Evaluation of PCLs Using Three EUS-FNA Needles

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Statistical Analysis Plan

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Randomized Evaluation of PCLs Using Three EUS-FNA Needles

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Approval Signatures	
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Revision History

Revision Number	Section	Change	Reason for Change

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1 PROTOCOL SUMMARY

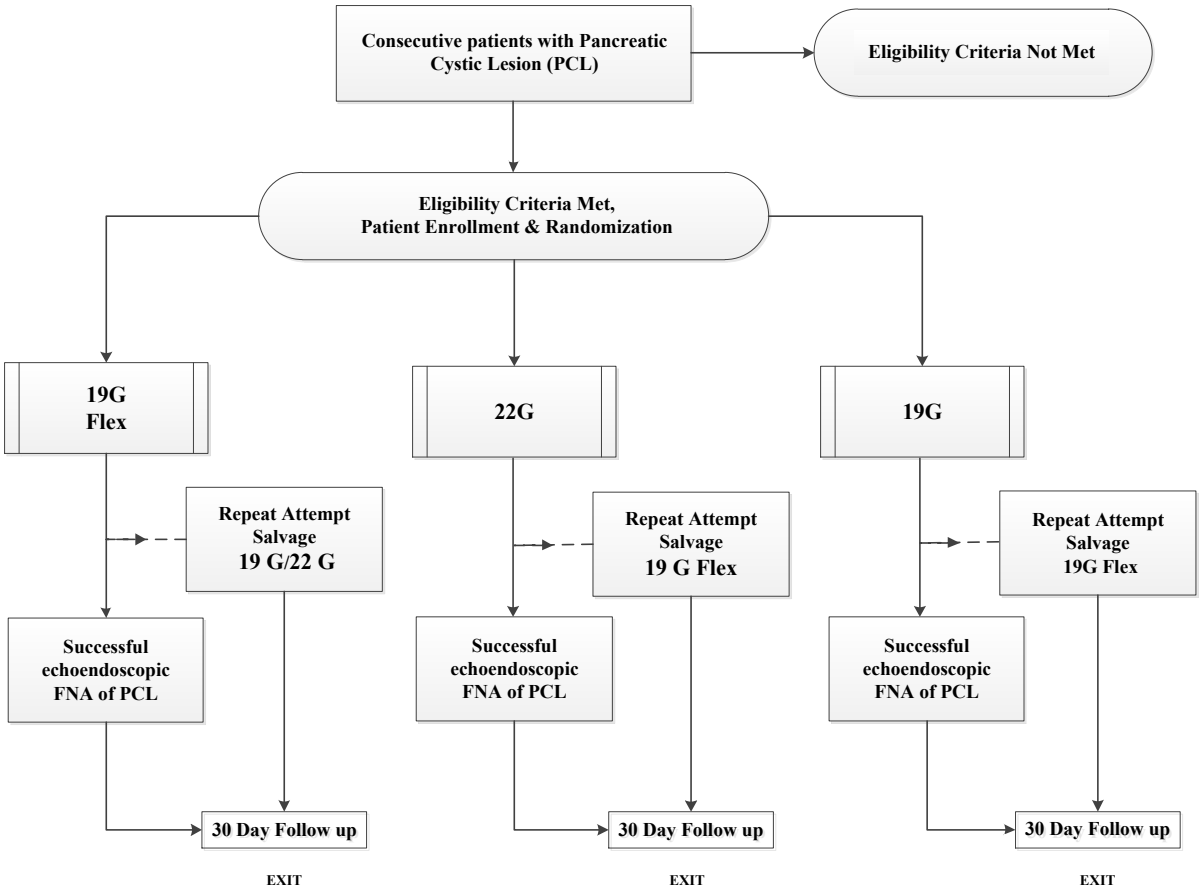
Primary Objective	To document impact of EUS-FNA needle gauge and flexibility on effectiveness of pancreatic cystic lesions (PCL) aspiration, on ability to obtain sufficient material for standard diagnostic testing, and on diagnostic accuracy of EUS-FNA aspirate for differentiation of mucinous (pre-malignant) and non-mucinous cysts.
Devices	Name Expect™ Endoscopic Ultrasound Aspiration Needle Cleared Indication The Expect™ Needle is designed to sample targeted sub mucosal and extramural gastrointestinal lesions through the accessory channel of a curvilinear echoendoscope. Device Categories • Expect 22 gauge (22 G) • Expect 19 gauge (19 G) • Expect 19 gauge Flex (19 G Flex)
Study Design	Prospective, multi-center study Three arm randomized (19 G Flex, 22 G, 19 G) in 2:1:1 ratio with salvage. Salvage procedure will use 19 G Flex for subjects originally randomized to 19 G or 22 G standard needles, and will use 19 G or 22 G standard needle at investigator discretion for subjects originally randomized to 19 G Flex needle. Phased • Phase I: 2 centers • Phase II: expanded to 7 to 12 centers
Number of Subjects	250 subjects (121 in 19 G Flex arm, and 61 subjects each in 22 G and 19G arm, rounded up) 30 out of the 250 subjects will be enrolled in Phase I, and 220 subjects will be enrolled in Phase II.
Number of Centers	7 to 12 centers
Primary Endpoint	Volume of aspirated cyst fluid as a function of estimated maximal volume based on pre-aspiration EUS measure of cyst diameter (s) (%

	aspiration of total estimated volume)
Secondary Endpoints	Safety Endpoint a. Occurrence, severity and relatedness of adverse events to the needle and procedure Other Endpoints b. Extent of cyst aspiration as measured by reduction of maximal cyst diameter before and after FNA (converted to volume by standard geometric formulation) c. Successful echoendoscopic fine needle aspiration of PCL, defined as complete cyst aspiration or collection of aspirate adequate to perform two standard assays: cytology and carcinoembryonic antigen (CEA) d. Ability to reach and penetrate the PCL, stratified by route of access and PCL location e. Number of EUS-FNA needles and EUS-FNA needle passes used f. Time needed for aspiration for each pass and time from first needle pass in to last needle pass out g. Ease of needle passage in and out of echoendoscope and quality of needle visualization inside the cyst, categorized as *Excellent, Very Good, Good, Fair,* and *Poor* h. Accuracy of two EUS-FNA-based standard assays (cytology and CEA) as a diagnostic measure of disease state categorized as one of the following: *serous cyst, mucinous cyst, intra papillary mucinous neoplasm (IPMN),* or *pseudocyst* i. Post EUS-FNA patient management plan (clinical and/or imaging surveillance, percutaneous aspiration, surgical removal) j. Impact of EUS-FNA based assays on diagnosis k. Rate of cross-over to salvage arm
Follow-up Schedule	• **Baseline:** Screening, Enrollment, Randomization, Demographics, Medical History, Lesion Location • **Procedure:** Lesion Characteristics, EUS-FNA Procedure Details (including needle use and times), Aspirate Characteristics, Salvage Procedure (if needed), Adverse Events assessment (through discharge or approximately 2hrs post-procedure) • **30-Day Follow-up:** Adverse events, End of Study
Inclusion	1. Pancreatic cystic lesion measuring 13mm or greater in largest

Criteria	diameter. 2. Indicated for EUS evaluation of the PCL including EUS-FNA. 3. Age 18 years of age or older. 4. Willing and able to comply with the study procedures and provide written informed consent form to participate in the study.
Exclusion Criteria	1. Cysts in which FNA is not indicated based on review by the clinician, including potential concern of blood vessel location relative to the cyst. 2. Requirement for anticoagulation using clopidogril, warfarin, or other long acting antiplatelet agents (with the exception of aspirin) that cannot be safely stopped according to institutional guidelines. 3. Standard contraindications for EUS. 4. Known pancreatic pseudocyst. 5. Pregnancy.
Statistical Methods and Sample Size	The sample size of this study was computed based on the following assumptions on the primary endpoint, as well as for the secondary endpoint c): <u>Primary endpoint considerations:</u> • The volume of actual aspirated cyst fluid as a function of estimated maximal volume either in 19 G or 19 G Flex Arm is superior by at least 10% compared to the 22 G Arm, detected with 90% conjunctive power, using multiple comparison Hochberg's step up method. • Requires 50 subjects in 19 G Flex Arm, and 25 subjects each in 22 G and 19 G Arms. <u>Secondary endpoint c) considerations:</u> • Superiority of EUS-FNA success rate in 19 G Flex Arm by 16% or more compared to both the success rate of the 19 G Arm and the 22 G Arm, detected with 80% conjunctive power, using multiple comparison Hochberg's step up method. • Requires 121 subjects in 19 G Flex Arm, and 61 subjects each in 22 G and 19G Arm, rounded up The final sample size was considered to be the maximum of required sample sizes of the above stated two endpoints to make sure that the both endpoints are properly powered, namely 250 subjects.

A flow chart illustrating the study design is provided in **Figure 1** below.

Figure 1 – Study Design Flow Chart



2 INTRODUCTION

This statistical plan addresses the planned analyses for the Randomized Evaluation of PCLs Using Three EUS-FNA Needles Trial based on the protocol with PDM 90869947. Specified analyses may be used for scientific presentations and/or manuscripts and may not all be provided to Competent Authorities.

3 ENDPOINT ANALYSIS

3.1 Primary Endpoint

3.1.1 Hypotheses

The primary endpoint is "Volume of aspirated cyst fluid as a function of estimated maximal volume based on pre-aspiration EUS measure of cyst diameter (s) (% aspiration of total estimated volume)."

Analysis Approach:

% aspiration of total estimated volume is calculated overall and for each arm (19 G Flex, 22 G, 19 G) and/or a combination of arms such as 19G Flex and 19G, for each and combined morphologic characteristics, and for no salvage subjects. If there is multilocular morphology, the primary endpoint is calculated for measurements of the largest locule.

Valid Data Sources:

- Procedure Form: sections "PCL Characteristics" and "Post-Aspiration Assessment"

Valid Data Points:

- Morphology: Unilocular (Pre-aspiration EUS measures)
  - Maximal diameter (long axis) (mm)
  - Perpendicular measurement (short axis) (mm)

- Morphology: Multilocular (Pre-aspiration EUS measures)
  - Measurements of the largest locule
    - Maximal diameter (long axis) of the largest locule (mm)
    - Perpendicular measurement (short axis) of largest locule (mm)

- Total volume of aspirate (Post-aspiration assessment)

Formula:

$$\%\ \text{aspiration of total estimated volume} = \frac{A-B}{\text{A}} \times 100\ \%$$

Volume is computed using the standard geometric formula $\left(\frac{4}{3}\pi R^3\right)$ where R is half the long axis. The volume unit is in cc. "A" is calculated by the image during pre-procedure measurements. "B" is calculated by the image during post-procedure measurements.

Hypotheses:

H_{0i}: $\mu_i - \mu_0 \leq 0$ Versus H_{1i}: $\mu_i - \mu_0 > 0$ for *i*=1, 2;

where μ_0 *is mean volume percentage of* 22 G Arm,
μ_1 *is mean volume percentage of* 19 G Flex Arm, and
μ_2 *is mean volume percentage of* 19 G Arm.

Superiority by 10% or more is deemed clinically significant.
The multiple comparison Hochberg's step up method will be used.
The analysis population for the primary endpoint will be the Intent to Treat and Per-protocol analysis sets.

3.1.2 Sample Size

The sample size was estimated based on the following assumptions:
Design parameters:
One-sided Type I Error=0.05
Multiple comparison method= Hochberg's step up
Rejection region=Right tail

Treatment parameters:

Arm	Mean volume percentage	Standard deviation	Sample size per group
22G	67	15.29	25
19G	77	4.67	25
19GFlex	77	4.67	50

Overall power:
Global (reject any H_{0i}): 0.912
Conjunctive (reject all H_{0i}): 0.901

Given the above assumptions, a total sample of 100 subjects will be required where 50 subjects will be in 19 G Flex arm, and 25 subjects in the 19 G and 22 G arms respectively.

3.1.3 Statistical Methods

Two-sample *t*-test statistic will be computed as follows.

$T_i = \frac{\bar{x}_i - \bar{x}_0}{s_{\bar{x}_i - \bar{x}_0}}$, for *i*=1, 2;

where $\bar{x}_0$ *is mean volume percentage of* 22 G Arm,
$\bar{x}_1$ *is mean volume percentage of* 19 G Flex Arm,
$\bar{x}_2$ *is mean volume percentage of* 19 G Arm,

$$s_{\bar{x}_i-\bar{x}_0} = \sqrt{\frac{s_i^2}{n_i} + \frac{s_0^2}{n_0}} \ ,$$

n_0 *is sample size of* 22 G Arm,
n_1 *is sample size of* 19 G Flex Arm,
n_2 *is sample size of* 19 G Arm.

Now, for each null hypothesis p-value will be computed using one-sided right tail test with type-1 error=0.05 using the following:

$$p_i = P(T_i > t_i) = 1 - \Phi(t_i)$$

where T_i follows standard normal distribution and $\Phi(.)$ is cumulative distribution function.

Next, let $p_{(1)} < p_{(2)}$ be the ordered p-values and $H_{(1)}$, $H_{(2)}$ be the associated null hypotheses. The Hochberg step up procedure will be carried out as following:

- Step 1: If $p_{(2)} > \alpha$ retain $H_{(2)}$, otherwise reject all hypotheses and stop.
- Step 2: If $p_{(1)} > \alpha/(3-1)$ retain $H_{(1)}$ and stop; otherwise reject $H_{(1)}$ and stop.

The adjusted p-values for individual hypotheses will be computed by:

$$\tilde{p}_i = \begin{cases} p_{(i)} & if \quad i = 2 \\ \min(\tilde{p}_{(i+1)}, 2p_{(i)}) & for \quad i = 1 \end{cases}$$

The adjusted p-value for the global hypothesis will be computed using the following:

$$\tilde{p} = \min(\tilde{p}_1, \tilde{p}_2)$$

3.2 Secondary Endpoint "c"

3.2.1 Hypotheses

The secondary endpoint "c" is "Successful echoendoscopic fine needle aspiration of PCL, defined as complete cyst aspiration or collection of aspirate adequate to perform two standard assays: cytology and carcinoembryonic antigen (CEA)."

NOTES: Endpoint c) success is defined as:

- complete aspiration, defined as reaching a final cyst maximal diameter of less than 5mm attained with a maximum of 3 needle passes; in the case of a multilocular cyst, complete aspiration of the largest locule is required,

or

- collection of aspirate adequate to perform two standard assays: cytology and CEA, attained with a maximum of 3 needle passes

Total aspirate volume should be 3cc or more. In cases where less than 3cc of aspirate is obtained but cytology and CEA assays can be adequately completed, the secondary endpoint is considered to have been met.

Analysis Approach:

Successful echoendoscopic fine needle aspiration of PCL is calculated overall and for each arm (19 G Flex, 22 G, 19 G).

Hypotheses:

H_{0i}: $\pi_2 - \pi_i \leq 0$ Versus H_{1i}: $\pi_2 - \pi_i > 0$ for *i*=0, 1;

where π_0 *is proportion of success in* 19 G Arm, and
π_1 *is proportion of success in* 22 G Arm, and
π_2 *is proportion of success in* 19 G Flex Arm.

It is the desire of this study to demonstrate superiority of the EUS-FNA 19 G Flex needle over two other needles:

- One offering the same needle diameter, but without added needle flexibility, namely the standard 19 G needle
- One offering better needle flexibility via a reduced needle diameter, namely the standard 22 G needle

Superiority by 16% or more is deemed clinically significant.
The multiple comparison Hochberg's step up method will be used.
The analysis population for the success endpoint will be the Intent to Treat and Per-protocol analysis sets.

3.2.2 Sample Size

The sample size was estimated based on the following assumptions:
Design Parameters:

One-sided Type I Error=0.05
Multiple comparison method= Hochberg's step up
Rejection region=Right tail

Treatment Parameters:

Arm	Response rate	Sample size per group
19GFlex	0.91	121
19G	0.75	61
22G	0.75	61

Overall Power:
Global (reject any H_{0i}): 0.947
Conjunctive (reject all H_{0i}): 0.808

Given the above assumptions, a total sample of 243 subjects will be required where 121 subjects will be in 19 G Flex arm, and 61 subjects in the 19 G and 22 G arms respectively. In order to account for attrition, a total of 250 randomized subjects will be enrolled.

3.2.3 Statistical Methods

Test statistics T_i with pooled variance will be computed as follows.

$$T_i = \frac{\hat{\pi}_2 - \hat{\pi}_i}{\sqrt{\tilde{\pi}_i(1-\tilde{\pi}_i)\left(\frac{1}{n_i}+\frac{1}{n_2}\right)}},\ i = 0,1$$

where $\hat{\pi}_2$ is the sample proportion for 19G Flex arm,

$\hat{\pi}_i$ are the sample proportions for 19G arm and for 22G arm, and

$\tilde{\pi}_i = \frac{n_2\hat{\pi}_2 + n_i\hat{\pi}_i}{n_2 + n_i}$.

Now, for each null hypothesis p-value will be computed using one-sided right tail test with type-1 error=0.05 using the following:

$$p_i = P(T_i > t_i) = 1 - \Phi(t_i)$$

where T_i follows standard normal distribution and $\Phi(.)$ is cumulative distribution function.
Next let $p_{(1)} < p_{(2)}$ be the ordered p-values and $H_{(1)}$, $H_{(2)}$ be the associated null hypotheses. The Hochberg step up procedure will be carried out as following:

- Step 1: If $p_{(2)} > \alpha$ retain $H_{(2)}$, otherwise reject all the null hypotheses and stop.

- Step 2: If $p_{(1)} > \alpha/(3-1)$ retain $H_{(1)}$ and stop; otherwise reject $H_{(1)}$ and stop.

The adjusted p-values for individual hypotheses will be computed by:

$$\tilde{p}_i = \begin{cases} p_{(i)} & if \quad i = 2 \\ \min(\tilde{p}_{(i+1)}, 2p_{(i)}) & for \quad i = 1 \end{cases}$$

The adjusted p-value for the global hypothesis will be computed using the following:

$$\tilde{p} = \min(\tilde{p}_1, \tilde{p}_2)$$

4 GENERAL STATISTICAL METHODS

4.1 Analysis Sets

The Intent-to-treat (ITT) cohort will consist of all randomized subjects, namely subjects who signed the ICF, met all eligibility criteria (see note below), were randomized to one of three study arms, and experienced echoendoscope insertion and attempted EUS-FNA procedure. Subjects in this cohort, independent of whether or not the EUS-FNA was successful, will be counted towards the enrollment ceiling. Any adverse events occurring or resulting from a treatment attempt will be collected.

NOTE:

Exclusion criteria #1 may be documented after a subject was randomized and the EUS procedure was started. In such cases, the subject will be excluded from the study and no longer be a part of the ITT cohort.

The Modified Intent-to-treat population will consist of ITT population with salvage, namely the subjects with 19G + Salvage, 22G + Salvage, and 19G Flex+ Salvage.

The per-protocol (PP) cohort will consist of the subset of the ITT subjects in whom the target lesion could be reached and EUS-FNA yielded any aspirate, used the assigned needle, and there were no major protocol deviations.

All endpoints will be analyzed for all subjects and stratified by EUS-FNA needle type (by randomization arm) for the ITT analysis set. Similarly, primary and selected secondary endpoints will be analyzed for the PP analysis set.

4.2 Demographics, Baseline Characteristics and Medical History

Subject demographic information will be summarized. The demographic summary includes subjects' gender and age. The baseline characteristics include: medical history, diabetes, chronic pancreatitis, and necrotizing pancreatitis.

4.3 Subject Disposition

The subject dispositions will be presented. Number and percentages of subjects will be presented who are:

- Enrolled
- ITT
- Per protocol (PP)

Site breakdown table will be given for Enrolled, ITT, and per protocol analysis sets.

4.4 PCL characteristics

PCL characteristics will be summarized. PCL characteristic include location of cystic lesion, morphology, connection to pancreatic duct, solid components, presence of visible mucin, distinct PCL wall and mural nodule.

4.5 Control of Systematic Error

Stratified randomization of needle types (with PCL Location strata) will be done to control for selection bias.

4.6 Number of Subjects per Investigative Site

A total of 250 subjects will be enrolled in this trial at 7 to 12 investigational sites. To avoid that enrollment be too heavily biased towards one site, an enrollment maximum by site will be instituted per clinical study agreement between each investigational site and the Sponsor.

4.7 Methods for Handling Missing Data

As per section 5.6, in fitting a multivariate model, if there are missing categorical covariates in the model, an appropriate statistical method (for example, for generalized linear model, Ibrahim (1990, JASA)) will be used. For missing values related to dates see section 7.4.

In the other data analysis (for example missing continuous covariates), the adjustments for missing data will be performed only if deemed necessary and will be described completely. In all such situations, a sensitivity analysis will be performed to justify all adjustments.

5 ADDITIONAL DATA ANALYSES

5.1 Other Endpoints/Measurements

5.1.1 Secondary Endpoints

Secondary endpoints are listed below. These endpoints will be summarized based on ITT and per protocol (PP) population using descriptive statistics (mean, standard deviation, n, minimum, maximum) for continuous variables and frequency statistics for discrete

variables. For a sample table based on ITT population or per protocol population see the appendix.

Safety Endpoint

- Occurrence, severity and relatedness of adverse events to the needle and procedure

Other Endpoints

- Extent of cyst aspiration as measured by reduction of maximal cyst diameter before and after FNA (converted to volume by standard geometric formulation)
- Successful echoendoscopic fine needle aspiration of PCL, defined as complete cyst aspiration or collection of aspirate adequate to perform two standard assays: cytology and carcinoembryonic antigen (CEA)
- Ability to reach and penetrate the PCL, stratified by route of access and PCL location
- Number of EUS-FNA needles and EUS-FNA needle passes used
- Time needed for aspiration for each pass and time from first needle pass in to last needle pass out
- Ease of needle passage in and out of echoendoscope and quality of needle visualization inside the cyst, categorized as *Excellent, Very Good, Good, Fair,* and *Poor*
- Accuracy of two EUS-FNA-based standard assays (cytology and CEA) as a diagnostic measure of disease state categorized as one of the following: *serous cyst, mucinous cyst, intra papillary mucinous neoplasm (IPMN),* or *pseudocyst*
- Post EUS-FNA patient management plan (clinical and/or imaging surveillance, percutaneous aspiration, surgical removal)
- Impact of EUS-FNA based assays on diagnosis
- Rate of cross-over to salvage arm

5.1.2 Diagnostic Accuracy Measures

Diagnostic accuracy is defined in terms of "disease state" and the following three disease state categories will be used in this study in order to assess diagnostic accuracy of EUS-FNA:

- "*Disease State per EUS-FNA*": This measure is derived from the results of the cytology and chemistry assays of the cyst aspirate. Cysts will be classified as *serous cyst, mucinous cyst, IPMN,* or *pseudocyst* based on the table below. If any of the aspirate assays are not performed or the results from the two aspirate-based assays do not follow the table below, then the Disease State per EUS-FNA will be classified as

Indeterminate. The Disease State per EUS-FNA will be recorded on the *Cytology and Chemistry Results CRF*.

Disease State per EUS-FNA	Cytology	Cyst Fluid CEA (ng/mL)
Serous	Bland PAS +*	< 0.5
Mucinous	Mucinous	> 200
IPMN	Mucinous	> 200
Pseudocyst	Pigmented histiocytes	< 200

* This cytological assay might be replaced by another cytological assay as needed

In order to assess the accuracy of EUS-FNA-based standard assays (cytology and CEA) as a diagnostic measure of disease state, the *Disease State per EUS-FNA* measure will be compared to a reference diagnosis. The reference diagnostic standard will be diagnosis from a surgical specimen histology, the true gold standard for cases that evolve to surgical resection within the 30 day follow-up time of the protocol. For cases that do not progress to surgery within 30 days of the EUS-FNA procedure, the reference diagnostic standard will be a composite diagnosis by a consensus board. The consensus board will consist of 3 to 5 Investigators who will be blinded to the type of needle used. The diagnosis of disease state will be based solely on baseline medical history and EUS and cross sectional imaging of the cyst. Information regarding aspirate characteristics and results from aspirate tests will not be provided to the consensus board. Diagnoses will be rendered at a separate Investigators Meeting or virtual meeting.

- "*Disease State per Histology*": This is the reference diagnostic standard. This measure is derived from the histology results of the surgical specimen in cases that progress to surgery within 30 days. Cysts will be classified as follows: *serous cyst, mucinous cyst, IPMN, pseudocyst,* or *Indeterminate.* As described above, this measure will serve as the gold standard for assessing diagnostic accuracy.

- "*Disease State per Consensus Board*": This is the surrogate for the reference diagnostic standard. This measure is derived by the consensus board as described above. Cysts will be classified as follows: *serous cyst, mucinous cyst, IPMN, pseudocyst,* or *Indeterminate.* The classification will be based on baseline medical history and EUS and cross sectional imaging. Medical history will include, but not be limited to presence or absence of prior necrotizing pancreatitis, trauma or other potential cause of pseudocyst. Imaging will pertain to cyst location and cyst appearance.

Degree of correlation between *Disease State per EUS-FNA* and *Disease State per Consensus Board or per Histology* is illustrated below.

Total number of subjects enrolled is sum of all entries, namely

TOTAL = A + B + C + …. + S + T+ U + V + W + X + Y.

PCL Type	Serous per Consensus Board or Histology	Mucinous per Consensus Board or Histology	IPMN per Consensus Board or Histology	Pseudocyst Per Consensus Board or Histology	Indeterminate per Consensus Board or Histology
Serous per EUS-FNA	A	E	F	G	U
Mucinous per EUS-FNA	K	B	H	I	V
IPMN per EUS-FNA	L	M	C	J	W
Pseudocyst Per EUS-FNA	N	O	P	D	X
Indeterminate Per EUS-FNA	Q	R	S	T	Y

Degree of correlation between *Disease State per EUS-FNA* and *Disease State per Consensus Board* or *per Histology*:

- [A+B+C+D+Y] / [TOTAL]

Diagnostic accuracy for Serous Cysts:

- Sensitivity

  = [A] / [A+K+L+N+Q]

- Specificity

  = [B+C+D+H+I+J+M+O+P+R+S+T+V+W+X+Y] /
  [B+C+D+E+F+G+H+I+J+M+O+P+R+S+T+U+V+W+X+Y]

- Positive Predictive Value (PPV)

  = [A] / [A+E+F+G+U]

- Negative Predictive Value (NPV)

  = [B+C+D+H+I+J+M+O+P+R+S+T+V+W+X+Y] /
  [B+C+D+H+I+J+K+L+M+N+O+P+Q+R+S+T+V+W+X+Y]

- Overall Accuracy
  = [A+ B+C+D+H+I+J+M+O+P+R+S+T+V+W+X+Y] / [TOTAL]

Diagnostic accuracy for Mucinous Cysts:

- Sensitivity

  = [B] / [B+M+O+E+R]

- Specificity

  = [A+C+D+Y+F+G+J+L+N+P+Q+S+T+U+W+X] /
  [A+C+D+F+G+H+I+J+K+L+N+P+Q+S+T+ U+W+X+Y+V]

- Positive Predictive Value (PPV)

  = [B] / [B+H+I+K+V]

- Negative Predictive Value (NPV)

  = [A+C+D+F+G+J+L+N+P+Q+S+T+Y+U+W+X] /
  [A+C+D+E+F+G+J+L+M+N+O+P+Q+R+S+T+U+W+X+Y]

- Overall Accuracy

  = [A+B+C+D+F+G+J+L+N+P+Q+S+T+U+W+X+Y] / TOTAL

Diagnostic accuracy for IPMN:

- Sensitivity

  = [C] / [C+F+H+P+S]

- Specificity

  = [A+B+D+E+G+I+K+N+O+Q+R+T+U+V+X+Y] /
  [A+B+D+E+G+I+J+K+L+M+N+O+Q+R+T+U+V+X+Y+W]

- Positive Predictive Value (PPV)

  = [C] / [C+J+L+M+W]

- Negative Predictive Value (NPV)

  = [A+B+D+E+G+I+K+N+O+Q+R+T+U+V+X+Y] /
  [A+B+D+E+F+G+H+I+K+N+O+P+Q+R+S+T+U+V+X+Y]

- Overall Accuracy
  = [A+B+C+D+E+G+I+K+N+O+Q+R+T+U+V+X+Y] / TOTAL

Diagnostic accuracy for Pseudocysts (in principle excluded from study, but a few cases may not have been identified at baseline as such):

- Sensitivity

  = [D] / [D+G+I+J+T]

- Specificity

  = [A+B+C+E+F+H+K+L+M+Q+R+S+U+V+W+Y] /
  [A+B+C+E+F+H+K+L+M+N+O+P+Q+R+S+U+V+W+Y+X]

- Positive Predictive Value (PPV)

  = [D] / [D+N+O+P+X]

- Negative Predictive Value (NPV)

  = [A+B+C+E+F+H+K+L+M+Q+R+S+U+V+W+Y] /
  [A+B+C+E+F+G+H+I+J+K+L+M+Q+R+S+T+U+V+W+Y+X]

- Overall Accuracy

  = [A+B+C+D+E+F+H+K+L+M+Q+R+S+U+V+W+Y] / TOTAL

5.2 Interim Analyses

The following analyses are planned:

- Informal interim analysis after study completion of approximately 30 subjects (Phase I) to assure that the data structure is robust for data analyses; no decisions pertaining to enrollment will be made based on findings of this interim analysis.
- Informal interim analysis after study completion of approximately 100 subjects (30 Phase I + 70 Phase II) to finalize endpoint programs and validate them for end of study data analysis; no decisions pertaining to enrollment will be made based on findings of this interim analysis.
- Formal final analysis after study completion of all subjects (30 Phase I + 220 Phase II).

5.3 Subgroup Analyses

Subgroup Analyses will be performed in the following subgroups:

- gender (male, female)

No adjustments for multiple comparisons will be made. Additional analyses may be performed as appropriate.

5.4 Justification of Pooling

Poolability of subjects from study sites will be based on the fact that each site follows the identical clinical protocol. At the end of the study, to justify pooling data across sites, the

comparability of baseline variables and demographic characteristics will be assessed across sites. Baseline demographic characteristics include age and gender. Also, the effect of study site will be investigated to determine the poolability of primary endpoint from different sites. If low enrolling sites exist (fewer than 5 subjects per site for example), they may be combined into one 'center' for purposes of poolability analysis. An analysis of variance will be used for the primary endpoint to assess differences between study centers. Each test will be done at a 5% significance level, with no adjustment for multiplicity of variables. If a significant difference is found among centers, then multiple comparisons of centers will be done with an overall significance level of at most 5%, to identify any sites significantly different from the rest. Subsequently, at the end of the study, the primary endpoint will be analyzed both with and without these sites.

5.5 Multivariable Analyses

Multivariate analyses will be carried out to assess potential correlations between the primary endpoint and PCL location, PCL size, route of access (duodenal, esophageal or gastric), needle used, and number of locules. To analyze the salvage cross-over rate, the mixed model multivariate analysis approach above or any other suitable analysis approach will be adopted. Other multivariate analysis (e.g. impact of different predictors to salvage or not) will be done (if necessary) to address any clinical need.

5.6 Other Analyses

5.6.1 Baseline Characteristics

Baseline data will be summarized overall and for each needle to assess subject demographics, clinical history and pre-procedure characteristics. Data will be summarized using descriptive statistics for continuous variables (mean, standard deviation, number of observations, minimum and maximum) and frequency statistics (percent of conformers, number of conformers, number of observations) for discrete variables.

5.6.2 Post-Procedure Endpoints

Post-procedure information will be collected as detailed in the clinical trial schedule in the protocol. Data will be summarized overall and for each needle using descriptive statistics for continuous variables (mean, standard deviation, number of observations, minimum and maximum) and frequency statistics (percent of conformers, number of conformers, number of observations) for discrete variables.

5.6.3 Subject Disposition

Subject disposition (e.g., number completing the study, number lost-to-follow-up) will be summarized overall and for each needle with frequency tables.

5.6.4 Time-to-Event Methods

The Kaplan-Meier product-limit method will be used to estimate event rates for time-to-event endpoints. Kaplan-Meier plots of time-to-event endpoints will be constructed.

5.7 Changes to Planned Analyses

This statistical analysis plan has been updated prior to performing the final statistical analyses due to updates in the protocol.

6 VALIDATION

All clinical data reports generated per this plan will be validated per 90702587, Global WI: Clinical Data Reporting Validation.

7 PROGRAMMING CONSIDERATIONS

7.1 Statistical Software

All statistical analyses will be done using The SAS System software, version 8 or higher (Copyright © 2000 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA. All rights reserved).

7.2 Format of Output

Results of analysis will be output programmatically to Word documents from SAS with no manual intervention. All output for the final statistical report will be in the form of a Word document containing tables, figures, graphs, and listings, as appropriate.

7.3 Rules and definitions for calculated variables

Rules for programming will be documented accordingly in the SAS code or in the specific table or listing footnotes. This may include references to CRF pages, manipulation of variables, or other appropriate identifiers

7.4 Variable calculations and definitions

Subject age (in years) is a derived variable that will be calculated based on the time from subject birth to subject ICF day. For example, if the subject birth date is 05/13/1975, and procedure date is 02/03/2006, then age = 30 years. Subject duration in the study (in days) is calculated as the difference between dates of enrollment and last known date of the subject in the study. For example, if enrollment date is 02/03/2006, and last known date of the subject in the study is 02/19/2006, then study subject duration is 16 days. When calculating rates of adverse events, missing and partial dates will be handled as follows:

Partial Date Description	Action Taken
Entire onset date is missing	The procedure date will be used for the onset date.

Partial Date Description	Action Taken
The month and the day of the month are missing but the year is available	January 1 will be used for the month and day of the onset date. However, if the imputed date falls before the procedure date, then the procedure date will be used for the onset date.
Day is missing, but the month and year are available	The 1st will be used as the day of the onset date. However, if the imputed date falls before the procedure date, then the procedure date will be used for the onset date.

In deriving the PP population, 'any aspirate' is defined as any reported aspirate; in other words if the 'total volume of aspirate' is greater than zero then there is an aspirate. Similarly, an ITT subject is supposed to have met all eligibility criteria; if they were inadvertently kept in the study, then that would be called as a major protocol violation.

If percentage is presented in the tables/listings, both numerators and denominators will be presented along with the percentage. The denominator will be based on the data which is not missing. A set of derived datasets will be created using SAS programs from the raw eCRF data. The derived datasets will be the basis for the creation of summary tables and statistical analysis.

8 APPENDIX