**ORIGINAL RESEARCH**

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

Huiyuan Tang,1 Katie Partyka,1 Peter Hsueh,1 Jessica Y. Sinha,1 Doron Kletter,2 Herbert Zeh,3 Ying Huang,3 Randall E. Brand,3 and Brian B. Haab1

1Van Andel Research Institute, Center for Cancer and Cell Biology, Grand Rapids, Michigan; 2Protein Metrics, Inc, San Carlos, California; 3University of Pittsburgh School of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Pittsburgh, Pennsylvania; 4Fred Hutchinson Cancer Research Center, Vaccine and Infectious Disease Division, Seattle, Washington

**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-marker 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...
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. The increase of sLeA in the blood likely results from accumulation in the stroma followed by leakage into the capillaries or lymph. 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. But the cause of low CA19-9 levels is not clear for patients with wild-type fucosyltransferase 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, and we and others 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, 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.

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 March 2016 Panel of Glycan Biomarkers 211.
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 Biopspecimen 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, Rockford, IL); 10× protease inhibitor (Complete Table; 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 (Sidelinprinter; 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.

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 pre-complexed CCL2/streptavidin required only a final wash and dry. We scanned the slides for fluorescence using 633-nm excitation (LS Reloaded; Tescan, 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 method20 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 1A), including variants of sialylLewis X, which we previously showed was increased in a subset of pancreatic cancer patients. To test for increases of glycans, we acquired lectins and antibodies targeting the glycans (Figure 1B 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 sLeA, sLeX, and sulfo-sLeX (Supplementary Figure 2), fucose, mainly Lewis X variants including sulfated Lewis X. We previously showed that CCL2 is specific for glycans with 3' fucose, 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 antibody (Figure 1C).

We did not have a reagent to optimally detect sialylated, nonfucosylated, type 1 N-acetyl-lactosamine structures (Siaα2,3Galβ1,3GlcNAcβ1,B-1). We did, however, have 2 antibodies, called TRA-1-60 and 7LE (Figure 1B), with good affinity to the nonsialylated variant. We therefore tested the use of sialidase to remove sialic acid before detecting with the antibodies (Figure 2A). 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 2B). The staining of tumor tissue in the regions of cancerous epithelia increased upon sialidase treatment (Figure 2D), and the differentiation of cases from controls in a set of plasma samples was enhanced after enzyme treatment (Figure 2C). 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 3A). 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 3B). The markers mentioned earlier showed significant improvement in AUC over sLeA in the discovery set (Figure 3C). The classification performance of sLeA in the validation set (Figure 3D) 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, 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 4A). The trends were similar in the validation cohort (Figure 4B). 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 4C). 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.
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 5A), and the ROC curve for a MUC5AC glycoform was improved significantly compared with sLeA (with an improvement in AUC of 0.14; 95% confidence interval).

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.
interval, 0.04–0.26) (Figure 5B). 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 5C). 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 5D). Furthermore, in 10-fold cross-validation averaged over 3 trials, the average accuracy of the
panel was 84%, whereas the average accuracy of the individual markers ranged from 43% to 60% (Figure 5D). 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.

---

**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.
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. 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. 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, but a systematic study still is required to...
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. 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, DNA, and tumor cells in the circulation; proteins in the urine; and various types of biomarkers in the pancreatic juice or stool (reviewed by Chari et al), 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 antibody, the nonsialylated version of the glycan, is an excellent marker for pluripotent stem cells. Previous research found sialyl-LacNac type 1 on glycolipids in malignant glioma and embryonal carcinoma. Pancreatic cancer cells frequently activate developmental pathways, 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 and are associated with an invasive phenotype in pancreatic cancer. Studies in mice support a role for sLeX in invasion and modulation of immune responses. Both sLeX and sLeA have the potential to promote metastasis through interactions with E-selectin receptors. 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.

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.

References
1. Magnani JL, Nilsson B, Brockhaus M, et al. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. J Biol Chem 1982;257:14365–14369.
2. Magnani JL, Brockhaus M, Smith DF, et al. A monosialoganglioside is a monoclonal antibody-defined antigen of colon carcinoma. Science 1981;212:55–56.
3. Herlyn M, Stepleski Z, Herlyn D, et al. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. Proc Natl Acad Sci U S A 1979;76:1438–1442.
4. Gooenettilleke KS, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. Eur J Surg Oncol 2007;33:266–270.
5. Haglund C, Lindgren J, Roberts PJ, et al. Gastrointestinal cancer-associated antigen CA 19-9 in histological specimens of pancreatic tumours and pancreatitis. Br J Cancer 1986;53:189–195.
6. Kalthoff H, Kreiker C, Schmiegel WH, et al. Characterization of CA 19-9 bearing mucins as physiological exocrine pancreatic secretion products. Cancer Res 1986;46:3605–3607.
7. Nishihara S, Yazawa S, Iwasaki H, et al. Alpha (1,3/1,4) fucosyltransferase (FucT-III) gene is inactivated by a single amino acid substitution in Lewis histo-blood type negative individuals. Biochem Biophys Res Commun 1993;196:624–631.
8. Pour PM, Tempero MM, Takasaki H, et al. Expression of blood group-related antigens ABH, Lewis A, Lewis B, Lewis X, Lewis Y, and CA 19-9 in pancreatic cancer cells in comparison with the patient’s blood group type. Cancer Res 1988;48:5422–5426.
9. Singh S, Pal K, Yadav J, et al. Upregulation of glycans containing 3’ fucose in a subset of pancreatic cancers uncovered using fusion-tagged lectins. J Proteome Res 2015;14:2594–2605.
10. Tang H, Singh S, Partyka K, et al. Glycan motif profiling reveals plasma sialyl-Lewis X elevations in pancreatic cancers that are negative for CA 19-9. Mol Cell Proteomics 2015;14:1323–1333.
11. Balmana M, Sarrats A, Llop E, et al. Identification of potential pancreatic cancer serum markers: increased sialyl-Lewis X on ceruloplasmin. Clin Chim Acta 2015;442C:56–62.
12. Metzgar RS, Gaillard MT, Levine SJ, et al. Antigens of human pancreatic adenocarcinoma cells defined by murine monoclonal antibodies. Cancer Res 1982;42:601–608.
13. Kawa S, Tokoo M, Oguchi H, et al. Epitope analysis of SPan-1 and DUPAN-2 using synthesized glycoconjugates sialyllact-N-fucopentaose II and sialyllact-N-tetraose. Pancreas 1994;9:692–697.
14. Takasaki H, Uchida E, Tempero MA, et al. Correlative study on expression of CA 19-9 and DU-PAN-2 in tumor tissue and in serum of pancreatic cancer patients. Cancer Res 1988;48:1435–1438.
15. Partyka K, Maupin KA, Brand RE, et al. Diverse monoclonal antibodies against the CA 19-9 antigen show variation in binding specificity with consequences for clinical interpretation. Proteomics 2012;12:2212–2220.
16. Chen S, LaRoche T, Hamelinck D, et al. Multiplexed analysis of glycan variation on native proteins captured by antibody microarrays. Nat Methods 2007;4:437–444.

17. Yue T, Goldstein IJ, Hollingsworth MA, et al. The prevalence and nature of glycan alterations on specific proteins in pancreatic cancer patients revealed using antibody-lectin sandwich arrays. Mol Cell Proteomics 2009;8:1697–1707.

18. Yue T, Maupin KA, Fallon B, et al. Enhanced discrimination of malignant from benign pancreatic disease by measuring the CA 19-9 antigen on specific protein carriers. PLoS One 2011;6:e29180.

19. Haab BB, Yue T. High-throughput studies of protein glycoforms using antibody-lectin sandwich arrays. Methods Mol Biol 2011;785:223–236.

20. Fallon BP, Curnutte B, Maupin KA, et al. The marker state space (MSS) method for classifying clinical samples. PLoS One 2013;8:e65905.

21. Haab BB, Huang Y, Balasenthil S, et al. Definitive characterization of CA 19-9 in resectable pancreatic cancer using a reference set of serum and plasma specimens. PLoS One 2015;10:e0139049.

22. Sina M, Cote GA, Korc M. Improving the diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration using microRNAs. Gastroenterology 2014;147:930–932.

23. Brody JR, Costantino CL, Potoczak M, et al. Adenosquamous carcinoma of the pancreas harbors KRAS2, DPC4 and TP53 molecular alterations similar to pancreatic ductal adenocarcinoma. Mod Pathol 2009;22:651–659.

24. Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015;523:177–182.

25. Cote GA, Gore AJ, McElyea SD, et al. A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile. Am J Gastroenterol 2014;109:1942–1952.

26. Zill OA, Greene C, Sebianovic D, et al. Cell-Free DNA next-generation sequencing in pancreaticobiliary carcinomas. Cancer Discov 2015;5:1040–1048.

27. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148:349–361.

28. Gentilioni N, Caradonna P, Costamagna B, et al. Pancreatic juice 90K and serum CA 19-9 combined determination can discriminate between pancreatic cancer and chronic pancreatitis. Am J Gastroenterol 1995;90:1069–1072.

29. Chari ST, Kelly K, Hollingsworth MA, et al. Early detection of sporadic pancreatic cancer: summative review. Pancreas 2015;44:693–712.

30. Natunen S, Satomaa T, Pitkanen V, et al. The binding specificity of the marker antibodies Tra-1-60 and Tra-1-81 reveals a novel pluripotency-associated type 1 lactosamine epitope. Glycobiology 2011;21:1125–1130.

31. Andrews PW, Banting G, Damjanov I, et al. Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells. Hybridoma 1984;3:347–361.

32. Barone A, Saljo K, Benkantder J, et al. Sialyl-lactotetra, a novel cell surface marker of undifferentiated human pluripotent stem cells. J Biol Chem 2014;289:18846–18859.

33. Fredman P, von Holst H, Collins VP, et al. Sialylactotetraosylceramide, a ganglioside marker for human malignant gliomas. J Neurochem 1988;50:912–919.

34. Fukuda MN, Bothner B, Lloyd KO, et al. Structures of glycosphingolipids isolated from human embryonal carcinoma cells. The presence of mono- and disialosyl glycolipids with blood group type 1 sequence. J Biol Chem 1986;261:5145–5153.

35. Mazur PK, Einwachter H, Lee M, et al. Notch2 is required for progression of pancreatic intraepithelial neoplasia and development of pancreatic ductal adenocarcinoma. Proc Natl Acad Sci U S A 2010;107:13438–13443.

36. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425:851–856.

37. Morris JP, Cano DA, Sekine S, et al. Beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. J Clin Invest 2010;120:508–520.

38. Galustian C, Lawson AM, Komba S, et al. Sialyl-Lewis(x) sequence 6-O-sulfated at N-acetylgalcosamine rather than at galactose is the preferred ligand for L-selectin and de-N-acetylation of the sialic acid enhances the binding strength. Biochem Biophys Res Commun 1997;240:748–751.

39. McEver RP. Selectin-carbohydrate interactions during inflammation and metastasis. Glycoconjug J 1997;14:585–591.

40. Takahashi S, Oda T, Hasebe T, et al. Overexpression of sialyl Lewis x antigen is associated with formation of extratumoral venous invasion and predicts postoperative development of massive hepatic metastasis in cases with pancreatic ductal adenocarcinoma. Pathobiology 2001;69:127–135.

41. Ohyama C, Tsuboi S, Fukuda M. Dual roles of sialyl Lewis X oligosaccharides in tumor metastasis and rejection by natural killer cells. EMBO J 1999;18:1516–1525.

42. Mitsuoka C, Sawada-Kasugai M, Ando-Furui K, et al. Identification of a major carbohydrate capping group of the L-selectin ligand on high endothelial venules in human lymph nodes as 6-sulfo sialyl Lewis X. J Biol Chem 1998;273:11225–11233.

43. Iwai K, Ishikura H, Kaji M, et al. Importance of E-selectin (ELAM-1) and sialyl Lewis(a) in the adhesion of pancreatic cancer cells. Hybridoma 1984;3:347–361.

Received October 26, 2015. Accepted December 10, 2015.

Correspondence
Address correspondence to: Brian B. Haab, PhD, Van Andel Research Institute, 333 Bostwick NE, Grand Rapids, Michigan 49503. e-mail: brian.haab@vai.org; fax: (616) 234–5269.

Acknowledgments
The authors thank Dr Galen Hostetter and members of the Van Andel Research Institute Pathology and Biorepository core for assistance with preparing tissue sections for immunohistochemistry.
Brian Haab, Randall Brand, and Herbert Zeh conceived and designed the study; Huiyuan Tang, Katie Partyka, Peter Hsueh, and Jessica Sinha performed the experiments; Huiyuan Tang, Katie Partyka, Ying Huang, and Doron Kletter analyzed the data; Katie Partyka prepared the figures; Ying Huang was responsible for the statistical analysis; Randall Brand and Herbert Zeh supplied samples; and Brian Haab and Huiyuan Tang wrote the paper.

Conflicts of interest
The authors disclose no conflicts.

Funding
Supported by the National Cancer Institute, Early Detection Research Network (U01CA152653), and the Alliance of Glycobiologists for Cancer Detection (U01CA168898).
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 CaCl₂, 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 μg/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 diaminobenzidine 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. 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, and for mining glycan array data to find particular lectins, we used the GlycanBinder database, which derives information from the CFG website.

Cross-Validation

We performed 10-folded cross validation 3 times on each individual marker and 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.
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.
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.
| 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     |
| 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         | 4     | 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     |
| 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         |       | Female  | 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 dilatation       | 0         |       | Female  | 75     |
| S06073   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Female  | 77     |
| S06083   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Female  | 69     |
| S06084   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Female  | 41     |
| S06093   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Male    | 34     |
| S06094   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Female  | 58     |
| S06105   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Male    | 55     |
| S06129   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Male    | 62     |
| S06134   | Test     | 14 benign stricture; biliary dilatation       | 0         |       | Female  | 44     |
| S06142   | Test     | 14 benign stricture; biliary dilatation       | 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     |
| IDs    | Set         | Disease                              | Diagnosis | Stage | Sex    | Age, y |
|--------|-------------|--------------------------------------|-----------|-------|--------|--------|
| S06138 | Test        | 3 chronic pancreatitis               | 0         | 0     | Male   | 63     |
| S06150 | Test        | 3 chronic pancreatitis               | 0         | 0     | Female | 70     |
| S06151 | Test        | 3 chronic pancreatitis               | 0         | 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         | 2     | 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     |
| S05212 | Validation  | 1 pancreatic adenocarcinoma          | 1         | 2     | Male   | 64     |
| 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     |
| 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 A | 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 |
| Heliz 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 | aFucose, all linkages | Recombinant production | N/A |
| Coprinopsis cinereus (linky 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 | NS |
| sLeX: sLeA/sLacNAc t1 (7LE) | 9.54E-05 | 1.22E-03 |
| MUC5AC: sulfo/sLeX (CCL2) | 3.88E-05 | 6.45E-03 |
| sLeA: 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 t12 (TRA-1-60) | 7.20E-05 | - |
| sLeX: sLeA/sLacNAc t11 (7LE) | 9.54E-05 | 1.22E-03 |
| sLeA: sLacNAc t112 (TRA-1-60) | 1.05E-04 | 8.32E-03 |
| MUC5AC(Ab2): sulfo/sLeX/sLeA (ESEL) | 1.46E-04 | - |
| MUC1: sLacNAc t112 (TRA-1-60) | 2.44E-04 | - |
| sLeA: sLeX | 3.43E-04 | 5.09E-03 |
| sLeX: sLeX | 3.78E-04 | NS |
| MUC16: sLacNAc t112 (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 t112 (TRA-1-60) | 1.21E-03 | 4.93E-02 |
| LeA: sLeX | 4.59E-03 | - |
| MUC5AC(Ab2): sLacNAc t112 (TRA-1-60) | 1.23E-02 | - |
| sLeX: sLacNAc t112 (TRA-1-60) | 1.81E-02 | NS |
| sLeA(Ab2): sLeX | 3.42E-02 | NS |
| LeX: sLacNAc t112 (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 t112 (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 t112 (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 t112 (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 |
| 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.**
| 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.