Patient LN, FN
DOB xx/xx/xxxx (Age: x)

PANCREASEQ® RESULTS SUMMARY

Genomic Alterations Identified:
- KRAS mutation p.G12D
- GNAS mutation p.R201C
- See Interpretation and Detailed Results

INTERPRETATION
The detection of KRAS (codons 12, 13 and/or 61) and GNAS (codons 201 and 227) mutations are associated with the presence of an intraductal papillary mucinous neoplasm (IPMN) (1-13, 15-17). In addition, studies have shown the absence of additional alterations that include TP53, PIK3CA, PTEN, AKT1 and SMAD4 correspond to a low-likelihood (<10%) of high-grade dysplasia and early invasive pancreatic ductal adenocarcinoma (1,2). However, correlation of molecular testing with cytology, imaging and other clinical data is recommended.

DETAILED RESULTS

<table>
<thead>
<tr>
<th>Marker Type</th>
<th>Marker Result</th>
<th>AF</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene mutations</td>
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<tr>
<td></td>
<td>KRAS</td>
<td>c.35G&gt;A 32% Tier 1/2</td>
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<tr>
<td></td>
<td>GNAS</td>
<td>c.601C&gt;T 32% Tier 1/2</td>
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<tr>
<td>Copy number alterations</td>
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AF=Variant Allele Frequency, Tier 1/2=Variants of Clinical or Potential Clinical Significance, VUS=Variants of Uncertain Significance
NOTE
The quantity and quality of isolated DNA was sufficient for the analysis. Amplification of controls was acceptable.

BACKGROUND
Both pancreatic cysts and pancreatic solid lesions represent a broad and diverse group of benign and malignant entities. Among pancreatic cysts, distinguishing one pancreatic cyst from another can be challenging on the basis of standard clinical findings, imaging parameters and ancillary fluid studies, such as cytology and CEA analysis. DNA sequencing studies of pancreatic cysts have identified a limited number of genetic alterations that can be used diagnostically and prognostically to classify pancreatic cysts.(1-4, 6-8) Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) represent mucinous pancreatic cystic neoplasms. Over 95% of IPMNs are characterized by mutations in the genes: KRAS (codons 12, 13 and/or 61), GNAS (codons 201 and 227), RNF43, BRAF, and CTNNB1. KRAS, RNF43, BRAF and CTNNB1 mutations can also be found in MCNs with a prevalence that ranges from 14% to 50%.(1-4, 6-7, 9) In contrast to IPMNs, MCNs do not harbor GNAS mutations, and, thus, genetic alterations in GNAS are highly specific for IPMNs.(4, 7) Other neoplastic cysts include serous cystadenomas and solid pseudopapillary neoplasms. Serous cystadenomas (SCAs) have an extremely low malignant potential and approximately 89% to 100% harbor mutations and/or deletions in VHL, but lack mutations in KRAS and GNAS.(1-3, 7) Finally, solid-pseudopapillary neoplasms (SPNs) are characterized by the presence of CTNNB1 mutations (within exon 3), and an absence of alterations in KRAS, GNAS, RNF43, BRAF and VHL.(3, 7)

IPMNs and MCNs are precursor neoplasms to pancreatic ductal adenocarcinoma; however, only a subset harbor or progress to malignancy. Studies have shown that IPMNs and MCNs with genetic alterations in TP53, SMAD4 and the phosphatidylinositol-3 kinase (PI3K) pathway, which include PIK3CA, PTEN, and AKT1, are associated with high-grade dysplasia and early invasive pancreatic ductal adenocarcinoma (advanced neoplasia). Kanda et al detected TP53 mutations in 56% of IPMNs with advanced neoplasia.(10) Similarly, 40% to 60% of IPMNs with advanced neoplasia harbor alterations in PIK3CA, PTEN, AKT1 and SMAD4.(11-13) Using EUS-FNA obtained pancreatic cyst fluid, Singh et al found 88% of IPMNs with advanced neoplasia have mutations in KRAS and/or GNAS with concurrent alterations in TP53, PIK3CA, PTEN or AKT1.(1-2)

Cystic pancreatic neuroendocrine tumors (PanNETs) are typically diagnosed by standard cytology, but the diagnosis may be facilitated by the presence of MEN1 and/or TSC2 mutations in a subset of these pancreatic cysts. Genetic alterations are absent in benign non-neoplastic cysts, such as pseudocysts, lymphoepithelial cysts, retention cysts, squamoid cysts or acinar cell cystadenomas.(1-3) However, adequate sampling and preservation of the specimen should always be considered when evaluating molecular testing results. And therefore, correlation of molecular testing with cytology, imaging and other clinical data is recommended. It is important to underscore that several of the aforementioned genetic alterations have been discussed by the International Consensus Fukuoka Guidelines for the management of IPMNs and MCNs, and the European Evidence-Based Guidelines on pancreatic cystic neoplasms.(15-16) Both guideline organizations highlight the utility of these DNA markers in the diagnosis of pancreatic cysts.

In addition, solid pancreatic lesions have a wide range of pathology, from chronic pancreatitis to pancreatic ductal adenocarcinoma (PDAC). Endoscopic ultrasound (EUS) with fine-needle aspiration (FNA) is an important diagnostic tool in the work up of a solid pancreatic lesion with sensitivities as high as 80% to 95% and specificities as high as 75% to 100%.(17-19) However, in a subset of cases, the preoperative diagnosis remains inconclusive due to limited cellularity, leading to a non-diagnostic, atypical or suspicious cytopathologic diagnoses.(20,21)

Next-generation sequencing (NGS) has been instrumental in our understanding of the genome of various solid lesions of the pancreas and can be used as an adjunct to the evaluation of solid pancreatic lesions.(22,23) For example, PDAC is characterized by frequent genomic alterations in KRAS, TP53 and/or SMAD4. Kameta et al demonstrated that NGS for KRAS, TP53 and SMAD4 alterations on EUS-FNA specimens is associated with a 96%, 44% and 11% sensitivity, respectively, and 100% specificity for PDAC.(24) Similarly, Young and colleagues found EUS-FNA specimens harboring mutations in KRAS, TP53 and/or SMAD4 were present in 95% of cases that correlated with PDAC.(25) Within a large cohort of EUS-FNA specimens, Gleeson et al. found KRAS, TP53 and SMAD4 alterations were present in 93%, 72% and 31% of PDACs.(26)

In contrast to PDAC, pancreatic neuroendocrine tumors (PanNETs) do not have KRAS mutations, but harbor frequent alterations in MEN1, VHL, and/or TSC2.(23, 14) Further, recurrent genomic alterations in several chromatin remodeling genes leads to numerous chromosomal copy number alterations, which is associated with decreased disease-free survival and decreased disease-specific survival.(14, 27) This is especially critical when evaluating small neuroendocrine tumors (27). Moreover, these prognostic findings can be extended to other neuroendocrine tumors of the gastrointestinal tract, such as those found in the colon, small intestine and stomach, and hence studies strongly support the utility of molecular profiling of all gastrointestinal tract well-differentiated neuroendocrine tumors.(28, 29)

References:
METHODOLOGY
Nucleic acids are isolated from pancreatic cyst fluid samples collected in the FNAPreserve solution or from fixed samples using standard laboratory procedures. The NGS analysis is applied to assay targeted regions of 20 pancreatic cancer-related genes (AKT1, APC, BRAF, CTNNB1, GNAS, HRAS, IDH1, IDH2, KRAS, MEN1, MET, NF2, NRAS, PIK3CA, PTEN, STK11, TERT, TP53, TSC2, VHL) and copy number alterations (CNAs) in the SMAD4, TP53, VHL, and RNF43 genes. The Torrent Suite Software v5.8 is used for data analysis. Analytical sensitivity (PPA) and analytical specificity (PPV) for SNVs/indels is >99%/99% at 3-5% AF (6-10% of tumor cells) and for CNA is 92%/100% with LOD 13-25% of tumor cells in freshly collected cyst fluid samples and 40-70% of tumor cells in fixed samples. The assay minimal required sequencing depth is 500x. For targets with sequencing depth >1000x, the lowest limit of detection for SNVs is 1%. Genetic regions that did not meet minimal sequencing coverage requirements are specified in the report.

GENES ASSAYED BY PANCREASEQ
Substitutions and indels:

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<thead>
<tr>
<th>Gene</th>
<th>Transcript</th>
<th>Genomic Position</th>
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<tbody>
<tr>
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<td>NM_004985.3</td>
<td>chr12:25398284C&gt;T</td>
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<td>GNAS</td>
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<td>chr20:57484420C&gt;T</td>
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Copy number alterations:

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<tr>
<td>RNF43</td>
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<tr>
<td>SMAD4</td>
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ADDITIONAL DETAILS OF DNA SEQUENCE VARIANTS

LOW COVERAGE HOTSPOTS OBSERVED IN THE FOLLOWING GENES
NONE

GROSS DESCRIPTION
1 part(s) labelled with patient name and identifiers received from Client.

Sample 1: One(1) FNA vial received and labeled with patient name and identifiers

DISCLAIMER
PancreaSeq is a diagnostic test that was developed and its performance characteristics determined by the UPMC Molecular and Genomic Pathology laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. PancreaSeq test does not sequence genes in their entirety and mutations outside of mutation hotspots, some insertions and deletions may not be detected. This test does not provide information on germline or somatic status of detected mutations. Certain sample characteristics may result in reduced sensitivity, including sample heterogeneity, low sample quality, and other causes. The information in this report must be used in conjunction with all relevant clinical information and does not intend to substitute clinical judgment. Decisions on patient care must be based on the independent clinical judgment of the treating physician. A treating physician's decision should not be based solely on this or any other single tests or the information in this report.

Electronically signed out by: