl-beta-D	EC Number Not in Uniprot DB; GLYCOLIPIDS
t-GalNAc, 3-GalNAc	2.4.1.88	globoside alpha-N-acetylgalactosaminyltransferase				UDP-N-acetyl-D-galactosamine +	EC Number Not in Uniprot DB; GLYCOLIPIDS
t-GalNAc, 3,4-Gal	2.4.1.92	Beta-1,4 N-acetylgalactosaminyltransferase 1	GM2/GD2 synthase	**B4GALNT1**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	GLYCOLIPIDS: Involved in the biosynthesis of gangliosides GM2, GD2 and GA2.
t-GlcNAc, 2-Man	2.4.1.101	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltrans	GNT-I	**MGAT1**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + 3-	MAJOR: Initiates complex N-linked carbohydrate formation. Essential for the conversion of high-mannose to hybrid and complex N-glycans
t-GlcNAc, 3,6-GalNAc	2.4.1.102	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucos	Core 2 GNT	**GCNT1**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	MAJOR: Forms critical branches in O-glycans.
t-GlcNAc, 3,6-GalNAc	2.4.1.102	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucos	C2GnT3	**GCNT4**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	Glycosyltransferase that mediates core 2 O-glycan branching, an important step in mucin-type biosynthesis. Does not have core 4 O-glycan or I-branching enzyme activity
t-Man	2.4.1.109	Protein O-mannosyl-transferase 1	Dolichyl-phosphate-mannose--pro	**POMT1**	Evidence at protein level	Dolichyl phosphate D-mannose +	Transfers mannosyl residues to the hydroxyl group of serine or threonine residues. Coexpression of both POMT1 and POMT2 is necessary for enzyme activity, expression of either POMT1 or POMT2 alone is insufficient
t-Man	2.4.1.109	Protein O-mannosyl-transferase 2	Dolichyl-phosphate-mannose--pro	**POMT2**	Evidence at protein level	Dolichyl phosphate D-mannose +	Transfers mannosyl residues to the hydroxyl group of serine or threonine residues. Coexpression of both POMT1 and POMT2 is necessary for enzyme activity, expression of either POMT1 or POMT2 alone is insufficient
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase sub	Integral membrane protein 1	**STT3A**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Catalytic component of oligosaccharyltransferase (OST) oligosaccharyltransferase (OST) complex which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. N-glycosylation occurs cotranslationally and t
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase sub	RPN-I	**RPN1**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Essential subunit of N-oligosaccharyl transferase enzyme which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase sub	RPN-II	**RPN2**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Essential subunit of N-oligosaccharyl transferase enzyme which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 k	Oligosaccharyl transferase 48 kDa	**DDOST**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Essential subunit of N-oligosaccharyl transferase enzyme which catalyzes the transfer of a high mannose oligosaccharide to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase sub	STT3-B	**STT3B**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Catalytic component of oligosaccharyltransferase (OST) oligosaccharyltransferase (OST) complex which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. N-glycosylation occurs cotranslationally and t
N-Glycans	2.4.1.119	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase sub	DAD-1	**DAD1**	Evidence at protein level	Dolichyl diphosphooligosaccharid	Component of the N-oligosaccharyl transferase enzyme which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. N-glycosylation occurs cotranslationally and the complex associates with the Sec61 co
t-Gal, 3-GalNAc	2.4.1.122	Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	Core 1 beta3-Gal-T1	**C1GALT1**	Evidence at protein level	UDP-alpha-D-galactose + glycopr	MAJOR: Glycosyltransferase that generates the core 1 O-glycan Gal-beta1-3GalNAc-alpha1-Ser/Thr (T antigen), which is a precursor for many extended O-glycans in glycoproteins. Plays a central role in many processes, such as angiogenesis, thrombopoiesis and kidney homeostasis development
t-Man, 2-Man (Gets removed)	2.4.1.131	GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase	Glycolipid 2-alpha-mannosyltransf	**ALG11**	Evidence at protein level	2 GDP-D-mannose + D-Man-alph	May not be Trimmed: Mannosyltransferase involved in the last steps of the synthesis of Man5GlcNAc(2)-PP-dolichol core oligosaccharide on the cytoplasmic face of the endoplasmic reticulum. Catalyzes the addition of the 4th and 5th mannose residues to the dolichol-linked oligosaccharide chain
3,6-Man	2.4.1.132 & 2.4.1.257	Alpha-1,3/1,6-mannosyltransferase ALG2	GDP-Man:Man(1)GlcNAc(2)-PP-do	**ALG2**	Evidence at transcript level	GDP-D-mannose + D-Man-beta-(1	MAJOR: Mannosylates Man$_2$GlcNAc(2)-dolichol diphosphate and Man$_1$GlcNAc(2)-dolichol diphosphate to form Man$_3$GlcNAc(2)-dolichol diphosphate
t-Gal, 4-Xyl	2.4.1.133	Beta-1,4-galactosyltransferase 7	b4Gal-T7	**B4GALT7**	Evidence at protein level	UDP-alpha-D-galactose + O-beta-	PROTEOGLYCANS: Required for the biosynthesis of the tetrasaccharide linkage region of proteoglycans, especially for small proteoglycans in skin fibroblasts
t-Gal, 3-Gal	2.4.1.134	Beta-1,3-galactosyltransferase 6	Beta3GalT6	**B3GALT6**	Evidence at transcript level	UDP-alpha-D-galactose + 4-beta-	PROTEOGLYCANS: Beta-1,3-galactosyltransferase that transfers galactose from UDP-galactose to substrates with a terminal beta-linked galactose residue. Has a preference for galactose-beta-1,4-xylose that is found in the linker region of glycosaminoglycans, such as heparan sulfate and chondroitin sulfate. Has no activity towards substrates wit
t-GlcA, 3-Gal	2.4.1.135	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1	GlcAT-P	**B3GAT1**	Evidence at protein level	UDP-glucuronate + 3-beta-D-gala	Involved in the biosynthesis of L2/HNK-1 carbohydrate epitope on glycoproteins. Can also play a role in glycosaminoglycan biosynthesis. Substrates include asialo-orosomucoid (ASOR), asialo-fetuin, and asialo-neural cell adhesion molecule. Requires sphingomyelin for activity: stearoyl-sphingomyelin was the most effective, followed by palmitoy
t-GlcA, 3-Gal	2.4.1.135	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 2	GlcAT-S	**B3GAT2**	Evidence at protein level	UDP-glucuronate + 3-beta-D-gala	Involved in the biosynthesis of L2/HNK-1 carbohydrate epitope on both glycolipids and glycoproteins
t-GlcA, 3-Gal	2.4.1.135	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 3	GlcAT-I	**B3GAT3**	Evidence at protein level	UDP-glucuronate + 3-beta-D-gala	PROTEOGLYCAN Specificity: Glycosaminoglycans biosynthesis. Involved in forming the linkage tetrasaccharide present in heparan sulfate and chondroitin sulfate. Transfers a glucuronic acid moiety from the uridine diphosphate-glucuronic acid (UDP-GlcUA) to the common linkage region trisaccharide Gal-beta-1,3-Gal-beta-1,4-Xyl covalently bou
4-GlcNAc	2.4.1.141	UDP-N-acetylglucosamine transferase subunit ALG13 homolog	Glycosyltransferase 28 domain-co	**ALG13**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + N	Probable MAJOR N-Glycan Core: Isoform 2 may be involved in protein N-glycosylation, second step of the dolichol-linked oligosaccharide pathway
t-Man (Gets Changed: Precursor to MAJOR 3,6-Man (Makes t-Man	2.4.1.142	Chitobiosyldiphosphodolichol beta-mannosyltransferase	MT-1	**ALG1**	Evidence at protein level	GDP-mannose + chitobiosyldipho	Participates in the formation of the lipid-linked precursor oligosaccharide for N-glycosylation. Involved in assembling the dolichol-pyrophosphate-GlcNAc(2)-Man$_5$ intermediate on the cytoplasmic surface of the ER
t-GlcNAc, 2-Man	2.4.1.143	Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltrans	GNT-II	**MGAT2**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 6-	MAJOR N-glycans: Catalyzes an essential step in the conversion of oligo-mannose to complex N-glycans
t-GlcNAc, 3-Gal	2.4.1.146	beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,3-N-acetylglucosaminyltransferase				UDP-N-acetyl-D-glucosamine + b	EC Number Not in Uniprot DB; O-glycans
t-GlcNAc, 3-GalNAc	2.4.1.147	acetylgalactosaminyl-O-glycosyl-glycoprotein beta-1,3-N-acetylgluc	beta3Gn-T6			UDP-N-acetyl-D-glucosamine + N	EC Number Not in Uniprot DB; O-glycans
t-GlcNAc, 3,6-GalNAc	2.4.1.148	acetylgalactosaminyl-O-glycosyl-glycoprotein beta-1,6-N-acetylgluc	core 2 beta 1,6 N-acetylglucosaminyltransferase			UDP-N-acetyl-D-glucosamine + N	EC Number Not in Uniprot DB; O-glycans
t-GlcNAc, 3-Gal	2.4.1.149	N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase	Poly-N-acetyllactosamine extensio	**B3GNT1**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + b	MAJOR: Can initiate the synthesis or the elongation of the linear poly-N-acetyllactosaminoglycans
t-GlcNAc, 3,6-Gal	2.4.1.150	N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase, is	N-acetylglucosaminyltransferase	**GCNT2**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	"Evidence at transcript level": Branching enzyme that converts linear into branched poly-N-acetyllactosaminoglycans. Introduces the blood group I antigen during embryonic development. It is closely associated with the development and maturation of erythroid cells. The expression of the blood group I antigen in erythrocytes is determined by
t-GlcNAc, 3,6-Gal	2.4.1.150	N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase, is	N-acetylglucosaminyltransferase	**GCNT2**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	"Evidence at transcript level": Branching enzyme that converts linear into branched poly-N-acetyllactosaminoglycans. Introduces the blood group I antigen during embryonic development. It is closely associated with the development and maturation of erythroid cells. The expression of the blood group I antigen in erythrocytes is determined by
t-GlcNAc, 3,6-Gal	2.4.1.150	N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase, is	N-acetylglucosaminyltransferase	**GCNT2**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	"Evidence at transcript level": Branching enzyme that converts linear into branched poly-N-acetyllactosaminoglycans. Introduces the blood group I antigen during embryonic development. It is closely associated with the development and maturation of erythroid cells. The expression of the blood group I antigen in erythrocytes is determined by
t-GlcNAc, 2,6-Man	2.4.1.155	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransf	GNT-V	**MGAT5**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 6-	MAJOR N-glycans: Catalyzes the addition of N-acetylglucosamine in beta 1-6 linkage to the alpha-linked mannose of biantennary N-linked oligosaccharides. It is one of the most important enzymes involved in the regulation of the biosynthesis of glycoprotein oligosaccharides
t-GlcNAc, 2,6-Man	2.4.1.155	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransf	GNT-Vb	**MGAT5B**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + 6-	Glycosyltransferase that acts on alpha-linked mannose of N-glycans and O-mannosyl glycans. Catalyzes the transfer of N-acetylglucosamine (GlcNAc) to the beta 1-6 linkage of the mannose residue of GlcNAcbeta1,2-Manalpha on both the alpha1,3- and alpha1,6-linked mannose arms in the core structure of N-glycan. Also acts on the GlcNAcbeta
t-GlcNAc, 3-Gal	2.4.1.163	beta-galactosyl-N-acetylglucosaminylgalactosylglucosyl-ceramide beta-1,3-acetylglucosaminyltransferase				UDP-N-acetyl-D-glucosamine + b	EC Number Not in Uniprot DB; GLYCOLIPIDS
t-GalNAc, 4-NeuNAc	2.4.1.165	N-acetylneuraminylgalactosylglucosylceramide beta-1,4-N-acetylgalactosaminyltransferase				UDP-N-acetyl-D-galactosamine +	EC Number Not in Uniprot DB; GLYCOLIPIDS
t-GalNAc, 4-GlcA	2.4.1.174	Chondroitin sulfate N-acetylgalactosaminyltransferase 1	CsGalNAcT-1	**CSGALNACT1**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Chondroitin Synthesis; Transfers 1,4-N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the non-reducing end of glucuronic acid (GlcUA). Required for addition of the first GalNAc to the core tetrasaccharide linker and for elongation of chondroitin chains. Important role in chondroitin chain biosynthesis in cartilage
t-GalNAc, 4-GlcA	2.4.1.174	Chondroitin sulfate N-acetylgalactosaminyltransferase 2	GalNAcT-2	**CSGALNACT2**	Evidence at transcript level	UDP-N-acetyl-D-galactosamine +	Chondroitin Synthesis: Transfers 1,4-N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the non-reducing end of glucuronic acid (GlcUA). Required for addition of the first GalNAc to the core tetrasaccharide linker and for elongation of chondroitin chains
t-GalNAc, 4-GlcA; 3-GalNAc	2.4.1.175 & 2.4.1.226	Chondroitin sulfate synthase 1	ChSy-1	**CHSY1**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Chondroitin Synthesis: Has both beta-1,3-glucuronic acid and beta-1,4-N-acetylgalactosamine transferase activity. Transfers glucuronic acid (GlcUA) from UDP-GlcUA and N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the non-reducing end of the elongating chondroitin polymer. Involved in the negative control of osteogenesis likely throu
t-GalNAc, 4-GlcA; 3-GalNAc	2.4.1.175 & 2.4.1.226	Chondroitin sulfate synthase 2	ChPF	**CHPF**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Chondrointin Synthesis; Has both beta-1,3-glucuronic acid and beta-1,4-N-acetylgalactosamine transferase activity. Transfers glucuronic acid (GlcUA) from UDP-GlcUA and N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the non-reducing end of the elongating chondroitin polymer
t-GalNAc, 4-GlcA; 3-GalNAc	2.4.1.175 & 2.4.1.226	Chondroitin sulfate synthase 3	ChSy-2	**CHSY3**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	Chondroitin Synthesis: Has both beta-1,3-glucuronic acid and beta-1,4-N-acetylgalactosamine transferase activity. Transfers glucuronic acid (GlcUA) from UDP-GlcUA and N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the non-reducing end of the elongating chondroitin polymer. Specific activity is much reduced compared to CHSY1
t-Gal, 3-Gal	2.4.1.179	lactosylceramide beta-1,3-galactosyltransferase					EC Number Not in Uniprot DB: Glycolipids
t-GlcNAc, 6-Inositol	2.4.1.198	Phosphatidylinositol N-acetylglucosaminyltransferase subunit A	PIG-A	**PIGA**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 1-	GPI Anchors: Necessary for the synthesis of N-acetylglucosaminyl-phosphatidylinositol, the very early intermediate in GPI-anchor biosynthesis
t-GlcNAc, 6-Inositol	2.4.1.198	Phosphatidylinositol N-acetylglucosaminyltransferase subunit C	PIG-C	**PIGC**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + 1-	GPI Anchors: Part of the complex catalyzing the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to phosphatidylinositol, the first step of GPI biosynthesis
t-GlcNAc, 6-Inositol	2.4.1.198	Phosphatidylinositol N-acetylglucosaminyltransferase subunit H	PIG-H	**PIGH**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 1-	GPI Anchors: Part of the complex catalyzing the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to phosphatidylinositol, the first step of GPI biosynthesis
t-GlcNAc, 6-Inositol	2.4.1.198	Phosphatidylinositol N-acetylglucosaminyltransferase subunit P	PIG-P	**PIGP**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 1-	GPI Anchors: Part of the complex catalyzing the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to phosphatidylinositol, the first step of GPI biosynthesis
t-GlcNAc, 6-Inositol	2.4.1.198	Phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	PIG-Q	**PIGQ**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + 1-	GPI Anchors: Part of the complex catalyzing the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to phosphatidylinositol, the first step of GPI biosynthesis.
t-GlcNAc, 3-Gal	2.4.1.206	Lactosylceramide 1,3-N-acetyl-beta-D-glucosaminyltransferase	BGnT-5	**B3GNT5**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + b	GLYCOLIPIDS: Beta-1,3-N-acetylglucosaminyltransferase that plays a key role in the synthesis of lacto- or neolacto-series carbohydrate chains on glycolipids, notably by participating in biosynthesis of HNK-1 and Lewis X carbohydrate structures. Has strong activity toward lactosylceramide (LacCer) and neolactotetraosylceramide (nLc$_4$Cer; paraglobo
3-GlcNAc, 4-GlcA	2.4.1.212	Hyaluronan synthase 1	HA synthase 1	**HAS1**	Evidence at protein level	UDP-alpha-N-acetyl-D-glucosami	Hyaluronan Synthsis: Plays a role in hyaluronan/hyaluronic acid (HA) synthesis. Also able to catalyze the synthesis of chito-oligosaccharide depending on the substrate
3-GlcNAc, 4-GlcA	2.4.1.212	Hyaluronan synthase 2	HA synthase 2	**HAS2**	Evidence at transcript level	UDP-alpha-N-acetyl-D-glucosami	Hyaluronan Synthsis: Plays a role in hyaluronan/hyaluronic acid (HA) synthesis
3-GlcNAc, 4-GlcA	2.4.1.212	Hyaluronan synthase 3	HA synthase 3	**HAS3**	Evidence at protein level	UDP-alpha-N-acetyl-D-glucosami	Hyaluronan Synthsis: Plays a role in hyaluronan/hyaluronic acid (HA) synthesis
t-GlcNAc, 3-Fuc	2.4.1.222	Beta-1,3-N-acetylglucosaminyltransferase lunatic fringe	O-fucosylpeptide 3-beta-N-acetylg	**LFNG**	Evidence at protein level	Transfers a beta-D-GlcNAc residu	Glycosyltransferase that initiates the elongation of O-linked fucose residues attached to EGF-like repeats in the extracellular domain of Notch molecules. Decreases the binding of JAGGED1 to NOTCH2 but not that of DELTA1. Essential mediator of somite segmentation and patterning
t-GlcNAc, 3-Fuc	2.4.1.222	Beta-1,3-N-acetylglucosaminyltransferase radical fringe	O-fucosylpeptide 3-beta-N-acetylg	**RFNG**	Evidence at transcript level	Transfers a beta-D-GlcNAc residu	Glycosyltransferase that initiates the elongation of O-linked fucose residues attached to EGF-like repeats in the extracellular domain of Notch molecules. May be involved in limb formation and in neurogenesis
t-GlcNAc, 3-Fuc	2.4.1.222	Beta-1,3-N-acetylglucosaminyltransferase manic fringe	O-fucosylpeptide 3-beta-N-acetylg	**MFNG**	Evidence at protein level	Transfers a beta-D-GlcNAc residu	Glycosyltransferase involved in the elongation of O-linked ligands to activate Notch signaling. Possesses fucose-specific beta-1,3-N-acetylglucosaminyltransferase activity
t-GlcNAc, 4-GlcA	2.4.1.223	Exostosin-like 2	Alpha-1,4-N-acetylhexosaminyltra	**EXTL2**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + b	Heparan Sulfate Synthesis: Glycosyltransferase required for the biosynthesis of heparan-sulfate and responsible for the alternating addition of beta-1-4-linked glucuronic acid (GlcA) and alpha-1-4-linked N-acetylglucosamine (GlcNAc) units to nascent heparan sulfate chains
t-GlcNAc, 4-GlcA	2.4.1.223	Exostosin-like 3	Glucuronyl-galactosyl-proteoglyca	**EXTL3**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	Probable glycosyltransferase
4-GlcNAc, 4-GlcA	2.4.1.224 & 2.4.1.225	Exostosin-1	Multiple exostoses protein 1	**EXT1**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + b	Heparan Sulfate Synthesis: Glycosyltransferase required for the biosynthesis of heparan-sulfate. The EXT1/EXT2 complex possesses substantially higher glycosyltransferase activity than EXT1 or EXT2 alone. Appears to be a tumor suppressor
4-GlcNAc, 4-GlcA	2.4.1.224 & 2.4.1.225	Exostosin-2	Multiple exostoses protein 2	**EXT2**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + b	Heparan Sulfate Synthesis: Glycosyltransferase required for the biosynthesis of heparan-sulfate. The EXT1/EXT2 complex possesses substantially higher glycosyltransferase activity than EXT1 or EXT2 alone. Appears to be a tumor suppressor
t-GlcNAc, 4-GlcA	2.4.1.224	Exostosin-like 1	Exostosin-L	**EXTL1**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + b	Probable glycosyltransferase
t-GlcA, 3-GalNAc	2.4.1.226	Chondroitin sulfate glucuronyltransferase	ChPF-2	**CHPF2**	Evidence at transcript level	UDP-alpha-D-glucuronate + N-ac	Chondroitin Synthesis: Transfers glucuronic acid (GlcUA) from UDP-GlcUA to N-acetylgalactosamine residues on the non-reducing end of the elongating chondroitin polymer. Has no N-acetylgalactosaminyltransferase activity
t-Gal, 4-Gal	2.4.1.228	Lactosylceramide 4-alpha-galactosyltransferase	Gb3 synthase	**A4GALT**	Evidence at transcript level	UDP-alpha-D-galactose + beta-D-	GLYCOLIPIDS: Necessary for the biosynthesis of the Pk antigen of blood histogroup P. Catalyzes the transfer of galactose to lactosylceramide and galactosylceramide. Necessary for the synthesis of the receptor for bacterial verotoxins
t-GalNAc, 4-GlcNAc	2.4.1.244	Beta-1,4-N-acetylgalactosaminyltransferase 3	B4GalNAcT3	**B4GALNT3**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	N- and O-Glycans: Transfers N-acetylgalactosamine (GalNAc) from UDP-GalNAc to N-acetylglucosamine-beta-benzyl with a beta-1,4-linkage to form N,N'-diacetyllactosediamine, GalNAc-beta-1,4-GlcNAc structures in N-linked glycans and probably O-linked glycans. Mediates the N,N'-diacetyllactosediamine formation on gastric mucosa
t-GalNAc, 4-GlcNAc	2.4.1.244	N-acetyl-beta-glucosaminyl-glycoprotein 4-beta-N-acetylgalactosam	Beta4GalNAc-T4	**B4GALNT4**	Evidence at protein level	UDP-N-acetyl-D-galactosamine +	N- and O-Glycans: Transfers N-acetylgalactosamine (GalNAc) from UDP-GalNAc to N-acetylglucosamine-beta-benzyl with a beta-1,4-linkage to form N,N'-diacetyllactosediamine, GalNAc-beta-1,4-GlcNAc structures in N-linked glycans and probably O-linked glycans
t-GlcNAc	2.4.1.255	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase	OGT	**OGT**	Evidence at protein level	UDP-N-acetyl-D-glucosamine + [p	O-GlcNAc: Catalyzes the transfer of a single N-acetylglucosamine from UDP-GlcNAc to a serine or threonine residue in cytoplasmic and nuclear proteins resulting in their modification with a beta-linked N-acetylglucosamine (O-GlcNAc). Glycosylates a large and diverse number of proteins including histone H2B, AKT1, PFKL, MLL5, MAPT/TAU and
t-GlcNAc	2.4.1.255	EGF domain-specific O-linked N-acetylglucosamine transferase	Extracellular O-linked N-acetylgluc	**EOGT**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + [p	O-GlcNAc: Catalyzes the transfer of a single N-acetylglucosamine from UDP-GlcNAc to a serine or threonine residue in extracellular proteins resulting in their modification with a beta-linked N-acetylglucosamine (O-GlcNAc). Specifically glycosylates the Thr residue located between the fifth and sixth conserved cysteines of folded EGF-like domains
t-GlcNAc	2.4.1.255	Glycosyltransferase-like domain-containing protein 2	Extracellular O-linked N-acetylgluc	**GTDC2**	Evidence at transcript level	UDP-N-acetyl-D-glucosamine + [p	O-GlcNAc
t-Man, 3-Man (Gets removed)	2.4.1.258	Dol-P-Man:Man(5)GlcNAc(2)-PP-Dol alpha-1,3-mannosyltransferase	Dol-P-Man-dependent alpha(1-3)-	**ALG3**	Evidence at protein level	Dolichyl beta-D-mannosyl phospl	Adds the first Dol-P-Man derived mannose in an alpha-1,3 linkage to Man5GlcNAc2-PP-Dol
t-Man, 2-Man (Gets removed)	2.4.1.259 & 2.4.1.261	Alpha-1,2-mannosyltransferase ALG9	Dol-P-Man:Man(6)GlcNAc(2)-PP-D	**ALG9**	Evidence at protein level	Dolichyl beta-D-mannosyl phospl	Catalyzes the transfer of mannose from Dol-P-Man to lipid-linked oligosaccharides
t-Man, 3,6-Man (Gets removed)	2.4.1.260	Dol-P-Man:Man(7)GlcNAc(2)-PP-Dol alpha-1,6-mannosyltransferase	hALG12	**ALG12**	Evidence at protein level	Dolichyl beta-D-mannosyl phospl	Adds the eighth mannose residue in an alpha-1,6 linkage onto the dolichol-PP-oligosaccharide precursor (dolichol-PP-Man$_7$GlcNAc$_2$) required for protein glycosylation
t-Glc, 3-Glc (Gets removed)	2.4.1.265	Probable dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-gluco	Dol-P-Glc:Glc(1)Man(9)GlcNAc(2)-	**ALG8**	Evidence at protein level	Dolichyl beta-D-glucosyl phospha	Adds the second glucose residue to the lipid-linked oligosaccharide precursor for N-linked glycosylation. Transfers glucose from dolichyl phosphate glucose (Dol-P-Glc) onto the lipid-linked oligosaccharide Glc$_1$Man$_9$GlcNAc(2)-PP-Dol
t-Glc, 3-Man (Gets removed)	2.4.1.267	Dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase	Dol-P-Glc:Man(9)GlcNAc(2)-PP-Do	**ALG6**	Evidence at protein level	Dolichyl beta-D-glucosyl phospha	Adds the first glucose residue to the lipid-linked oligosaccharide precursor for N-linked glycosylation. Transfers glucose from dolichyl phosphate glucose (Dol-P-Glc) onto the lipid-linked oligosaccharide Man$_9$GlcNAc(2)-PP-Dol

onding 1,4-galactosyl derivative, forming 1,3-L-fucosyl link

gulation of the biosynthesis of glycoprotein oligosacchar

y N-linked sugar chains. Involved in glucose transport by mediating SLC2A2/GLUT2 glycosylation, thereby controlling cell-surface expression of SLC2A2 in pancreatic beta c

ntennary N-linked sugar chains. Has lower affinities for donors or acceptors than MGAT4A, suggesting that, under physiological conditions, it is not the main contributor in N-glycan biosynth

on of tri- and tetra-antennary N-linked sugar chains By similarity. Does not catalyze the transfer of GlcNAc to the Manalpha1-6 arm to form GlcNAcBeta1-4Manalpha1-6 linkage ('GnT-VI' activ

has weak or no activities toward glycoproteins and glycolip

lood group I antigen during embryonic developm

riety of cell to cell and cell to matrix interactions, as those occurring during development and egg fertilization, by binding to specific oligosaccharide ligands on opposing cells or in the extracellular

of UDP-Gal), whereas O individuals lack such act

inge reg

de, suggesting that it can fill vicinal Thr/Ser residues in cooperation with other GALNT proteins. Prefers Muc1a as subs

phate homeost

he Gal-NAc-Muc5AC glycopeptide, but no detectable activity to mono-GalNAc-glycosylated Muc1a, Muc2, Muc7 and EA2. May play an important role in the initial step of mucin-type oligosaccharide biosynthesis in digestive or

thesis of Tn antigen in neuronal c

he complex associates with the Sec61 complex at the channel-forming translocon complex that mediates protein translocation across the endoplasmic reticulum (ER). SST3A seems to be involved in complex substrate specificity. STT3A is present in the majority of OST complexes and mediates cotranslational N-glycosylation of most sites on target proteins, while STT3B-containing complexes are required for efficient cotranslational glycosylation and mediate glycosylation of sites that have been skipped b

he complex associates with the Sec61 complex at the channel-forming translocon complex that mediates protein translocation across the endoplasmic reticulum (ER). STT3B is present in a small subset of OST complexes and mediates both cotranslational and post-translational N-glycosylation of target proteins: STT3B-containing complexes are required for efficient cotranslational glycosylation and while they are less competent than STT3A-containing complexes for cotranslational glycosylation, they have the ability to mediate glycosylation of some nascent sites that are not accessible for STT3A. STT3B-containing complexes also act post-translationally and mediate m

mplex at the channel-forming translocon complex that mediates protein translocation across the endoplasmic reticulum (ER). Loss of the DAD1 protein triggers apop

n terminal glucosamine or galactosamine resid

l-sphingomyelin and lignoceroyl-sphingomyelin. Activity was demonstrated only for sphingomyelin with a saturated fatty acid and not for that with an unsaturated fatty acid, regardless of the length of the acy

nd to a Ser residue at the glycosaminylglycan attachment site of proteoglycans. Can also play a role in the biosynthesis of l2/HNK-1 carbohydrate epitope on glycoproteins. Shows strict specificity for Gal-beta-1,3-Gal-beta-1,4-Xyl, exhibiting negligible incorporation into other galactoside substrates including Galbeta1-3Gal beta1-O-benzyl, Galbeta1-4GlcNAc and Galbeta1

isofor

isofor

isofor

1,2-Manalpha1-Ser/Thr moiety, forming a 2,6-branched structure in brain O-mannosyl glycan. Plays an active role in modulating integrin and laminin-dependent adhesion and migration of neuronal cells via its activity in the O-mannosyl glycan pat

gh the modulation of NOTCH signal

side), resulting in the synthesis of Lc_3Cer and neolactopentaosylceramide (nLc_5Cer), respectively. Probably plays a central role in regulating neolacto-series glycolipid synthesis during embryonic development

HCFC1. Can regulate their cellular processes via cross-talk between glycosylation and phosphorylation or by affecting proteolytic processing. Involved in insulin resistance in muscle and adipocyte cells via glycosylating insulin signaling components and inhibiting the 'Thr-308' phosphorylation of AKT1, enhancing IRS1 phosphorylation and attenuating insulin signaling. Involved in glycolysis regulation by mediating glycosylation of 6-phosphofructokinase PFKL, inhibiting its activity. Component of a THAP1/THAP3-HCFC1-OGT complex that is required for the regulation of the transcriptional activity of RRM1. As part of the NSL complex it may be involved in acetylation of nu


odifi

Supporting Information Table S3: Clinical characteristics and the risk of lung cancer mortality in the Lung Cancer in Central and Eastern Europe study.

	Total Patients	Deaths	HR*	CI	5-year survival	Median survival (months)
Grade						
Grade 1	29	18	ref		0.48	43.5
Grade 2	83	68	1.89	1.11, 3.21	0.23	18.0
Grade 3	114	101	2.57	1.54, 4.28	0.16	17.3
Grade 4	55	51	3.29	1.90, 5.70	0.09	11.9
not applicable	604	531	2.88	1.75, 4.73	0.15	15.0
missing	950	714				
Histology						
Squamous cell carcinoma	713	586	ref		0.22	15.9
Small cell carcinoma	360	312	1.24	1,07, 1.43	0.15	14.9
Adenocarcinoma	387	282	0.94	0.82, 1.09	0.31	21.9
Large cell carcinoma	55	41	1.08	0.77, 1.49	0.3636	20.5
Mixed	73	50	0.70	0.52, 0.94	0.33	24.4
Other/Unspecified	245	210	1.22	1.04, 1.45	0.19	12.9
TNM stage						
1	96	59	ref		0.54	79.0
2	75	60	1.93	1.34, 2.77	0.28	21.7
3	305	281	3.16	2.36, 4.22	0.09	15.7
4	287	265	3.85	2.87, 5.16	0.08	12.3

Abbreviations: HR = hazard ratios, adjusted for the variable in the table; CI = confidence intervals; ref = reference category.

Supporting Information Table S4: Individual patient details from the Lung Cancer in Central and Eastern Europe (CEE) Study

Lung Cancer Case or Control	Gender	Age (yrs.)	Type of Smoker	Age at which Started Smoking	Average Daily Tobacco Consumption (# of cigarettes)	Cumulative Smoking History (Tobacco pack yrs.)	Age at which Quit Smoking	Time Since Quit Smoking	Type of Tobacco (1=Never smoker, 2=Cigarettes only, 3=Never cigarettes, 4=Cigarettes + other)	Lung Histology (0=controls 1=Adenocarcinoma, 2=Squamous cell carcinoma, 3=Small cell, 4=Others/mixed)
Control	Female	59	Never Smoker	.	0.0	0.0	.	.	1	0
Control	Female	70	Never Smoker	.	0.0	0.0	.	.	1	0
Lung Cancer	Male	41	Current Smoker	21	20.0	20.0	.	0	2	2
Lung Cancer	Female	46	Ex-Smoker	18	14.5	16.0	40.0	6	2	1
Lung Cancer	Female	66	Ex-Smoker	25	5.0	8.8	60.0	6	2	1
Lung Cancer	Female	65	Current Smoker	23	48.6	102.0	65.0	0	2	4
Lung Cancer	Female	68	Current Smoker	25	17.6	37.0	67.0	1	2	4
Lung Cancer	Male	68	Ex-Smoker	20	15.0	15.0	40.0	28	2	2
Control	Male	58	Current Smoker	26	12.0	19.2	.	0	2	0
Control	Female	73	Current Smoker	34	24.6	48.0	.	0	2	0
Control	Male	69	Ex-Smoker	25	10.0	12.0	49.0	20	2	0
Control	Female	62	Current Smoker	30	9.3	14.9	.	0	2	0
Lung Cancer	Male	44	Ex-Smoker	17	17.1	18.0	38.0	6	2	4
Lung Cancer	Male	64	Ex-Smoker	19	20.0	39.0	58.0	6	2	4
Control	Female	52	Current Smoker	25	10.0	13.5	.	0	2	0
Control	Female	70	Never Smoker	.	0.0	0.0	.	.	1	0
Control	Female	50	Never Smoker	.	0.0	0.0	.	.	1	0
Control	Female	60	Current Smoker	19	18.8	38.5	.	0	2	0
Lung Cancer	Female	51	Current Smoker	19	16.6	26.5	51.0	0	2	1
Control	Female	58	Current Smoker	17	16.5	33.9	.	0	2	0
Control	Male	56	Never Smoker	.	0.0	0.0	.	.	1	0
Lung Cancer	Female	65	Current Smoker	18	15.0	35.3	65.0	0	2	4
Lung Cancer	Male	71	Ex-Smoker	12	17.3	45.0	64.0	7	2	2
Lung Cancer	Female	72	Never Smoker	.	0.0	0.0	.	.	1	2
Control	Male	67	Current Smoker	18	14.7	36.8	.	0	2	0
Control	Male	71	Current Smoker	20	17.1	43.7	.	0	4	0
Lung Cancer	Female	45	Never Smoker	.	0.0	0.0	.	.	1	4
Control	Male	63	Current Smoker	17	10.1	23.3	.	0	4	0
Lung Cancer	Male	70	Ex-Smoker	15	22.8	40.0	50.0	20	4	2
Lung Cancer	Male	60	Current Smoker	20	18.0	36.0	.	0	2	2
Lung Cancer	Female	74	Current Smoker	20	19.9	53.6	.	0	4	4
Lung Cancer	Male	51	Current Smoker	10	17.4	35.7	.	0	4	1
Lung Cancer	Female	70	Never Smoker	.	0.0	0.0	.	.	1	4
Lung Cancer	Male	58	Current Smoker	20	15.9	30.9	.	0	4	2
Lung Cancer	Male	56	Current Smoker	17	14.0	27.3	.	0	2	2
Control	Male	61	Current Smoker	8	10.0	26.5	.	0	2	0
Control	Male	77	Ex-Smoker	18	6.8	3.7	29.0	48	3	0
Control	Male	68	Ex-Smoker	16	10.5	11.6	38.0	30	3	0
Control	Female	64	Never Smoker	.	0.0	0.0	.	.	1	0
Control	Male	72	Ex-Smoker	17	10.5	4.2	25.0	47	3	0
Control	Male	71	Current Smoker	20	9.3	23.6	.	0	4	0
Control	Male	69	Current Smoker	22	20.0	47.0	.	0	2	0
Lung Cancer	Male	68	Ex-Smoker	21	10.0	6.0	33.0	35	2	2
Lung Cancer	Female	73	Ex-Smoker	22	9.9	10.9	44.0	29	2	1
Lung Cancer	Male	53	Current Smoker	15	11.7	22.3	.	0	2	2
Lung Cancer	Male	45	Current Smoker	15	19.6	29.4	.	0	2	1
Lung Cancer	Male	68	Ex-Smoker	24	7.1	11.4	56.0	12	4	1
Control	Male	68	Current Smoker	13	10.5	28.9	.	0	3	0
Control	Male	61	Ex-Smoker	15	15.0	11.3	30.0	31	2	0
Lung Cancer	Female	46	Ex-Smoker	18	14.5	16.0	40.0	6	2	1
Control	Female	55	Current Smoker	20	8.0	14.0	.	0	2	0
Control	Female	49	Ex-Smoker	16	9.2	10.1	38.0	11	2	0
Control	Female	54	Ex-Smoker	20	3.0	1.5	30.0	24	2	0
Lung Cancer	Female	48	Current Smoker	20	6.0	8.4	48.0	0	2	1
Control	Male	68	Ex-Smoker	22	13.6	19.0	50.0	18	2	0
Lung Cancer	Female	52	Current Smoker	20	17.7	28.3	52.0	0	2	3
Lung Cancer	Male	70	Current Smoker	20	19.6	50.0	.	0	2	2
Control	Female	54	Current Smoker	25	12.0	17.4	.	0	2	0
Lung Cancer	Male	72	Ex-Smoker	15	20.0	41.0	56.0	16	2	2

Supporting Information Table S5: Clinical and analytical performance of individual glycan nodes (iGNs). Left-hand columns provide statistical comparisons of cancer cases (n = 30) vs. controls (n = 26)[a] in the CEE Lung Cancer cohort. Raw data from which these values were calculated were the ratio of glycan node peak area / stable-isotope-labeled-monosaccharide-internal-standard peak area. All iGNs showed an increasing trend in the cancer patient samples, even if there was not a statistically significant difference. Right-hand columns illustrate the relatively poor intra- and inter-assay precision[b] of iGNs (indicated by grey cells). This analytical feature probably contributed to the weaker diagnostic performance of iGNs relative to GNRs (Table 1): Despite strong statistical significance, none of these iGNs produced an ROC c-statistic of over 0.75.

	CEE Lung Cancer vs. CEE Controls t-test p-value[b]	ROC c-statistic (AUC ± SE)	Intra-Assay Precision (%CV)	Inter-Assay Precision (%CV)
t-Fuc	0.0022	0.697 ± 0.067	14.89	27.66
t-Gal	0.0398	0.643 ± 0.072	16.22	28.51
2-Man	0.0116	0.663 ± 0.070	18.69	27.14
4-Glc	0.6773	NS[d]	37.46	61.07
3-Gal	0.00204	0.713 ± 0.066	14.91	28.23
6-Glc &/or 6-Man	0.178	NS[d]	24.29	34.62
6-Gal	0.00086	0.737 ± 0.064	16.69	29.15
3,4-Gal	0.0064	0.679 ± 0.069	27.75	35.4
2,4-Man	0.00034	0.747 ± 0.063	18.72	30.88
2,6-Man	0.00099	0.731 ± 0.065	18.96	27.64
3,6-Man	0.00142	0.719 ± 0.065	15.15	25.17
3,6-Gal	0.0125	0.697 ± 0.068	22.49	37.86
3,4,6-Man	0.213	NS[d]	16.58	24.72
t-GlcNAc	0.205	NS[d]	7.55	9.92
4-GlcNAc	0.00412	0.704 ± 0.069	12.03	16.33
3-GlcNAc	0.43293	NS[d]	30.57	33.08
3-GalNAc	0.1295	NS[d]	10.74	12.06
3,4-GlcNAc	0.00245	0.713 ± 0.069	15.85	17.78
4,6-GlcNAc	0.07692	NS[d]	13.85	15.21
3,6-GalNAc	0.23354	NS[d]	27.36	29.63

[a] Data for three control samples are missing because internal standard was mistakenly omitted from them during sample processing.

[b] n = 6 samples analyzed on three different days

[c] Two-sided Student's t-test. GNs for which $0.005 < p < 0.05$ are highlighted in light blue; $p < 0.005$ highlighted in dark yellow.

[d] NS indicates not statistically significant.

Supporting Information Table S6: Stability of the top 12 lung cancer glycan node ratios when plasma samples were left at room temperature overnight. Twelve aliquots of the same sample were created and 6 were placed at -80 °C overnight and the remaining 6 were kept out a room temperature. For the two cases in which a statistically significant difference is noted, the overall difference between the sample sets was less than 12%.

Glycan Node Ratio	Kept in Freezer (Avg. ± SE)	Room Temp. Overnight (Avg. ± SE)	T-Test (p-value)
t-Gal/6-Gal	0.378 ± 0.0106	0.386 ± 0.0108	0.195
t-Gal/3,6-Man	0.221 ± 0.0166	0.223 ± 0.0203	0.877
2,4-Man/3-GalNAc	4.57 ± 0.629	4.26 ± 0.590	0.426
t-Gal/2,4-Man	1.52 ± 0.118	1.47 ± 0.0777	0.440
2,4-Man/3,4,6-Man	1.86 ± 0.165	2.05 ± 0.190	0.107
2-Man/2,4-Man	12.2 ± 0.958	11.6 ± 0.649	0.274
6-Gal/3-GalNAc	18.2 ± 1.12	16.3 ± 1.33	0.0292
2,4-Man/t-GlcNAc	2.43 ± 0.165	2.31 ± 0.242	0.405
6-Gal/3,4,6-Man	7.46 ± 0.297	7.79 ± 0.630	0.287
2,6-Man/3,4,6-Man	1.87 ± 0.103	1.82 ± 0.163	0.517
3,6-Man/3,4,6-Man	12.7 ± 0.295	13.5 ± 1.02	0.127
t-Gal/2,6-Man	1.50 ± 0.0590	1.65 ± 0.0510	0.000897

Supporting Information Table S7: Results from an investigation of the effect of blood collection type on the top 12 performing GNRs. Analysis by repeated measures ANOVA followed by the REGW multiple comparison test demonstrated no significant differences between the blood collection types (see second worksheet).

Donor #	Type of Plasma or Serum	t-Gal/6-Gal	t-Gal/3,6-Man	2,4-Man/3-GalNAc	t-Gal/2,4-Man	2,4-Man/3,4,6-Man	2-Man/2,4-Man	6-Gal/3-GalNAc	2,4-Man/t-GlcNAc	6-Gal/3,4,6-Man	2,6-Man/3,4,6-Man	3,6-Man/3,4,6-Man	t-Gal/2,6-Man
1	3.8% Sodium Citrate	0.362713272	0.202384212	2.790598291	1.47151608	2.304977056	13.41914242	11.32136752	2.879188713	9.35121779	2.195905401	16.7592658	1.544606976
2	3.8% Sodium Citrate	0.294000091	0.190024659	5.893050354	0.914200975	2.531462281	8.746910529	18.32459426	2.848149638	7.871648194	2.2813729	12.17876296	1.014417803
3	3.8% Sodium Citrate	0.279203973	0.217763714	4.655549481	0.93752202	2.694620253	9.772284204	15.63258611	2.847826087	9.048101266	2.348417722	11.60094937	1.075731034
4	3.8% Sodium Citrate	0.31508079	0.183495831	5.366336634	1.177457229	2.540264167	10.67108353	20.0540054	2.958808933	9.492969749	2.419258628	16.30038347	1.236350828
5	3.8% Sodium Citrate	0.353101772	0.20550042	4.45039019	1.547031563	1.920846866	13.45384519	19.49832776	2.159296822	8.415734392	2.01335258	14.46036329	1.475951485
6	3.8% Sodium Citrate	0.411984645	0.26762958	4.670395869	1.522756587	2.990082645	14.59572508	17.26247849	1.748952626	11.05179063	3.059504132	17.01294766	1.488204574
7	3.8% Sodium Citrate	0.251085296	0.17536004	5.193008553	0.894657691	2.469755925	10.94464337	18.50353291	2.561731792	8.800141493	1.890166254	12.60028299	1.168990362
8	3.8% Sodium Citrate	0.286054827	0.205988491	3.386671833	1.20809976	1.839049022	12.12252603	14.30298334	2.990420801	7.766884073	1.641489586	10.78581948	1.353499103
9	3.8% Sodium Citrate	0.299550753	0.224731946	3.516912198	1.146275259	2.645244216	10.02000603	13.45798468	2.328235937	10.12241823	2.33915433	13.49242088	1.296271032
10	3.8% Sodium Citrate	0.355009352	0.263830065	2.845105773	1.530812854	1.715675676	11.74744802	12.26819649	2.624875951	7.398054054	1.530810811	9.954810811	1.715677966
11	3.8% Sodium Citrate	0.25856888	0.177642296	7.123659306	0.975467186	2.590272998	10.27703481	26.87444795	3.139004726	9.771966047	2.601743519	14.22367515	0.971166564
12	3.8% Sodium Citrate	0.324973248	0.188867727	3.899791232	1.300588865	2.062948647	13.9001606	15.60751566	1.6	8.256212038	2.081722805	14.20596356	1.288859416
13	3.8% Sodium Citrate	0.324222659	0.212325981	3.892098093	1.315457855	2.83863275	10.88840661	15.79128065	3.098481562	11.51709062	2.601748808	17.58664547	1.43522762
14	3.8% Sodium Citrate	0.267972262	0.195972002	5.450871852	0.892809993	2.797443182	9.539352087	18.16080819	3.749809596	9.3203125	2.171022727	12.74460227	1.150418739
15	3.8% Sodium Citrate	0.34761601	0.206070861	6.887453247	1.076174961	2.988492181	9.389267378	21.32267936	4.271615352	9.251991738	2.096341104	15.60696371	1.534168485
16	3.8% Sodium Citrate	0.375865911	0.233451679	2.75513347	1.445686603	1.845598349	11.81032234	10.59702259	2.832189974	7.09869326	1.981086657	11.42916094	1.346814789
17	3.8% Sodium Citrate	0.277022811	0.183099237	4.932092004	0.887297357	3.573809524	9.22673773	15.7973713	3.392090395	11.4468254	2.70515873	17.31865079	1.172216518
18	3.8% Sodium Citrate	0.280323815	0.208873789	5.459614596	1.089591469	2.554383273	10.93954641	21.22099221	2.703207471	9.928639939	2.028198734	13.32495684	1.372268987
19	3.8% Sodium Citrate	0.30747087	0.194500568	2.828885135	1.33934436	2.646491783	13.79882964	12.32263514	2.202972902	11.52812895	2.249051833	18.22392541	1.576025857
20	3.8% Sodium Citrate	0.321050314	0.234621638	5.641184269	1.08514805	3.15911903	11.61898794	19.06716748	2.716170213	10.67780252	2.203414996	14.61123484	1.55581761
21	3.8% Sodium Citrate	0.286980246	0.175082911	3.652777778	1.208952644	2.663291139	12.72450743	15.38794192	2.681807648	11.21956272	2.328653625	18.39010357	1.382684325
22	3.8% Sodium Citrate	0.377033775	0.20566332	4.141838019	1.422796353	2.982774252	12.41970618	15.6298783	2.976926557	11.25596857	2.489876095	20.63508613	1.704454424
1	DiSodium EDTA	0.335724283	0.201549166	3.730608592	1.227668932	2.693881947	12.01839264	13.64200477	2.692721792	9.850926325	2.248384317	16.40887548	1.470920763
2	DiSodium EDTA	0.297351208	0.209312957	2.495173197	1.458807465	1.648162041	12.45926263	12.24134015	2.110470701	8.085896474	2.257689422	11.48687172	1.064960957
3	DiSodium EDTA	0.292546369	0.162327232	4.133073361	0.995223493	2.565894992	10.84951056	14.06044358	2.711111111	8.729005901	1.754728401	15.73142684	1.455290161
4	DiSodium EDTA	0.330836847	0.205154223	7.928725702	1.07395805	2.628714644	10.83056388	25.73812095	3.226543617	8.53329753	2.303437164	13.76100967	1.225615917
5	DiSodium EDTA	0.359637423	0.238630206	5.161796537	1.385050844	2.222765933	12.30742216	19.879329	2.154002484	8.560410113	2.219853198	12.90131656	1.38686821
6	DiSodium EDTA	0.345956839	0.200732446	4.18588137	1.233409839	2.870935396	12.29932143	14.9235589	1.771277066	10.23549635	2.362555508	17.6405959	1.49881768
7	DiSodium EDTA	0.271425075	0.20161896	5.08253643	1.142569157	2.199261084	9.175697167	21.39503643	2.56168235	9.257832512	2	12.46315271	1.256403941
8	DiSodium EDTA	0.261413778	0.176104826	1.810932798	1.607864857	1.269244288	14.39047355	11.13841525	2.249844237	7.806678383	1.891388401	11.5884007	1.078981602
9	DiSodium EDTA	0.265195858	0.189240326	3.21209564	0.928452579	2.589929689	10.55372625	11.24556962	2.392917016	9.067362214	1.897255614	12.70673622	1.26742379
10	DiSodium EDTA	0.359050632	0.25187288	3.078674424	1.403410025	2.055916885	11.72350041	12.03351885	2.578936215	8.035898302	1.625137258	11.45535941	1.7754158
11	DiSodium EDTA	0.267535414	0.168898215	6.69878391	0.944188428	2.896063629	10.58125029	23.64140942	3.490900227	10.22081424	2.504044217	16.18980857	1.092005384
12	DiSodium EDTA	0.320057798	0.18003556	5.338742394	1.346504559	1.960278054	13.66660334	22.46044625	1.802122561	8.247020854	2.306603774	14.66112214	1.144333226
13	DiSodium EDTA	0.316800715	0.204519231	4.964441672	1.069112842	3.667281106	10.22932898	16.75358702	3.175578611	12.37603687	2.485714286	19.17050691	1.57730812
14	DiSodium EDTA	0.298490712	0.18891447	3.888649812	1.066952552	2.426653239	10.05311938	13.89994621	3.903347732	8.674051695	1.654246391	13.70527022	1.565137987
15	DiSodium EDTA	0.344725525	0.214738897	7.190801294	0.989406356	3.426712329	10.15970418	20.63851958	4.032238565	9.83510274	2.17859589	15.7885274	1.556236737
16	DiSodium EDTA	0.319443435	0.275001877	2.972519572	1.180919108	2.227876901	10.86315507	10.9888161	2.195539297	8.236019638	1.912705065	9.566997964	1.375508671
17	DiSodium EDTA	0.262578324	0.183961124	6.092585116	0.873768609	4.091270558	7.506560057	20.27398735	3.634422413	13.61431412	2.972799566	19.43249593	1.202510867
18	DiSodium EDTA	0.278067623	0.177090827	7.70575693	1.236537397	2.267908863	8.65625133	34.26668854	3.150194448	10.08515157	1.99121452	15.83568256	1.408363636
19	DiSodium EDTA	0.295641426	0.178619953	4.043712898	1.149205926	3.231830925	12.91131723	15.71856449	2.978139905	12.56264826	2.161742506	20.79296959	1.718076616
20	DiSodium EDTA	0.291509792	0.223335271	4.129000492	1.191032674	2.489904988	11.91569282	16.87001477	2.291883028	10.17309976	1.973574822	13.27850356	1.502632767
21	DiSodium EDTA	0.302387114	0.220376335	4.02886836	1.490398395	2.063886424	13.21696761	19.8573903	2.326775592	10.17243419	1.808044957	13.95800959	1.701292328
22	DiSodium EDTA	0.371142896	0.228501023	8.047153781	1.297207412	3.177822513	11.99044502	28.12616822	3.47004946	11.10702902	2.474249287	18.04059722	1.666079056
1	K2 EDTA	0.338223017	0.225215209	3.320650595	1.240269396	2.48922273	12.13653459	12.17688063	2.853969046	9.128020901	2.499237971	13.70825169	1.235299242
2	K2 EDTA	0.330997071	0.227108954	4.04974271	1.076803614	2.691109422	10.69737399	13.17467124	2.540075309	8.75474924	2.354483283	12.75949848	1.230756818
3	K2 EDTA	0.290671	0.179447083	3.69032707	1.146897982	2.159201954	10.94625684	14.56089074	2.763418447	8.519543974	1.890065147	13.80008143	1.310211116
4	K2 EDTA	0.302166407	0.186265092	7.724687933	0.887781668	3.174408663	9.794954664	22.69556172	2.999192246	9.326588772	1.959247649	15.12995155	1.4384
5	K2 EDTA	0.36833658	0.233746004	6.448360347	1.254303668	2.406456604	9.596142043	21.95872597	2.394499265	8.19475313	2.036151357	12.91327894	1.482417962
6	K2 EDTA	0.334872401	0.196434945	3.564724085	1.353587963	2.541176471	12.33029514	14.4089737	1.884405671	10.27169118	2.992647059	17.51066176	1.149385749
7	K2 EDTA	0.293146683	0.175929985	4.692101021	1.378950985	1.795599424	13.24587723	22.07146695	3.664288712	8.446432243	1.447254781	14.07402838	1.710855357
8	K2 EDTA	0.304262776	0.207827636	2.956835977	1.26731396	1.975056993	13.08758827	12.31580004	2.815522845	8.226498592	1.882526485	12.04371731	1.329605357
9	K2 EDTA	0.288863362	0.217729641	5.007850414	1.168305544	2.450754401	9.376485678	20.25421068	3.138473924	9.91205644	2.135722269	13.15039117	1.340637776
10	K2 EDTA	0.333110671	0.264140592	1.925960637	1.6986618	1.528165086	12.74136253	9.8212277141	2.228246137	7.7927124	1.538390035	9.827477226	1.687371601
11	K2 EDTA	0.237022699	0.159462801	4.379358929	0.975066356	2.548790488	11.17920051	18.01585065	2.602679506	10.48523985	2.286592866	15.58507585	1.086874664
12	K2 EDTA	0.315001768	0.207038108	3.822291022	1.299044225	1.843787336	14.67212052	15.7628483	1.476441043	7.603643967	1.813620072	11.56869773	1.320652174
13	K2 EDTA	0.312516228	0.223331169	5.427059424	1.074696304	3.75631068	8.011912874	18.66284111	3.290729143	12.91738747	2.787202118	18.07581642	1.448367586
14	K2 EDTA	0.279324157	0.188819022	6.032515209	0.955454324	2.898891129	9.376847376	20.63478078	4.055994358	9.915927419	2.090322581	14.66885081	1.32503858
15	K2 EDTA	0.32391351	0.208265793	7.201611763	1.036692385	3.328998105	7.254962012	23.04891842	3.914316453	10.65453238	2.542186785	16.57087772	1.357550517
16	K2 EDTA	0.312696726	0.218342096	5.963846813	1.092584162	2.377520793	8.416940218	20.8380966	2.962029808	8.307223425	2.013729065	11.89711747	1.28996577
17	K2 EDTA	0.28304685	0.184187791	5.014290957	1.202363681	2.527192735	10.98593432	17.34468398	2.895528289	12.01238548	2.70240923	18.45978962	1.258161728
18	K2 EDTA	0.299300802	0.19755632	10.14041827	1.032366682	2.817718216	9.927690559	34.97695262	4.202157764	9.71904649	2.190583491	14.7245019	1.327919441
19	K2 EDTA	0.296381501	0.195256551	3.708359133	1.408999833	2.567906528	12.50571882	17.62956656	2.557762118	12.2078465	2.258762997	18.53038911	1.601841306
20	K2 EDTA	0.325645011	0.191659272	4.130517195	1.373541366	2.172934473	12.59059919	17.42215001	2.240599295	9.165242165	2.046581197	15.57250712	1.458342034
21	K2 EDTA	0.3145908	0.230087819	5.602205882	1.382399265	2.359554041	12.59502559	24.61764706	2.797503213	10.36853515	2.129451843	14.17652524	1.531777196
22	K2 EDTA	0.321209376	0.22961356	5.510685663	1.145915812	3.187483904	10.220732	19.65939448	3.423789765	11.37136235	2.921194952	15.90754571	1.25037468
1	K3 EDTA	0.334091648	0.193881785	3.446756426	1.216856061	2.705957719	11.67897727	12.55405957	3.003199431	9.855861627	2.155028828	16.98334401	1.527942925
2	K3 EDTA	0.297989593	0.209778717	4.086759505	1.101143954	2.598159078	9.030207967	15.10156941	2.447835297	9.600829117	2.402262507	13.63792861	1.190938606
3	K3 EDTA	0.290451189	0.182283218	6.243596688	0.934511563	3.509918218	5.82553355	20.08844696	3.519256771	11.29297895	2.349617192	17.99430137	1.39599726
4	K3 EDTA	0.268917232	0.160585329	8.122455626	0.880914194	3.345405768	7.056936648	26.60739293	3.100447539	10.95881958	2.41750503	18.35171026	1.219031766
5	K3 EDTA	0.380149115	0.222804861	4.442260442	1.504056047	2.076569678	13.67336529	17.57575758	1.940376819	8.215926493	1.77143951	14.01799387	1.763129458
6	K3 EDTA	0.3460631	0.232700574	3.32718894	1.455909511	2.665026146	13.97506925	13.99769585	1.467728274	11.21193479	2.795755152	16.67394648	1.387831445
7	K3 EDTA	0.281522588	0.170896034	3.207705193	0.983115753	2.733111323	11.22872063	11.20174949	2.331890137	9.544402157	2.102283539	15.72280368	1.278117221
8	K3 EDTA	0.322556626	0.221393162	5.296926291	1.22028169	2.200595091	12.80822535	20.03909281	3.564972886	8.325192165	1.874659063	12.12930821	1.432444944
9	K3 EDTA	0.2841919	0.210448827	4.908716961	1.019416264	2.591581185	7.34926097	17.60791178	3.035503599	9.296183347	2.384391461	12.55364569	1.107997597
10	K3 EDTA	0.369629066	0.240941644	4.68459069	1.164296728	2.1353576	10.2009594	14.75601926	3.459988145	6.726175233	1.624108286	10.31863911	1.530803018
11	K3 EDTA	0.288971282	0.205305359	4.898432602	1.137207219	2.600865513	11.33380264	19.27711599	3.244601329	10.23535286	2.658455393	14.40645806	1.112572001
12	K3 EDTA	0.309393516	0.19396205	2.582880435	1.437489041	1.563751028	14.90303349	12.0004529	1.289104882	7.265423636	2.134631204	11.58925144	1.053050739
13	K3 EDTA	0.325227863	0.212957738	6.426049618	1.092805702	3.285121951	9.539646596	21.5923187	4.067955301	11.03841463	2.77097561	16.85780488	1.295572573
14	K3 EDTA	0.273302316	0.199271219	6.678870543	0.936548314	2.87682952	9.391673534	22.88705432	3.863648948	9.858276644	2.265512266	13.52071738	1.189262966
15	K3 EDTA	0.393863651	0.239575811	2.601427722	1.441344615	2.53801509	11.66384633	9.519928614	3.353527607	9.287869994	1.546140453	15.26929774	2.365990991
16	K3 EDTA	0.355564883	0.21705213	2.409909192	1.511932306	1.646565799	13.26918755	10.24741118	2.243696233	7.001523892	1.752040927	11.46957658	1.420912028
17	K3 EDTA	0.273609602	0.186377094	4.219855305	1.055243357	2.705927835	10.18963711	16.27491961	3.134029851	10.43608247	2.572938144	15.32061856	1.109786637
18	K3 EDTA	0.266346789	0.177769064	5.039339485	1.048573631	2.481109517	10.55609098	19.83924235	2.852900742	9.767814443	1.93926351	14.6348637	1.341553637
19	K3 EDTA	0.306142764	0.182868516	2.920596905	1.396770327	2.624668874	14.04056015	13.32516581	2.243243243	11.975	2.360430464	20.04751656	1.553131795
20	K3 EDTA	0.311982626	0.192700815	3.976162866	1.284811562	2.539626368	10.36566318	16.37469398	2.325295758	10.45872768	2.076948399	16.93268044	1.571026667
21	K3 EDTA	0.300351654	0.189692391	7.173510343	1.179987088	2.634353741	10.9797719	28.18246372	3.586201574	10.34954649	2.245578231	16.38707483	1.384277492
22	K3 EDTA	0.360473958	0.245727372	9.375814863	1.104154354	3.53682528	9.367877629	28.71870926	3.77443905	10.83351777	2.659166359	15.89241362	1.46858094
1	Serum	0.341268593	0.195134231	1.451891639	1.745536432	1.803597331	14.9892231	7.426202709	2.167712692	9.225123296	2.053089643	16.13373948	1.533418115
2	Serum	0.307860359	0.25731321	2.944607843	1.149492259	2.1339254	10.52322291	10.99460784	2.285768645	7.967673179	2.418472469	9.53285968	1.014247944
3	Serum	0.28857157	0.177658894	6.183887391	0.847588144	3.160085706	9.566156528	18.16322252	3.480782198	9.281756964	2.005050505	15.07636976	1.335852225
4	Serum	0.299259882	0.181950948	4.161205767	1.255685039	2.498033045	12.28037795	17.46028834	2.440430438	10.48166798	2.248151062	17.23949646	1.395254427
5	Serum	0.347933419	0.210156719	4.90143481	1.441050443	2.095386257	11.96750244	20.30047827	2.08962766	8.678549204	1.899279936	14.36812161	1.589843201
6	Serum	0.426172887	0.226182596	2.406950673	2.068933395	1.973345588	15.77503493	11.68497758	1.498255408	9.579963235	2.227941176	18.05055147	1.832508251
7	Serum	0.270251631	0.176063315	3.697521097	1.090007845	2.24336	12.32037658	14.9132384	2.411592707	9.04816	2.02176	13.88864	1.209480848
8	Serum	0.315595844	0.199641476	2.837806301	1.305098684	2.118466899	13.55180921	11.735317	2.562247586	8.760598142	1.75159698	13.8488676	1.578450062
9	Serum	0.28246333	0.185942492	2.989616811	1.082857852	2.096203848	11.4610105	11.46106304	2.1652641	8.036054776	1.783151326	12.2074883	1.272965879
10	Serum	0.368838753	0.275057314	3.958479532	1.412074654	2.056405063	11.9745408	15.15477583	2.813383209	7.872810127	1.54521519	10.55706329	1.879218822
11	Serum	0.258009502	0.161728772	4.22410148	1.06006006	2.653386454	11.41324658	17.3551797	2.720835225	10.90172643	2.2478973	17.39176627	1.251280032
12	Serum	0.342510121	0.221622858	5.65917782	1.268392601	2.223286385	12.36578258	20.95721797	1.840783643	8.233333333	2.10685446	12.72431925	1.338488279
13	Serum	0.329528205	0.198057019	2.895779369	1.427782163	2.364100012	11.94754012	12.54685357	2.569979716	10.24318945	2.269436497	17.042667	1.487338303
14	Serum	0.272592334	0.165091596	4.203153316	0.942267063	2.357731016	10.07109904	14.52899601	3.638688422	8.149944089	1.741181254	13.4568466	1.275922466
15	Serum	0.454350371	0.250161117	4.191082803	1.415805471	2.74624374	10.74285714	13.05987261	3.095014111	8.557595993	1.717028381	15.54257095	2.26446281
16	Serum	0.292393702	0.240345382	2.041836516	1.225491226	1.93160138	11.70925712	8.55782021	2.0124762	8.09579866	2.086056424	9.848995332	1.134753843
17	Serum	0.259399604	0.159106463	6.038265306	0.743677953	3.389729951	8.138813447	17.31122449	4.082039911	9.718085106	2.811988543	15.84390344	0.896471444
18	Serum	0.238269795	0.163270017	7.106366928	0.765907306	3.185082499	6.943483153	22.84308992	3.685524257	10.23830133	2.340681634	14.94137138	1.042208355
19	Serum	0.301412727	0.183106544	3.354028436	1.374735057	2.144545455	12.52663558	15.29763033	2.560419682	9.781212121	2.194848485	16.10090909	1.343227944
20	Serum	0.294982757	0.175320511	4.162644494	1.291825002	2.210662359	11.99727175	18.22956804	2.382865103	9.681206247	1.701238557	16.28896069	1.678652823
21	Serum	0.304358826	0.196020099	2.507930053	1.511837198	2.314505536	12.52772823	12.45760472	2.173009161	11.49680991	1.921748921	17.85100394	1.820818279
22	Serum	0.346652898	0.229341175	4.064737243	1.446692899	2.717413442	12.25388795	16.9634425	2.965	11.34063136	2.096232179	17.14154786	1.875394705

Supporting Information Table S8: Top performing GNRs in a second cohort of lung cancer patients vs. age and gender-matched nominally healthy individuals. Serum rather than EDTA plasma was employed in this sample set. GNRs with 3-GalNAc as the denominator are greyed-out as they may simply indicate smoking status (Table 3).

	Lung Cancer (n = 16) vs. Healthy (n = 16)	Trend in Cancer	Intra-Assay Precision	Inter-Assay Precision
Glycan Node Ratio	**ROC AUC ± SE**	**(Increased or Decreased, I/D)**	**(%CV)**	**(%CV)**
3-Gal/3-GalNAc	0.977 ± 0.026	I	5.28	8.28
t-Gal/2,4-Man[a]	0.922 ± 0.031	D	7.06	7.38
2,4-Man/3-GalNAc[a]	0.922 ± 0.043	I	7.76	11.97
6-Gal/3-GalNAc[a]	0.918 ± 0.042	I	5.29	9.56
2,4-Man/4,6-GlcNAc	0.914 ± 0.038	I	8.95	12.0
2-Man/2,4-Man[a]	0.906 ± 0.037	D	6.08	8.93
3,6-Man/3-GalNAc	0.910 ± 0.045	I	3.83	10.94
t-Gal/6-Gal[a]	0.887 ± 0.052	D	2.34	3.76

[a] GNR overlaps with the top 12-performing GNRs in the Lung Cancer in Central and Eastern Europe cohort.

Supporting Information Table S9: Top performing GNRs in a cohort of colorectal cancer patients vs. age and gender-matched nominally healthy individuals. Serum rather than EDTA plasma was employed in this sample set.

	Colorectal Cancer (n = 10) vs. Healthy (n = 16)	Trend in Cancer	Intra-Assay Precision	Inter-Assay Precision
Glycan Node Ratio	**ROC AUC ± SE**	**(Increased or Decreased, I/D)**	**(%CV)**	**(%CV)**
2-Man/6-Gal	0.969 ± 0.031	D	4.76	6.84
t-Gal/6-Gal[a]	0.969 ± 0.031	D	2.34	3.76
t-GlcNAc/3,4-GlcNAc	0.938 ± 0.018	D	9.88	15.5
t-Gal/2,4-Man[a]	0.925 ± 0.036	D	7.06	7.38
t-Gal/2,6-Man[a]	0.919 ± 0.041	D	5.90	9.82
t-Gal/3,6-Man[a]	0.888 ± 0.062	D	4.35	5.69
2-Man/2,4-Man[a]	0.850 ± 0.063	D	6.08	8.93

[a] GNR overlaps with the top 12-performing GNRs in the Lung Cancer in Central and Eastern Europe cohort.

Supporting Information Table S10: Top performing GNRs in a cohort of prostate cancer patients vs. age-matched nominally healthy males. Serum rather than EDTA plasma was employed in this sample set. As a common theme in these GNRs, 4-Glc (which, in serum may largely be derived from glycolipids) was found to increase.

	Prostate Cancer (n = 59) vs. Healthy (n = 75)	Trend in Cancer	Intra-Assay Precision	Inter-Assay Precision
Glycan Node Ratio	**ROC AUC ± SE**	**(Increased or Decreased, I/D)**	**(%CV)**	**(%CV)**
Fuc/4-Glc	0.729 ± 0.044	D	14.2	19.8
t-Gal/4-Glc	0.714 ± 0.045	D	11.0	17.7
4-Glc/3,4,6-Man	0.710 ± 0.044	I	10.1	16.0
2-Man/4-Glc	0.688 ± 0.046	D	11.3	17.9