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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Glycans Related to the CA19-9 Antigen Are Increased in Distinct Subsets of Pancreatic Cancers and Improve Diagnostic Accuracy Over CA19-9

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SUMMARY

The cancer antigen 19-9 (CA19-9) blood test is a useful biomarker for pancreatic cancer in certain situations but is not increased in a substantial percentage of patients. This article reports that glycan biomarkers related to CA19-9 are increased in subsets of pancreatic cancer patients with prevalence similar to CA19-9. The detection of a 3-biomarker panel of glycans resulted in improved diagnostic accuracy over CA19-9.

BACKGROUND & AIMS: The cancer antigen 19-9 (CA19-9) is the current best biomarker for pancreatic cancer, but it is not increased in approximately 25% of pancreatic cancer patients at a cut-off value that provides a 25% false-positive rate. We hypothesized that antigens related to the CA19-9 antigen, which is a glycan called *sialyl-Lewis A* (sLeA), are increased in distinct subsets of pancreatic cancers.

METHODS: We profiled the levels of multiple glycans and mucin glycoforms in plasma from 200 subjects with either pancreatic cancer or benign pancreatic disease, and we validated selected findings in additional cohorts of 116 and 100 subjects, the latter run with the investigators blinded to diagnoses and including cancers that exclusively were early stage.

RESULTS: We found significant increases in 2 glycans: an isomer of sLeA called sialyl-Lewis X, present both in sulfated and nonsulfated forms, and the sialylated form of a marker for pluripotent stem cells, type 1 N-acetyl-lactosamine. The glycans performed as well as sLeA as individual markers and were increased in distinct groups of patients, resulting in a 3-marker panel that significantly improved upon any individual biomarker. The panel showed 85% sensitivity and 90% specificity in the combined discovery and validation cohorts, relative to 54% sensitivity and 86% specificity for sLeA; and it showed 80% sensitivity and 84% specificity in the independent test cohort, as opposed to 66% sensitivity and 72% specificity for sLeA.

CONCLUSIONS: Glycans related to sLeA are increased in distinct subsets of pancreatic cancers and yield improved diagnostic accuracy compared with CA19-9. *(Cell Mol Gastroenterol Hepatol 2016;2:210–221; http://dx.doi.org/10.1016/j.jcmgh.2015.12.003)*

Keywords: Biomarkers; Sialyl-Lewis A; Antibody Arrays; Lectins.

Many pancreatic cancers secrete glycoproteins and glycolipids that bear a glycan called *sialyl-Lewis A* (sLeA).[1,2] The sLeA glycan forms the basis for the Food and Drug Administration–approved cancer antigen 19-9 (CA19-9) test, named after the monoclonal antibody first developed against the sLeA antigen.[3] The test is used as an approximate indicator of extent of disease recurrence, but a problem with CA19-9 is that it is not increased in a substantial proportion of patients. By using a typical cut-off value of 37 U/mL, approximately 25%–35% of patients do not show increases,[4] rendering the test inconclusive for the diagnosis or monitoring of cancer in many patients. However, the test is very specific for cancer at high cut-off values.[4] Therefore, CA19-9 represents an important marker for pancreatic cancer and a good basis on which to build molecular indicators for cancer, but it needs to be improved. After many years of research since the discovery of CA19-9, a biomarker validated to perform better than CA19-9 for pancreatic cancer detection is not yet available. Identifying another marker to detect cancer among patients with low CA19-9 levels potentially could lead to an improved diagnostic test.

The sLeA glycan is part of a family of glycans called the Lewis antigens, named after the discoverer of a series of antigens found on red blood cells comprising a system of blood types. The Lewis glycans generally appear on the termini of oligosaccharides attached to both proteins and lipids. The common feature among the family members is a core N-acetyl-lactosamine (LacNAc), which is a disaccharide

Abbreviations used in this paper: **AUC, area under the ROC curve; BSA, bovine serum albumin; CA19-9, cancer antigen 19-9; CCL2, *Coprinopsis cinerea* lectin 2; LacNAc, N-acetyl-lactosamine; PBS, phosphate-buffered saline; ROC, receiver operating characteristic; sLeA, sialyl-Lewis A; sLeX, sialyl-Lewis X.**

Most current article

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of galactose linked to N-acetylglucosamine. The monosaccharides fucose and sialic acid can be attached to the LacNAc in various linkages. A sulfate group also can be attached to the Galactose or N-Acetylglucosamine. In the normal pancreas, sLeA appears on the epithelial surfaces of the ducts, and in the cancerous pancreas, it can be heavily secreted into the lumen of the proliferating ducts.[5] The increase of sLeA in the blood likely results from accumulation in the stroma followed by leakage into the capillaries or lymph.[6] One reason for the lack of increases is genetics. A glycosyltransferase enzyme that is critical for the biosynthesis of sLeA, fucosyltransferase 3, is inactive in approximately 5% of the North American population as a result of homozygous mutations in the active part of the gene.[7] But the cause of low CA19-9 levels is not clear for patients with wild-type fucosytransferase 3.

Other members of the Lewis glycans besides sLeA also appear both in the normal and cancerous pancreas. An isomer of sLeA called sialyl Lewis X (sLeX) is up-regulated in the tissue of some pancreatic cancers,[8] and we[9,10] and others[11] found it increased in the circulation of many pancreatic cancer patients. Some patients have an increase in a glycan detected by the DUPAN-2 monoclonal antibody,[12,13] identified primarily as type 1 sialyl-LacNAc,[14,15] and our previous research also found indirect evidence for additional glycans by comparing patient increases between anti-sLeA antibodies with either broad or narrow specificity.[15]

These observations raise the possibility that diversity exists between pancreatic cancers in the type of glycans they make and secrete into the blood. Potentially, a variety of glycans is secreted, with differences between individual cancers. Thus, to encompass the full range of pancreatic cancers, we may need to detect the various antigens that pancreatic cancers are expressing in addition to sLeA, and that are not normally increased under healthy or benign conditions. Assays to detect the additional cancer-associated glycans potentially could be used to identify a higher percentage of pancreatic cancer patients than sLeA alone. Therefore, in this research, we tested the hypothesis that certain glycans related to sLeA are increased in the plasma of pancreatic cancer patients and that they detect patients that have low levels of sLeA.

Materials and Methods

Human Plasma and Tissue Samples

All collections took place at the University of Pittsburgh Medical Center after obtaining informed consent from the participants and before any surgical or medical procedures. The donors consisted of patients with pancreatic cancer, pancreatitis, or benign biliary obstruction, and from healthy subjects (Table 1 and Supplementary Table 1). Resectable cancer included stages I and II, and nonresectable cancer included stages III and IV. The pancreatitis patients were a mixture of chronic and acute, and the healthy subjects had no evidence of pancreatic, biliary, or liver disease. All blood samples (EDTA plasma) were collected according to the standard operating procedure from the Early Detection

Table 1. Sample Characteristics

Discovery	N	Age, *y* (SD)	Male, %	Validation	N	Age, *y* (SD)	Male, %	Test	N	Age, *y* (SD)	Male, %
All cancer	108	68.1 (9.8)	48.1	All cancer	48	65.9 (9.3)	58.3	All cancer	50	66.1 (12.0)	46.0
Stage I	2			Stage I	0			Stage I	3		
Stage II	36			Stage II	21			Stage II	47		
Stage III	32			Stage III	6			Stage III	0		
Stage IV	32			Stage IV	20			Stage IV	0		
Unknown stage	6			Unknown stage	0			Unknown stage	0		
Neuroendocrine tumor	0			Neuroendocrine tumor	1			Neuroendocrine tumor	0		
All control	91	57.5 (15.3)	49.5	All control	69	54.0 (15.4)	40.6	All control	50	59.8 (14.8)	36.0
Pancreatitis	61			Pancreatitis	12			Pancreatitis	30		
Benign stricture	30			Benign stricture	9			Benign stricture	10		
Abnormal Imaging	0			Abnormal Imaging	48			Abnormal Imaging	10		
P value[a]		<.05	NS	*P* value[a]		<.05	NS	*P* value[a]		<.05	NS

[a]*P* value was computed based on a 2-sample *t* test for continuous variables (age) and the Fisher exact test for binary variable (sex).

Research Network and were frozen at -70°C or colder within 4 hours of time of collection. Aliquots were shipped on dry ice and thawed no more than 3 times before analysis.

In addition, the Van Andel Research Institute Biospecimen Facility provided formalin-fixed, paraffin-embedded tissue from patients who underwent pancreatic resections at a regional hospital affiliate in Grand Rapids, Michigan. The Institutional Review Boards at the University of Pittsburgh Medical Center and the Van Andel Research Institute approved this research project (protocol #12008).

Biological Reagents

The buffers and biological solutions used in the microarray assays included the following: 1X phosphate-buffered saline (PBS) + 0.5% or 0.1% Tween-20 (PBST 0.5 or 0.1); 10× sample buffer (1× PBS + 1% Tween-20 + 1% Brij-35; Thermo Scientific, Rockford, IL); 4× IgG blocking cocktail (400 μg/mL each of mouse, sheep, and goat IgG, 800 μg/mL rabbit IgG in 1× PBS, antibodies from Jackson Immunoresearch, West Grove, PA); 10× protease inhibitor (Complete Tablet; Roche Applied Science, Indianapolis, IN); and 2× sample dilution buffer (2× sample buffer + 2× protease inhibitor + 2× IgG cocktail in 1× PBS).

The antibodies and lectins were acquired from various sources (Supplementary Table 2). The capture antibodies to be printed onto microarray slides were purified by dialysis (Slide-A-Lyzer; Pierce Biotechnology, Rockford, IL) to 1× PBS and ultracentrifuged. Biotinylation was performed using the EZ-Link-sulfo-NHS-LC-Biotin kit (Pierce Biotechnology) according to the manufacturer's instructions.

Antibody Array Fabrication and Use

The antibody array methods followed those presented earlier,[16–18] with slight modifications. We printed 48 identical arrays containing various antibodies (Supplementary Table 2) onto glass microscope slides coated with ultrathin nitrocellulose (PATH Slides; Grace BioLabs, Bend, OR) using a contact printer (Aushon 2470; Aushon BioSystems, Billerica, MA). We printed 6 replicates of each antibody in randomized positions within each array. After printing, hydrophobic borders were imprinted onto the slides (SlideImprinter; The Gel Company, San Francisco, CA) to segregate the arrays and allow for individual sample incubations on each array. The arrays were blocked using 1% bovine serum albumin (BSA) in PBS plus 0.5% Tween-20 for 1 hour at room temperature. The slides were rinsed in 1× PBS plus 0.5% Tween-20, washed in the same buffer for 15 minutes, and dried by brief centrifugation at 160 × g, with printed arrays facing outside.

The plasma samples were diluted 2-fold into PBS containing 0.1% Tween-20, 0.1% Brij-35, an IgG blocking cocktail (200 μg/mL mouse and rabbit IgG and 100 μg/mL goat and sheep IgG; Jackson ImmunoResearch), and protease inhibitor (Complete Mini EDTA-free Tablet, Roche Applied Science). We applied 6 μL of each plasma sample to each array and let the sample incubate overnight at 4°C. Each unique sample was applied to 3 separate arrays. The arrays were washed in 3 changes of PBS/0.1% Tween-20 for 3 minutes each and dried by centrifugation (Eppendorf 5810R, Hauppauge, NY rotor A-4-62, 1500 × g for 3 minutes), and a biotinylated lectin or antibody was incubated on the arrays for 1 hour at room temperature. The lectins and antibodies were prepared at 3 μg/mL in PBS with 0.1% BSA and 0.1% Tween-20, except for the anti-LeA (clone 7LE) antibody, which was at 15 μg/mL. For *Coprinopsis cinerea* lectin 2 (CCL2) detection, we pre-incubated the CCL2 with Cy5-conjugated streptavidin at a 4:1 molar ratio as described.[9]

After washing and drying the arrays as described earlier, Cy5-conjugated streptavidin (Roche Applied Science) prepared at 2 μg/mL in PBS with 0.1% BSA and 0.1% Tween-20 was incubated for 1 hour at room temperature, followed by a final wash and dry. The arrays detected with precomplexed CCL2/streptavidin required only a final wash and dry. We scanned the slides for fluorescence using 633-nm excitation (LS Reloaded; Tecan, San Jose, CA).

We quantified the resulting images using in-house software written in Matlab (version R2014a; Mathworks, Natick, MA). We used a custom script to remove any outliers from the 6 replicate spots according to the Grubbs test. The script calculates the Grubbs statistic for the spot farthest from the mean of the replicates and rejects the spot if the Grubbs statistic exceeds a preset threshold, using $P < .1$ here. The script repeatedly removes spots until no outliers remain or to a minimum of 4 spots. It then calculates the geometric mean of the remaining replicate spots as the final output for each array.

The program also averages values between replicate arrays and reports the associated coefficient of variation. We repeated assays for measurements that had a CV greater than 0.4 for signals in the quantifiable response range of the assay (determined by dilution series of pooled samples).[19]

Statistics and Analysis Methods

To characterize classification performance of individual biomarkers, nonparametric estimates of the receiver operating characteristic (ROC) curves were generated. Performance of each biomarker was compared with CA19-9 based on the area under the ROC curve (AUC). In particular, a nonparametric bootstrap procedure stratified on case and control status was performed with 500 bootstrap samples. Two-sided P values for testing the equivalence in AUC between a pair of biomarkers were computed based on a Wald test and bootstrap estimated standard error. Also reported were 95% confidence intervals of the difference in AUC based on bootstrap samples. All statistical calculations were performed using R program R-3.2.2 (https://cran.r-project.org/).

We selected marker panels using the Marker State Space method[20] with 10-fold cross-validation to select individual markers. The program limits the initial size of panels to 3 markers, with the option of adding markers iteratively. Marker State Space software is available upon request. We used GraphPad Prism (San Diego, CA) and Microsoft (Redmond, WA) Excel for graph preparation, and Canvas XIV (ACD Systems, Victoria, Canada) for figure preparation.

Immunohistochemistry, Glycan Array Analysis, and Cross-Validation

See Supplementary Materials and Methods for more detail.

Results

Candidate Glycan Biomarkers for sLeA-Low Cancers

Several glycans are structurally similar to the CA19-9 antigen, sLeA (Figure 1*A*), including variants of sialyl-Lewis X, which we previously showed was increased in a subset of pancreatic cancer patients.[9,10] To test for increases of glycans, we acquired lectins and antibodies targeting the glycans (Figure 1*B* and Supplementary Table 2). Glycan array data were helpful for determining the specificities of the reagents. Some bind only 1 motif with high specificity, but others bind more, such as the 7LE antibody, which binds both Lewis A and nonfucosylated LacNAc type 1 (Supplementary Figure 1). The mouse E-selectin protein binds sLeA, sLeX, and sulfo-sLeX (Supplementary Figure 2), and we validated its use as a detection reagent using cell line and tissue specimens (Supplementary Figure 3). We previously showed that CCL2 is specific for glycans with 3' fucose,[9] mainly Lewis X variants including sulfated Lewis X.

We incubated each plasma sample on a microarray of antibodies targeting various mucins and glycans and then probed the glycans on the captured material with a glycan-binding antibody or lectin. Each sample was incubated on multiple arrays, with each array receiving a different detection reagent (Figure 1*C*).

We did not have a reagent to optimally detect sialylated, nonfucosylated, type 1 N-acetyl-lactosamine structures (Siaα2,3Galβ1,3GlcNAcβ1-). We did, however, have 2 antibodies, called *TRA-1-60* and *7LE* (Figure 1*B*), with good affinity to the nonsialylated variant. We therefore tested the use of sialidase to remove sialic acid before detecting with the antibodies (Figure 2*A*). We confirmed the ability to remove sialic acid on a captured glycoprotein and detect the underlying structure using a protein mixture with a high level of Mucin16 showing the sLeA glycan (Figure 2*B*). The staining of tumor tissue in the regions of cancerous epithelia increased upon sialidase treatment (Figure 2*D*), and the differentiation of cases from controls in a set of plasma samples was enhanced after enzyme treatment (Figure 2*C*). Therefore, in subsequent experiments we used sialidase treatment before detection using the TRA-1-60 and 7LE antibodies.

We acquired measurements of candidate biomarkers in 3 sample cohorts, comprising discovery, validation, and test sets (Table 1 and Supplementary Table 1). Each measurement consisted of a capture antibody and a detection reagent, so with 9 capture antibodies and 12 detection reagents (Supplementary Table 2), we acquired 108 unique measurements of capture/detection pairs.

In the discovery cohort, 34 individual biomarkers had significant increases (Supplementary Table 3). Representative markers included 2 distinct glycoforms of MUC5AC, one showing type 1 sialyl-LacNAc, and the other showing sulfated and/or sialylated sLeA/sLeX (Figure 3*A*). We tested a reduced set of 5 capture antibodies and 5 detection reagents (25 unique assays) in the validation cohort and observed significant increases in 19 (Supplementary Table 3), including the glycoforms of MUC5AC (Figure 3*B*). The markers mentioned earlier showed significant improvement in AUC over sLeA in the discovery set (Figure 3*C*). The classification performance of sLeA in the validation set (Figure 3*D*) was higher than in previous studies. A recent definitive characterization of CA19-9 showed an AUC of 0.77 for discriminating pancreatic cancer from chronic pancreatitis, with lower performance when including benign biliary obstruction,[21] so we viewed the performance in the validation set as an aberration.

Because the cancer patients tended to be older than the control subjects (Table 1), we tested associations with age for each marker within the cancer patients and within the control subjects. None showed an association with age except for the sLeA sandwich (the standard CA19-9 assay), with moderate significance (Supplementary Table 4). Thus, the markers examined here were not increased as a consequence of age.

Complementary Increases in the Markers

We next tested whether the individual markers provided complementary information to sLeA and to one another—that is, whether they showed increases in distinct subsets of patients and few increases in the controls. For each marker, we set a threshold to provide one false-positive increase, thus providing a view of increases that were specific to cancer. At such a threshold, CA19-9 was increased in only 22% of the cases in the discovery cohort. In contrast, several other markers showed a greater percentage of increases in the stages I–II and stages III–IV cancers, with differences between the markers in the patients with increases (Figure 4*A*). The trends were similar in the validation cohort (Figure 4*B*). These results suggested that the markers have increases in distinct groups of patients, independent of stage.

The results also suggested that a biomarker panel would perform better than any individual marker. By using all 316 samples from the combined discovery and validation cohorts, we found that a panel of 3 markers provided better sensitivity and specificity than sLeA (Figure 4*C*). The panel (panel 1) consisted of a glycoform of MUC5AC showing sulfated- and sialyl-Lewis X (detected by CCL2); another glycoform of MUC5AC showing sialyl-LacNAc type 1 and sLeA (detected by the 7LE antibody after desialylation); and a sandwich assay consisting of the capture of sLeA and the detection of sulfated and/or sialylated sLeA/sLeX (detected by mouse E-selectin). An alternate panel (panel 2) differed by 1 marker. The marker selection program did not choose sLeA for inclusion in the panel, indicating that sLeA at best provided only marginal additional diagnostic information beyond what already was detected by the 3 markers. A notable feature of the panel is that it contains 3 classes of glycans: Lewis X variants, Lewis A/X variants, and sialylated type 1 N-acetyl-lactosamine.

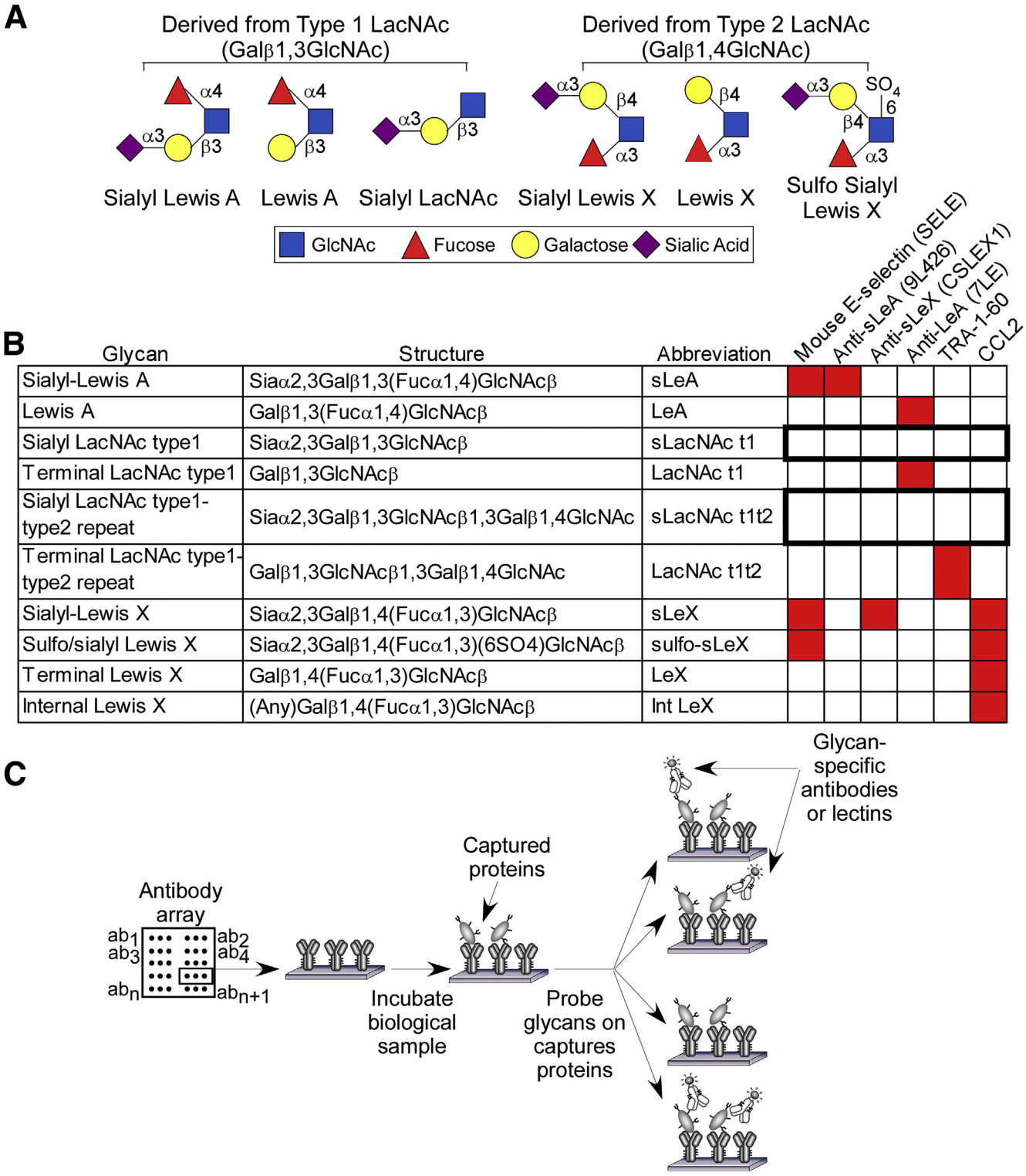


Figure 1. Testing candidate glycans related to sLeA. (*A*) Glycans with structures similar to sLeA. (*B*) Reagents to detect the glycan structures. A *red square* indicates specificity for a glycan, and the *bolded boxes* indicate structures for which we had no detection reagent. (*C*) Antibody-lectin sandwich arrays for parallel testing of candidate biomarkers.

Testing the Marker Panel in Blinded Samples

We applied the marker panels to a new, blinded set of 100 samples (ie, the test set), consisting of stages I–II cancer cases and patients with benign pancreatic diseases. The individual markers had robust and specific increases in cancer (Figure 5*A*), and the ROC curve for a MUC5AC glycoform was improved significantly compared with sLeA (with an improvement in AUC of 0.14; 95% confidence

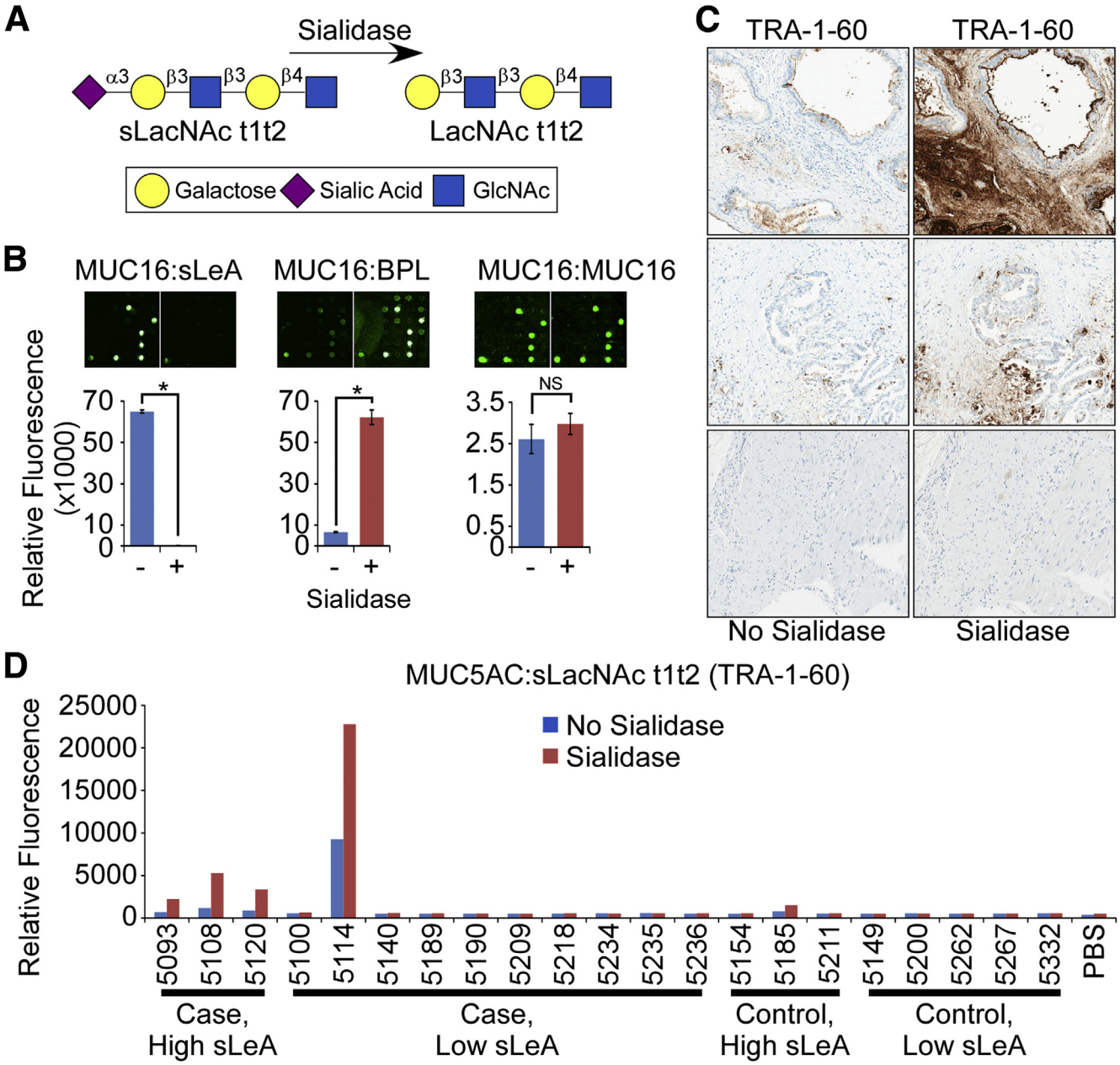


Figure 2. Using sialidase to expose underlying glycans. (*A*) Treatment with sialidase to expose terminal, type 1 N-acetyllactosamine. (*B*) Sialidase treatment of captured MUC16 eliminated the sLeA epitope (*left*); exposed terminal galactose, as detected by the *Bauhinea purpurea* lectin (BPL, *middle*); and did not affect the amount of retained MUC16 (*right*). (*C*) Sialidase treatment resulted in increased staining of selected regions of cancer tissue by the TRA-1-60 antibody. (*D*) Sialidase treatment of captured MUC5AC in a series of plasma samples exposed the TRA-1-60 epitope and resulted in improved discrimination between cancer and control samples.

interval, 0.04–0.26) (Figure 5*B*). Furthermore, the relationships between the markers were similar to the previous sets; increases in the new markers occurred in patients who did not have sLeA increases (Figure 5*C*). These observations confirmed the cancer-associated increases of the new biomarkers and their independent contributions to the patterns of increase.

In the blinded application of the panels to classify the samples, both panels 1 and 2 had higher sensitivity than sLeA, but without statistically significant improvement in overall performance (Supplementary Table 5). We reasoned that the thresholds defining increases for each individual marker were not set optimally, owing to the limited number of samples used for training. When we adjusted the thresholds, while keeping the classification rule the same, the accuracy was 82% for panel 1 compared with 69% for sLeA at its best threshold. All 3 markers of the panel showed increases in cancer patient samples that were not increased in sLeA even at the lower sLeA threshold (Figure 5*D*). Furthermore, in 10-fold cross-validation averaged over 3 trials, the average accuracy of the

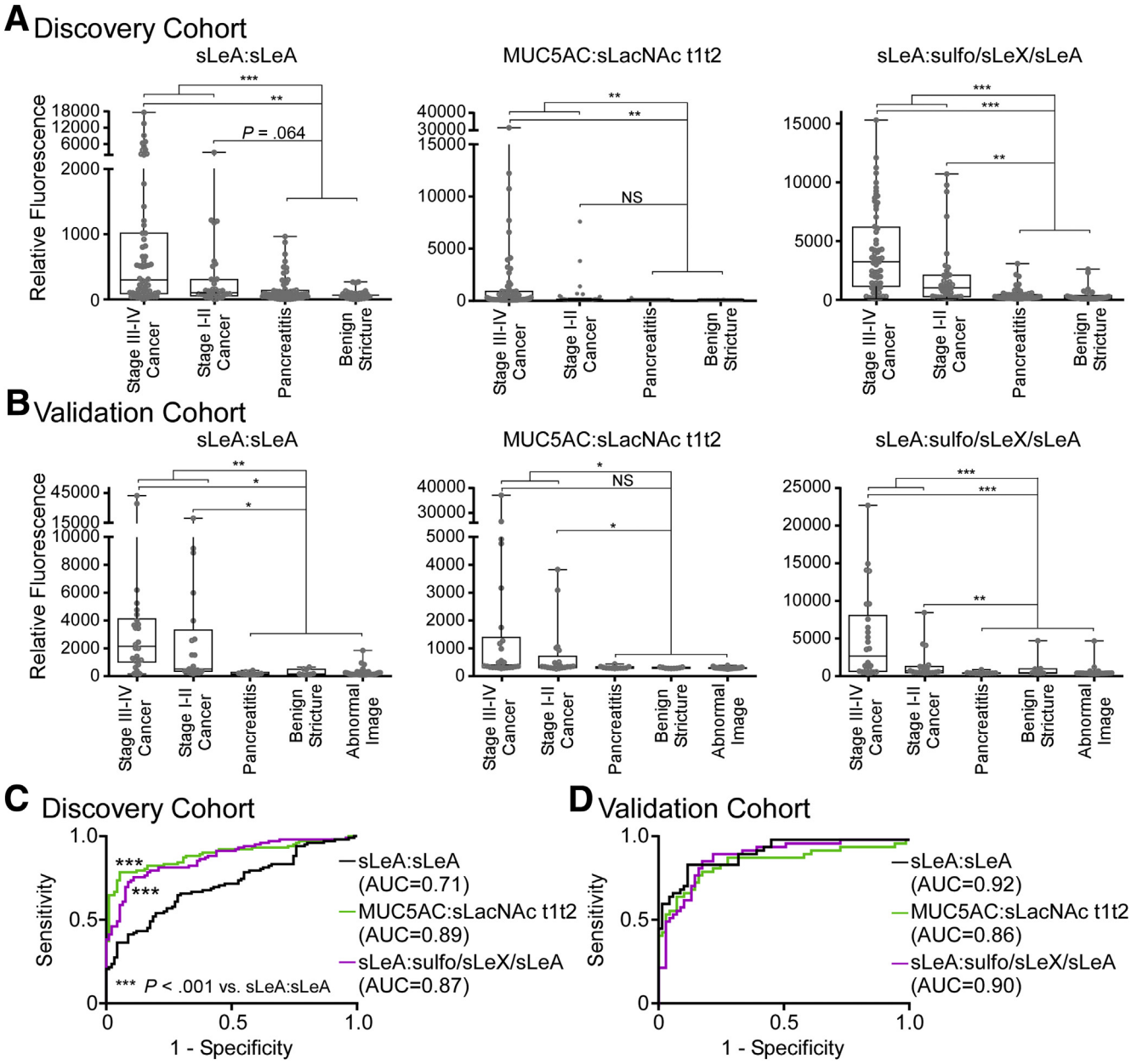
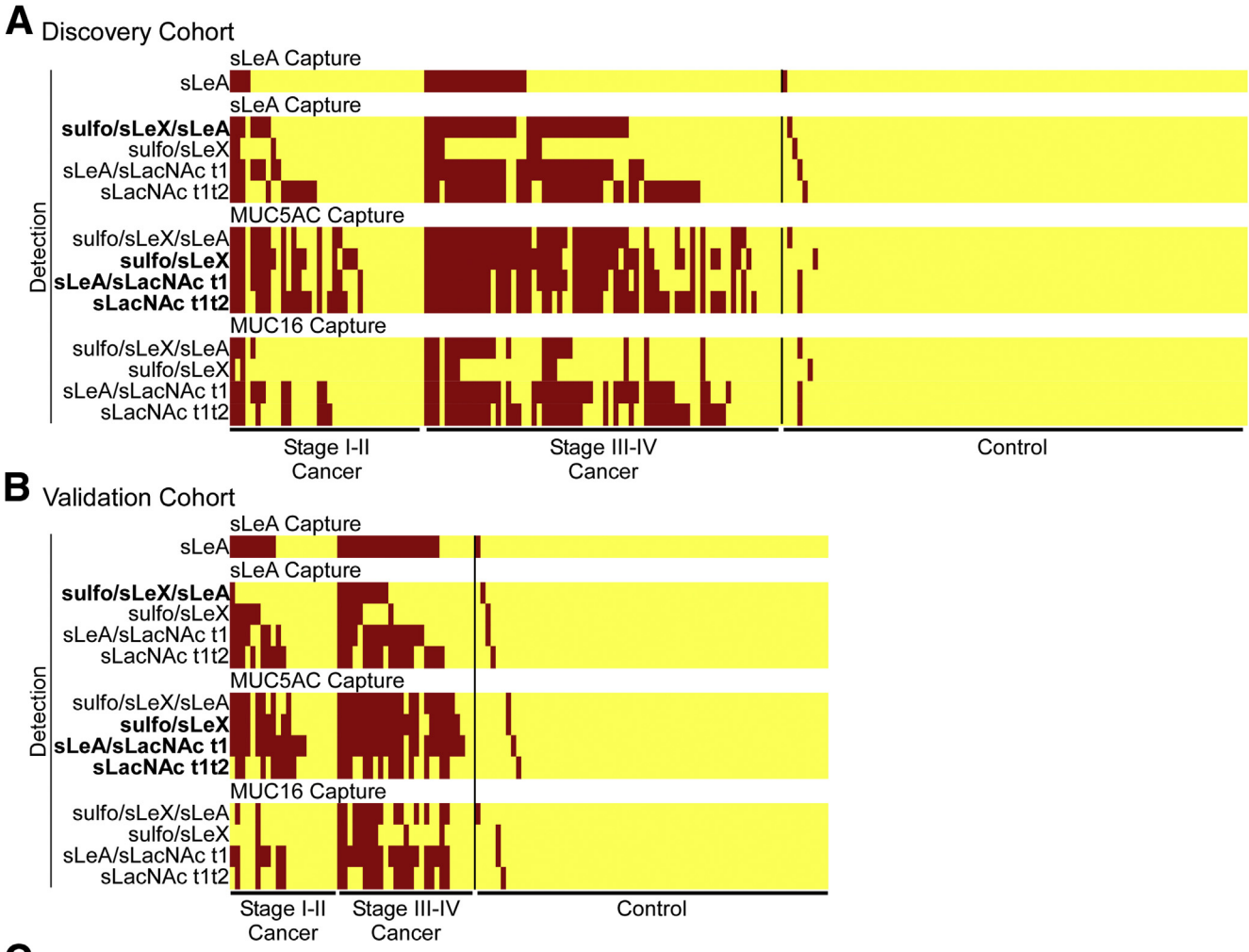


Figure 3. Novel glycan biomarkers of pancreatic cancer. (*A*) Discovery cohort. The heading of each graph indicates the capture and detection targets, separated by a colon. A glycoform of MUC5AC showing type 1 sialyl-LacNAc (detected by TRA-1-60 after desialylation) and a sandwich assay of sLeA capture and sulfated and/or sialylated sLeA/sLeX detection (detected by mouse E-selectin) showed significant increases in cancer. (*B*) Validation cohort. We observed similar increases in the next set of samples. The receiver-operator characteristic curves showed (*C*) improvement over sLeA in the discovery cohort and (*D*) comparable performance in the validation cohort.

panel was 84%, whereas the average accuracy of the individual markers ranged from 43% to 60% (Figure 5*D*). We concluded from these analyses that each of the new biomarkers was increased independently of sLeA at least in some patients, and that together they formed a biomarker panel with improved accuracy compared with sLeA.

Discussion

In this work we identified glycan biomarkers in addition to the CA19-9 antigen, sLeA, that characterizes subgroups of pancreatic cancer patients. Because the glycans do not have identical increases across patients, they can be used in combination to provide better biomarker performance than any individual marker including sLeA. The glycans can be divided into 3 structural categories, consisting of sialyl-Lewis X variants, sulfated and/or sialylated sLeA/sLeX variants, and nonfucosylated sialyl-LacNAc type 1. Each category has its own biosynthetic pathways, cell types on which the glycans are shown, and protein receptors, suggesting that the glycans reflect biological subtypes of cancer.



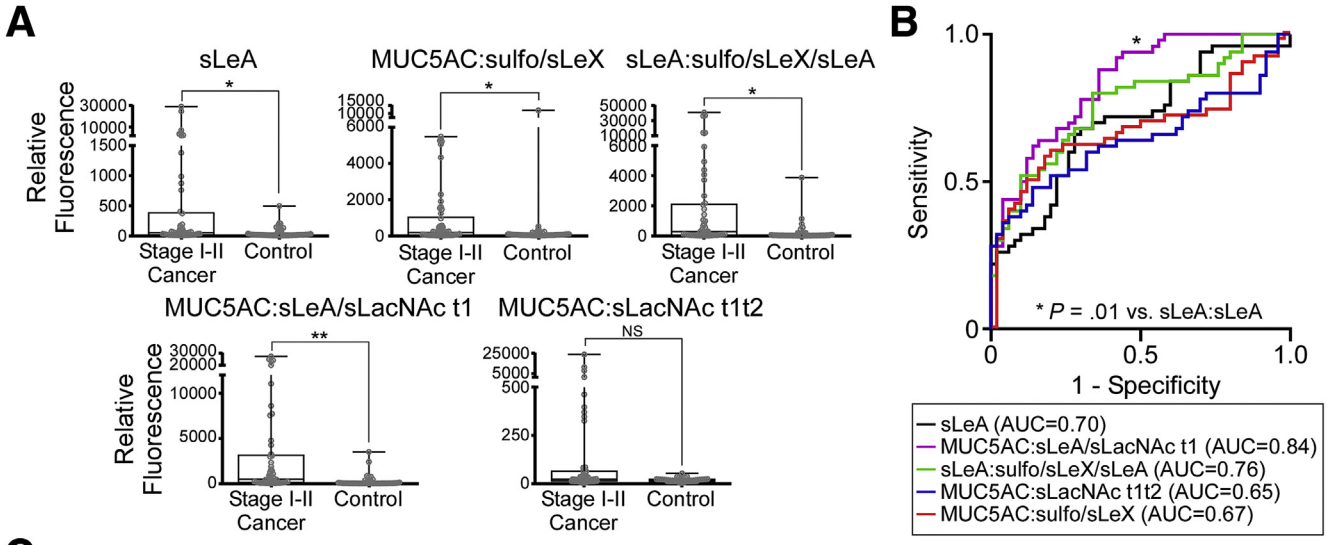
C Combined Cohorts

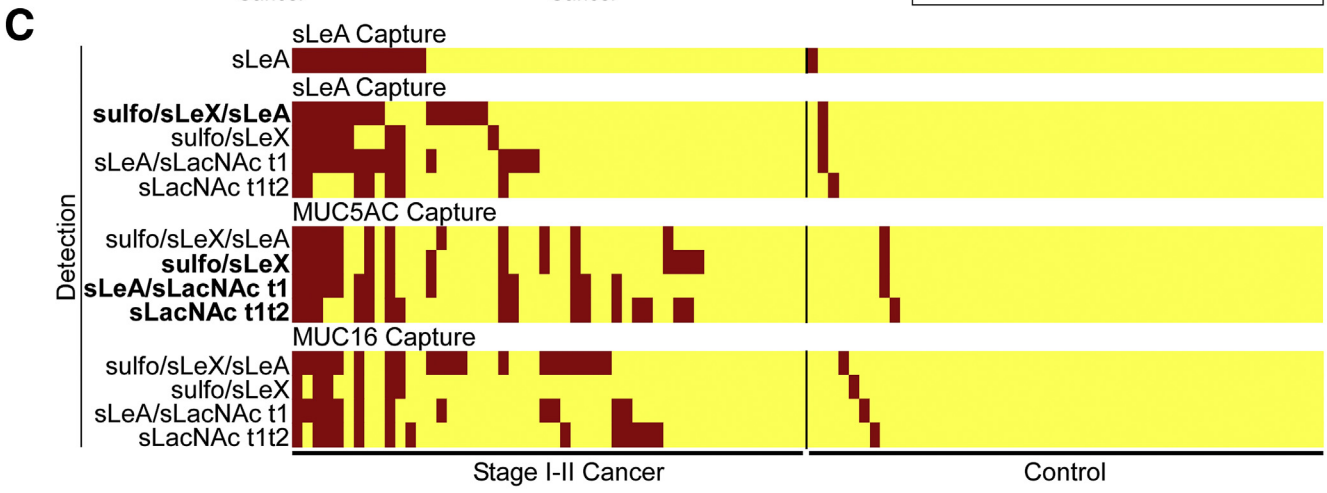
	Markers	Sensitivity	Specificity	Accuracy
sLeA	sLeA:sLeA	54%	86%	70%
Panel 1	MUC5AC:sLeA/sLacNAc t1, sLeA:sulfo/sLeX/sLeA, MUC5AC:sulfo/sLeX	85%	90%	88%
Panel 2	MUC5AC:sLacNAc t1t2, sLeA:sulfo/sLeX/sLeA, MUC5AC:sulfo/sLeX	88%	85%	86%

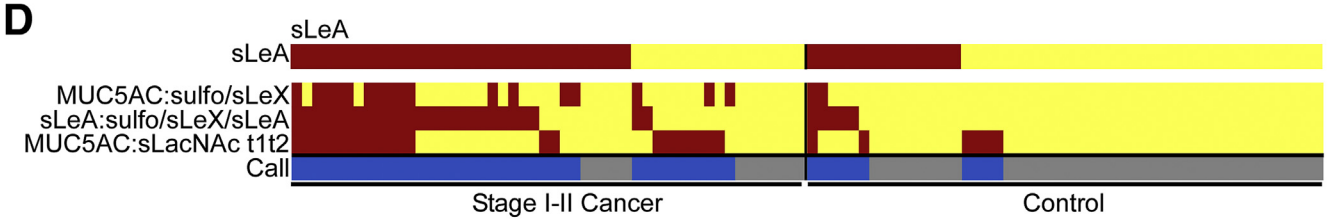
Figure 4. Complementary increases in early-stage and late-stage cancers. (*A*) Discovery cohort. The *rows* present data from the indicated capture and detection targets, and the *columns* represent individual plasma samples. For each biomarker, we set a threshold to provide 1 increase in the control samples. A *red box* indicates a measurement greater than the threshold, and a *yellow box* is a measurement below the threshold. Several markers were increased at this high-specificity threshold in both stages I–II and stages III–IV patient samples, including samples without sLeA increases. (*B*) The validation samples showed similar patterns of increases. (*C*) Two candidate biomarker panels provided improved performance compared with sLeA in the combined sample sets.

Thus, their combined use could have value not only for improved diagnostic accuracy, but also for enhanced information about the disease. Such a capability could meet the need for improved diagnostic accuracy among symptomatic people.[22] Further research could address other needs in clinical practice, including surveillance among people with an increased risk for cancer, improving the determining likelihood of rapid progression after surgery, and monitoring the course of the disease after treatment.

Markers to subclassify pancreatic cancer cells would meet a gap in the application of molecular medicine to pancreatic cancer. Pancreatic cancers show huge diversity in histomorphologies and clinical courses, and finding a molecular basis for the differences has been difficult. For example, adenosquamous carcinomas harbor the same genetic mutations as the more common ductal adenocarcinomas.[23] Particular glycans may be better molecular indicators of the state of a cell than specific genetic alterations; DNA alterations provide information about the inception of the neoplasm, but glycans may indicate changes more clearly in cell identity and cell-environment interactions. We previously found evidence that the tumors showing high sLeA were better differentiated than tumors with high sLeX,[10] but a systematic study still is required to







	Sensitivity	Specificity	Accuracy
sLeA:sLeA	66%	72%	69%
Panel	80%	84%	82%

	sLeA:sLeA	MUC5AC: sulfo/sLeX	MUC5AC: sLeA/sLacNAc t1	MUC5AC: sLacNAc t1t2	sLeA:sulfo/sLeX/sLeA	Panel 1
Avg. Accuracy	0.43	0.50	0.60	0.46	0.49	0.84

Figure 5. Blinded testing of the individual and combined biomarkers. (*A*) The individual assays showed increases similar to those observed in the previous cohorts. (*B*) The ROC curves were consistent with previous performance. The MUC5AC glycoform showing type 1 sLacNAc had significantly better performance than sLeA. (*C*) At high-specificity thresholds, the patterns of increase were similar to those in the previous cohorts. Several assays were increased in patient samples that were not increased in sLeA. (*D*) We classified a sample as a case if it showed an increase in at least 1 of the 3 markers. The *bottom row* indicates the classification, where blue is a case, and gray is a control. The average accuracy of the panel (calculated as correct classifications divided by the number of samples) in 10-fold cross-validation performed 3 times exceeded that of sLeA and any individual marker.

examine the molecular characteristics and clinical course of cancer cells showing the various glycans found here.

Additional research will help determine the relationship between the glycan biomarkers and other promising candidates for the detection of resectable and early stage pancreatic cancer. A recent study showed that exosomes coated with the proteoglycan glypican-1 were increased in patients with resectable pancreatic cancer and may represent a viable biomarker for early diagnosis or detection.[24] Considering that the glycan side chains of glypican-1 are important in epithelial function and signaling, an interesting possibility is that the glycans found in the present work also are on cancer exosomes and could improve the information content of exosome detection. Other promising biomarkers include micro-RNAs,[25] DNA,[26] and tumor cells[27] in the circulation; proteins in the urine[28]; and various types of biomarkers in the pancreatic juice or stool (reviewed by Chari et al[29]), all of which could help define biological subtypes of pancreatic cancer.

Previous studies have shown possible origins and functions in cancer of the glycans found in this work. Particularly interesting is sialyl-LacNAc type 1, as detected by the TRA-1-60 and 7LE antibodies after desialylation. The target of the TRA-1-60 antibody, the nonsialylated version of the glycan, is an excellent marker for pluripotent stem cells.[30–32] Previous research found sialyl-LacNAc type 1 on glycolipids in malignant glioma[33] and embryonal carcinoma.[34] Pancreatic cancer cells frequently activate developmental pathways,[35–37] potentially leading to the expression of the sialyl-LacNAc type 1 epitope. Future research could test whether cancer cells showing sLacNAc t1 have active sonic hedgehog, notch, or β-catenin pathways.

Sulfated and sialylated Lewis X is found on activated and migrating lymphocytes[38,39] and are associated with an invasive phenotype in pancreatic cancer.[40] Studies in mice support a role for sLeX in invasion and modulation of immune responses.[41] Both sLeX and sLeA have the potential to promote metastasis through interactions with E-selectin receptors,[42,43] therefore the relative levels of sLeX and sLeA could affect cancer cell behavior, disease progression, and metastasis. In future work we hope to define the glycan structures and the level of sulfation more precisely, because sulfated versions of sLeX have increased affinity for E-selectin receptors.[42]

In summary, we show here that glycans besides sLeA—the antigen detected by the CA19-9 assay—are increased in distinct groups of patients and contribute to the improved accuracy of a biomarker panel. The 3 types of glycans—sLeA, sLeX variants, and sialylated type 1 LacNAc—possess structures and functions associated with particular differentiation states. Thus, the new glycan biomarkers have the potential to improve the accuracy of diagnosing pancreatic cancer and to shed light on the molecular differences between tumors.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Materials and Methods

Immunohistochemistry With Sialidase Treatment

We used automated staining (Ventana Discovery Ultra) to perform immunohistochemistry (IHC) on sections cut from formalin-fixed, paraffin-embedded blocks. We performed antigen retrieval using the Ventana CC1 buffer for 36 minutes at 95°C. For the slides treated with sialidase, we incubated a 1:200 dilution of sialidase (α2-3,6,8 Neuraminidase , NEB P0720L, 50,000 U/mL) in 1X GlycoBuffer (5 mmol/L $CaCl_2$, 50 mmol/L pH 5.5 sodium acetate) overnight at 37°C. The control slides received only the 1× GlycoBuffer under the same conditions. The slides then were incubated with the TRA-1-60 antibody (NB100-730, Novus Biologicals, 500 ìg/mL diluted at 1:100) for 1 hour at RT, followed by the secondary antibody (Ventana Umap HRP-conjugated anti-mouse) for 12 minutes at 37°C. The development step used the diaminobenzadine chromagen according to preset parameters in the Ventana platform.

Glycan Array Analysis

The glycan synthesis and array core facility of the Consortium for Functional Glycomics (CFG) performed the glycan array experiments and the primary analysis according to the methods presented previously.[27] We downloaded data from www.functionalglycomics.org that previously had been obtained using lectins and glycan-binding antibodies supplied by various investigators. In addition, we sent the recombinant version of CCL2 with biotinylation at the C-terminus to the CFG core facility for processing on their glycan array version 5.2. For detailed analyses of the datasets, we used the GlycoSearch analysis program,[28] and for mining glycan array data to find particular lectins, we used the GlycanBinder database,[23] which derives information from the CFG website.

Cross-Validation

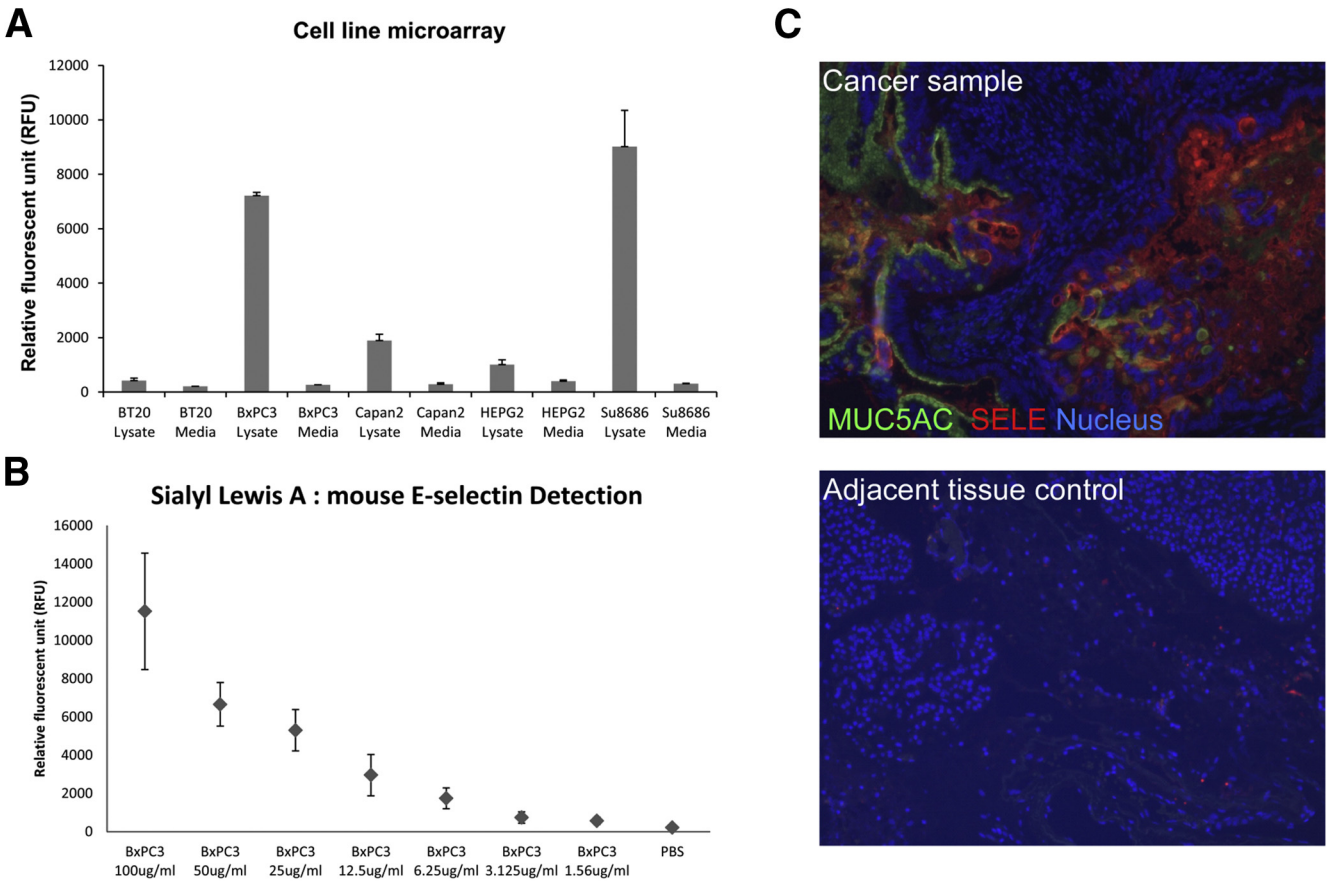
We performed 10-folded cross validation 3 times on each individual marker an on the panel, using the MSS program described in the main text. The program divides samples randomly into 10 groups; uses the samples from 9 groups to define optimal thresholds for discriminating cases from controls; and applies the thresholds to the remaining group to determine the accuracy of discrimination (calculated as the number of correct classifications divided by the total number of samples). The program repeats the process for each possible group of 9 (10 times in all), calculating an accuracy for each split and for each marker. For each marker, we averaged the accuracy over the 10 splits and over 3 repeats of the 10-fold cross validation.

v5.0 GlycanID	9L426 2 ug/ml	9L426 20 ug/ml	7LE 20 ug/ml	Glycan Structure
332	24737.7	33518.0		Neu5Aca2-3Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-3(Fuca1-4)GlcNAcb-Sp0
240	11819.5	37934.5		Neu5Aca2-3Galb1-3(Fuca1-4)GlcNAcb-Sp8
241	32815.1	34101.9		Neu5Aca2-3Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
281		89.2		Neu5Gca2-3Galb1-3(Fuca1-4)GlcNAcb-Sp0
400		1391.7		Neu5Aca2-3Galb1-3GlcNAcb1-3GalNAca-Sp14
295		1591.4		Neu5Aca2-3Galb1-3GlcNAcb1-3Galb1-3GlcNAcb-Sp0
248		631.0		Neu5Aca2-3Galb1-3GlcNAcb1-3Galb1-4GlcNAcb-Sp0
494		71.5		Galb1-4(Fuca1-3)GlcNAcb1-6(Neu5Aca2-6(Neu5Aca2-3Galb1-3)GlcNAcb1-3)Galb1-4Glc-Sp21
126			28245.4	Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
127			16500.4	Galb1-3GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
277			9179.0	Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-3(Fuca1-4)GlcNAcb-Sp0
382			4738.1	Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3)Galb1-4Glcb-Sp0
				Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-
571			4450.2	6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24
386			4308.6	Galb1-3GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb1-6(Galb1-3GlcNAcb1-3)Galb1-4Glc-Sp21
				Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-
572			3834.9	2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24
383			3808.9	Galb1-4(Fuca1-3)GlcNAcb1-6(Galb1-3GlcNAcb1-3)Galb1-4Glc-Sp21
391			2997.1	Galb1-3GlcNAcb1-3GalNAca-Sp14
148			2612.6	Galb1-3GlcNAcb1-3Galb1-4Glcb-Sp10
289			2090.0	Galb1-3GlcNAcb1-3Galb1-3GlcNAcb-Sp0
551			1338.1	Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAc-Sp25
147			993.0	Galb1-3GlcNAcb1-3Galb1-4GlcNAcb-Sp0
477			929.7	Neu5Aca2-6Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3)Galb1-4Glcb-Sp21
			744.3	Neu5Aca2-6(Galb1-3)GlcNAcb1-4Galb1-4Glcb-Sp10
			741.5	Galb1-4GlcNAcb1-6(Fuca1-4(Fuca1-2Galb1-3)GlcNAcb1-3)Galb1-4Glc-Sp21
			635.2	Galb1-3GlcNAcb1-6Galb1-4GlcNAcb-Sp0
			496.2	Galb1-3(Fuca1-4)GlcNAc-Sp8
			483.5	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp12
			442.8	Galb1-3(Fuca1-4)GlcNAc-Sp0
			273.2	Galb1-3(Fuca1-4)GlcNAcb-Sp8
254				Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
256				Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb-Sp8
253				Neu5Aca2-3Galb1-4(Fuca1-3)(6S)GlcNAcb-Sp8
257				Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb-Sp8
231				Neu5Aca2-3(6S)Galb1-4(Fuca1-3)GlcNAcb-Sp8

Supplementary Figure 1. Binding specificities of anti-Lewis A (clone 7LE) and anti-sialyl-Lewis A (clone 9L426). The highlighted numbers are the relative fluorescence of the indicated lectins binding to the listed glycans, with the glycans grouped by motif. The red text in the glycan names indicates the sLeA motif, blue indicates nonfucosylated LacNAc type 1/ type 2, and green indicates nonfucosylated LacNAc type 1. 7LE does not bind where sialic acid is present, but it does bind LacNAc type 1 without fucose. Anti-sLeA clone 9L426, on the other hand, mainly binds sLeA, but has weak binding when the fucose is missing. Neither antibody binds sialyl-Lewis X, shown at *bottom*.

ID	hE-sel 2 ug/ml	hE-sel 10 ug/ml	mE-sel 0.5 ug/ml	mE-sel 5 ug/ml	Glycan Name
v3.0	1001654	1001653	1001665	1001664	Dataset ID
257	35871.0	51830.2	10939.9	37095.5	Neu5Gca2-3Galb1-3(Fuca1-4)GlcNAcb-Sp0
217	24986.1	49732.2	9080.4	40518.0	Neu5Aca2-3Galb1-3(Fuca1-4)GlcNAcb-Sp8
218	4965.7	18110.2	2754.2	42049.1	Neu5Aca2-3Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
232	3133.2	12591.3	2063.4	41704.9	Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb-Sp8
31	2615.9	5867.7	9071.3	39851.2	[3OSO3]Galb1-3(Fuca1-4)GlcNAcb-Sp8
265	2162.4	4248.2	10507.5	38438.3	[3OSO3]Galb1-4(Fuca1-3)[6OSO3]Glc-Sp0
267	2449.6	6542.8	3649.6	23237.4	[3OSO3]Galb1-4(Fuca1-3)[6OSO3]GlcNAc-Sp8
26	2762.0	7394.6			[3OSO3][6OSO3]Galb1-4[6OSO3]GlcNAcb-Sp0
288		5756.2			[6OSO3]Galb1-4[6OSO3]GlcNAcb-Sp0
271		5562.3			Fuca1-2[6OSO3]Galb1-4[6OSO3]Glc-Sp0
35		4789.0			[3OSO3]Galb1-4[6OSO3]GlcNAcb-Sp8
45		4373.5			[6OSO3]Galb1-4[6OSO3]Glcb-Sp8
39		3391.2			[4OSO3][6OSO3]Galb1-4GlcNAcb-Sp0
27		4610.8			[3OSO3][6OSO3]Galb1-4GlcNAcb-Sp0
29		4955.4			[3OSO3]Galb1-4[6OSO3]Glcb-Sp0
287	2636.7	4558.5			[3OSO3][4OSO3]Galb1-4GlcNAcb-Sp0
30		3362.5			[3OSO3]Galb1-4[6OSO3]Glcb-Sp8
227	3055.0	3884.5			Neu5Aca2-3Galb1-4[6OSO3]GlcNAcb-Sp8
244					Neu5Aca2-6Galb1-4[6OSO3]GlcNAcb-Sp8
228		3775.1	2153.1	47873.8	Neu5Aca2-3Galb1-4(Fuca1-3)[6OSO3]GlcNAcb-Sp8
231		3411.8	2986.2	45569.2	Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb-Sp8
233			2126.5	44456.1	Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4GlcNAcb-Sp8
259			2058.8	35006.2	Neu5Gca2-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
229			3128.6	37277.0	Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
230				38951.8	Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
293				29502.2	Galb1-3(Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb1-6)GalNAc-Sp14
247					Neu5Aca2-6Galb1-4GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
47		3237.0			[6OSO3]GlcNAcb-Sp8
40	2294.5	3778.3			[4OSO3]Galb1-4GlcNAcb-Sp8
139					Galb1-4[6OSO3]Glcb-Sp0
37		3504.4			[3OSO3]Galb1-4GlcNAcb-Sp8
38	2117.2	4509.9			[3OSO3]Galb-Sp8
36		3764.0			[3OSO3]Galb1-4GlcNAcb-Sp0
272					Fuca1-2[6OSO3]Galb1-4Glc-Sp0
117				12977.7	Galb1-3(Fuca1-4)GlcNAc-Sp0
115				11950.2	Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb-Sp0
274				11543.5	Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-3(Fuca1-4)GlcNAcb-Sp0
119				11261.2	Galb1-3(Fuca1-4)GlcNAcb-Sp8
118				9249.1	Galb1-3(Fuca1-4)GlcNAc-Sp8
57				6565.6	Fuca1-2Galb1-3(Fuca1-4)GlcNAcb-Sp8
116					Galb1-3(Fuca1-4)GlcNAcb1-3Galb1-4GlcNAcb-Sp0
266			2234.2	50836.0	[3OSO3]Galb1-4(Fuca1-3)Glc-Sp0
34				32021.5	[3OSO3]Galb1-4(Fuca1-3)GlcNAcb-Sp8
268				14917.2	[3OSO3]Galb1-4(Fuca1-3)GlcNAc-Sp0
281				17427.5	Galb1-4(Fuca1-3)[6OSO3]Glc-Sp0
280				3260.5	Galb1-4(Fuca1-3)[6OSO3]GlcNAc-Sp0

Supplementary Figure 2. Binding specificities of mouse and human E-selectin. Both mouse and human E-selectins bind sLeA and sulfated Lewis A. Only the mouse E-selectin has high binding to sLeX, sulfo-sLeX, and sulfo-LeX (shown at *bottom*). Human E-selectin can bind disulfated LacNAc type 2 at a high lectin concentration.



Supplementary Figure 3. Validation of mouse E-selectin (mSELE) as a detection reagent. (*A*) Cell line microarray. We spotted lysates and conditioned media of cell lines known to express sLeA (BxPC3, Capan2, and Su8686) or to not express sLeA (BT20 and HEPG2), and probed the lysates with biotinylated mSELE followed by Cy5-labeled streptavidin. The fluorescence values show binding mainly on the cell lines expressing sLeA. (*B*) Antibody-lectin sandwich arrays. We spotted anti-sLeA, incubated dilutions of a lysate from BxPC3, and probed with mSELE. The fluorescence shows a good response curve with low nonspecific binding at the spot incubated with PBS. (*C*) Validation in immunofluorescence. Cy3-labeled anti-MUC5AC (green), Cy5-labeled mESEL (red), and 4′,6-diamidino-2-phenylindole (blue) were incubated on sections of pancreatic cancer (*top*) and adjacent control tissue (*bottom*). E-selectin binding appears on various proteins near the cancer cells, as expected.

Supplementary Table 1. Details of Sample Characteristics

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05093	Discovery	1 pancreatic adenocarcinoma	1		Male	76
S05094	Discovery	1 pancreatic adenocarcinoma	1	4	Female	59
S05097	Discovery	1 pancreatic adenocarcinoma	1	2	Female	78
S05098	Discovery	1 pancreatic adenocarcinoma	1	4	Male	79
S05099	Discovery	1 pancreatic adenocarcinoma	1	4	Female	57
S05100	Discovery	1 pancreatic adenocarcinoma	1	2	Female	86
S05101	Discovery	1 pancreatic adenocarcinoma	1	2	Male	84
S05102	Discovery	1 pancreatic adenocarcinoma	1	2	Female	68
S05104	Discovery	1 pancreatic adenocarcinoma	1	2	Male	49
S05106	Discovery	1 pancreatic adenocarcinoma	1	2	Male	67
S05107	Discovery	1 pancreatic adenocarcinoma	1	2	Male	62
S05108	Discovery	1 pancreatic adenocarcinoma	1	4	Male	60
S05109	Discovery	1 pancreatic adenocarcinoma	1	4	Male	71
S05112	Discovery	1 pancreatic adenocarcinoma	1	2	Female	69
S05113	Discovery	1 pancreatic adenocarcinoma	1	2	Male	77
S05114	Discovery	1 pancreatic adenocarcinoma	1	2	Female	84
S05115	Discovery	1 pancreatic adenocarcinoma	1	4	Female	79
S05116	Discovery	1 pancreatic adenocarcinoma	1	3	Male	80
S05117	Discovery	1 pancreatic adenocarcinoma	1	3	Female	56
S05118	Discovery	1 pancreatic adenocarcinoma	1		Female	80
S05119	Discovery	1 pancreatic adenocarcinoma	1		Male	65
S05120	Discovery	1 pancreatic adenocarcinoma	1	4	Female	66
S05121	Discovery	1 pancreatic adenocarcinoma	1	3	Male	64
S05122	Discovery	1 pancreatic adenocarcinoma	1	3	Male	72
S05123	Discovery	1 pancreatic adenocarcinoma	1	3	Female	53
S05124	Discovery	1 pancreatic adenocarcinoma	1	2	Female	62
S05125	Discovery	1 pancreatic adenocarcinoma	1	2	Male	61
S05126	Discovery	1 pancreatic adenocarcinoma/528 pseudopapillary tumor	1	2	Female	65
S05129	Discovery	1 pancreatic adenocarcinoma	1	2	Female	56
S05131	Discovery	1 pancreatic adenocarcinoma	1	4	Male	74
S05132	Discovery	1 pancreatic adenocarcinoma/11 common bile duct stones	1	3	Female	56
S05134	Discovery	1 pancreatic adenocarcinoma	1		Female	82
S05137	Discovery	1 pancreatic adenocarcinoma	1	2	Male	66
S05140	Discovery	1 pancreatic adenocarcinoma	1	4	Female	49
S05141	Discovery	1 pancreatic adenocarcinoma	1	4	Male	78
S05142	Discovery	1 pancreatic adenocarcinoma	1	4	Female	67
S05143	Discovery	1 pancreatic adenocarcinoma	1	2	Female	72
S05171	Discovery	1 pancreatic adenocarcinoma	1	3	Male	53
S05173	Discovery	1 pancreatic adenocarcinoma	1	4	Female	88
S05175	Discovery	1 pancreatic adenocarcinoma	1	3	Female	71
S05179	Discovery	1 pancreatic adenocarcinoma	1	3	Male	79
S05181	Discovery	1 pancreatic adenocarcinoma	1	4	Female	65
S05182	Discovery	1 pancreatic adenocarcinoma	1	2	Male	60
S05183	Discovery	1 pancreatic adenocarcinoma	1	4	Female	82
S05187	Discovery	1 pancreatic adenocarcinoma	1	4	Male	71
S05189	Discovery	1 pancreatic adenocarcinoma	1	2	Female	67
S05195	Discovery	1 pancreatic adenocarcinoma	1		Female	57
S05196	Discovery	1 pancreatic adenocarcinoma	1	4	Male	79

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05197	Discovery	1 pancreatic adenocarcinoma	1	2	Male	78
S05198	Discovery	1 pancreatic adenocarcinoma	1	4	Female	71
S05202	Discovery	1 pancreatic adenocarcinoma	1	2	Male	74
S05204	Discovery	1 pancreatic adenocarcinoma	1	3	Female	72
S05207	Discovery	1 pancreatic adenocarcinoma	1	3	Female	71
S05208	Discovery	1 pancreatic adenocarcinoma	1	3	Female	83
S05209	Discovery	521 intraductal papillary mucinous neoplasm degenerated into adenocarcinoma	1	2	Female	65
S05213	Discovery	1 pancreatic adenocarcinoma	1	3	Male	64
S05214	Discovery	1 pancreatic adenocarcinoma	1	4	Male	56
S05216	Discovery	1 pancreatic adenocarcinoma	1	3	Male	54
S05217	Discovery	1 pancreatic adenocarcinoma	1	3	Female	80
S05218	Discovery	1 pancreatic adenocarcinoma	1	2	Female	82
S05221	Discovery	1 pancreatic adenocarcinoma	1	2	Female	76
S05223	Discovery	1 pancreatic adenocarcinoma	1	3	Female	69
S05226	Discovery	1 pancreatic adenocarcinoma	1	3	Female	65
S05230	Discovery	1 pancreatic adenocarcinoma	1	2	Female	72
S05234	Discovery	1 pancreatic adenocarcinoma	1	2	Male	67
S05235	Discovery	1 pancreatic adenocarcinoma	1	2	Female	52
S05236	Discovery	1 pancreatic adenocarcinoma	1	3	Male	74
S05238	Discovery	1 pancreatic adenocarcinoma	1	4	Female	72
S05239	Discovery	1 pancreatic adenocarcinoma	1	4	Female	65
S05243	Discovery	1 pancreatic adenocarcinoma	1	3	Male	66
S05247	Discovery	1 pancreatic adenocarcinoma	1	3	Male	70
S05250	Discovery	1 pancreatic adenocarcinoma	1	4	Female	74
S05251	Discovery	1 pancreatic adenocarcinoma	1		Male	52
S05258	Discovery	1 pancreatic adenocarcinoma/9 unknown cyst (clinical)	1	4	Female	57
S05259	Discovery	1 pancreatic adenocarcinoma	1	4	Female	81
S05266	Discovery	1 pancreatic adenocarcinoma	1	2	Male	62
S05270	Discovery	1 pancreatic adenocarcinoma	1	2	Female	67
S05272	Discovery	1 pancreatic adenocarcinoma	1	4	Male	60
S05279	Discovery	1 pancreatic adenocarcinoma	1	4	Female	79
S05281	Discovery	1 pancreatic adenocarcinoma	1	2	Male	66
S05284	Discovery	1 pancreatic adenocarcinoma/5 intraductal papillary mucinous neoplasm (surgical)	1	2	Female	74
S05286	Discovery	1 pancreatic adenocarcinoma	1	3	Male	37
S05287	Discovery	1 pancreatic adenocarcinoma	1	4	Female	64
S05293	Discovery	1 pancreatic adenocarcinoma	1	2	Male	63
S05306	Discovery	1 pancreatic adenocarcinoma	1	3	Male	57
S05309	Discovery	1 pancreatic adenocarcinoma	1	3	Male	57
S05311	Discovery	1 pancreatic adenocarcinoma	1	3	Male	74
S05318	Discovery	1 pancreatic adenocarcinoma	1	3	Male	67
S05324	Discovery	1 pancreatic adenocarcinoma	1	2	Male	69
S05325	Discovery	1 pancreatic adenocarcinoma	1	3	Male	86
S05331	Discovery	1 pancreatic adenocarcinoma	1	3	Male	63
S05336	Discovery	1 pancreatic adenocarcinoma	1	3	Male	52
S05340	Discovery	1 pancreatic adenocarcinoma	1	2	Male	79
S05342	Discovery	1 pancreatic adenocarcinoma	1	4	Female	59
S05346	Discovery	1 pancreatic adenocarcinoma/55 intraductal papillary mucinous neoplasm (clinical)	1	1	Male	65
S05352	Discovery	1 pancreatic adenocarcinoma	1	3	Male	72

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05355	Discovery	1 pancreatic adenocarcinoma	1	3	Male	58
S05356	Discovery	1 pancreatic adenocarcinoma	1	2	Female	56
S05357	Discovery	1 pancreatic adenocarcinoma	1	3	Male	65
S05360	Discovery	1 pancreatic adenocarcinoma	1	2	Female	55
S05372	Discovery	1 pancreatic adenocarcinoma	1	4	Male	69
S05392	Discovery	1 pancreatic adenocarcinoma	1	2	Female	79
S05396	Discovery	1 pancreatic adenocarcinoma	1	4	Female	70
S05397	Discovery	1 pancreatic adenocarcinoma	1	4	Female	70
S05398	Discovery	1 pancreatic adenocarcinoma	1	4	Male	73
S05400	Discovery	1 pancreatic adenocarcinoma	1	4	Female	65
S05401	Discovery	1 pancreatic adenocarcinoma	1	3	Female	76
S05403	Discovery	1 pancreatic adenocarcinoma	1	1	Female	79
S05149	Discovery	10 acute pancreatitis	0		Male	67
S05151	Discovery	10 acute pancreatitis	0		Male	43
S05154	Discovery	10 acute pancreatitis	0		Female	73
S05156	Discovery	10 acute pancreatitis	0		Female	53
S05200	Discovery	10 acute pancreatitis	0		Male	46
S05215	Discovery	10 acute pancreatitis	0		Female	50
S05222	Discovery	10 acute pancreatitis	0		Male	54
S05233	Discovery	10 acute pancreatitis	0		Male	70
S05242	Discovery	10 acute pancreatitis	0		Male	47
S05257	Discovery	10 acute pancreatitis	0		Male	56
S05262	Discovery	10 acute pancreatitis	0		Male	49
S05267	Discovery	10 acute pancreatitis	0		Male	55
S05305	Discovery	10 acute pancreatitis	0		Male	58
S05332	Discovery	10 acute pancreatitis	0		Female	53
S05339	Discovery	10 acute pancreatitis	0		Male	83
S05361	Discovery	10 acute pancreatitis	0		Female	76
S05371	Discovery	10 acute pancreatitis	0		Female	49
S05399	Discovery	10 acute pancreatitis	0		Male	57
S05162	Discovery	11 common bile duct stones	0		Female	66
S05163	Discovery	11 common bile duct stones	0		Male	72
S05166	Discovery	11 common bile duct stones	0		Male	69
S05244	Discovery	11 common bile duct stones	0		Male	71
S05261	Discovery	11 common bile duct stones	0		Female	87
S05273	Discovery	11 common bile duct stones	0		Male	82
S05274	Discovery	11 common bile duct stones	0		Female	75
S05295	Discovery	11 common bile duct stones	0		Female	36
S05328	Discovery	11 common bile duct stones	0		Female	64
S05347	Discovery	11 common bile duct stones	0		Female	35
S05351	Discovery	11 common bile duct stones	0		Female	81
S05370	Discovery	11 common bile duct stones	0		Male	55
S05389	Discovery	11 common bile duct stones	0		Female	21
S05394	Discovery	11 common bile duct stones	0		Female	82
S05153	Discovery	14 benign stricture; biliary dilation	0		Female	86
S05240	Discovery	14 benign stricture; biliary dilation	0		Female	74
S05253	Discovery	14 benign stricture; biliary dilation	0		Female	47
S05283	Discovery	14 benign stricture; biliary dilation	0		Female	77
S05298	Discovery	14 benign stricture; biliary dilation	0		Male	58
S05307	Discovery	14 benign stricture; biliary dilation	0		Female	84

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05315	Discovery	14 benign stricture; biliary dilation	0		Male	68
S05326	Discovery	14 benign stricture; biliary dilation	0		Male	52
S05348	Discovery	14 benign stricture; biliary dilation	0		Female	59
S05353	Discovery	14 benign stricture; biliary dilation	0		Female	53
S05354	Discovery	14 benign stricture; biliary dilation	0		Female	73
S05369	Discovery	14 benign stricture; biliary dilation	0		Female	38
S05385	Discovery	14 benign stricture; biliary dilation	0		Female	66
S05406	Discovery	14 benign stricture; biliary dilation	0		Female	60
S05290	Discovery	14 benign stricture; biliary dilation/15 gallstones	0		Female	52
S05145	Discovery	14 benign stricture; biliary dilation/55 intraductal papillary mucinous neoplasm	0		Male	38
S05150	Discovery	3 chronic pancreatitis	0		Female	54
S05158	Discovery	3 chronic pancreatitis	0		Male	76
S05161	Discovery	3 chronic pancreatitis	0		Male	30
S05167	Discovery	3 chronic pancreatitis	0		Male	51
S05185	Discovery	3 chronic pancreatitis	0		Female	60
S05194	Discovery	3 chronic pancreatitis	0		Female	33
S05199	Discovery	3 chronic pancreatitis	0		Male	41
S05210	Discovery	3 chronic pancreatitis	0		Male	81
S05211	Discovery	3 chronic pancreatitis	0		Female	40
S05220	Discovery	3 chronic pancreatitis	0		Male	50
S05225	Discovery	3 chronic pancreatitis	0		Female	51
S05227	Discovery	3 chronic pancreatitis	0		Male	46
S05229	Discovery	3 chronic pancreatitis	0		Male	52
S05232	Discovery	3 chronic pancreatitis	0		Male	44
S05241	Discovery	3 chronic pancreatitis	0		Male	58
S05246	Discovery	3 chronic pancreatitis	0		Female	56
S05248	Discovery	3 chronic pancreatitis	0		Male	65
S05249	Discovery	3 chronic pancreatitis	0		Male	57
S05260	Discovery	3 chronic pancreatitis	0		Male	55
S05263	Discovery	3 chronic pancreatitis	0		Male	37
S05268	Discovery	3 chronic pancreatitis	0		Female	76
S05277	Discovery	3 chronic pancreatitis	0		Female	46
S05278	Discovery	3 chronic pancreatitis	0		Female	70
S05280	Discovery	3 chronic pancreatitis	0		Female	39
S05282	Discovery	3 chronic pancreatitis	0		Female	44
S05297	Discovery	3 chronic pancreatitis	0		Male	59
S05303	Discovery	3 chronic pancreatitis	0		Female	77
S05308	Discovery	3 chronic pancreatitis	0		Male	40
S05314	Discovery	3 chronic pancreatitis	0		Female	42
S05317	Discovery	3 chronic pancreatitis	0		Male	70
S05327	Discovery	3 chronic pancreatitis	0		Female	73
S05329	Discovery	3 chronic pancreatitis	0		Male	67
S05334	Discovery	3 chronic pancreatitis	0		Female	73
S05338	Discovery	3 chronic pancreatitis	0		Male	67
S05349	Discovery	3 chronic pancreatitis	0		Female	29
S05350	Discovery	3 chronic pancreatitis	0		Male	45
S05364	Discovery	3 chronic pancreatitis	0		Male	58
S05366	Discovery	3 chronic pancreatitis	0		Female	55
S05367	Discovery	3 chronic pancreatitis	0		Male	45
S05381	Discovery	3 chronic pancreatitis	0		Male	28

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05402	Discovery	3 chronic pancreatitis	0		Female	42
S05291	Discovery	3 chronic pancreatitis/15 gallstones	0		Male	76
S05254	Discovery	3 chronic pancreatitis/57 pseudocyst (clinical)	0		Female	55
S06059	Test	1 pancreatic adenocarcinoma	1	2	Male	49
S06061	Test	1 pancreatic adenocarcinoma	1	2	Male	62
S06062	Test	1 pancreatic adenocarcinoma	1	2	Female	78
S06063	Test	1 pancreatic adenocarcinoma	1	2	Female	70
S06064	Test	1 pancreatic adenocarcinoma	1	2	Male	76
S06066	Test	1 pancreatic adenocarcinoma	1	2	Male	64
S06067	Test	1 pancreatic adenocarcinoma	1	2	Female	63
S06068	Test	1 pancreatic adenocarcinoma	1	2	Female	78
S06072	Test	1 pancreatic adenocarcinoma	1	2	Male	69
S06074	Test	1 pancreatic adenocarcinoma	1	2	Male	88
S06081	Test	1 pancreatic adenocarcinoma	1	2	Female	72
S06082	Test	1 pancreatic adenocarcinoma	1	2	Male	76
S06085	Test	1 pancreatic adenocarcinoma	1	2	Female	74
S06087	Test	1 pancreatic adenocarcinoma	1	2	Female	62
S06088	Test	1 pancreatic adenocarcinoma	1	2	Male	65
S06089	Test	1 pancreatic adenocarcinoma	1	2	Female	69
S06090	Test	1 pancreatic adenocarcinoma	1	2	Male	74
S06091	Test	1 pancreatic adenocarcinoma	1	1	Male	58
S06092	Test	1 pancreatic adenocarcinoma	1	2	Male	65
S06095	Test	1 pancreatic adenocarcinoma	1	2	Male	76
S06096	Test	1 pancreatic adenocarcinoma	1	2	Male	71
S06097	Test	1 pancreatic adenocarcinoma	1	1	Male	77
S06099	Test	1 pancreatic adenocarcinoma	1	2	Male	70
S06100	Test	1 pancreatic adenocarcinoma	1	2	Female	51
S06103	Test	1 pancreatic adenocarcinoma	1	2	Female	81
S06107	Test	1 pancreatic adenocarcinoma	1	2	Female	56
S06108	Test	1 pancreatic adenocarcinoma	1	2	Female	63
S06109	Test	1 pancreatic adenocarcinoma	1	2	Female	78
S06111	Test	1 pancreatic adenocarcinoma	1	2	Female	70
S06112	Test	1 pancreatic adenocarcinoma	1	2	Male	50
S06115	Test	1 pancreatic adenocarcinoma	1	2	Male	58
S06116	Test	1 pancreatic adenocarcinoma	1	2	Female	61
S06117	Test	1 pancreatic adenocarcinoma	1	2	Female	79
S06119	Test	1 pancreatic adenocarcinoma	1	2	Male	56
S06121	Test	1 pancreatic adenocarcinoma	1	2	Male	85
S06122	Test	1 pancreatic adenocarcinoma	1	1	Male	69
S06127	Test	1 pancreatic adenocarcinoma	1	2	Female	52
S06128	Test	1 pancreatic adenocarcinoma	1	2	Female	72
S06135	Test	1 pancreatic adenocarcinoma	1	2	Female	37
S06136	Test	1 pancreatic adenocarcinoma	1	2	Male	61
S06137	Test	1 pancreatic adenocarcinoma	1	2	Female	68
S06140	Test	1 pancreatic adenocarcinoma	1	2	Female	52
S06143	Test	1 pancreatic adenocarcinoma	1	2	Female	75
S06145	Test	1 pancreatic adenocarcinoma	1	2	Male	66
S06146	Test	1 pancreatic adenocarcinoma	1	2	Female	65
S06147	Test	1 pancreatic adenocarcinoma	1	2	Female	29
S06148	Test	1 pancreatic adenocarcinoma	1	2	Male	57

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S06153	Test	1 pancreatic adenocarcinoma	1	2	Female	82
S06155	Test	1 pancreatic adenocarcinoma	1	2	Female	71
S06158	Test	1 pancreatic adenocarcinoma	1	2	Female	54
S06069	Test	10 acute pancreatitis	0		Male	63
S06070	Test	10 acute pancreatitis	0		Male	51
S06071	Test	10 acute pancreatitis	0		Female	75
S06075	Test	10 acute pancreatitis	0		Female	61
S06076	Test	10 acute pancreatitis	0		Male	68
S06086	Test	10 acute pancreatitis	0		Female	35
S06098	Test	10 acute pancreatitis	0		Male	63
S06101	Test	10 acute pancreatitis	0		Female	70
S06102	Test	10 acute pancreatitis	0		Male	34
S06104	Test	10 acute pancreatitis	0		Female	62
S06106	Test	10 acute pancreatitis	0		Male	82
S06126	Test	10 acute pancreatitis	0		Male	60
S06149	Test	10 acute pancreatitis	0		Male	56
S06152	Test	10 acute pancreatitis	0		Male	64
S06156	Test	10 acute pancreatitis	0		Male	42
S06060	Test	14 benign stricture; biliary dilation	0		Female	75
S06073	Test	14 benign stricture; biliary dilation	0		Female	77
S06083	Test	14 benign stricture; biliary dilation	0		Female	69
S06084	Test	14 benign stricture; biliary dilation	0		Female	41
S06093	Test	14 benign stricture; biliary dilation	0		Male	34
S06094	Test	14 benign stricture; biliary dilation	0		Female	58
S06105	Test	14 benign stricture; biliary dilation	0		Male	55
S06129	Test	14 benign stricture; biliary dilation	0		Male	62
S06134	Test	14 benign stricture; biliary dilation	0		Female	44
S06142	Test	14 benign stricture; biliary dilation	0		Female	85
S06065	Test	20 abnormal imaging test (benign)	0		Female	38
S06077	Test	20 abnormal imaging test (benign)	0		Male	57
S06078	Test	20 abnormal imaging test (benign)	0		Female	63
S06079	Test	20 abnormal imaging test (benign)	0		Male	56
S06080	Test	20 abnormal imaging test (benign)	0		Female	56
S06139	Test	20 abnormal imaging test (benign)	0		Female	75
S06141	Test	20 abnormal imaging test (benign)	0		Female	72
S06144	Test	20 abnormal imaging test (benign)	0		Female	56
S06157	Test	20 abnormal imaging test (benign)	0		Female	27
S06154	Test	20 abnormal imaging test (benign)	0		Female	50
S06110	Test	3 chronic pancreatitis	0		Female	52
S06113	Test	3 chronic pancreatitis	0		Female	58
S06114	Test	3 chronic pancreatitis	0		Female	71
S06118	Test	3 chronic pancreatitis	0		Male	75
S06120	Test	3 chronic pancreatitis	0		Female	67
S06123	Test	3 chronic pancreatitis	0		Female	61
S06124	Test	3 chronic pancreatitis	0		Female	36
S06125	Test	3 chronic pancreatitis	0		Female	84
S06130	Test	3 chronic pancreatitis	0		Female	90
S06131	Test	3 chronic pancreatitis	0		Female	35
S06132	Test	3 chronic pancreatitis	0		Male	59
S06133	Test	3 chronic pancreatitis	0		Female	59

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S06138	Test	3 chronic pancreatitis	0		Male	63
S06150	Test	3 chronic pancreatitis	0		Female	70
S06151	Test	3 chronic pancreatitis	0		Female	75
S05090	Validation	1 pancreatic adenocarcinoma	1	2	Male	68
S05091	Validation	1 pancreatic adenocarcinoma	1	2	Male	66
S05092	Validation	1 pancreatic adenocarcinoma	1	2	Male	66
S05095	Validation	1 pancreatic adenocarcinoma	1	4	Male	71
S05096	Validation	1 pancreatic adenocarcinoma	1	3	Female	71
S05395	Validation	1 pancreatic adenocarcinoma	1	4	Male	53
S05103	Validation	1 pancreatic adenocarcinoma	1	4	Female	59
S05105	Validation	1 pancreatic adenocarcinoma	1	4	Male	74
S05110	Validation	1 pancreatic adenocarcinoma	1	4	Male	78
S05111	Validation	1 pancreatic adenocarcinoma	1	2	Female	82
S05127	Validation	1 pancreatic adenocarcinoma	1	4	Male	73
S05128	Validation	1 pancreatic adenocarcinoma	1	4	Female	63
S05130	Validation	1 pancreatic adenocarcinoma	1	2	Male	56
S05133	Validation	1 pancreatic adenocarcinoma	1	3	Male	65
S05135	Validation	1 pancreatic adenocarcinoma	1	3	Male	58
S05136	Validation	1 pancreatic adenocarcinoma	1	4	Female	82
S05138	Validation	1 pancreatic adenocarcinoma	1	4	Male	45
S05139	Validation	1 pancreatic adenocarcinoma	1	4	Male	45
S05169	Validation	1 pancreatic adenocarcinoma	1	2	Male	51
S05172	Validation	1 pancreatic adenocarcinoma	1	2	Female	51
S05176	Validation	1 pancreatic adenocarcinoma	1	4	Male	65
S05174	Validation	1 pancreatic adenocarcinoma	1	2	Female	80
S05201	Validation	1 pancreatic adenocarcinoma	1	4	Female	62
S05180	Validation	1 pancreatic adenocarcinoma	1	4	Female	70
S05186	Validation	1 pancreatic adenocarcinoma	1	2	Female	75
S05191	Validation	1 pancreatic adenocarcinoma	1	2	Female	75
S05205	Validation	1 pancreatic adenocarcinoma	1	2	Female	71
S05271	Validation	2 neuroendocrine tumor	1		Male	60
S05192	Validation	1 pancreatic adenocarcinoma	1	2	Male	78
S05255	Validation	1 pancreatic adenocarcinoma	1	2	Female	63
S05299	Validation	1 pancreatic adenocarcinoma	1	3	Male	70
S05294	Validation	1 pancreatic adenocarcinoma	1	4	Male	65
S05212	Validation	1 pancreatic adenocarcinoma	1	2	Male	71
S05245	Validation	1 pancreatic adenocarcinoma	1	2	Male	68
S05219	Validation	1 pancreatic adenocarcinoma	1	2	Female	76
S05228	Validation	1 pancreatic adenocarcinoma	1	2	Male	60
S05237	Validation	1 pancreatic adenocarcinoma	1	4	Female	60
S05252	Validation	1 pancreatic adenocarcinoma	1	2	Male	81
S05296	Validation	1 pancreatic adenocarcinoma	1	2	Female	59
S05300	Validation	1 pancreatic adenocarcinoma	1	2	Female	64
S05301	Validation	1 pancreatic adenocarcinoma	1	4	Male	53
S05285	Validation	1 pancreatic adenocarcinoma	1	4	Male	54
S05323	Validation	1 pancreatic adenocarcinoma	1	4	Male	72
S05405	Validation	1 pancreatic adenocarcinoma	1	3	Female	63
S05322	Validation	1 pancreatic adenocarcinoma	1	2	Female	71
S05359	Validation	1 pancreatic adenocarcinoma	1	3	Male	71
S05363	Validation	1 pancreatic adenocarcinoma	1	4	Female	64

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05377	Validation	1 pancreatic adenocarcinoma	1	4	Male	64
S05148	Validation	10 acute pancreatitis	0		Male	37
S05157	Validation	10 acute pancreatitis	0		Male	33
S05275	Validation	10 acute pancreatitis	0		Female	60
S05302	Validation	10 acute pancreatitis	0		Female	53
S05310	Validation	10 acute pancreatitis	0		Male	61
S05313	Validation	10 acute pancreatitis	0		Male	39
S05316	Validation	10 acute pancreatitis	0		Male	23
S05188	Validation	11 common bile duct stones	0		Female	58
S05341	Validation	11 common bile duct stones	0		Female	78
S05393	Validation	11 common bile duct stones	0		Male	48
S05144	Validation	14 benign stricture; biliary dilation	0		Female	65
S05146	Validation	14 benign stricture; biliary dilation	0		Male	73
S05147	Validation	14 benign stricture; biliary dilation	0		Male	23
S05152	Validation	14 benign stricture; biliary dilation	0		Female	70
S05231	Validation	14 benign stricture; biliary dilation	0		Female	63
S05375	Validation	14 benign stricture; biliary dilation	0		Male	57
S05335	Validation	16 primary sclerosing cholangitis	0		Male	75
S05155	Validation	20 abnormal imaging test (benign)	0		Female	65
S05165	Validation	20 abnormal imaging test (benign)	0		Female	59
S05159	Validation	20 abnormal imaging test (benign)	0		Female	59
S05160	Validation	20 abnormal imaging test (benign)	0		Male	46
S05170	Validation	20 abnormal imaging test (benign)	0		Female	58
S05177	Validation	20 abnormal imaging test (benign)	0		Female	56
S05178	Validation	20 abnormal imaging test (benign)	0		Female	75
S05193	Validation	20 abnormal imaging test (benign)	0		Female	42
S05269	Validation	20 abnormal imaging test (benign)	0		Female	67
S05203	Validation	20 abnormal imaging test (benign)	0		Female	28
S05184	Validation	20 abnormal imaging test (benign)	0		Female	90
S05206	Validation	20 abnormal imaging test (benign)	0		Female	73
S05224	Validation	20 abnormal imaging test (benign)	0		Female	43
S05264	Validation	20 abnormal imaging test (benign)	0		Female	55
S05276	Validation	20 abnormal imaging test (benign)	0		Female	48
S05304	Validation	20 abnormal imaging test (benign)	0		Female	73
S05292	Validation	20 abnormal imaging test (benign)	0		Female	32
S05319	Validation	20 abnormal imaging test (benign)	0		Female	54
S05321	Validation	20 abnormal imaging test (benign)	0		Female	67
S05337	Validation	20 abnormal imaging test (benign)	0		Male	56
S05404	Validation	20 abnormal imaging test (benign)	0		Female	32
S05343	Validation	20 abnormal imaging test (benign)	0		Female	43
S05344	Validation	20 abnormal imaging test (benign)	0		Male	61
S05345	Validation	20 abnormal imaging test (benign)	0		Female	44
S05358	Validation	20 abnormal imaging test (benign)	0		Male	51
S05362	Validation	20 abnormal imaging test (benign)	0		Male	65
S05380	Validation	20 abnormal imaging test (benign)	0		Female	56
S05382	Validation	20 abnormal imaging test (benign)	0		Male	52
S05373	Validation	20 abnormal imaging test (benign)	0		Female	64
S05374	Validation	20 abnormal imaging test (benign)	0		Male	41
S05383	Validation	20 abnormal imaging test (benign)	0		Female	30
S05384	Validation	20 abnormal imaging test (benign)	0		Male	56

Supplementary Table 1. Continued

IDs	Set	Disease	Diagnosis	Stage	Sex	Age, *y*
S05376	Validation	20 abnormal imaging test (benign)	0		Male	69
S05386	Validation	20 abnormal imaging test (benign)	0		Male	42
S05388	Validation	20 abnormal imaging test (benign)	0		Male	74
S05390	Validation	20 abnormal imaging test (benign)	0		Female	54
S05391	Validation	20 abnormal imaging test (benign)	0		Female	20
S05379	Validation	20 abnormal imaging test (benign)	0		Female	45
S05168	Validation	3 chronic pancreatitis	0		Male	43
S05256	Validation	3 chronic pancreatitis	0		Female	43
S05312	Validation	3 chronic pancreatitis	0		Female	43
S05330	Validation	3 chronic pancreatitis	0		Male	57
S05378	Validation	3 chronic pancreatitis	0		Male	26
S05164	Validation	5 intraductal papillary mucinous neoplasm (surgical)	0		Male	64
S05333	Validation	522 panc surgery (pathology showed chronic pancreatitis)	0		Male	52
S05265	Validation	55 intraductal papillary mucinous neoplasm (clinical)	0		Male	53
S05368	Validation	55 intraductal papillary mucinous neoplasm (clinical)	0		Female	76
S05387	Validation	55 intraductal papillary mucinous neoplasm (clinical)	0		Female	83
S05288	Validation	57 pseudocyst (clinical)	0		Female	48
S05289	Validation	57 pseudocyst (clinical)	0		Male	65
S05320	Validation	9 unknown cyst (clinical)	0		Female	45
S05365	Validation	9 unknown cyst (clinical)	0		Female	68

Supplementary Table 2. Capture Antibodies and Detection Reagents

Name	ID	Primary target	Source	Catalog No.
Capture antibodies				
Anti-MUC1	CM1	MUC1	GeneTex (Irvine, CA)	GTX10114
Anti-MUC16	X325	MUC16	Abcam (Cambridge, MA)	AB10033
Anti-MUC16 (Ab2)	X306	MUC16	Novus Biologicals (Littleton, CO)	NB120-10032
Anti-MUC5AC	45M1	MUC5AC	ThermoScientific (Waltham, MA)	MS-145-P1ABX
Anti-MUC5AC (Ab2)	2-11M1	MUC5AC	Affinity BioReagents (Golden, CO)	MA1-35704
Anti-sialyl Lewis A (CA19-9, Ab1)	9L426	Sialyl Lewis A	USBio (Salem, MA)	C0075-03A
Anti-sialyl Lewis A (CA19-9, Ab2)	121SLE	Sialyl Lewis A	Abcam	AB3982
Anti-sialyl Lewis X	CSLEX1	Sialyl Lewis X	BD Pharmingen (San Jose, CA)	551344
Anti-Lewis X	P12	Lewis X	Abcam	3358
Mouse IgG, biotin labeled	N/A	N/A	Jackson ImmunoResearch (West Grove, PA)	015-000-003
Detection antibodies and lectins				
Anti-sialyl Lewis A (CA19-9, Ab1)	9L426	Sialyl Lewis A	USBio	C0075-03A
TRA-1-60	TRA-1-60	Terminal N-acetyl-lactosamine, type 1	Novus Biologicals	NB100-730
Anti-sialyl Lewis X	CSLEX1	Sialyl Lewis X	BD Pharmingen	551344
DUPAN2	DUPAN2	Sialyl Lewis A and sialyl Lewis C	Dr Hollingsworth (Nebraska)	N/A
Recombinant mouse E-selectin/CD62E Fc chimera, CF	ESEL	Sulfated Lewis structure	R&D Systems (Minneapolis, MN)	575-ES-100
Anti-blood group Lewis A	7LE	Lewis A and terminal N-acetyl-lactosamine, type 1	Abcam	ab3967
Erythrina cristagalli lectin	ECL	Terminal Galβ	Vector Labs (Burlingame, CA)	BK-3000
Helix aspersa agglutinin	HAA	Terminal GlcNAcα, GalNAcα, GalNAcβ	Sigma-Aldrich (St. Louis, MO)	L8764
Ricinus communis agglutinin I	RCA-1	Terminal galactose	Vector Labs	BK-1000
Ralstonia solanacearum lectin	RSL	αFucose, all linkages	Recombinant production	N/A
Coprinopsis cinerea (Inky cap fungus) lectin 2	CCL2	Lewis X variants: sialylated, sulfated, internal	Recombinant production	N/A
Sclerotia rolfsii lectin	SRL	Terminal GlcNAc	Wako (Richmond, VA)	199-17271
Bauhinea purpurea lectin	BPL	Terminal Galβ	Vector Labs	BK-1285

Supplementary Table 3. *P* Values of the Individual Assays in the Discovery and Validation Cohorts

Assay	Discovery	Validation
sLeA: sulfo/sLeX/sLeA (ESEL)	*6.06E-14*	*1.81E-04*
MUC5AC: sulfo/sLeX/sLeA (ESEL)	1.30E-11	1.44E-05
sLeA: sLeA/sLacNAc t1 (7LE)	8.62E-11	1.04E-06
MUC5AC: sulfo/sLeX (CCL2)	*3.66E-10*	*2.80E-05*
MUC5AC: sLeA/sLacNAc t1 (7LE)	*5.02E-10*	*6.46E-05*
sLeX: sulfo/sLeX/sLeA (ESEL)	9.01E-09	NS
sLeA: sLeA	**1.02E-07**	**2.00E-03**
MUC16: sulfo/sLeX/sLeA (ESEL)	3.54E-07	7.58E-04
MUC16: sLeA/sLacNAc t1 (7LE)	1.17E-06	5.09E-04
sLeA(Ab2): sLeA/sLacNAc t1 (7LE)	5.45E-06	6.88E-05
MUC16(Ab2): sulfo/sLeX/sLeA (ESEL)	9.56E-06	-
LeA: sulfo/sLeX/sLeA (ESEL)	1.73E-05	-
MUC16: sLeA	3.29E-05	3.83E-02
sLeA(Ab2): sLeA	3.88E-05	6.45E-03
sLeA(Ab2): sLacNAc t1t2 (TRA-1-60)	5.76E-05	NS
sLeX: sulfo/sLeX (CCL2)	5.90E-05	7.11E-05
LeA: sLacNAc t1t2 (TRA-1-60)	7.20E-05	-
sLeX: sLeA/sLacNAc t1 (7LE)	9.54E-05	1.22E-03
sLeA: sLacNAc t1t2 (TRA-1-60)	1.05E-04	8.32E-03
MUC5AC(Ab2): sulfo/sLeX/sLeA (ESEL)	1.46E-04	-
MUC1: sLacNAc t1t2 (TRA-1-60)	2.44E-04	-
sLeA: sLeX	3.43E-04	5.09E-03
sLeX: sLeX	3.78E-04	NS
MUC16: sLacNAc t1t2 (TRA-1-60)	7.66E-04	1.46E-02
MUC16: sulfo/sLeX (CCL2)	8.75E-04	5.78E-03
sLeA: sulfo/sLeX (CCL2)	1.19E-03	1.86E-04
MUC5AC: sLacNAc t1t2 (TRA-1-60)	*1.21E-03*	*4.93E-02*
LeA: sLeX	4.59E-03	-
MUC5AC(Ab2): sLacNAc t1t2 (TRA-1-60)	1.23E-02	-
sLeX: sLacNAc t1t2 (TRA-1-60)	1.81E-02	NS
sLeA(Ab2): sLeX	3.42E-02	NS
LeX: sLacNAc t1t2 (TRA-1-60)	3.79E-02	-
LeX: sulfo/sLeX/sLeA (ESEL)	4.90E-02	-

NOTE. The assays that were in the biomarker panels are shown in italics, and the CA19-9 assay (capture and detection of sLeA) is shown in bold.

Supplementary Table 4. Associations Between Marker levels and Age Within Patient Groups

Cohort	Young cancer patients vs old	Young control patients vs old
Discovery		
MUC5AC: sLacNAc t1t2 (TRA-1-60)	NS	NS
sLeA: sulfo/sLeX/sLeA (ESEL)	NS	NS
MUC5AC: sulfo/sLeX (CCL2)	NS	NS
MUC5AC: sLeA/sLacNAc t1 (7LE)	NS	NS
sLeA: sLeA	NS	NS
Validation		
MUC5AC: sLacNAc t1t2 (TRA-1-60)	NS	NS
sLeA: sulfo/sLeX/sLeA (ESEL)	NS	NS
MUC5AC: sulfo/sLeX (CCL2)	NS	NS
MUC5AC: sLeA/sLacNAc t1 (7LE)	NS	NS
sLeA: sLeA	NS	NS
Test		
MUC5AC: sLacNAc t1t2 (TRA-1-60)	NS	NS
sLeA: sulfo/sLeX/sLeA (ESEL)	NS	NS
MUC5AC: sulfo/sLeX (CCL2)	NS	NS
MUC5AC: sLeA/sLacNAc t1 (7LE)	NS	NS
sLeA: sLeA	NS	$P < .05$ (higher in older patients)

NOTE. Within either just the cancers or just the controls, we divided the subjects by age, with the oldest third in one group and the youngest third in another group. We then compared the levels of each marker between the groups. Only one comparison showed a statistical difference.

Supplementary Table 5. Performance of the Panels and sLeA in the Blinded Samples

Panel	Sensitivity	*P* value	Specificity	*P* value	(Sen+Spe)/2	*P* value
sLeA:sLeA	0.54 (0.40–0.67)		0.84 (0.71–0.92)		0.69 (0.60–0.77)	
Panel 1	0.66 (0.52–0.78)	NS	0.80 (0.67–0.89)	NS	0.73 (0.64–0.81)	NS
Panel 2	0.72 (0.58–0.83)	.02	0.70 (0.56–0.81)	.06	0.71 (0.61–0.79)	NS
sLeA:sLeA	.66		.72	.69		
Panel 1	.80		.84	.82		
Panel 2	.76		.80	.78		

NOTE. Top: performance based on the blinded classifications; middle: *P* value of comparisons between the panels and the CA19-9 assay (capture and detection of sLeA); bottom: performance after adjusting the thresholds of the individual markers.