Evaluation of PCLs Using Three EUS-FNA Needles

NCT 01711294

Clinical Protocol

12 August 2016

Confidential

A Multicenter, Prospective, Randomized Study on Endosonographic Evaluation of Pancreatic Cystic Lesions Using 22 G, 19 G, and Flexible 19 G Fine Needle Aspiration

Randomized Evaluation of PCLs Using Three EUS-FNA Needles

CLINICAL PROTOCOL

90869947/E7084

12 August 2016

Sponsored By

Boston Scientific Corporation
100 Boston Scientific Way
Marlborough, MA 01752-1234
USA

Investigator's Signature Page

STUDY TITLE: A Multicenter, Prospective, Randomized Study on Endosonographic Evaluation of Pancreatic Cystic Lesions Using 22 G, 19 G, and Flexible 19 G Fine Needle Aspiration

PROTOCOL VERSION AG

STUDY CENTER: _____

(Print name of study center)

We, the undersigned, have read and understand the protocol specified above and agree on its content. We agree to perform and conduct the study as described in the protocol. In addition, when applicable, we agree to enlist sub-Investigators who also agree to perform and conduct the study as described in the protocol.

_____ _____
Principal Investigator (Print Name) Date

_____ _____
Co-Principal Investigator (Print Name) Date

_____ _____
Co-Principal Investigator (Print Name) Date

_____ _____
Co-Principal Investigator (Print Name) Date

Contact Information

Role	Contact
Clinical Contact	Nguyet Labenski Associate Project Manager Endoscopy Boston Scientific Corporation 100 Boston Scientific Way Marlborough, MA 01752-1234 Phone: +1-508-683-4065 Fax: +1-508-683-5042 Email: nguyet.labenski@bsci.com
Coordinating Principal Investigator	Michael B. Wallace, MD, MPH Professor of Medicine Chair, Division of Gastroenterology Mayo Clinic 4500 San Pablo Road Jacksonville, FL 32224 Phone: +1-904 953-7382 Fax: +1-904 953-6225 Email: Wallace.Michael@mayo.edu

Original Release: April 13, 2012

Current Version: August 12, 2016

Protocol Synopsis

Full Title	**A Multicenter, Prospective, Randomized Study on Endosonographic Evaluation of Pancreatic Cystic Lesions Using 22 G, 19 G, and Flexible 19 G Fine Needle Aspiration**
Short Title	Randomized Evaluation of PCLs Using Three EUS-FNA Needles
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 patients originally randomized to 19 G or 22 G standard needles, and will use 19 G or 22 G standard needle at investigator discretion for patients originally randomized to 19 G Flex needle. Phased • Phase I: 2 centers • Phase II: expanded to 7 to 12 centers
Number of Subjects	250 patients (121 in 19 G Flex arm, and 61 patients each in 22 G and 19G arm, rounded up)
Number of Centers	7 to 12 centers
Primary	Volume of aspirated cyst fluid as a function of estimated maximal volume

Endpoint	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 • **Long Term Clinical Follow-Up Chart Review:** Assess patient

	status, Chart Review Details, Morphological Status, Cytopathological Status, Biochemical Status, Surgical Pathology
Inclusion Criteria	1. Pancreatic cystic lesion measuring 13mm or greater in largest 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 patients in 19 G Flex Arm, and 25 patients 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 patients in 19 G Flex Arm, and 61 patients 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 patients.

1. Introduction

Endoscopic ultrasound fine needle aspiration (EUS-FNA) has become the reference standard for preoperative diagnosis of pancreatic cystic lesions (PCLs).[1,2] Limitations of EUS-FNA persist however and include poor cytological yield, modest sensitivity to detection of malignancy of neoplastic cysts, and incomplete aspiration of cyst fluid which limits the ability to perform tumor marker and cytological testing. Additionally, EUS-FNA may increase the risk of infectious complications compared to EUS without FNA. Adverse events may include but are not limited to pancreatitis, infection, and bleeding.[2, 3] Overall, however, EUS-FNA is considered a safe and effective procedure.

Aspiration with a larger diameter needle, particularly in neoplastic cysts with viscous mucoid contents, may improve EUS-FNA diagnostic yield and hence favorably impact treatment decisions, assuming no unfavorable impact on procedural safety. Traditionally, however, larger diameter EUS-FNA needles are stiffer than lower diameter EUS-FNA needles. This makes their use difficult or even impossible in lesions requiring significantly flexed positioning of the echoendoscope.

Qualitatively, the study hypothesis is based on the assumption that a larger diameter, yet flexible EUS-FNA needle may favorably combine lesion accessibility and viscous lesion aspiration. More specifically, the suggestion is that the 19 G Flex EUS-FNA needle combines lesion accessibility attributes of a 22 G standard EUS-FNA needle and aspiration attributes of a 19 G standard EUS-FNA needle. Thus, the hypothesis is that the EUS-FNA 19 G Flex needle will be superior to the EUS-FNA 22 G standard and 19 G standard needles for successful EUS-FNA procedures to assess PCLs. A successful procedure requires lesion access or aspirate volume adequate for three widely accepted assays for the assessment of PCLs.

The study design, accordingly, prescribes 3-way randomization with conjunctive superiority of performance of the 19 G Flex EUS-FNA needle compared to both the 19 G standard and 22 G standard EUS-FNA needles. A salvage procedure is offered in case access or complete aspiration of the PCL is not attained.

2. Primary Objective

To document impact of EUS-FNA needle gauge and flexibility on effectiveness of 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.

3. Study Design

- Prospective
- Multi-center
- Three arm randomized:

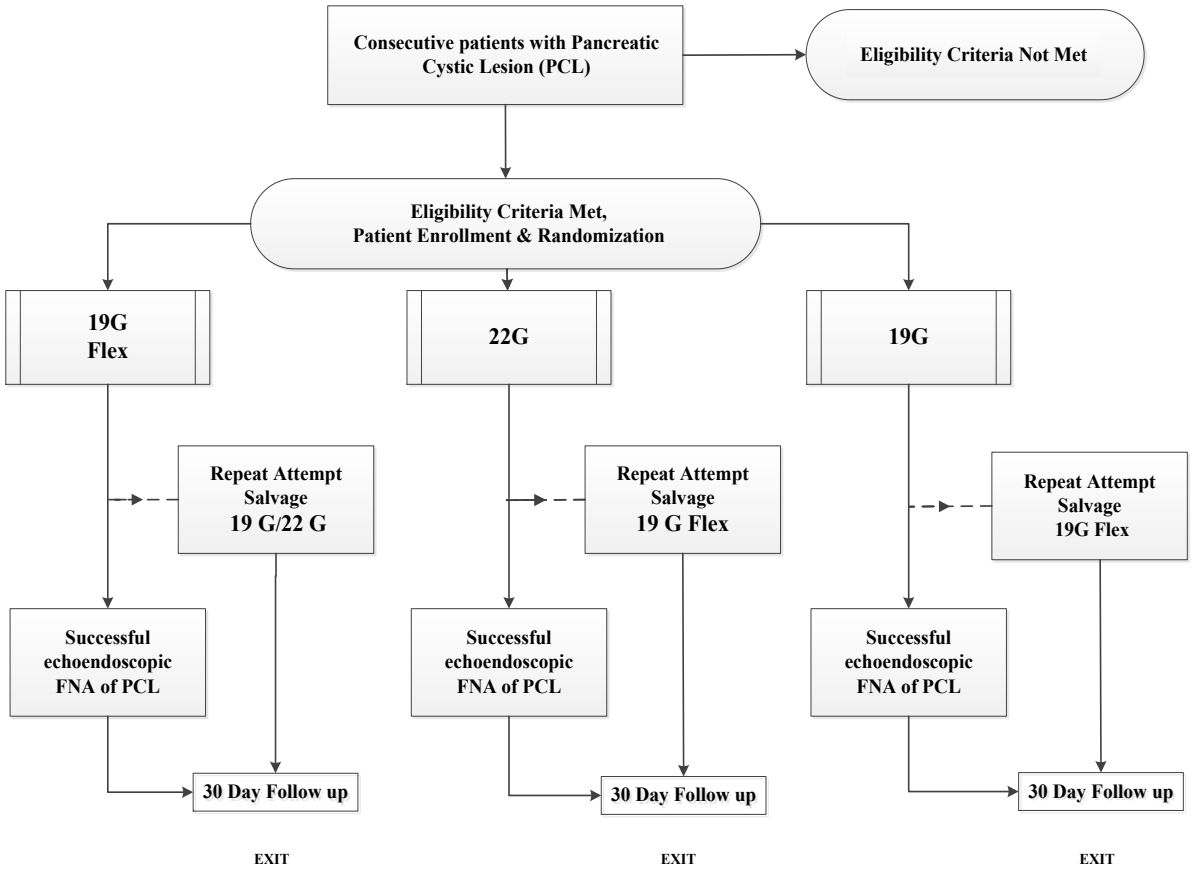
  - Arm 1: 19 G Flex, Arm 2: 22 G, Arm 3: 19 G in a 2:1:1 ratio
  - Salvage to 19G or 22G at investigator discretion for Arm 1 failures, and to 19 G Flex for Arm 2 and 3 failures
- Number of patients:
  - 250, namely 121 patients in 19 G Flex and 61 patients each in 22 G and 19 G arm, rounded up
- Number of centers:
  - Phase I: 2 centers, 30 patients
  - Phase II: expanded to 7 to 12 centers, 220 patients

  The Phase I centers are at different locations overseen by one common Investigational Review Board (IRB). The Lead Principal Investigator (Lead PI) is at one of these centers. The small Phase I preamble to further expansion of the study will allow for further definition of data collection points in Case Report Forms (CRFs). No formal analysis is planned for the Phase I data.

- Anticipated study duration:
  - Patients will have a study Follow-up Visit up to 30 days post-EUS-FNA procedure and will exit the study. No further study visits will occur.
  - A retrospective Long Term Clinical Follow-Up Chart Review will be performed to collect up to 24 months of standard of care clinical information (if applicable).

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

Figure 1 – Study Design Flow Chart



4. Endpoints

4.1. 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).

4.2. 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).

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 primary endpoint is considered to have been met. Aspirate handling procedures are provided in Section 7.4 below.

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€.

DEFINITIONS:

**Needle pass* -- from needle insertion through needle removal outside the cyst lumen, but not including needle repositioning within a single of multi-compartment cyst.

Note: A maximum of 3 needle passes are allowed, after which the EUS-FNA procedure will be considered failed.

†*Time of first needle pass in* -- time of the first needle puncture into the cyst lumen.

€*Time of last needle pass out* -- time of removal of needle outside the cyst lumen at the end of the last fluid aspiration from a single or multi-loculated cyst, or after 10 seconds of failed attempt to aspirate fluid after the last needle re-repositioning in a single or multi-compartment cyst.

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.*

NOTES:

- Determination of disease state per EUS-FNA will be based on results from cytology and CEA and will follow published criteria.[4] Definitions of the diagnostic accuracy measures are provided in **Appendix C.3**.
- Samples that do not have both diagnostic assay results (cytology and CEA) will be labeled as *Indeterminate*.

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.

5. Subject Selection

5.1. Study Population and Eligibility

All patients who are scheduled to undergo an EUS-FNA procedure of a PCL by a study Investigator will be offered the opportunity to enroll in the study (see **Section 8.2** below). A count of such consecutive patients will be kept at each participating center from the time the center is enrollment-ready to when enrollment is closed for the center. Subjects will be asked to sign an Informed Consent form (ICF) before any study-specific tests or procedures are performed. Pregnant women will be excluded as per routine clinical care.

5.2. Inclusion Criteria

1. Pancreatic cystic lesion measuring 13mm or greater in largest 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.

   NOTE: * In a multilocular cyst, the largest locule should measure 13mm or greater in its largest diameter.

5.3. 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.

6. Study Devices

Three versions of the Expect™ Endoscopic Ultrasound Aspiration Needle will be used in this study. The Expect™ Needle is a market-approved device designed to sample targeted sub mucosal and extramural gastrointestinal lesions through the accessory channel of a curvilinear echoendoscope.

Study devices are labeled on the box and inner pouch and include name of the legal manufacturer, device name and dimensions, lot number, and expiration date. Device labeling will be provided in local language(s) as per national regulations.

For a detailed description of the Expect™ Endoscopic Ultrasound Aspiration Needle, please reference the Directions for Use (DFU) included in each device package.

Devices with the specifications outlined in **Figure 2** below will be used in the study:

Figure 2 – Device Chart

Device Name	Delivery System Working Length	Needle Size	Needle Diameter	Needle Length
Expect™ Endoscopic Ultrasound Aspiration Needle	137.5 cm to 141.5 cm adjustable	- 22 G - 19 G - 19 G Flex	- 0.72 mm - 1.10 mm - 1.14 mm	0 cm to 8 cm adjustable

7. Study Procedures

Endoscopic ultrasound will be performed using standard linear sector scanning EUS equipment (e.g., Olympus, Pentax, or Fujinon). Study procedures will be followed as outlined in the sections below.

7.1. Antibiotic Treatment

All patients will be given broad spectrum antibiotics such as fluoroquinolone for at least 3 days starting on the day of the EUS-FNA procedure.

7.2. Needle Puncture and Aspirate Requirements

The following procedural rules will be followed:

a. Initial needle puncture with stylet in place (with time stamp). Remove stylet and apply 10cc suction. If no aspirate visible in syringe lumen after 5 seconds of suction: reposition needle and re-apply suction. If no aspirate after 5 seconds of suction- lesion classified as FNA failure. Switch to salvage method.

b. If aspirate visible within syringe lumen, hold suction until no additional material accumulated for 10 seconds. If cyst completely collapses, note final time stamp. If cyst is not completely collapsed, then repositioning is allowed until all cyst locules have been punctured, and no additional fluid is obtained for 10 seconds. Note final time stamp. If cysts is not completely collapsed after attempts, above, note final maximal and perpendicular diameter.

c. In case of a multi-compartment, loculated lesion, all locules accessible with the same needle pass will be aspirated, with priority to the largest locule. In cases where a locule is positioned in front of another along the axis of the needle, the one most proximal to the needle tip should be fully aspirated before accessing the locules located in a more distal position.

d. In the event that cyst components cannot be reached without repuncture, remove needle completely from cyst (note time stamp for pass completion). Repuncture for pass 2 (or more) as needed until criteria (see "b" above) for complete aspiration or failure is met.

e. If maximal cyst diameter is > 5mm after efforts described in "b" above, cyst aspiration is considered "failed." Switch to salvage method (19 Flex, if first method was not 19 Flex).

7.3. Salvage Aspiration

In case of inability to access the PCL or to attain complete cyst aspiration, a salvage aspiration procedure, or a repeated attempt to aspirate the PCL with another needle, should be performed.

Patients randomized to Arm 1 (19G Flex) should undergo a salvage procedure with a 22 G or 19 G needle (needle choice at discretion of physician); patients randomized to Arm 2 (22 G) and Arm 3 (19 G) should undergo a salvage procedure with a 19 G Flex needle. Salvage procedure will follow the needle puncture and aspirate requirements guidelines outlined in **Section 7.2** above.

7.4. Post-Procedure Handling of Cyst Aspirate

a. Record final cyst maximum diameter and perpendicular diameter.

b. Record total volume of cyst aspiration by suctioning all material, including what is in the needle lumen, into the syringe, inverting it, and removing air.

- If sample volume is > 3cc, send 2cc to chemistry laboratory for CEA. Send remainder to cytology lab.
- If sample volume is < 3cc, divide the sample into two aliquots guided by volumetric requirements of the local cytology and chemistry labs.
- Sequential portions of the aspirate will be used for the two assays as follows:
    - First aliquot (preferably at least 1cc) – Cytology
    - Second aliquot (preferably at least 1cc) – CEA
    - All remaining fluid is to be added to the first aliquot and sent for cytology

- In case the total aspirate volume is insufficient to satisfy local cytology and chemistry lab requirements, then the aliquot order outlined above should be used to prioritize which subset of assays will be performed.
- In cases in which the aspirate volume is more than 1 cc but less than 3 cc, the first cc should be sent for cytology and the remainder should be sent for CEA. Some laboratories may have the ability to perform CEA assays on less than 1 cc of fluid for assay.
- In cases in which the aspirate volume is less than 1 cc, the aspirate will nevertheless be sent for cytology.

7.5. Adverse Event Assessment

- Immediate Adverse Event assessment: Assessment for immediate complications during the procedure and prior to discharge (approximately 2hrs post-procedure) or planned hospital observation transfer will be done and will be recorded on the *Adverse Event (AE) CRF*.
- Delayed Adverse Event assessment: Assessment for delayed complications will be done by telephone at 30 days (± 5 days) and will be recorded on the *AE CRF*. If a scheduled office visit is made during the same time window, it may substitute for the telephone call. If surgery or other major intervention (e.g., ERCP, other invasive biopsy) is performed prior to 30 days, events will be censored at the date of the procedure.

Adverse event details including definitions, types, and reporting requirements are provided in **Section 10**.

8. Study Visits

8.1. Visit Schedule and Data Collection

The visit and data collection schedule is outlined in **Figure 3** below.

Figure 3 – Visit and Data Collection Schedule

Procedure/Assessment	Screening, Enrollment, Baseline	Procedure	30 Day Follow-up Visit (± 5 days)	Long Term Clinical Follow-Up Chart Review (Up to 24 months from Procedure)
ICF	X			
Eligibility Criteria Assessment	X			
Demographics	X			
Medical History	X			X
Lesion Location	X			X
Randomization	X			
Lesion Characteristics		X		X
EUS-FNA Procedure Details (including Needle Use and Times)		X		
Aspirate Characteristics		X		
Salvage EUS-FNA Procedure (if applicable)		X		
Adverse Events Assessment		X	X	
Additional Fluid Testing Results (if applicable)				X
Surgical Pathology (if applicable)				X

8.2. From Screening to Baseline

8.2.1. *Screening and Informed Consent*

On an all-comer basis, Investigators will keep a count of the number of patients to whom study participation was offered (see also **Section 5.1** above). No study-specific testing will be conducted before the subject has signed an Informed Consent Form (ICF). The ICF is study-specific and must be approved by the study Institutional Review Board (IRB)/Ethics Committee (EC) (see also **Appendix B.2** below). Study personnel should explain that even if a subject agrees to participate in the study and signs the ICF, the inclusion/exclusion criteria may demonstrate that the subject is not a suitable candidate for the study.

8.2.2. *Enrollment*

Patients who signed the ICF, met all inclusion criteria, and met none of the exclusion criteria will be considered enrolled. A *Screen Failure/Enrolled Log* will be maintained of patients who signed the ICF but did not satisfy enrollment criteria.

Only enrolled patients will be randomized and count toward the enrollment ceiling.

8.2.3. *Baseline*

Patient information will be collected on the *Baseline CRF* pertaining to:

- Demographics
- Medical History
- Lesion Location (head/uncinate, body, tail)

8.2.4. *Randomization*

Enrolled patients will be assigned to the 19G Flex, 22 G or 19 G Arm in a 2:1:1 ratio by randomization schedules which will be computer-generated using a pseudo-random number generator. An Interactive Web Response System (IWRS) will be utilized.

Randomization will be stratified (block randomization) by the following strata:

- Investigational center
- PCL location: head/uncinate, other

8.3. Procedure

8.3.1. *Lesion Characteristics*

The following lesion characteristics will be assessed by EUS prior to the start of the FNA procedure and will be recorded on the *Procedure CRF*:

a. Cyst size (maximal diameter, and cross-sectional diameter)

   NOTE: In the case of a multilocular cyst, size will be measured both as the aggregate size of all cyst locules and the long and short perpendicular axis of the largest locule.

b. Number of locules (1, 2, 3, 4, 5, 6-10, > 10)

c. Presence of visible mucin (floating, or mobile filling defects)

d. Presence of mural nodule (non-mobile filling defect) and size

e. Presence of solid components

f. Presence of distinct PCL wall

g. Connectivity to pancreatic duct

h. Maximal pancreatic duct diameter

An EUS image of the patient's PCL will be saved during the procedure.

8.3.2. *EUS-FNA Procedure*

The EUS-FNA procedure will be carried out in line with the guidelines provided in **Section 7** above, and the following data will be collected and recorded on the *Procedure CRF*:

a. Route of access (gastric, duodenal, esophageal)

b. Ease of insertion of EUS-FNA needle through echoendoscope

c. Ability to reach target lesion

d. Ability to penetrate into target lesion

e. Quality of needle visualization

f. Number of needle passes

g. Ease of removal of EUS-FNA needle from echoendoscope

h. Aspirate characteristics and adequacy of aspirate volume to perform two assays (cytology and CEA)

i. Adverse Event assessment during procedure and through 2 Hours Post-Procedure or discharge from endoscopy unit

8.4. Cytology and Chemistry Results

The results of cyst aspirate cytology and CEA assessments will be provided by the cytology and chemistry labs and will be recorded on the *Cytology and Chemistry Results CRF*. Results will be categorized as follows[4] and will be used for determination of Disease State per EUS-FNA:

- Cytology
  - Bland PAS +
  - Mucinous
  - Pigmented histiocytes
- CEA (ng/mL)
  - < 0.5
  - > 0.5 and < 200
  - > 200

8.5. 30-Day Follow-up

The post-procedure 30-day Follow-up Visit will be conducted over the telephone. The visit window allows for this visit to occur anytime from 25 days to 35 days post procedure. Patients will be assessed for potential adverse events that might have occurred from discharge until 30 days post-procedure. AE information will be recorded on the *Adverse Event CRF*.

End-of-Study information including information about loss to follow-up or any other circumstances that might have led to the patient's study participation ending will be collected on the *End of Study CRF*. A subject will be considered lost to follow-up after 3 documented attempts of contact by study staff.

Protocol deviations will also be documented throughout the study.

8.6. Long Term Clinical Follow-Up Chart Review

After completion of the 30-day Follow-up Visit, a retrospective Long Term Clinical Follow-Up Chart Review will be performed to collect up to 24 months of standard of care clinical information (if applicable). There are no office or telephone visits required. The Investigator or study coordinator will review the hospital charts to assess patient status and extract PCL characteristic pertaining to Morphological Status, Cytopathological Status, Biochemical Status, and Surgical Pathology. No new Adverse Event information will be captured after the 30 Day Post Procedure.

9. Statistical Considerations

9.1. Study Hypothesis and Sample Size Calculation

Sample size was calculated based on assumptions for the primary endpoint as well as for secondary endpoint c):

9.1.1. *Primary endpoint considerations:*

The study hypothesis states that for either of the 19 G Flex and 19 G Arms, the volume of actual aspirated cyst fluid as a ratio (%) of estimated maximal volume is superior by at least 10% compared to that of the 22 G Arm.

The study investigators provided the following experience-based estimate of the primary endpoint (aspirate volume as % of max volume based on EUS image of the cyst):

Figure 4 – Hypothesis Generating References (Primary Endpoint)

Site Investigator	**Mean Volume Ratio (%) for 19G and 19Flex needles**	**Mean Volume Ratio (%) for 22G needles**
Investigator 1	80%	50%
Investigator 2	90%	70%
Investigator 3	90% Unilocular cysts, 50% Multi-locular	80%

	cysts, 80% Blended mean	
Investigator 4	90%	90%
Investigator 5	90%	50%
Investigator 6	85%	65%

From the above table, bootstrap method with 10,000 resamples yielded an estimated mean of 67.44% and standard deviation of 15.29% for 22G, and a mean of 85.9% and standard deviation of 4.67% for 19G/19G Flex.

The assumption was made that the mean of the "control group", namely the 22G arm, will be 67% with a standard deviation of 15.29%.

Two null hypotheses are made:

- H_{01} : Mean volume ratio of 19 G Flex Arm and 22 G Arm are the same
- H_{02} : Mean volume ratio of 19 G Arm and 22 G Arm are the same

The alternative hypothesis is:

- H_1 : Mean volume ratio of 19 G Flex/19G Arm is superior to 22 G Arm

If 50 patients are enrolled in the 19 G Flex Arm and 25 patients each in the 19 G and 22 G Arm, the study will have 90% conjunctive power to detect the rejection of both null hypotheses to demonstrate superiority of the mean volume ratio of 19 G Flex Arm and 19 G Arm to that of 22 G Arm by 10% or more. Thus, a minimum of 100 patients (50+25+25=100) are required.

9.1.2. *Secondary endpoint c) considerations:*

The study hypothesis states that the EUS-FNA 19 G Flex needle is superior to the EUS-FNA 22 G standard and 19 G standard needles in the secondary endpoint c) measure, which requires access or adequate aspirate volume for two standard assays expected from successful EUS-FNA procedures in PCL assessment indications.

To create a quantitative hypothesis for the study, a meta-analysis was completed of nine literature reports of EUS-FNA success levels in this indication, using standard, non-flexible EUS-FNA needles. The following references outlined in **Figure 5** were used:

Figure 5 – Hypothesis Generating References (Secondary Endpoint c)

Reference	Success ratio	Success Rate
(1) Brugge	109/112	97.3%
(3) Al-Haddad	31/37	83.8%
(5) Larsen	132/251	52.6%

(6) Khalid	76/113	67.3%
(7) Sawhney	84/100	84.0%
(8) Rogart	75/107	70.1%
(9) Hong	60/69	87.0%
(10) de Jong	80/128	62.5%
(11) Repak	38/38	100%

This yields a success rate point estimate of 75% with 95% CI [67% - 90%] (see **Appendix C.1** for details).

The assumption was made that the success level of both "control groups", namely the 22 G arm and the 19 G Arm, will be 75%.

Two null hypotheses are made:

- H_{01} : Success rate of 19 G Flex Arm and 22 G Arm are the same
- H_{02} : Success rate of 19 G Flex Arm and 19 G Arm are the same

The alternative hypothesis is:

- H_1 : Success rate of 19 G Flex Arm is superior to both 22 G Arm and 19 G Arm

If 121 patients are enrolled in the 19 G Flex Arm and 61 patients each in the 19 G and 22 G Arm, the study will have 80% conjunctive power to detect the rejection of both null hypotheses to demonstrate superiority of the success rate of 19 G Flex Arm to that of both other arms by 16% or more. Thus, a minimum of 243 patients (121+61+61=243) are required.

Details of the sample size calculation are provided in **Appendix C.2.**

9.2. Enrollment per Investigational Site

A total of 250 patients 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.

Enrollment controls are discussed in **Appendix B.13**.

9.3. Data Analysis

The Intent-to-treat (ITT) cohort will consist of all randomized patients, namely patients 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 EUS-FNA was successful or not, 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 patient was randomized and the EUS procedure was started. In such cases, the patient 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 patients with 19G + Salvage, 22G + Salvage, and 19G Flex+ Salvage.

The per-protocol (PP) cohort will consist of the subset of the ITT patients 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 the ITT and PP study populations, for all patients and stratified by EUS-FNA needle type (by randomization arm).

Justification for pooling of data from all study sites will be assessed using standard poolability tests.

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.

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).

A statistical analysis plan will be in place prior to enrollment. Changes to the plan or additional analyses conducted will be identified as *ad hoc* analyses.

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). Subject demographics, medical history, risk factors, pre- and post-procedure characteristics, and outcome variables 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.

10. Potential Risks

10.1. Anticipated Adverse Effects

As per the commercial DFU included with the study devices, the potential complications associated with the use of the Expect Needle may include, but are not limited to:

- Bleeding
- Perforation
- Pancreatitis
- Infection
- Peritonitis
- Inflammation
- Aspiration
- Fever
- Allergic Reaction to Medication
- Hypotension
- Respiratory Depression or Arrest
- Cardiac Arrhythmia or Arrest
- Tumor Seeding

10.2. Definitions and Classification of Adverse Events

Adverse event definitions provided below are extracted from References ISO 14155-2011 and MEDDEV 2.7/3 12/2010 as indicated.

Figure 6 - Adverse Event Definitions

Term	Definition
Adverse Event (AE) *Ref: ISO 14155-2011*	Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal lab findings) in subjects, users or other persons, whether or not related to the investigational medical device. This includes events related to: • The investigational medical device or comparator • The procedures involved (study-required) For users/other persons, this definition is restricted to events related to the investigational device **Note:** In this study, reported AEs will include unanticipated events such as unplanned hospital admission or overnight observation, laboratory or radiological investigation which confirms pancreatitis,

Figure 6 - Adverse Event Definitions

Term	Definition
	bleeding, or perforation, or any other event deemed by the Investigator to be unanticipated and attributable to the EUS-FNA procedure.
Adverse Device Effect (ADE) *Ref: ISO 14155-2011*	Adverse event related to the use of an investigational medical device: • This includes adverse events resulting from insufficient or inadequate instructions for the use, deployment, implantation, installation or operation, or any malfunction of the investigational medical device. • This includes any event resulting from use error or from intentional misuse of the investigational medical device.
Serious Adverse Event (SAE) *Ref: ISO 14155-2011*	An adverse event that: • Led to death • Led to a serious deterioration in the health of the subject that either resulted in: o a life-threatening illness or injury, or o a permanent impairment of a body structure or a body function, or o in-patient hospitalization or prolonged hospitalization (of an existing hospitalization), or o medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function • Led to fetal distress, fetal death, or a congenital abnormality or birth defect. **Note**: Planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered a serious adverse event.
Serious Adverse Device Effect (SADE) *Ref: ISO 14155-2011*	An adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event. **Note**: All SAEs that could have led to a SADE if suitable action had not been taken or if circumstances had been less fortunate shall be reported as required by the local IRB/EC, national regulations, or the protocol. If applicable, see MEDDEV 2.7/3 12/2010 for reporting timeline requirements.
Unanticipated Serious Adverse Device Effect (USADE)	A serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report. **Note**: An anticipated serious adverse device effect (ASADE) is an

Figure 6 - Adverse Event Definitions

Term	Definition
Ref: ISO 14155-2011	effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report.
Device Deficiency *Ref: ISO 14155-2011* *Ref: MEDDEV 2.7/3 12/2010*	A device deficiency is any inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. **Note**: Device deficiencies include malfunctions, use errors, and inadequate labeling. **Note**: All device deficiencies that could have led to a SADE if suitable action had not been taken or if circumstances had been less fortunate shall be reported as required by the local IRB/EC, national regulations, or the protocol. If applicable, see MEDDEV 2.7/3 12/2010 for reporting timeline requirements.

Abbreviations: EC=Ethics Committee; IRB=Institutional Review Board

Underlying diseases are not reported as AEs unless there is an increase in severity or frequency during the course of the investigation. Death should not be recorded as an AE, but should only be reflected as an outcome of a specific SAE.

Reporting requirements are provided in S**ection 10.4** below.

Refer to S**ection 10.1** above for the known risks associated with the study device(s) and EUS-FNA procedure.

10.3. Relationship to Study Device(s)

The Investigator must assess the relationship of the AE to the study devices as related or unrelated using criteria outlined below.

- AE Unrelated to Study Device:
    - The adverse event is determined to be due to a concurrent illness or effect of another device/drug and is not related to the investigational product.
- AE Related to Study Device:
    - The adverse event is determined to be potentially related to the investigational product, and an alternative etiology is equally or less likely compared to the potential relationship to investigational product.
    - There is a strong relationship to investigational product, or recurs on re-challenge, and another etiology is unlikely.
    - There is no other reasonable medical explanation for the event.

Unrelated AEs will not be documented.

10.4. Reporting Requirements to Sponsor

10.4.1. *Communication Method*

Reporting of adverse events and device deficiencies or malfunctions as outlined by category and as applicable during the entire duration of the study, from signing of the ICF through completion of an end-of-study report, should be made by entering pertinent information into the associated study data base fields. In case access to the electronic case report forms is not possible, the Investigator or site study staff members should contact the Sponsor Global Safety Office and Sponsor Project Manager by e-mail. If e-mail communications are also impossible, then one of these Boston representatives should be contacted by telephone. Contact information for the Sponsor Global Safety Office and Sponsor Project Manager will be provided and kept up to date by Sponsor at all times.

Upon receipt of these communications, associated source documentation may be requested by the Sponsor Global Safety Office and Sponsor Project Manager.

10.4.2. *Communication Timelines*

The following should be reported to Sponsor within 2 business days:

- All Serious Adverse Events (SAEs), whether related to the EUS-FNA devices and/or procedure or not
- Device Deficiencies, Failures, Malfunctions, and Product Nonconformities that led to any Adverse Event (AE) or Serious Adverse Event (SAE).

The following should be reported to Sponsor **within 10 business days**:

- All non-serious Adverse Events (AEs) related to the EUS-FNA devices and/or procedure
- Device Deficiencies, Failures, Malfunctions, and Product Nonconformities that did not lead to an Adverse Event (AE) or Serious Adverse Event (SAE).

10.5. Reporting Requirements to EC/IRB

The Principal Investigator is responsible for informing the EC/IRB of AEs and SAEs as required by local procedure. These reporting requirements may vary from one EC/IRB to another.

The Sponsor is responsible for reporting AE and SAE information to participating Investigators as required by local procedures at individual Investigational sites.

10.6. Return of Devices to Sponsor

All device deficiencies (including but not limited to failures, malfunctions, product nonconformities, and labeling errors) will be documented and reported to the Sponsor as

detailed in S**ection 10.4** above. If possible, the device(s) related to an observed device deficiency should be returned to the Sponsor for analysis. Instructions for returning the investigational device(s) are provided in **Appendix B.9**.

10.7. Potential Risks and Benefits Associated with Study Participation

Patients enrolled in this study were scheduled to undergo a EUS-FNA procedure for assessment of a PCL. The EUS-FNA procedures in this study are performed per DFU and standard of practice. The EUS-FNA needles are commercially available devices. Thus, there is no known added risk resulting from undergoing the EUS-FNA procedure in this study compared to outside of this study.

Patients randomized to the standard 19 G or 22 G EUS-FNA needles and in whom the PCL could not be accessed or complete cyst aspiration cannot be attained, will receive a second attempt at adequate PCL aspiration using the marketed, novel 19 G Flex EUS-FNA needle design. Thus, in a subset of patients, there is an added potential benefit to undergoing the EUS-FNA procedure in this study compared to outside of this study.

10.8. Safety Reviews

On a pre-established frequency, listings of all device and/or procedure related adverse events and of all serious adverse events will be reviewed by the Sponsor. Incidence of key types of adverse events will be statistically compared to pre-established safety thresholds. These thresholds will be based on literature reports of these types of adverse events and will also be guided by an ASGE guideline[12] on complications associated with EUS.

Thresholds will be established for:

- Pancreatitis
- Infection
- Hemorrhage

The Sponsor will capture frequency of safety reviews and of the three above-mentioned thresholds in a Safety Plan placed on file prior to first patient enrollment.

APPENDIX A: BIBLIOGRAPHY

1. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, Fernandez del Castillo C, Warshaw AL, The Investigators of the CPC Study; *Diagnosis of Pancreatic Cystic Neoplasms: A Report of the Cooperative Pancreatic Cyst Study* Gastroenterology 2004; 126: 1330-1336
2. Sakamoto H, Kitano M, Kamata K, El-Masry M, Kudo M; *Diagnosis of pancreatic tumors by endoscopic Ultrasonography* World J Radiol 2010; 2(4): 122-134
3. Al-Haddad M, Gill KRS, Raimondo M, Woodward TA, Krishna M, Crook JE, Skarvinko LN, Jamil LH, Hasan M, Wallace MB; *Safety and efficacy of cytology brushing versus standard fine-needle aspiration in evaluating cystic pancreatic lesions: a controlled study* Endoscopy 2010; 42: 127-132
4. Brugge WR; *The use of EUS to diagnose cystic neoplasms of the pancreas* GIE 2009; 69: S 203-9
5. Larsen MC, Binmoeller KF, Shaw RE, Jaffee IM, Shah JN; *Factors Affecting Diagnostic Yield of EUS-FNA of Pancreatic Cystic Lesions* GIE 2010; 71: AB282 (T1456)
6. Khalid A, Zahid M, Finkelstein SD, LeBlanc JK, Kaushik N, Ahmad N, Brugge WR, Edmundowicz SA, Hawes RH, McGrath KM; *Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study* GIE 2009; 69: 1095-102
7. Sawhney MS, Devarajan S, O'Farrel P, Cury MS, Kundu R, Vollmer CM, Brown A, Chuttani R, Pleskow DK; *Comparison of carcinoembryonic antigen and molecular analysis in pancreatic cyst fluid* GIE 2009; 69: 1106-110
8. Rogart JN, Loren DE, Singu BS, Kowalski TE; *Cyst wall puncture and aspiration during EUS-guided fine needle aspiration may increase the diagnostic yield of mucinous cysts of the pancreas* J Clin Gastroenterol 2011; 45(2): 164-9
9. Hong S-KS, Loren DE, Rogart JN, Siddiqui AA, Sendecki JA, Bibbo M, Coben RM, Meckes DP, Kowalski TE; *Targeted cyst wall puncture and aspiration during EUS-FNA increases the diagnostic yield of premalignant and malignant pancreatic cysts* GIE 2012; 75(4): 775-82
10. de Jong K, Poley JW, van Hooft JE, Visser M, Bruno MJ, Fockens P; *Endoscopic ultrasound-guided fine-needle aspiration of pancreatic cystic lesions provides inadequate material for cytology and laboratory analysis: initial results from a prospective study* Endoscopy 2011; 43(7): 585-90
11. Repak R, Rejchrt S, Bartova J, Malirova E, Tycova V, Bures J; *Endoscopic ultrasonography (EUS) and EUS-guided fine-needle aspiration with cyst fluid analysis in pancreatic cystic neoplasms* Hepatogastroenterol 2009; 56(91-92): 629-35
12. Jacobson BC, The ASGE Standards of Practice Committee; *ASGE guideline: complications of EUS* GIE 2005; 61: 8-12

APPENDIX B: ADDITIONAL STUDY CONDUCT REQUIREMENTS

This section provides additional details on items that are mentioned or described more succinctly in the main body of the protocol above. These sections are included per Sponsor protocol template requirements.

B.1 Compliance

This study will be conducted in accordance with relevant sections of the International Standard (ISO) 14155: Clinical Investigation of Medical devices for Human Subjects – Good Clinical Practice, the relevant parts of the ICH Guidelines for Good Clinical Practices, ethical principles that have their origins in the Declaration of Helsinki, and pertinent individual country laws and regulations.

B.2 Ethics Committee/Investigational Review Board Review

The study will not begin until the required approval from the EC or IRB has been obtained. Approval needs to be gained for the protocol and the ICF.

Prior to gaining approval to participate in the study, the investigational center will provide to the Sponsor documentation verifying that their EC/IRB meets applicable requirements. This typically consists of a detailed list of members of the EC/IRB, including their titles and roles.

A copy of the written EC/IRB approval of the protocol and ICF must be received by the Sponsor before recruitment of subjects into the study and shipment of investigational product/equipment.

Annual EC/IRB approval renewals will be obtained throughout the duration of the study as required by local/country or EC/IRB requirements. Copies of the Investigator's reports and the EC/IRB continuance of approval must be provided to the Sponsor.

If a protocol revision is necessary which affects the rights, safety or welfare of the subject or scientific integrity of the data, an amendment is required. Appropriate EC/IRB approvals of the revised protocol must be obtained prior to implementation.

B.3 Informed Consent

Subject participation in this clinical study is voluntary. Informed Consent is required from all subjects or their legally authorized representative. The Investigator is responsible for ensuring that Informed Consent is obtained prior to the use of any investigational devices, study-required procedures and/or testing, or data collection.

The obtaining and documentation of Informed Consent must be in accordance with the principles of the Declaration of Helsinki, ISO 14155, any applicable national regulations, and local EC/IRB and/or Regulatory authority body, as applicable. The ICF must be approved by the EC/IRB as described in **Section B.2** above.

The Sponsor will provide a study-specific template of the ICF to Investigators participating in this study. The ICF template may be modified to meet the requirements of the investigative center's EC/IRB. Any modification requires approval from the Sponsor prior to use of the form. The ICF must be in a language understandable to the subject and if needed, the Sponsor will assist the center in obtaining a written consent translation. Translated consent forms must also have EC/IRB approval prior to their use. Privacy language shall be included in the body of the form or as a separate form as applicable.

The process of obtaining Informed Consent shall:

- be conducted by the Principal Investigator or designee authorized to conduct the process,
- include a description of all aspects of the clinical study that are relevant to the subject's decision to participate throughout the clinical study,
- avoid any coercion of or undue influence of subjects to participate,
- not waive or appear to waive subject's legal rights,
- use native language that is non-technical and understandable to the subject or his/her legal representative,
- provide ample time for the subject to consider participation and ask questions if necessary,
- ensure important new information is provided to new and existing subjects throughout the clinical study.

The ICF shall always be signed and personally dated by the subject or legal representative and by the Investigator or an authorized designee responsible for conducting the informed consent process. If a legal representative signs, the subject shall be asked to provide informed consent for continued participation as soon as his/her medical condition allows. The original signed ICF will be retained by the center and a copy of the signed and dated document and any other written information must be given to the person signing the form.

Failure to obtain subject consent and any violations of the informed consent process must be reported as deviations to the Sponsor and to the EC/IRB, as appropriate.

If new information becomes available that can significantly affect a subject's future health and medical care, that information shall be provided to the affected subject(s) in written form via a revised ICF or, in some situations, enrolled subjects may be requested to sign and date an addendum to the ICF. In addition to new significant information during the course of a study, other situations may necessitate revision of the ICF, such as if there are amendments to the protocol, a change in Principal Investigator, administrative changes, or following annual review by the EC/IRB. The new version of the ICF must be approved by the EC/IRB. Sponsor approval is required if changes to the revised ICF are requested by the center's EC/IRB. The EC/IRB will determine the subject population to be re-consented.

B.4 Withdrawal of Consent

Patients have the right to withdraw from study participation at any time.

If a subject withdraws from the clinical investigation, the reason(s) shall be reported. If such withdrawal is due to problems related to investigational device safety or performance, the Investigator shall ask for the subject's permission to follow his/her status/condition outside of the clinical study.

While study withdrawal is discouraged, subjects may withdraw from the study at any time, with or without reason, and without prejudice to further treatment. Additional data may no longer be collected after the point at which a subject has been withdrawn from the study or withdraws consent. Withdrawn subjects will not be replaced. All open adverse events should be closed or documented as ongoing. Data collected up to the point of subject withdrawal may still be used.

B.5 Data Management

Subject data will be recorded in a limited access secure electronic data capture (EDC) system. All changes made to the clinical data will be captured in an electronic audit trail and available for review by the Sponsor or its representative. Database backups are performed regularly.

The Investigator provides his/her electronic signature on the appropriate electronic case report forms (eCRFs). Changes to data previously submitted to the Sponsor require a new electronic signature by the Investigator acknowledging and approving the changes.

Visual and/or electronic data review will be performed to identify possible data discrepancies. Manual and/or automatic queries will be created in the EDC system and will be issued to the site for appropriate response. Site staff will be responsible for resolving all queries in the database.

B.6 Document Retention

The Investigator will maintain, at the investigative site, in original format all essential study documents and source documentation that support the data collected on the study subjects in compliance with ICH/GCP guidelines. Documents must be retained for at least 2 years after completion of the study. The Sponsor will also retain study documents for 2 years after completion of the study, defined as the date on which a final study report is issued. The Investigator will take measures to ensure that essential documents are not accidentally damaged or destroyed. If for any reason the Investigator withdraws responsibility for maintaining these essential documents, custody must be transferred to an individual who will assume responsibility and the Sponsor must receive written notification of this custodial change.

B.7 Deviations

An Investigator must not make any changes or deviate from this protocol, except to protect the life and physical well-being of a subject in an emergency. An Investigator shall notify the Sponsor and the reviewing EC/IRB of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency, and those deviations which affect the scientific integrity of the clinical investigation. Such notice shall be given as soon as possible, but no later than 5 working days after the emergency occurred, or per prevailing local requirements, if sooner than 5 working days.

All deviations from the investigational plan, with the reason for the deviation, must be documented and reported to the Sponsor using the EDC Protocol Deviation CRF in the database. Sites may also be required to report deviations to the EC/IRB, per local guidelines and government regulations.

Deviations will be reviewed and evaluated on an ongoing basis and, as necessary, appropriate corrective and preventive actions (including notification, center re-training, or discontinuation) will be put into place by the Sponsor.

B.8 Device Accountability

There are no investigational devices used in this study. The Expect™ Endoscopic Ultrasound Aspiration Needle is available for commercial use in the geographical areas in which this clinical study is taking place, and therefore no requirements for device accountability exist for this study. Any country/region requirements that depart from the aforementioned will be implemented on a case-by-case basis.

Device Lot information must be maintained in the subject's medical record and recorded on the Procedure CRF.

B.9 Device Failures and Product Nonconformities

All device deficiencies (including but not limited to failures, malfunctions, use errors, product nonconformities, and labeling errors) will be documented and reported to the Sponsor. If possible, the device(s) should be returned to the Sponsor for analysis. Instructions for returning the investigational device(s) will be provided by the Sponsor. If it is not possible to return the device, the Investigator should document why the device was not returned and the final disposition of the device. Device failures and malfunctions should also be documented in the subject's medical record.

Device deficiencies, failures, malfunctions, and product nonconformities are not to be reported as adverse events. However, if there is an adverse event that results from a device failure or malfunction, that specific event would be recorded on the appropriate eCRF.

B.10 List of Investigator Responsibilities

The Principal Investigator's responsibilities include, but are not limited to, the following.

- Prior to beginning the study, sign the Investigator Agreement and Protocol Signature page documenting his/her agreement to conduct the study in accordance with the protocol.
- Provide his/her qualifications and experience to assume responsibility for the proper conduct of the study and that of key members of the center team through up-to-date curriculum vitae or other relevant documentation and disclose potential conflicts of interest, including financial, that may interfere with the conduct of the clinical study or interpretation of results.
- Make no changes in or deviate from this protocol, except to protect the life and physical well-being of a subject in an emergency; document and explain any deviation from the approved protocol that occurred during the course of the clinical investigation.
- Create and maintain source documents throughout the clinical study and ensure their availability with direct access during monitoring visits or audits; ensure that all clinical-investigation-related records are retained per requirements.
- Ensure the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor in the CRFs and in all required reports.
- Record, report, and assess (seriousness and relationship to the device/procedure) every adverse event and observed device deficiency.
- Report to the Sponsor, per the protocol requirements, all SAEs and device deficiencies that could have led to a SADE.
- Report to the EC/IRB any SAEs and device deficiencies that could have led to a SADE, if required by the national regulations or this protocol or by the EC/IRB, and supply the Sponsor with any additional requested information related to the safety reporting of a particular event.
- Maintain the device accountability records as needed.
- Allow the Sponsor to perform monitoring and auditing activities, and be accessible to the monitor and respond to questions during monitoring visits.
- Ensure that informed consent is obtained in accordance with this protocol and local EC/IRB requirements.
- Provide adequate medical care to a subject during and after a subject's participation in a clinical study in the case of adverse events, as described in the ICF.
- Inform the subject of the nature and possible cause of any adverse events experienced.
- Inform the subject of any new significant findings occurring during the clinical investigation, including the need for additional medical care that may be required.

- Ensure that clinical medical records are clearly marked to indicate that the subject is enrolled in this clinical study.
- Inform, with the subject's approval or when required by national regulations, the subject's personal physician about the subject's participation in the clinical investigation.
- Make all reasonable efforts to ascertain the reason(s) for a subject's premature withdrawal from clinical investigation while fully respecting the subject's rights.

Ensure that an adequate investigation site team and facilities exist and are maintained and documented during the clinical investigation.

When specific tasks are delegated by an Investigator, included but not limited to conducting the informed consent process, the Investigator is responsible for providing appropriate training and adequate supervision of those to whom tasks are delegated. The Investigator is accountable for regulatory violations resulting from failure to adequately supervise the conduct of the clinical study.

B.11 Confidentiality

All information and data sent to the Sponsor concerning subjects or their participation in this study will be considered confidential by the Sponsor. Only authorized Sponsor personnel or a Sponsor representative will have access to these confidential records. Study data collected during this study may be used by the Sponsor for the purposes of this study, publication, and to support future research and/or other business purposes. All data used in the analysis and reporting of this study will be without identifiable reference to specific subject name.

The Sponsor will keep subjects' health information confidential in accordance with all applicable laws and regulations. The Sponsor may use subjects' health information to conduct this research, as well as for additional purposes, such as overseeing and improving the performance of its device, new medical research and proposals for developing new medical products or procedures, and other business purposes. Information received during the study will not be used to market to subjects; subject names will not be placed on any mailing lists or sold to anyone for marketing purposes.

B.12 Monitoring

Regular on site monitoring by the Sponsor will not be planned during the study, but occasional visits by the Sponsor may be conducted as a measure of quality assurance. However, the study database will be monitored to ensure that data are reported in a satisfactory manner with respect to timeliness, adequacy, and accuracy. Sites will be queried in the case of any missing, incomplete or inconsistent data.

The Investigator/institution guarantees direct access to original source documents by Sponsor personnel and their designees if required during a monitoring visit or quality assurance audit.

B.13 Enrollment Controls

The Sponsor Project Manager will have responsibility to make every effort possible to avoid over-enrollment. To that end, the Sponsor Project Manager will keep all participating centers informed of enrollment status on a regular basis. An enrollment ceiling will be established for each center as well as for the study as a whole.

When an investigational site is within 3 patients of its enrollment ceiling, the Sponsor Project Manager will ask that the site contact him/her PRIOR to each new patient enrollment.

When overall enrollment of the study is within 15 patients of the enrollment ceiling, the Sponsor Project Manager will ask that each site contact him/her PRIOR to each new patient enrollment. Alerts will be sent to all enrolling sites after each new enrollment starting when overall enrollment of the study is within 5 patients of the enrollment ceiling.

When the last patient is enrolled, all investigational sites will immediately be notified of with an "Enrollment complete. Stop Enrollment" message by the Sponsor Project Manager.

B.14 Suspension or Termination

The Sponsor reserves the right to terminate the study at any stage but intends to exercise this right only for valid scientific or administrative reasons and reasons related to protection of subjects. Investigators and associated ECs/IRBs will be notified in writing in the event of study termination. Possible reasons for premature study termination include, but are not limited to, the following:

- The occurrence of unanticipated adverse device effects that present a significant or unreasonable risk to subjects enrolled in the study.
- An enrollment rate far below expectation that prejudices the conclusion of the study.

Any Investigator or EC/IRB in this study may discontinue participation in the study or withdraw approval of the study, respectively, with suitable written notice to the Sponsor. Investigators and associated ECs/IRBs will be notified in writing in the event of these occurrences.

In the event of premature study termination a written statement as to why the premature termination has occurred will be provided to all participating centers by the Sponsor. The ECs/IRBs will be notified. Detailed information on how enrolled subjects will be managed thereafter will be provided.

Detailed information on how enrolled subjects will be managed thereafter will be provided by the Sponsor.
In the event an Investigator terminates participation in the study, study responsibility will be transferred to a co-Investigator, if possible. In the event there are no opportunities to transfer Investigator responsibility, detailed information on how enrolled subjects will be managed thereafter will be provided by the Sponsor.

The Investigator must return all documents and investigational product to the Sponsor, unless this action would jeopardize the rights, safety, or welfare of the subjects.

The Sponsor reserves the right to stop the inclusion of subjects at a study center at any time after the study initiation visit if no subjects have been enrolled for a period beyond 2 months after center initiation, or if the center has multiple or severe protocol violations/non-compliance without justification and/or fails to follow remedial actions. In the event of termination of Investigator participation, the EC, as applicable, should be notified.

B.15 Publication Policy

In accordance with the Corporate Policy on the Conduct of Human Subject Research, the Sponsor requires disclosure of its involvement as a Sponsor or financial supporter in any publication or presentation relating to a Sponsor study or its results. In accordance with the Corporate Policy for the Conduct of Human Subject Research, the Sponsor will submit study results for publication (regardless of study outcome) following the conclusion or termination of the study. The Sponsor adheres to the Contributorship Criteria set forth in the Uniform Requirements of the International Committee of Medical Journal Editors (ICMJE; http://www.icmje.org). In order to ensure the public disclosure of study results in a timely manner, while maintaining an unbiased presentation of study outcomes, Sponsor personnel may assist authors and Investigators in publication preparation provided the following guidelines are followed.

- All authorship and contributorship requirements as described above must be followed.
- Sponsor involvement in the publication preparation and the Sponsor Publication Policy should be discussed with the Coordinating Principal Investigator(s) at the onset of the project.
- The First and Senior authors are the primary drivers of decisions regarding publication content, review, approval, and submission.

APPENDIX C: STATISITICS AND DIAGNOSTIC ACURACY MEASURES

C.1 Meta-Analysis of Nine References

Based on published success rates in references 1, 3, 5, 6, 7, 8, 9, 10 and 11 as provided in **Figure 5** in **Section 9.1** above:

	proportion	95%-CI %	W(fixed) %	W(random)
1	0.9732	[0.9237; 0.9944]	11.72	11.31
2	0.8378	[0.6799; 0.9381]	3.94	10.41
3	0.5259	[0.4621; 0.5890]	26.14	11.59
4	0.6726	[0.5779; 0.7579]	11.83	11.32
5	0.8400	[0.7532; 0.9057]	10.48	11.25
6	0.7009	[0.6048; 0.7856]	11.20	11.29
7	0.8696	[0.7668; 0.9386]	7.26	11.01
8	0.6250	[0.5351; 0.7090]	13.38	11.37
9	1.0000	[0.9075; 1.0000]	4.05	10.44

Number of trials combined: 9

	proportion	95%-CI	z	p.value
Fixed effect model	0.7393	[0.7111; 0.7665]	NA	--
Random effects model	0.8038	[0.6746; 0.9066]	NA	--

C.2 Sample Size Calculation

Primary endpoint considerations:

Two null hypotheses are made:

- H_{01} : Mean volume ratio of 19 G Flex Arm and 22 G Arm are the same
- H_{02} : Mean volume ratio of 19 G Arm and 22 G Arm are the same

The alternative hypothesis is:

- H_1 : Mean volume ratio of 19 G Flex/19G Arm is superior to 22 G Arm

Superiority by 10% or more is deemed clinically significant. Statistical power of 90% is chosen using the multiple comparison Hochberg's method. Test results are shown below, resulting in the requirement for 50 patients for 19 G Flex Arm and 25 patients each for 19 G and 22G Arm.

Hypothesis:

H_i: $\mu_i - \mu_0 \leq 0$ Versus K_i: $\mu_i - \mu_0 > 0$ for i=1,2.

Design Parameters:

One-sided Type I Error=0.05
Multiple comparison procedure= Hochberg's step up
Total Sample required=100
Rejection region=Right tail

Treatment parameters:

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

Overall:
Global (reject anyH_i): 0.912
Conjunctive (reject all H_i): 0.901

Secondary endpoint c) considerations:

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. Statistical power of 80% is chosen using the multiple comparison Hochberg's method. Test results are shown below, resulting in the requirement for 121 patients for 19 G Flex arm and 61 patients each for 19 G and 22G arm.

Hypothesis:

H_i: $\pi_i - \pi_0 \leq 0$ Versus K_i: $\pi_i - \pi_0 >$ for i=1,2

Design Parameters:

One-sided Type I Error=0.05
Multiple comparison procedure= Hochberg's step up
Total Sample required=243
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:
Global (reject any H_i): 0.947
Conjunctive (reject all H_i): 0.808

C.3 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. [4] 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 patients 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