Clinical Study Protocol

Study Title: Evaluation of Applaud Medical's Acoustic Enhancer with Laser Lithotripsy in the Treatment of Urinary Stones

Short Title: Acoustic Enhancer Research on Laser Lithotripsy (AEROLITH) Clinical Study

Protocol No: CIP-0001, **Revision:** E

IDE Number: G200183

Study Sponsor
Applaud Medical, Inc.
953 Indiana Street
San Francisco, CA 94107

Study Contact
Tessa Yamut
Executive Vice President of Regulatory, Clinical, and Quality Affairs
Applaud Medical, Inc.
Tel. (408) 768-5125
E-mail: tessa.yamut@applaudmedical.com

Investigator Protocol Signature Page

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I agree to conduct the investigation in accordance with the Acoustic Enhancer Research on Laser Lithotripsy Clinical Study Protocol.

Investigator Signature: _____

Investigator Name (Please Print or Type): _____

Date: _____

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PROTOCOL SYNOPSIS

Protocol Title:	EVALUATION OF APPLAUD MEDICAL'S ACOUSTIC ENHANCER WITH LASER LITHOTRIPSY SYSTEM IN THE TREATMENT OF URINARY STONES **Short Title:** Acoustic Enhancer Research on Laser Lithotripsy (AEROLITH) Clinical Study
Protocol Number:	CIP-0001, Rev. E
Study Objective:	A pivotal study to evaluate the safety and effectiveness of Applaud Acoustic Enhancer when used in conjunction with conventional ureteroscopic laser lithotripsy (URS-LL) in the treatment of subjects with urinary stones
Study Design:	Prospective, multi-center, two-arm, randomized, double blinded study
Device Name:	Applaud Acoustic Enhancer
Intended Use:	The Applaud Acoustic Enhancer is indicated for use in ureteroscopic laser lithotripsy for the fragmentation of calcium-based urinary stones. When the Acoustic Enhancer is used in a laser lithotripsy procedure, Acoustic Enhancer microparticles generate additional stone-fragmenting shockwaves.
Device Description:	Applaud's Acoustic Enhancer is provided in a lyophilized form. After reconstitution, it is a liquid, containing micron-scale particles that are made of a perfluoroalkane gas core with a lipid shell. The device is intended to be used with cleared/approved pulsed laser systems in fragmenting urinary stones (calculi) in the upper (superior) pole, lower (inferior) pole, and interpolar region of the kidney, pelvis of the kidney, and proximal ureter.
Study Groups	Investigational Arm: Acoustic Enhancer with URS-LL Control Arm: URS-LL
Sample Size and Number of Sites:	196 randomized subjects will be enrolled in this study at up to 18 investigational sites located in the U.S. Each site may enroll no more than 20% of the total sample size of 196 subjects.
Study Duration:	The anticipated timeline for this study is as follows: Subject Enrollment: 12 months Follow up Visits: ● 14 Day telephone call follow up for adverse event monitoring ● 30 Day on site follow up visit ● 90 Day on site follow up visit for subjects who have unresolved hydronephrosis at the 30-Day visit
Patient Population:	Male or female subjects 18 to 75 years of age with a diagnosis of urinary stone disease

Inclusion / Exclusion Criteria	**Inclusion Criteria:** 1. Male or female aged ≥ 18 years to ≤ 75 years 2. Provides written informed consent 3. Patients with at least one urinary stone measuring 6mm or greater (but no more than a cumulative diameter of 20mm) located proximally to the iliac vessels on one side may be treated. ***Note 1:*** *Punctate stones measuring ≤ 2mm do not count in in the cumulative diameter limit.* ***Note 2:*** *Some corroborating imaging may be required to exclude a patient if the investigator suspects that the stone has moved to middle/distal ureter (e.g. change in symptoms, an increase in serum creatinine).* 4. Urinary stone(s) should be apparent on a CT scan within 60days prior to study enrollment. Stone assessment will be conducted using CT imaging following CLIN-0025: AEROLITH IDE Study Screening Guide. ***Note 1:*** *Soft tissue window will be used for stone measurement. Window width/level adjustments will be applied as necessary by the reviewer to improve stone evaluation. A bone window may help distinguish stones from ureteral stents. The region of interest will be magnified (approximately 400%) by the reviewer to clearly visualize the stone outline. For details, please refer to CLIN-0003.* ***Note 2:*** *If CT imaging has not been conducted yet for the patient, please follow CT image acquisition parameters detailed in CLIN-0002: Image Acquisition Protocol, AEROLITH Study* 5. Patients with bilateral stones are allowed but only one side may be treated. Only the treated side will be evaluated for safety and effectiveness. 6. Patients may enter the study with a stent in place. 7. Patients presenting with absence of a Urinary Tract Infection as confirmed using urinalysis **Note:** If the urinalysis is more than 7 days removed on the day of the procedure, a urinalysis and confirmation that the patient is free of UTI symptoms should be performed on the day of the procedure for the patient to be eligible to participate in the study.

	Exclusion Criteria: 1. Patients with stones exceeding a cumulative diameter of 20mm on the side to be treated. ***Note:*** Punctate stones measuring ≤ 2mm do not count in the cumulative diameter limit. 2. Patients with ureteral stones located distal to the iliac vessels on the side to be treated 3. Diagnosis of radiolucent stones (on the side to be treated) on KUB or Scout CT Imaging 4. For patients who have a ureteral stent in place, patient is excluded if stent is calcified or encrusted as verified using standard of care imaging (e.g., KUB X-ray) 5. Patients who have had prior URS-LL within 3 months on the side to be treated at the time of consent 6. History of cystinuria 7. Urine pH is < 5.5. ***Note:*** If a patient's largest stone measures a mean stone intensity of ≥ 500HU and the patient has a historical pH value of ≥ 5.5, a recently tested (within 30 days of consent) urine pH showing <5.5 in this case does not exclude a subject. 8. Patients with known history of recurrent uric acid stones 9. Untreated urinary tract infection (UTI) 10. History of drug resistant chronic UTI 11. If female, pregnant as confirmed using urine or serum test to be conducted on the day of the procedure 12. Patient has an American Society of Anesthesiologists (ASA) physical classification level of 4 or greater. 13. Known sensitivity to possible medications used before, during, or after the URS Laser Lithotripsy procedure, including but not limited to the following: sedative agents, general anesthetics, topical anesthetics, and opioid analgesics 14. Stones suspected in calyceal diverticula 15. Horseshoe kidney 16. Congenitally ectopic pelvic kidneys 17. Full staghorn calculi >2cm 18. Patients with elevated serum creatinine > 1.5mg/dl 19. Patients with a solitary kidney 20. Malrotated kidney on the side with urinary stone 21. Duplicated collecting system or duplicated ureters 22. Patients who are currently involved in any investigational drug or device trial or have been enrolled in such trials within 30 days of index procedure 23. Patients who are actively taking antiplatelet and anti-coagulation except low dose aspirin 24. Prostate biopsy within the last 3 months 25. History of radiation therapy of abdomen and pelvis 26. History of urinary tract reconstruction

	27. Other factors that the investigator feels would interfere with the participation and completion of the study such as: ● Inability to provide voluntary consent ● Inability to understand the clinical investigation or cooperate with investigational procedures ● Planned relocation or unable to return for required follow-up visits ● Vulnerable individuals (mentally disabled, physically disabled, prisoner, etc.)
Primary Safety Endpoint:	The rate of occurrence of each arm of clinically significant serious adverse events defined as: • Serious infection (urosepsis, pyelonephritis) requiring hospitalization and treatment through 90 days post index procedure • New urinary tract stricture formation requiring surgical intervention through 90 days post index procedure Confidence intervals will be presented to aid in the evaluation of the incidence of clinically significant serious adverse events, both for each treatment group individually, and for the difference in the rates between the two treatment groups.
Primary Efficacy Endpoint:	Treatment success is defined as the proportion of study subjects who have a complete absence of stones or have residual fragments measuring ≤ 2 mm on the treatment side, as assessed by CT imaging through 30-day follow-up post index procedure. The primary efficacy endpoint will demonstrate that the investigational arm (Acoustic Enhancer with URS-LL) is superior in the fragmentation of stones to ≤ 2mm versus the control arm (URS-LL).
Secondary Efficacy Endpoint:	Total radiant energy (TRE): Mean value of laser energy, measured for each subject in kilojoules divided by the stone cross-sectional area. The secondary endpoint will demonstrate non-inferiority of the mean TRE used between the investigational arm and the control arm.
Additional Observations:	● Mean total lasering time ● Additional interventional stone procedures for stone(s) that were treated as part of the AEROLITH study. ● Adverse Events by type, over time, severity, seriousness, and relatedness. AEs will be tabulated and summarized as counts and percentages. AEs will also be cross tabulated according to: - Severity - Unanticipated Adverse Device Effect (UADE) - Seriousness (Serious Adverse Event (SAE), Non-serious AE) - Device-Relatedness (Unrelated, Possibly Related, Probably Related, Definitely Related) - Procedure-Relatedness (Unrelated, Possibly Related, Probably Related, Definitely Related)
Statistical Analyses / Sample Size	A sample size of 196 subjects randomized 1:1 to the investigational arm (Acoustic Enhancer with URS-LL) and the control arm (URS-LL) was selected based on consideration of the primary endpoint. Assuming no more than 10% non-evaluable subjects, this

Justification:	provides a randomized sample size of 176 subjects (88 per arm). The primary hypothesis test for the treatment success outcome is as follows: H_0: $p_{AE} \leq p_{Control}$ vs H_1: $p_{AE} > p_{Control}$ A one-sided p-value of 0.025 on a Farrington-Manning test will be used test will be used to indicate statistical significance. Assuming a response rate of 55% in the control arm and 75% in the investigational arm, the sample size of 88 per group provides 80% power for the primary hypothesis. Adjusted for a 10% drop-out, a total sample size of 196 was proposed.
Study Success	The study is considered a success if the primary effectiveness endpoint is met with statistical significance and any safety findings are offset by the benefit in improved response when risks and benefits are weighed. Statistical significance for the primary effectiveness endpoint is achieved when the one-sided p-value for the Farrington-Manning test when comparing the Treatment Success rates between the investigational arm (Acoustic Enhancer with URS-LL) and the control arm (URS-LL) is less than 0.025. Statistical significance among any of the other effectiveness endpoints is ***not*** needed for Study Success.
References:	CLIN-0001: Acoustic Enhancer Patient Informed Consent CLIN-0002: Image Acquisition Protocol, Acoustic Enhancer Research in Laser Lithotripsy CLIN-0003: Radiographic Evaluation Protocol of Applaud Medical's Acoustic Enhancer with Laser Lithotripsy System in the Treatment of Urinary Stones LBL-014: Acoustic Enhancer Instructions for Use CLIN-0004: Acoustic Enhancer Clinical Study Investigator Agreement CLIN-0005: Interim Clinical Report for Applaud Acoustic Enhancer with Ureteroscopic Laser Lithotripsy

ABBREVIATIONS

AE	Adverse Event
ASA	American Society of Anesthesiologists
ASADE	Anticipated Serious Adverse Device Effect
BMI	Body Mass Index
CIP	Clinical Investigation Plan
CT	Computed Tomography
DMP	Data Management Plan
EC	Ethics Committee
eCRF	Electronic Case Report Form
IFU	Instructions for Use
IRB	Institutional Review Board
ISO	International Organization for Standardization
PCNL	Percutaneous Nephrolithotomy
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SWL	Shockwave Lithotripsy
TGA	Therapeutic Goods Administration
UADE	Unanticipated Adverse Device Effect
USADE	Unanticipated Serious Adverse Device Effect
URS	Ureteroscopy

PRINCIPAL CONTACTS

	Name, Address and Contact
Principal Investigator	TBD
Study Sponsor Contact	Tessa Yamut Executive Vice President of Regulatory, Clinical, and Quality Affairs Applaud Medical, Inc. 953 Indiana St. San Francisco, CA 94107 Tel. (408) 768-5125 E-mail: tessa.yamut@applaudmedical.com
Study Statistician	Advanced Research Associates 2350 Mission College Blvd, #825 Santa Clara, CA 95054
Medical Monitor	J. Stuart Wolf, Jr, MD, FACS Professor Executive Associate Chair Associate Chair for Clinical Integration and Operations Chief, Division of Surgical Subspecialties Department of Surgery and Perioperative Care Dell Medical School The University of Texas at Austin
Data Management	Advanced Research Associates 2350 Mission College Blvd, #825 Santa Clara, CA 95054
Imaging Core Lab	Medical Metrics, Inc. 2121 Sage Road, Suite 300 Houston, TX 77056

1. INTRODUCTION

1.1. Background Information

Urinary stone disease is a prominent and growing public health priority, both in the U.S. and globally [1], [2]. Lifetime prevalence of urinary stones is approximately 1 in 11 in the U.S. general population [1], with rates of emergency department visits for urinary stone disease more than doubling in the past twenty-five years [3]. Some one-half of subjects with previous urinary stones experience a recurrence within 10 years [2], [4]. Stone disease is associated with significant morbidity: many stones require surgical intervention, while those that pass without surgery routinely take many weeks to do so, a period of debilitating pain and risks including life-threatening sepsis from stone-associated obstruction [5].

Stones typically form in the renal collecting system (as urinary stones), with onset of severe—and frequently excruciating—flank pain and other symptoms triggered by one or more stones passing from the kidney into the ureter and being retained at a narrowing such as the ureteropelvic junction, over the iliac vessels, or at the ureteric meatus [5]. Imaging-based diagnosis of urinary stones is typically by non-contrast CT, although ultrasound can be effective as a radiation-sparing diagnostic imaging modality [6]. Clinical management of ureteric stones includes observation, typically with pain management; observation with medical expulsive therapy; and surgical exposure of ureter and endoscopic stone removal (ureterolithotomy); factors including ureteral anatomy and degree of obstruction; symptom severity and symptom manageability through pain medication; occurrence of infection or other comorbidities; and stone size, composition, and location are taken into account in physician-patient decision making around the urgency of surgical intervention and evaluating among possible surgical intervention options [5], [7].

Current mainstay minimally invasive surgical interventions for urinary stones—shockwave lithotripsy (SWL), ureteroscopy (URS) with laser lithotripsy, and percutaneous nephrolithotomy (PCNL)— can be effective in shortening the time required for many patients to become stone-free, while at the same time having significant drawbacks. PCNL, the most invasive of the mainstream non-open-surgery methods, is associated with risk of injury to organs nearby the kidney and ureter including the lung, liver, spleen, and bowel and has been reported to have a 4% transfusion rate. URS almost invariably entails general anesthesia, with its associated risks for the patient as well as its implications for healthcare system cost and need for potentially scarce operating theatre infrastructure; the ureteral manipulation in URS, moreover, is associated with long recovery times and significant post-operative pain as well as risk of urinary stricture and other complications [8], [9]. These downsides of URS are counterbalanced by the procedure having generally high effectiveness even after only a single treatment [10], although recent studies have noted that, for patients with stones larger than 5 mm, some 40-55% in fact have residual fragments 30 days after URS with laser lithotripsy when evaluated in follow-up by CT [11], [12].

SWL avoids both PCNL's percutaneous access and URS's ureteral manipulation, allowing it to be performed without general anesthesia in some cases and conferring further advantages—potentially significant ones—in minimizing recovery time [13], [14]. SWL's noninvasiveness is conducive to multiple treatments of a single stone over the course of several weeks without undue patient burden; the widely cited SWL treatment success rate of 64% [10], [15] reflects as many as three sequential SWL treatments. SWL is associated with rates of gross hematuria around 20% and reports of significant intra- and post-operative pain from the large (up to 3000) number of intense shockwaves administered to the patient by the SWL system's treatment head [7], [16], [17], partially offsetting the advantages associated with SWL's noninvasiveness. Taking into account the many and varied trade-offs with both URS and SWL, guidelines committees in the U.S., Europe, and elsewhere have generally elected to continue to recommend both as suitable first-line surgical interventions for most stone patients [8], [10].

For patients for whom immediate surgical intervention is not an absolute imperative, the prospect of achieving stone-free status quickly through URS or SWL is evaluated against the risks and costs of these interventions. Compelling intermediate options between surgical intervention (by URS or SWL) and watchful waiting with pain management are highly prized by urologists, patients, and other healthcare system stakeholders, but have remained elusive. Therapeutic regimens referred to as medical expulsive therapy (MET), typically incorporating the α-adrenoceptor antagonist tamsulosin and the calcium channel stabilizer nifedipine (a) can be used with the intention of increasing the likelihood of and shortening the time from symptom onset until spontaneous stone passage [8], [10]. Accumulating evidence from recent large-scale clinical trials, however, increasingly suggests that current mainstream MET regimens have little effect for most patients [18], [19].

1.2. Introduction to Applaud Acoustic Enhancer with Laser Lithotripsy and Study Rationale

Acoustic Enhancer is a product that is designed to improve patient outcomes in ureteroscopic laser lithotripsy (URS-LL). This improvement in patient outcomes derives from a shockwave multiplication effect: in a laser lithotripsy procedure that includes Acoustic Enhancer, many additional shockwaves are directed into the stone beyond the shockwaves directly produced by the laser pulse. The additional shockwaves emanate from pulsating microparticles contained within Acoustic Enhancer. The microparticles are engineered to pulsate in response to acoustic impulses associated with the laser pulse. A metric of particular importance, from the standpoint of improving patient outcomes in URS-LL with Acoustic Enhancer, is the absence of the residual fragments that can so readily lead to additional stone events.

The Acoustic Enhancer is a single-use device designed to be incrementally placed in the patient's urinary tract during a stone procedure. Reconstituted Acoustic Enhancer is a milky liquid with viscosity similar to water, physical properties that are conducive to placing small volumes of Acoustic Enhancer in the patient's urinary tract via the working channel of the ureteroscope at intervals over the course of the procedure. As in conventional URS-LL, a laser fiber is passed through the ureteroscope as the energy source.

The Acoustic Enhancer is designed to be used in procedures where ureteroscopic intervention is appropriate and to work with all commercially available lasers and laser fibers designed for URS-LL procedures. With these lasers designed to produce light at wavelengths readily absorbed by urine, each pulse of laser energy triggers the propagation of a burst of shockwaves into the urine around the fiber tip, with these bursts of shockwaves bringing about stone erosion, pitting and fragmentation [20]. In URS-LL procedures, the shockwave burst triggered by each pulse of laser energy also interacts with microparticles in Acoustic Enhancer, which are engineered to be acoustically responsive, emitting additional shockwaves toward stone surfaces and enhancing stone erosion, pitting and fragmentation. In bench studies, the presence of Acoustic Enhancer has been seen to confer additional stone erosion, pitting, and fragmentation functionality beyond that of the laser energy alone. While ureteroscopy with laser lithotripsy is routinely performed on stones spanning a wide range of sizes, residual fragments are particularly common in patients with stones that are greater than 6mm in any dimension [11], motivating the selection of a 6mm size threshold for inclusion in this study.

Applaud Medical is conducting feasibility studies to evaluate the safety and efficacy of its Acoustic Enhancer when used with URS-LL in patients with urinary stone disease. Ongoing clinical study (protocol number 2017-02) in India, has demonstrated preliminary evidence of safety and efficacy of the Acoustic Enhancer when used with conventional laser lithotripsy. A total of twenty-seven (N=27) subjects were treated with Applaud Acoustic Enhancer with URS-LL. Although there were 3 adverse events (AEs) in a total of 2 subjects that were reported, none of these events were serious in nature and none were related to the device and/or procedure as assessed by the investigator. All adverse events resolved within 5 days of onset.

The study also demonstrated preliminary evidence of stone fragmentation. In the cohort of subjects (n=16) that were treated with standardized procedure and laser parameters, 13 subjects had evaluable CT imaging. All 13 subjects (100%) demonstrated fragmentation of stones with a baseline average stone size of 8.53mm, and a post procedure average residual stone size of 1.44mm. Ten subjects (76.9%) showed significant stone fragmentation with residual fragments measuring < 2mm post procedure.

These preliminary results demonstrate that the use of Applaud Acoustic Enhancer in this study was safe for use with URS-LL.

Refer to the Device Description section for a more in-depth description and also refer to the IFU for technical details and procedure for operation.

2. DEVICE DESCRIPTION

2.1. Applaud Acoustic Enhancer

Applaud Acoustic Enhancer is supplied lyophilized in a vial. After reconstitution at the clinic, Applaud Acoustic Enhancer is a liquid containing micron-scale particles within a matrix consisting mostly of water. The presence of the particles gives Acoustic Enhancer a milky appearance Figure 1, although the particles are sufficiently small and sparse as to have a minimal effect on viscosity; Acoustic Enhancer's viscosity is similar to that of water alone. As will also be further described below, to use Acoustic Enhancer in a urinary stone procedure, multiple small volumes of Acoustic Enhancer are placed in the patient's urine over the course of the procedure. Acoustic Enhancer's comparatively low viscosity facilitates the placement of Acoustic Enhancer in the patient's urine by attaching a syringe to a ureteroscope.

Each Acoustic Enhancer microparticle has a perfluoroalkane gas core with a lipid shell. Acoustic Enhancer lipids closely resembles naturally occurring lung surfactants, while perfluoroalkane gases are routinely used as tamponades in surgery to correct retinal detachment. The microparticle lipid shell has also been engineered to mimic certain structures in the naturally occurring category of molecules known as pyrophosphates. Collectively, the design of the microparticle structure confers two key functions: a capacity to dramatically expand and contract in response to changes in the local pressure environment and a tendency to accumulate on the surfaces of urinary stones containing biomineralized calcium.

The Acoustic Enhancer is supplied in vials in a lyophilized form referred to as Lyophilized Acoustic Enhancer (AM-005). The Acoustic Enhancer material is prepared at the clinical site immediately prior to use through dilution and mixing of the supplied product. See the Appendix of the IFU for preparation instructions of the Applaud Acoustic Enhancer material.

2.2. Mechanism of Action

In laser lithotripsy treatments for urinary stones, pulses of laser energy vaporize volumes of urine, producing shockwaves rippling toward the stone. Because stones are hard and brittle, they are susceptible to progressive erosion, pitting and fragmentation from shockwaves produced by laser pulse urine vaporization. This is the normal mechanism of action by which stones are broken up in laser lithotripsy [20][26].

Acoustic Enhancer is designed to improve patient outcomes in laser lithotripsy by a shockwave multiplication effect: in a laser lithotripsy procedure that includes Acoustic Enhancer, many additional shockwaves are directed into the stone beyond the shockwaves produced by the original urine vaporization by each laser pulse. The additional shockwaves emanate from pulsation of specially engineered microparticles contained within Acoustic Enhancer.

Acoustic Enhancer is a liquid with a milky appearance. The milky appearance comes from millions of micron-scale particles, each with a lipid shell and a gas core—the microparticles that pulsate to produce additional shockwaves beyond those produced by laser-pulse urine vaporization. Acoustic Enhancer microparticles are spaced, on average, at approximately four-micron intervals within Acoustic Enhancer. The remainder of Acoustic Enhancer is mostly water, and the microparticles have a minimal effect on viscosity; Acoustic Enhancer's viscosity is similar to that of water alone. Acoustic Enhancer's comparatively low viscosity facilitates its placement in the patient's urine via the working channel of standard ureteroscopes used in laser lithotripsy procedures.

Using Acoustic Enhancer in a laser lithotripsy procedure entail placing multiple small volumes of Acoustic Enhancer in the patient's urine over the course of the procedure. Acoustic Enhancer improves erosion, pitting and fragmentation through the action of the laser energy on Acoustic Enhancer microparticles on or near urinary stone surfaces.

The lipid shell-gas core structure of Acoustic Enhancer microparticles has been engineered to confer a specific mechanical response—rapid, large-amplitude expansion and contraction—when the particle is subjected to longitudinal pressure waves with high-frequency components. The shell structure incorporates a core building block phospholipid material along with two phospholipid-polymer materials, while the core is a gas similar to the gases used as ocular tamponades.

During a conventional laser lithotripsy procedure, transient urine vaporization produces pronounced changes in the local pressure field from intense, rapidly propagating longitudinal pressure waves (shockwaves) produced by the motion of the urine phase boundary. As noted above, hard, brittle urinary stones are susceptible to progressive erosion, pitting and fragmentation from these shockwaves; the laser achieves its intended effect through mechanical effects.

Placement of small volumes of Acoustic Enhancer around the stone at intervals over the course of the procedure has a shockwave multiplication effect, as the laser-generated shockwaves (from the laser pulse's transient urine vaporization) act on both the stone and on the Acoustic Enhancer microparticles. For the microparticles, the changes in local pressure can bring about many-fold expansion and contraction—50-fold microparticle expansion followed by collapse is routinely observed in Applaud's labs using high-speed video microscopy [27]. This rapid large amplitude microparticle pulsation (expansion and contraction) itself produces shockwaves; these shockwaves, described as cavitation effects, enhance stone erosion, pitting and fragmentation. Results from experiments using a benchtop model system for URS-LL demonstrate a reduction in stone size when Acoustic Enhancer is added to laser lithotripsy (unpublished Applaud bench data and Wiener et al., 2019). Note that because Acoustic Enhancer's action on stones is wholly mechanical and occurs upon energization from the laser-generated shockwaves, there is no stone mass reduction from placing Acoustic Enhancer around a stone without laser energization.

Acoustic Enhancer is supplied in a lyophilized form. It undergoes a short preparation process at the clinic that includes mixing with Sterile Water for Injection to yield a defined quantity available to achieve a defined number of microparticles per volume of final mixed product. Preparation instructions are described in the Acoustic Enhancer Instructions for Use.

2.3. Device Accountability

Investigational product will be packaged in order to maintain product integrity during shipping. All investigational product will be labeled as being for Investigational Use Only. Device accountability will be maintained on a dedicated log at the site of product storage including the date and quantities of Acoustic Enhancer vials received, dispensed, and returned. Information regarding the specific identification numbers for Acoustic Enhancer vials used are to be recorded onto the appropriate case report form (CRF) for each study subject undergoing the treatment procedure throughout the course of the study. Only an appropriately qualified person may dispense the study device to subjects in the study. The study device is to be used in accordance with the protocol under the direct supervision of the study investigator. The Acoustic Enhancer must be stored and used per the Instructions for Use (IFU).

Unused Acoustic Enhancer vials will be returned to the Sponsor at the completion of the study.

3. INTENDED USE STATEMENT

The Acoustic Enhancer is indicated for use in ureteroscopic laser lithotripsy for the fragmentation of calcium-based urinary stones. When the Acoustic Enhancer is used in a laser lithotripsy procedure, Acoustic Enhancer microparticles generate additional stone-fragmenting shockwaves.

4. PRIOR CLINICAL INVESTIGATION

Applaud Medical is conducting feasibility studies to evaluate the safety and efficacy of its Acoustic Enhancer when used with ureteroscopic laser lithotripsy in patients with urinary stone disease. An ongoing clinical study (protocol number 2017-02) in India at Muljibhai Patel Urological Hospital (MPUH) has demonstrated preliminary evidence of safety and efficacy of the Acoustic Enhancer when used with conventional laser lithotripsy. A total of twenty-seven (N=27) subjects were treated with Applaud Acoustic Enhancer with URS-LL. Although there were 3 adverse events (AEs) in a total of 2 subjects that were reported, none of these events were serious in nature and none were related to the device and/or procedure as assessed by the investigator. All adverse events resolved within 5 days of onset.

The study also demonstrated preliminary evidence of stone fragmentation. A first cohort of six (n=6) were treated with variations of the procedure and were relied upon to establish a set of standardized procedures and laser parameters for the next sixteen (n=16) subjects.
A second cohort of sixteen (n=16) subjects were treated with standardized procedures and laser parameters. In this cohort, 13 of the 16 subjects treated had evaluable post-procedure CT imaging. All 13 demonstrated fragmentation of stones with a baseline average stone size of 8.53 mm, and a post procedure average stone size of 1.44mm. 76.9% (n=10) showed significant stone fragmentation with all residual fragments measuring ≤ 2mm post procedure.

A third cohort of five (n=5) subjects was treated with variations of the procedures and laser power and pulse timing parameters enabled by availability of a new laser at MPUH. These subjects were treated with combinations of shorter pulses with differing energy levels to help refine procedures for the FDA IDE clinical study.

These results demonstrate preliminary evidence that the use of Applaud Acoustic Enhancer in this study was safe for laser settings encompassing fragmentation and dusting.

Please refer to Interim Clinical Report for Applaud Acoustic Enhancer with ureteroscopic laser lithotripsy (CLIN-0005) for details.

5. PROTOCOL

5.1. Study Objective

The purpose of this pivotal study is to evaluate the safety and effectiveness of Applaud Acoustic Enhancer when used in conjunction with conventional ureteroscopic laser systems in the treatment of subjects with urinary stones.

5.1.1. Study Design Overview

The AEROLITH Clinical Study will evaluate the safety and effectiveness of Applaud Acoustic Enhancer when used in conjunction with conventional ureteroscopic laser lithotripsy (URS-LL) in the treatment of subjects with urinary stones. The clinical study is a prospective, multicenter, two-arm, randomized, double blinded study. A total of 196 subjects will be enrolled in this study at up to 18 investigational sites located in the U.S.

5.2. Study Design

The Acoustic Enhancer is a prospective, multi-center, two-arm, randomized, double blinded study. The subject population for this study is male or female subjects 18 to 75 years of age with a diagnosis of stone disease.

5.3. Study Scope and Participating Institutions

The Acoustic Enhancer Clinical study will include a maximum of 18 investigational sites within the United States. A separate list of participating institutions will be updated every 6 months and will be provided to regulatory authorities as required.

5.4. Patient Population

All subjects 18 to 75 years of age presenting with urinary stone disease may be eligible for study participation. Potential study candidates will be approached for consent prior to any data collection. Screening and enrollment data will be collected and monitored via an Electronic Data Capture (EDC) System. Subjects will be identified and recruited by site investigators and/or their designated research staff at facilities affiliated with one of the 18 investigational sites.

For the purposes of this study, enrollment is defined as at that time when the patient signs an Institutional Review Board (IRB)- approved informed consent form (thereafter becomes a study subject). All enrolled subjects will be evaluated for the primary endpoint at 30 days post procedure.

Any subject consented, whether treated or not, will be assigned a study identification number. The reason for failure to treat (i.e., screen failure, subject withdrawal, missed enrollment) will be recorded in his/her study records.

5.5. Number of Subjects

A sample size of 196 enrolled and randomized subjects (98 per arm) randomized 1:1 to the control arm and the investigational arm. Assuming no more than 10% non-evaluable subjects, this provides a randomized sample size of 176 subjects (88 subjects per arm). Each site may enroll no more than 20% of the total sample size of 196 subjects.

5.6. Study Duration

All subjects will be followed at 14 and 30 days post procedure. Subjects with new and unresolved device and/or procedure related serious adverse events at 30-days post procedure will continue to be followed until event has resolved or is deemed stable by the investigator.

Subjects with device and/or related hydronephrosis or pre-existing (prior to treatment) hydronephrosis that has worsened or recurred at 30 days will be followed through 90 days post- procedure.

At 90-days post procedure, subjects who have on-going device and/or procedure-related serious adverse events (including device and/or procedure related hydronephrosis) and subjects with pre- existing (prior to treatment) hydronephrosis that have worsened or recurred will continue to be followed until the event has resolved or is deemed stable by the investigator prior to study exit. For subjects with on-going, including stable, device and/or procedure-related serious adverse events (including device and/or procedure-related hydronephrosis and worsening/recurring hydronephrosis from baseline) at 90-days post procedure, the investigator should refer the subject for further care to the subject's primary care provider or urological specialist upon exiting the study.

5.7. Inclusion Criteria

5.7.1. Male or female aged ≥ 18 years to ≤ 75 years

5.7.2. Provides written informed consent

5.7.3. Patients with at least one urinary stone measuring 6mm or greater (but no more than a cumulative diameter of 20mm) located proximally to the iliac vessels on one side may be treated.

Note 1: *Punctate stones measuring ≤ 2mm do not count in the cumulative diameter limit.*

Note 2: *Some corroborating imaging may be required to exclude a patient if the investigator suspects that the stone has moved to middle/distal ureter (e.g. change in symptoms, an increase in serum creatinine).*

5.7.4. Urinary stone(s) should be apparent on a CT scan within 60 days prior to study enrollment.

Stone assessment will be conducted using CT imaging following CLIN-0025: AEROLITH IDE Study Screening Guide

Note 1: *Soft tissue window will be used for stone measurement. Window width/level adjustments will be applied as necessary by the reviewer to improve stone evaluation. A bone window may help distinguish stones from ureteral stents. The region of interest will be magnified (approximately 400%) by the reviewer to clearly visualize the stone outline. For details, please refer to CLIN-0003.*

Note 2: *If CT imaging has not been conducted yet for the patient, please follow CT image acquisition parameters detailed in CLIN-0002: Image Acquisition Protocol, AEROLITH Study*

5.7.5. Patients with bilateral stones are allowed but only one side may be treated. Only the treated side will be evaluated for safety and effectiveness.

5.7.6. Patient may enter the study with a stent in place.

5.7.7. Patients presenting with absence of a Urinary Tract Infection as confirmed using urinalysis

Note: *If the urinalysis is more than 7 days removed on the day of the procedure, a urinalysis and confirmation that the patient is free of UTI symptoms should be performed on the day of the procedure for the patient to be eligible to participate in the study.*

5.8. <u>Exclusion Criteria</u>

5.8.1. Patients with stones exceeding 20 mm in cumulative diameter on the side to be treated. ***Note:*** Punctate stones measuring ≤ 2mm do not count in the cumulative diameter limit.

5.8.2. Patients with ureteral stones located distal to the iliac vessels on the side to be treated

5.8.3. Diagnosis of radiolucent stones (on the side to be treated) on KUB or Scout CT Images

5.8.4. For patients who have a ureteral stent in place, patient is excluded if stent is calcified or encrusted as verified using standard of care imaging (e.g., KUB X-ray)

5.8.5. Patients who have had prior URS-LL within 3 months on the side to be treated at the time of consent

5.8.6. History of cystinuria

5.8.7. Urine pH is < 5.5. ***Note:*** If a patient's largest stone measures a mean stone intensity of ≥500 HU and the patient has a historical pH value of ≥ 5.5, a recently tested (within 30 days of consent) urine pH showing <5.5 in this case does not exclude a subject.

5.8.8. Patients with known history of recurrent uric acid stones.

5.8.9. Untreated urinary tract infection (UTI)

5.8.10. History of drug resistant chronic UTI

5.8.11. If female, pregnant as confirmed using urine or serum test to be conducted on the day of the procedure

5.8.12. Patient has an American Society of Anesthesiologists (ASA) physical classification level of 4 or greater.

5.8.13. Known sensitivity to possible medications used before, during, or after the URS laser lithotripsy procedure, including but not limited to the following: sedative agents, general anesthetics, topical anesthetics, and opioid analgesics

5.8.14. Stones suspected in calyceal diverticula

5.8.15. Horseshoe kidney

5.8.16. Congenital ectopic pelvic kidneys

5.8.17. Full staghorn calculi >2cm

5.8.18. Patients with elevated serum creatinine > 1.5mg/dl

5.8.19. Patients with solitary kidney

5.8.20. Malrotated kidney on the side with urinary stone

5.8.21. Duplicated collecting system or duplicated ureters

5.8.22. Patients who are currently involved in any investigational drug or device trial or have been enrolled in such trials within 30 days of index procedure

5.8.23. Patients who are actively taking antiplatelet and anti-coagulation except low dose aspirin

5.8.24. Prostate biopsy within the last 3 months

5.8.25. History of radiation therapy of abdomen and pelvis

5.8.26. History of urinary tract reconstruction

5.8.27. Other factors that the investigator feels would interfere with the participation and completion of the study such as:

- Inability to provide voluntary consent
- Inability to understand the clinical investigation or cooperate with investigational procedures
- Planned relocation or unable to return for required follow-up visits
- Vulnerable individuals (mentally disabled, physically disabled, prisoner, etc.)

6. STUDY ENDPOINTS

6.1. Primary Safety Endpoint

The rate of occurrence of each arm of clinically significant serious adverse events defined as:

- Serious infection (urosepsis, pyelonephritis) requiring hospitalization and treatment through 90 days post index procedure
- New urinary tract stricture formation requiring surgical intervention through 90 days post index procedure

Confidence intervals will be presented to aid in the evaluation of the incidence of clinically significant serious adverse events, both for each treatment group individually, and for the difference in the rates between the two treatment groups.

6.2. Primary Efficacy Endpoint

Treatment success: Proportion of study subjects who have a complete absence of stone(s), or have residual fragments measuring to ≤2mm, as assessed by CT imaging through 30 days follow-up post index procedure. CT imaging will be assessed by an independent core lab to determine treatment success. Treatment success, as defined by an absence of residual fragments >2mm on any dimension, has been identified as a priority in stone care in recent publications including Margaret Pearle's widely cited "Is Ureteroscopy as Good as We Think", published in the Journal of Urology in 2016 [12]. Increasing awareness and clinical prioritization of residual fragments in ureteroscopic laser lithotripsy derives in large part from expanded use of CT follow-up imaging in stone subjects. CT imaging has allowed characterization of rates of residual fragments and full appreciation of their clinical significance, with rigorous studies of residual fragment natural history having established a 2mm threshold as a critical size above which additional stone events are particularly frequent [22], [23]. Therefore, the primary efficacy endpoint will demonstrate that the investigational arm (Acoustic Enhancer +_URS-LL) is superior in the fragmentation of stones to ≤2mm versus the control arm (URS-LL).

6.3. Secondary Efficacy Endpoint

Total radiant energy: Mean value of laser energy, measured in kilojoules, with laser energy values recorded and displayed by the pulsed laser unit used in the procedure, with the laser energy value for each procedure normalized by the stone cross-sectional area. For this purpose, the stone cross- sectional area is calculated by taking the product of the two largest stone size measurements on the pre-procedure CT; for subjects with more than one stone treated in the index procedure, the stone cross-sectional area is calculated as the sum of the stone cross-sectional areas calculated individually for each treated stone. The mean value of laser energy for each arm is calculated by considering the normalized measured values

for all study subjects in that arm.

Total radiant energy is an indicator of how effectively the laser has eroded, pitted and fragmented the stone over the course of the procedure, reflecting the contributions of the diverse mechanisms through which the laser acts on the stone [24]. With Acoustic Enhancer's being designed to improve stone erosion, pitting and fragmentation through the generation of additional stone-fragmenting shockwaves; therefore, the secondary efficacy endpoint will demonstrate non-inferiority of the mean TRE used between the investigational arm and the control arm.

6.4. Additional Observations

6.4.1. Mean total lasering time. While total radiant energy is a more rigorous and broadly applicable indicator of improved stone erosion, pitting and fragmentation through the generation of additional stone-fragmenting shockwaves, a demonstration of lower mean lasering time in the Acoustic Enhancer arm compared to the standard laser arm could be clinically important.

6.4.2. Additional interventional stone procedure(s) on stone(s) that were treated as part of the AEROLITH study.

6.4.3. Adverse Events (AEs) by type over time, severity, seriousness, and relatedness. AEs will be tabulated and summarized as counts and percentages. AEs will also be cross tabulated according to:

- Severity
- Unanticipated Adverse Device Effect (UADE)
- Seriousness (Serious Adverse Event (SAE), Non-serious AE)
- Device-Relatedness (Unrelated, Possibly Related, Probably Related, Definitely Related)
- Procedure-Relatedness (Unrelated, Possibly Related, Probably Related, Definitely Related)

6.5. Measures to Increase Validity and Minimize Bias

6.5.1. Eligible subjects will be consecutively enrolled at each site. Reasons for screen failures will be documented.

6.5.2. Subjects will be blinded of their treatment allocation through index procedure.

6.5.3. Multiple sites will be utilized to ensure broad generalizability of results and minimize site effect.

6.5.4. CT image analysis will be performed by an independent core laboratory and they will be blinded to the treatment assignment. CT images will be obtained in accordance with guidelines specified in advance by the core laboratory.

6.5.5. The study monitor will review and verify data collection forms against source documentation to assure there are no missing, illegible, or incorrect data. Missing, illegible or incorrect data will be corrected following ICH/GCP guidelines.

6.5.6. The investigative site personnel will contact subjects prior to their scheduled study visits to increase compliance with the subject follow up protocol.

6.6. Study Success

The study is considered a success if the primary effectiveness endpoint is met with statistical significance and any safety findings are offset by the benefit in improved response when risks and benefits are weighed.

Statistical significance for the primary effectiveness endpoint is achieved when the one-sided p-value for the Farrington-Manning test when comparing the Treatment Success rates between the investigational arm (Acoustic Enhancer with URS-LL) and the control arm (URS-LL) is less than 0.025.

Statistical significance among any of the other effectiveness endpoints is *not* needed for Study Success.

7. STUDY PROCEDURES

7.1. Eligibility Review

Patients presenting with urinary stone disease will be screened for this study. Initial eligibility criteria for the study will be determined by the investigator based upon review of their medical history, presentation and diagnostic imaging. If the patient appears to meet the study criteria, the investigator will consent the patient accordingly.

7.2. Informed Consent Process

The informed consent document must comply with applicable regulatory guidelines (21CFR Part 50 and the Declaration of Helsinki). The investigator, or authorized designee, is responsible for obtaining written informed consent from each Patient using the Institutional Review Board (IRB) approved consent form prior to initiation of any study specific assessments or procedures.

The background of the proposed study, the potential benefits and risks, and all other legally required elements shall be carefully explained to the patient.

Before undergoing protocol required assessments, the subject or their legal representative must give witnessed written consent to participate in the study using the informed consent form approved by the Sponsor and clinical site's IRB.

A copy of the signed consent form shall be given to the subject with the original filed at the site.

Patients will be given adequate time to review the consent form, ask questions, and consider their options before being asked to sign the form. Informed consent shall be obtained under circumstances that minimize the possibility of coercion or undue influence. The information that is given to the patient shall be in language understandable to the patient. The patient will not be led to believe that they are waiving their rights as a study patient or the liability of the sponsor or investigator.

Patients will be informed that the Sponsor and regulatory authorities will have access to personally identifying information for the purposes of monitoring data against source documentation. However, all data stored and presented by the Sponsor will be de-identified.

If new information regarding the investigational device becomes available and/or the clinical investigational plan (CIP) changes and the information can significantly affect a Patient's future health and medical care, patients will be informed of the information and may be asked to sign a revised informed consent form.

The Sponsor and/or the IRB for the study site may alter or amend the text as appropriate, but both the IRB and the Sponsor must approve the final text of the informed consent before subject enrollment can begin. Any modification to the study sample informed consent form made by the Investigational Site must be approved by the sponsor and the IRB before use. Each Investigational Site will provide the sponsor with a copy of the IRB approved consent forms.

7.3. Randomization Assignment: Acoustic Enhancer with Ureteroscopic Laser Lithotripsy (Investigational Arm) vs. Standard Ureteroscopic Laser Lithotripsy (Control Arm)

Following consent and if a subject meets the study's inclusion/exclusion criteria, the subject will be randomly assigned to the investigational or control arm on the day of the procedure. If the potential subject does not meet the study procedure eligibility criteria, he/she will NOT be randomly assigned to treatment or the control arm. Random assignment will be conducted using an allocation ratio of 1:1 with one subject randomized to the Investigational Arm for each subject randomized to the Control Arm. Random assignment will be conducted using a stratified permuted block design generated separately for each clinical site. Subjects will be blinded to treatment allocation through index procedure.

7.4. Double Blinding

This study is a double blinded study in which:

- Study subjects will not be informed of their treatment allocation through the index procedure.
- In assessing stone fragmentation using a CT image as defined in the primary endpoint, an independent radiologist will conduct the assessment and will be blinded to the treatment allocation of each subject's CT image.

7.5. Point of Enrollment

After the patient signs the Informed Consent Form, all inclusion and exclusion criteria will be verified. If the potential subject meets all of the inclusion and none of the exclusion criteria, the potential subject will then become eligible for randomization. All subjects who sign the informed consent form will be entered in the Electronic Data Capture (EDC) system. Each site will assign a unique subject ID for each subject that signs an informed consent using three-digit site number and three-digit subject number. Each subject number will be assigned in consecutive order and will not be reused. Assigned subject numbers will be recorded on the Screening and Informed Consent Log (CAP-004-03). The Subject ID number will remain with the subject throughout the course of their involvement in the study. Those subjects who do not meet inclusion/exclusion criteria, withdraw consent prior enrollment, or were in the screening process while enrollment closed (also known as missed enrollment) will be appropriately documented in the EDC system.

Enrollment is defined as the time when the patient signs an IRB-approved informed consent form (thereafter becomes a study subject).

Potential subjects who fail to meet screening assessment requirements will be counted as a screen failure and will not be treated. Screen failure subjects will be exited from the study, and not evaluated for safety or effectiveness. Screen failures will be documented at the site on a screening log.

7.6. Subject Withdrawal or Discontinuation

Subjects may voluntarily withdraw from the study at any time for any reason. The Investigator(s) may elect at any time to withdraw a subject from the study for any reason unrelated to the study treatment if such a decision is in the subject's best medical interest. If a subject discontinues the study, or is withdrawn by the Investigator(s), as much follow-up data as possible will be obtained. The primary reason for termination or discontinuation will be documented on the Study Exit Form. Study subjects will be discontinued from the study if their stone could not be accurately assessed on the CT image. This subject would be considered a screen failure. Subjects who prematurely discontinue the study or are withdrawn from the study by the investigator after they receive general anesthesia will not be replaced.

Anticipated reasons for early termination of subject participation include:

- **Subject Lost to Follow-Up:** Unable to locate subject despite at least three documented attempts to notify the subject via telephone or e-mail, and by certified mail. A subject will not be considered lost to follow-up until the last scheduled follow-up visit (30 day follow up visit or 90 day follow up visit if subject has unresolved hydronephrosis).
- **Subject's Withdrawal of Consent:** The subject requests to terminate his/her involvement in the study, therefore withdrawing his/her consent to participate in the study (the investigator must thoroughly document the reasons for termination). Attempts will be made to retrieve any follow-up data, prior to the time point at which voluntary consent was withdrawn, when available, in particular regarding possible adverse events (AEs) at the time of study discontinuation.
- **Subject Withdrawn by Investigator:** Subjects who are withdrawn by the Investigator for any reason prior to study completion.
- **Subject Death:** If possible, an autopsy and/or death certificate should be obtained in order to document the cause of death.

Any subjects discontinued prior to receiving general anesthesia will be classified as screen failures and exited from the study and will not be included in any endpoint analysis.

8. SUBJECT ASSESSMENT AND STUDY VISIT SCHEDULE AND PROCEDURES

8.1. Pre-Treatment Visit

After the subject has completed the informed consent process, research staff will review potential subject's medical history, including available images, to verify study subject's eligibility. In addition, research staff will conduct protocol required assessments prior to the index procedure to obtain baseline information and verify subject eligibility.

- As part of routine care, study subjects may be prescribed medications such as analgesics, alpha blockers, calcium channel blockers, antibiotics to take as needed.
- Research staff will review subject's medical history, including available images, to verify study eligibility.
- Subjects will complete a baseline health status and undergo focused urogenital calculus physical exam and vital signs.

- Subjects will undergo a CT scan to measure stone dimensions (in mm), and location (e.g., the upper (superior) pole, lower (inferior) pole, and interpolar region of the kidney, pelvis of the kidney, and proximal ureter). This will be noted on the Electronic Case Report Form (eCRF). If there are multiple stones, each one will need to be measured and its location (i.e., pelvis, ureter), size will be documented.
    - CT image acquisition parameters will follow CLIN-0002: Image Acquisition Protocol, AEROLITH Study. A CT image collected prior to screening may be used as baseline CT image. The CT image will be used to measure stone size and the location of the stone.
    - Measuring baseline stone size(s) will be conducted by the investigator following CLIN- 0003: Radiographic Evaluation Protocol of Applaud's Acoustic Enhancer with Laser Lithotripsy System in the Treatment of Urinary Stones
    - A Stone Size Measurement Verification Electronic Case Report Form (eCRF) will be completed by the investigational site and the baseline CT image will be uploaded to the core lab for analysis following CLIN-0003.
- Subjects will undergo a urinalysis to verify absence of Urinary Tract Infection (UTI) and verify pH level is ≥ 5.5. ***Note:*** If a patient's largest stone intensity is ≥ 500HU and has a historical pH value of ≥ 5.5, a recently tested (within 30 days of consent) urine pH showing <5.5 in this case is acceptable.
- Serum creatinine to verify renal function and serum creatinine is < 1.5mg/dl.
- Subject's usage of all prescription medications (type, dose, and frequency) will be documented. In addition, any non-prescription medication (type, dose, and frequency) that may impact stone disease, acting as stone formation promoters (e.g., calcium or ascorbic acid) or stone formation inhibitors (e.g., magnesium or citric acid) will also be documented, whether such medications were taken in connection with the scheduled study procedure or otherwise.

8.2. Study Treatment – Investigational Arm and Control Arm

The investigator and designated co-investigator(s) will be trained to the Acoustic Enhancer Instructions for Use (IFU) before performing the investigational procedure.

On the day of the procedure and prior to randomization, the investigator will verify:

- If urinalysis is more than 7 days removed from the procedure, a negative urinalysis, with an absence of UTI symptoms, should be obtained on the day of the procedure.
- Female subjects are not pregnant using urine or serum testing.

The subject will be placed under general anesthesia and positioned following standard procedures for URS-LL. The investigational procedure involves the use of Acoustic Enhancer to augment the URS-LL that is done per standard practice. Procedures will be performed by the investigator (PI) and/or designated sub-investigator(s).

To facilitate reliable comparisons of treatment success between the Acoustic Enhancer with URS-LL (investigational arm) and URS Laser Lithotripsy (control arm), technique and equipment will be standardized as follows for all procedures:

- Ho:YAG lasers approved for ureteroscopic laser lithotripsy (30W to 120W) will be used
    - Moses mode or Thulium laser equipment is **NOT ALLOWED**
- Laser fibers of < 300µ diameter will be used.
- At investigator's discretion, ureteral access sheaths (10-16Fr outer diameter) may be used.

- Laser setting at 0.2 J to 1.5J, (**DO NOT** exceed 1.5J), frequency 5 Hz to 60 Hz.
    - **DO NOT** exceed a time-averaged power of 25 W (the product of the pulse energy and pulse repetition rate at any point during the procedure).
- A flexible ureteroscope (disposable or non-disposable) will be used; no semi-rigid ureteroscopes are allowed for the laser lithotripsy portion of the procedure.
- Use standard irrigation. To prevent unintentional suctioning of the Acoustic Enhancer microbubbles, **DO NOT** use continuous suction devices (e.g., ClearPetra, LithAssist).
- If possible, perform retrograde stone displacement into the pelvis/calyx following institution standard protocol.

During laser lithotripsy, small volumes of Acoustic Enhancer are placed in the urine near the stone at periodic intervals (refer to the IFU).

Lasering is continued until any visible remaining fragments are estimated to be 1-2 mm in size or smaller.

For the purposes of this study, no basketing will be performed for stone retrieval. If basketing is performed, this will be captured and recorded as a major protocol deviation. No other concomitant procedures, including diagnostic procedures, are allowed during the treatment. Any concomitant procedure (i.e., basketing) being conducted will be documented as a major protocol deviation.

Post-treatment, ureteral stents will be placed in all subjects for 3 to 14 days. If a stent cannot be placed, this will be captured and recorded as a minor protocol deviation.

Investigators must record any stents remaining >14 days post procedure and document any observations of calcification or encrustation. If a stent is removed outside of the window (3-14 days), this will be captured and recorded as a minor protocol deviation. If calcification and/or encrustation is observed, an Adverse Event CRF should be completed.

Document any adverse events observed during the procedure (see Adverse Events section below).

8.3. Recovery and Discharge

Recovery and discharge procedures will follow standard protocol at the treating institution. Any adverse events will be documented.

8.4. Follow up call at Day 14 (14 days +/- 4 days after each procedure)

- Conduct a follow-up call after the treatment.
- Document any reported AEs since last observation was performed (see Adverse Events section below).
- If symptoms of Urinary Tract Infection (UTI) are reported by subjects, conduct a urinalysis and subsequent urine culture if clinically indicated to verify presence/absence of UTI.
- Document all prescription medications. In addition, document any non-prescription medication that may impact stone disease, acting as stone formation promoters (e.g., calcium or ascorbic acid) or stone formation inhibitors (e.g., magnesium or citric acid).
- Record date of stent removal in the 14-day Follow Up Evaluation CRF

***Note**: If a stent is removed at >14 days but <30 or <90 days post procedure, please complete Follow Up (On-site) Evaluation CRF and select unscheduled visit for visit type.*

8.5. 30-day follow-up (30 +14 days after procedure)

- Conduct focused urogenital calculus physical exam and vital signs.
- Measure serum creatinine to assess renal function.
- Document any reported AEs since last visit (see Adverse Events section below)
- If symptoms of Urinary Tract Infection (UTI) are reported by subjects, conduct a urinalysis and subsequent urine culture if clinically indicated to verify presence/absence of UTI.
- Document all prescription medications. In addition, document any non-prescription medication that may impact stone disease, acting as stone formation promoters (e.g., calcium or ascorbic acid) or stone formation inhibitors (e.g., magnesium or citric acid)
- Conduct CT imaging following acquisition parameters delineated in CLIN-0002: Image Acquisition Protocol, AEROLITH Study
    - **Note 1:** As presence of ureteral stents may obscure image analysis, remove stents from the treated kidney/ureter prior to collecting the CT images.
    - **Note 2:** If a stent is removed outside of the window (3-14 days post procedure), this will be captured and documented using Protocol Deviation CRF as minor protocol deviation.
    - **Note 3:** If a stent is removed at >14 days, verify absence or presence of calcification and/or encrustation via standard of care imaging (e.g., KUB X-ray). If calcification and/or encrustation is observed, complete Adverse Event CRF.
- Upload the CT images to the core lab for analysis.
- Complete Study Exit CRF
    - **Note 1:** For those subjects with new or on-going device and/or procedure-related serious adverse events at 30 days post procedure, they will be followed until the event has resolved or is deemed stable by the investigator.
    - **Note 2:** For those subjects with new or on-going device and/or procedure related hydronephrosis and those subjects with pre-existing (prior to treatment) hydronephrosis that have worsened or recurred since baseline, they will be followed through 90-days post procedure (see below).

8.6. 90-day follow-up and Study exit (90 +/- 21 days after last treatment) only for those subjects with new and unresolved device and/or procedure related hydronephrosis and subjects with pre-existing (prior to treatment) hydronephrosis that have worsened or recurred at 30 days

- Conduct focused urogenital calculus physical exam and vital signs.
- Document any reported AEs since last visit (see Adverse Events section below).
- If symptoms of Urinary Tract Infection (UTI) are reported by subjects, conduct a urinalysis and subsequent urine culture if clinically indicated to verify presence/absence of UTI.
- Document all prescription medications. In addition, document any non-prescription medication that may impact stone disease, acting as stone formation promoters (e.g. calcium or ascorbic acid) or stone formation inhibitors (e.g. magnesium or citric acid).
- Perform diagnostic renal-bladder ultrasound to verify presence/absence of hydronephrosis.

- Complete Study Exit CRF
    - **Note 1:** For those subjects with on-going device and/or procedure-related serious adverse events (including device and/or procedure-related hydronephrosis and worsening/recurring hydronephrosis from baseline) at 90 days post procedure, they will be followed until the event has resolved or is deemed stable by the investigator. For subjects with on-going, including stable, device and/or procedure-related serious adverse events (including device and/or procedure-related hydronephrosis and worsening/recurring hydronephrosis from baseline) at 90-days post procedure, the investigator should refer the subject for further care to the subject's primary care provider or urological specialist upon exiting the study.

Table 2: Schedule of Events Table

Study Visit Day	Screening	Treatment	Day 14 (+/- 4 days) after procedure	Day 30 (+14 days) after procedure	*Day 90 (+/- 21 days) after procedure* ***
Informed Consent	X				
Patient Eligibility Review	X				
Medical History	X				
Phone Call			X		
Site Visit	X	X		X	X
Urine or serum pregnancy test		X+			
Focused Urogenital Calculus Physical Exam and Vital Signs	X			X	X
Serum creatinine	X			X	
Urinalysis	X		X • Urinalysis if symptomatic for UTI • Urine culture if clinically indicated	X • Urinalysis if symptomatic for UTI • Urine culture if clinically indicated	X • Urinalysis if symptomatic for UTI • Urine culture if clinically indicated
CT scan (non-contrast) stone presence, size & location	X*			X**	
Treatment: Investigational or Control Arm		X			
Ultrasound (renal bladder)					X
Other Assessments					
Adverse Event		X	X	X	X
Concomitant Prescription Medications. In addition, any non-prescription medication that may impact stone disease, acting as stone formation promoters (e.g., calcium or ascorbic acid) or stone formation inhibitors (e.g. magnesium or citric acid).	X	X	X	X	X
Protocol Deviation (if appropriate)	X	X	X	X	X

*CT within 60 days of subject signing consent. If CT imaging has not been conducted yet for the patient, please follow CT image acquisition parameters detailed in CLIN-0002.

Stone measurement will follow the Radiographic Evaluation Protocol of Applaud Medical's Acoustic Enhancer with Laser Lithotripsy System in the Urinary Treatment of Stones (Doc. No. CLIN-0003)

+ Pregnancy test to be done on the day of treatment prior to randomization

** See Imaging Acquisition Protocol, Acoustic Enhancer Research in Laser Lithotripsy (Doc. No. CLIN-0002)

***Only those subjects who have unresolved hydronephrosis at 30 days post-procedure.

9. RISKS AND BENEFITS OF THE INVESTIGATIONAL DEVICE AND CLINICAL INVESTIGATION

9.1. Potential Risks Related to Participating in this Clinical Investigation

Potential Risks of General Anesthesia

The Acoustic Enhancer with URS-LL procedure and URS-LL are performed under general anesthesia and are associated with potential anesthesia risks:

- Adverse reaction to anesthesia
- Bleeding at anesthesia instrumentation sites
- As with any procedure performed with sedation or anesthesia, there is risk associated that includes heart attack, stroke, and even death.

Potential Risks of the Acoustic Enhancer with Laser Lithotripsy Procedure

The potential risks associated with the Acoustic Enhancer with URS-LL procedure are expected to be similar to those of traditional URS-LL procedures used to treat urinary stones, with the addition of the risks for Acoustic Enhancer described in "Acoustic Enhancer Risks" described below. Potential risks for ureteroscopic laser lithotripsy generally are:

- Common (greater than 10% of subjects)
  - Mild burning sensation or hematuria for a short period after operation
  - Need for temporary insertion of a bladder catheter
  - Need for insertion of ureteric stent with a further procedure to remove it
  - As with other treatments for urinary stones, sloughed tissue found in or on urinary stones may be released as a result of a URS-LL treatment.
- Occasional (2% to 10% of subjects)
  - Kidney injury or infection needing further treatment
  - Failure to pass the ureteroscope if the ureter is narrow
- Rare (less than 2% of subjects)
  - Damage to the ureter with need for open surgical intervention or nephrostomy tube placement
- Scarring or stricture with persistent hydronephrosis/hydroureter requiring intervention

For the purposes of this protocol:

- Acute ureteral obstruction is defined by the presence of severe pain, persistent severe nausea or vomiting with per os intolerance requiring hospital admission with treatment requiring intravenous analgesics, or insertion of a ureteral stent or nephrostomy tube. Evidence of stone fragments in the ureter in diagnostic images is not sufficient for a diagnosis of urinary tract obstruction.
- Chronic ureteral obstruction is defined as persistent hydronephrosis identified on two sequential imaging studies from the same subject performed 4-6 weeks apart.

Potential Risks of the Acoustic Enhancer Material

The potential risks associated with the Acoustic Enhancer in the urinary tract during the investigational study procedure are:

Remote risks (≤ .01% of subjects)

- Acoustic Enhancer may cause an allergic reaction, which may range from minor itching or rash to major reaction such as anaphylactic reaction that could result in hospitalization or death. Excessive intraluminal pressure created by injection, causing backflow of urine and urinary tract rupture or urinoma formation

There is a potential risk associated with Acoustic Enhancer of an allergic reaction (less than 1 in a 10,000 subjects) or anaphylactic reaction (less than 1 in 20,000 subjects).

The placement of the Acoustic Enhancer closely resembles the manner in which irrigation fluid is delivered into the upper urinary tract in standard URS-LL. As such, there is a very low risk of adverse events associated with the increased pressures in the urinary tract arising during Acoustic Enhancer placement. Differential pressure risks include the pressure from backflow of urine in the urinary tract system, and in rare cases rupture in the urinary tract or urinoma formation.

As with any procedures in which ureteroscopes and other instruments are inserted into the urinary tract and materials placed using a cystoscope (narrow tube) into the urinary tract, in very rare cases, portions of the urinary tract could become inflamed or infected. While it is common practice to push a ureteral stone into the kidney prior to laser lithotripsy, there is also the potential risk of inadvertently doing so and of ureteral perforation from passage of the ureteroscope or other aspects of ureteroscope usage.

Significant new findings that may affect the subject's safety or willingness to continue in the study will be communicated to the study doctors and study subjects. An updated informed consent may be administered if appropriate.

9.2. Potential Benefits

URS-LL is routinely performed to treat urinary stones. This use of Acoustic Enhancer in URS-LL has the potential benefit of reducing the residual fragment burden. Residual fragments following URS-LL are associated with post-operative morbidity, potential readmission, and longer-term sequelae, thus reducing residual fragments in URS-LL is increasingly prioritized in the endourology community. [8] [9].

Placing Acoustic Enhancer through the working channel of the ureteroscope prior to administration of laser energy and at additional intervals during the procedure also has the potential benefit of shortening the required lasering time, with associated benefits of reduced heat generation and shortened time under general anesthesia during URS-LL procedures.

9.3. Mitigation of Risks

Risk to study subjects are mitigated in this study by the following:

- All investigations are conducted by investigators who are qualified by training and experience in the treatment of patients with urinary stones.
- All assessments are procedures performed in the controlled context of a clinical trial.
- Monitoring of the clinical trial to ensure compliance and with a focus on potential safety issues.
- All investigators will be trained to the Acoustic Enhancer IFU.

10. STATISTICAL METHODS

10.1. General Considerations

The study results will be reported using descriptive summaries and data will be made available in listings or electronic format as required. The data will be summarized according to randomized treatment group (investigational arm and control arm). Inferential analyses will be performed for the primary endpoints and other endpoints as outlined below. For continuous variables, descriptive statistics will include means, standard deviations, medians and ranges. Categorical variables will be summarized in frequency distributions and reported with the count and percentages. Percentages will be calculated based on observed values. Except for the primary effectiveness endpoint analyses, a two-sided p-value less than or equal 0.05 will be considered statistically significant. The primary effectiveness analyses p-values associated with statistical significance are outlined below. Where reported, all confidence intervals will be two-sided 95% CIs.

A separate Statistical Analysis Plan will be constructed with additional detail on statistical considerations and content of tables, listing and figures from the various data analyses. Statistical analyses will be performed using SAS (version 9.4 or higher, SAS Institute Inc. Cary, NC), R (version 3.0 or higher, R Foundation for Statistical Computing, Vienna, Austria) or other widely accepted statistical or graphical software.

Results will additionally be stratified according to stone location (the upper (superior) pole, lower (inferior) pole, and interpolar region of the kidney, pelvis of the kidney, and proximal ureter) and stone diameter (≤ median, > median where the median is based on all ITT subjects).

10.2. Analysis Population

The study results will be summarized based on three populations:

Intent-to-Treat (ITT): The ITT population consists of all randomized subjects who meet the study inclusion/exclusion criteria. Subjects who develop UTI or having their stone move to the middle/distal ureter between the time they were screened and were found to be eligible at the time they were randomized, or who no longer meet any of the other inclusion/exclusion criteria at the time of the procedure, will not be treated under the protocol. These subjects will not be included in the ITT analysis. The ITT population will be used for all effectiveness analyses with groups based on the randomized treatment.

Safety Population: The Safety population consists of all subjects who had a procedure performed. The Safety population will be used for the safety summaries (adverse events or strictures) with groups based on the actual treatment received.

Per-Protocol Population (PP): The Per-Protocol population will consist of all subjects who complete the primary endpoint information without a major protocol deviation; PP will include subjects who have evaluable CTs both at baseline and at 30-day follow-up. The PP population analyses will be completed based on the randomized treatment arm.

The definition of major protocol deviations will be defined prior to any final analysis. Subjects who are screened but not randomized will not be included in analysis. Any information on these subjects will be made available in a listing if requested by the IRB or FDA.

10.3. Sample Size Justification

A sample size of 196 enrolled and randomized subjects randomized 1:1 to the investigational arm (Acoustic Enhancer with URS-LL) and the control arm (URS-LL) was selected based on consideration of the primary endpoint. Assuming no more than 10% non-evaluable subjects, this provides a randomized sample size 176 subjects (88 per arm).

The primary hypothesis test for the treatment success outcome is as follows:

H_0: $p_{AE} \leq p_{Control}$ vs H_1: $p_{AE} > p_{Control}$

Where p_{AE} is the acoustic enhanced response rate and $p_{Control}$ is the control response rate (no acoustic enhancement). Using a one-sided 0.025 Farrington-Manning test, a 55% response rate in the control arm, and a 75% response rate in the investigational arm, the sample size provides 80% power for the analysis. The 55% control arm response rate is based on reported results for laser lithotripsy with CT- based follow-up as described in section 1.1, while the 75% response rate in the investigational arm is based on exploratory clinical studies.

10.4. Handling of Missing Data

The primary analysis of effectiveness will be based on data multiple imputation (MI) approach using the ITT Population. For the primary effectiveness endpoint, the observed results and a tipping point sensitivity analysis will also be completed as outlined in Section 10.6. No imputation or adjustment for missing data will be used for the other endpoints.

10.5. Adjustments for Multiplicity

The study will include a single primary effectiveness endpoint to be tested at the one-sided 0.025 level. If that hypothesis is met, two other endpoints will be considered sequentially. If the first secondary hypothesis is statistically significant, the second hypothesis will also be assessed. Hence, the overall Type I error for the study is controlled for the specified hypotheses.

Secondary Hypothesis 1:

Evaluation of non-inferiority of the normalized total radiant energy (TRE) will be evaluated based on the difference in the means of the log transformed values of the TRE for the investigational and control subjects, respectively. Normalized TRE is the total procedure radiant energy divided by the stop area. A non-inferiority bound of 0.223 will be considered evidence of non-inferiority (see Section 10.7 for discussion of non-inferiority bound). The hypothesis is as follows:

H_0: $\mu_{AE} \geq \mu_{Control} + \delta$ vs H_1: $\mu_{AE} < \mu_{Control} + \delta$

Where μ_{AE} is the mean of the log of the total radiant energy values for the Acoustic Enhancer, $\mu_{Control}$ is the mean of the log of the total radiant energy values for the Control group, and δ is the non-inferiority bound ($\delta = 0.223$). This will be analyzed using a one- sided 0.025 T-test.
The geometric means for each group will also be displayed by showing the exponentiation of the mean of the log transformed values. Similarly the geometric mean ratio (GMR) and associated 95% CI will be provided by showing the exponentiation of the difference means of the log values and the associated 95% CI values.

Secondary Hypothesis 2:

Reduction in normalized TRE will be analyzed by considering difference in the means log of normalized TRE. The hypothesis is as follows:

$$H_0: \mu_{AE} \geq \mu_{Control} \text{ vs } H_1: \mu_{AE} < \mu_{Control}$$

Where μ_{AE} is the mean of the log of the total radiant energy values for the Acoustic Enhancer and $\mu_{Control}$ is the mean of the log of the total radiant energy values for the Control group. This will be analyzed using a one- sided 0.025 test.

10.6. Primary Endpoint Analysis

The primary endpoint is defined as:

Treatment success: Proportion of study subjects who have a complete absence of stone(s), or have any residual fragments measuring less than or equal to 2 mm, as assessed by CT imaging through 30 days follow-up post index procedure. CT imaging will be assessed by an independent core lab to determine treatment success. Treatment success, as defined by an absence of residual fragments >2mm on any dimension, has been identified as a priority in stone care in recent publications including Margaret Pearle's widely cited "Is Ureteroscopy as Good as We Think", published in the Journal of Urology in 2016 [12]. Increasing awareness and clinical prioritization of residual fragments in ureteroscopic laser lithotripsy derives in large part from expanded use of CT follow-up imaging in stone subjects. CT imaging has allowed characterization of rates of residual fragments and full appreciation of their clinical significance, with rigorous studies of residual fragment natural history having established a 2mm threshold as a critical size above which additional stone events are particularly frequent [22], [23]. Therefore, a demonstration of a lower rate of >2mm residual fragments in the Acoustic Enhancer arm compared to the control arm would be clinically important.

Treatment success will be summarized descriptively as a categorical variable by treatment group. Exact binomial 95% CIs for success rates within each group will be provided

The primary analysis is to be completed on the ITT population for the hypothesis identified in Section 10.3 (H0: pAE ≤ pControl vs H1: pAE > pControl). In order to account for possible missing data, the primary analysis will be completed using a multiple imputation (MI) approach and estimates of treatment differences and standard errors for the differences obtained for each MI sample using Farrington-Manning estimates. The MI model for response will be fit using a logistic approach by treatment group and include stone size, and number of stones. The estimated variance for the difference in groups will be based on combining the average variance from the imputed samples and the variance

between the samples. The analysis may be completed using SAS MI and MIANALYZE procedures or through the R MICE package. The primary analysis results will include the two-sided 95% CI the difference and the one-sided p-value for the hypothesis test.

In addition, the Farrington-Manning test will be used to complete the analysis using the observed data. The two-sided 95% CI for the difference in observed data rates will be provided as well as the p-value. Note that all Farrington-Manning tests variances will be based on the sample variance under the null assumption the null groups results are equal. A tipping point analysis will be provided for evaluating the impact of missing data by imputing at the possible distribution of unavailable responses in the ITT populations. The treatment success analysis will also be completed in the per- protocol population.

The homogeneity of the treatment response will be evaluated using the available data and a logistic model of treatment success as a function of treatment, study site, and a site-treatment interaction. A p-value of less than or equal to 0.15 in the site-treatment term will be considered evidence of heterogeneity of the treatment effect across sites. In addition, as an exploratory analysis, a logistic model will be used to if stone location is associated with response or a location-treatment interaction.

10.7. Secondary Endpoint Analysis

The secondary endpoint is defined as:

Total radiant energy (TRE): Mean value of laser energy, measured in kilojoules, with laser energy values recorded and displayed by the pulsed laser unit used in the procedure, with the laser energy value for each procedure normalized by the stone cross-sectional area. For this purpose, the stone cross-sectional area is calculated by taking the product of the two largest stone size measurements on the pre-procedure CT; for subjects with more than one stone treated in the index procedure, the stone cross-sectional area is calculated as the sum of the stone cross-sectional areas calculated individually for each treated stone. The mean value of laser energy for each arm is calculated by considering the normalized measured values for all study subjects in that arm.

Normalized TRE will be summarized as a numeric variable. In addition, as the primary analysis is based on ratio of the means, the geometric mean and 95% CIs based on the back-transformation of the log values will be provided. The log-transformed values will also be summarized descriptively. All analyses of TRE will be based on available data. TRE is to be collected as part of the procedure and it few if any missing values are expected. If more than 4 subjects have missing TRE values, a MI analysis will be completed as a sensitivity analysis.

In support of the secondary hypotheses (Section 10.5), a t-test will be used to assess the difference in between groups in the means of the log-transformed normalized TRE value. If the two-sided 95% CI upper bound of the difference in the means is less than 0.223 (log 1.25) then secondary hypothesis 1 will be considered met and if bound is less than -0.0, the secondary hypothesis 2 will also be considered met. The one-sided p-values associated with the hypothesis will also be provided.

The ratio cut-off of 1.25 for the non-inferiority assessment was obtained based of review of papers describing total energy (Molina et al (2014), Humphreys et al (2018), and Rana, et al (2020)). Further, discussion with investigators indicated the endpoint could have considerable variability based on uniqueness of the individual subject, stone size, stone composition, and stone location. The Molina (2014) paper included analyses of total energy and found several significant predictors but at best the explained about half the variance (R2 = 0.524 for a multivariate model). Molina (2014) presented an exploration of total energy normalized by stone volume.

Based on normalized TRE from the paper it appears as the ratio of the upper quartile to the median was approximately 1.6. The ratio of 1.25 would reflect a ratio of AE to control that would be close the control mean and well within variability associated with a single standard deviation. Hence, given the inherent variability of the measure, the use of 1.25 as a ratio to indicate non-inferiority normalized TRE is justified.

The Wilcoxon Rank-Sum test will be used as a confirmatory analysis of the t-test for a comparison of the observed normalized TRE. If more than 4 subjects have missing TRE values, a multiple-imputation analysis will be performed including group and the total stone area as predictive covariates. The homogeneity of the treatment response will be evaluated using a ANOVA model of normalized total radiant power as a function of treatment, study site, and a site-treatment interaction. A p-value of less than or equal to 0.15 in the site-treatment term will be considered evidence of heterogeneity of the treatment effect across sites. In addition, as an exploratory analysis, an ANOVA model will be used if stone location is associated with response or a location-treatment interaction.

10.8. Additional Observations

Mean total lasering time will be analyzed as a numeric endpoint using the ITT Population. A two- sided t-test with equal variance assumed will be used to evaluate the null hypothesis that mean time is similar in both groups. A 95% two-sided CI for the mean difference will be provided. An ANOVA will be used to evaluate mean total laser time as a function of location, treatment, and location x treatment interaction. Least square means will be provided with ANOVA p-values.

The number and percentage of subjects receiving subsequent additional interventional stone procedures for stone(s) that were treated in the AEROLITH study will be summarized. A Likelihood-Ratio chi-square test will be used to evaluate the null hypothesis that the proportion of subjects with at least one secondary stone treatment is equal in both groups. In addition, the total number of secondary stone treatments will be summarized and a Cochran- Armitage trend test will be used to evaluate the null hypothesis that there is no trend in the number of stones between the groups.

Use and quantity of prescription pain medication for managing stone-associated pain will be characterized by timing and type of medication and will be summarized descriptively for the ITT and PP Population.

Residual stone size will be summarized descriptively as a continuous variable and rate of absence of residual stones as a categorical variable. These will be summarized for the ITT and PP Populations.

11. SAFETY REPORTING

Safety outcomes will be organized into adverse events related to the device, or procedure or unrelated to either, and the severity. All procedure, and post-procedure complications, whether device-related or not will be recorded and all reportable events will be reported. Subject symptoms including pain, neurological, and functional symptoms are expected and are considered adverse events when these symptoms result in an unscheduled visit, new or worsening symptoms, as compared to baseline.

11.1. Adverse Event

An adverse event (AE) is any undesired clinical response or complication experienced by a subject. Monitoring and documenting of adverse events will begin at the start of the index procedure. When an adverse event is recorded as ongoing, an effort will be made to follow-up on the event at subsequent subject visits until the event is resolved or the subject exits the study.

In this study, adverse events should be reported beginning at the time of administration of general anesthesia for the index procedure. Pre-existing conditions (recorded on the Medical History CRF), which occur during the study at the same severity as in the subject's history, should not be recorded as adverse events. Pre-existing conditions (recorded on the Medical History CRF) *that worsen during the study OR a new episode begins* must be recorded as AE(s). The onset date should be the start of the current episode or the date of the change in severity, not the onset date recorded on the Medical History CRF.

Upon enrollment, if a new adverse event is reported and subsequently determined to be medical history not previously documented, the information should be added to the medical history CRF. The event should be recorded as an AE only if there was a change in severity.

Intermittent Events

An AE that occurs intermittently at the same severity should be aggregated and recorded as one event unless, in the opinion of the Investigator, the events should be reported as discrete events (medical diagnosis terms).

- The onset date is the start of the first episode during the study.
- The resolution date is the end of the last episode (or may be persistent, if ongoing at the end of the study).
- The site should record "intermittent" in the AE description. If an intermittent event meets the regulatory criteria for seriousness, the associated frequency of the adverse event term (e.g., 4x/week) should be captured in the narrative/comment section of the CRF.
- If the event changes in severity or relationship to study device, it should be recorded as an AE update.

All peri- and post- procedural AEs, whether device- or procedure related or not, will be recorded as an Adverse Event. Data to be collected includes the description of the AE, onset and resolution dates (or whether the AE is ongoing), severity, actions taken, outcome, and determination of the event relationship to the device and/or procedure. In general, AEs should be reported and classified by the Investigator using a diagnosis and not an action taken. The diagnosis should be confirmed through specific signs, symptoms, and (if necessary) laboratory tests.

The Investigator, on the basis of his or her clinical judgment, will determine the relationship of the AE to the device and/or procedure based on the following categories:

1. **Unrelated:** The adverse event is determined to be solely caused by the underlying disease, disorder or condition of the subject, or attributable solely to other extraneous causes (unrelated to the device, device malfunction, or the procedure).
2. **Possibly related:** The adverse event has onset within a clinically relevant temporal relationship to exposure to the device or procedure, and is plausibly at least partially caused by or aggravated by the use of the device, device malfunction, or the procedure. It must also meet one of the following criteria: (1) follows a known or easily foreseen pattern of response to device use or procedure and (2) is not fully attributable to the underlying disease, disorder or condition of the subject, or attributable to other extraneous causes.
3. **Probably related:** The adverse event has onset within a clinically relevant temporal relationship to exposure to the device or procedure, and is more likely than not to be at least partially caused by or aggravated by the use of the device, device malfunction, or the procedure. It must also meet both of the following criteria: (1) follows a known or easily foreseen pattern of response to device use or procedure; and (2) is not fully attributable to the underlying disease, disorder or condition of the subject, or attributable to other extraneous causes. In addition, if the adverse effect is reversible upon reoperation or device exchange, and such a procedure is done, the effect disappears or lessens in severity within the expected time interval.

4. **Definitely related:** The adverse event is clearly caused by the use of the device, device malfunction, or the procedure. It must meet all following criteria: (1) has a clear temporal relationship between device exposure and onset of the event; (2) follows a known pattern of response to device use or procedure; and (3) is not reasonably attributable to the underlying disease, disorder or condition of the subject, or attributable to other extraneous causes.

Severity of an AE will be determined by the Investigator following Common Terminology Criteria for Adverse Events (CTCAE):

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental Activities of Daily Living (ADL)*.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

Note: *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

The term "severe" is used to describe intensity (severity) of a specific event. An event itself, may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on subject/event outcomes or action criteria usually associated with events that pose a threat to the subject's life or functioning.

For the purposes of safety reporting in this study, the following definitions will be applied:

- An **Adverse Event** (AE) is any untoward medical occurrence in a subject whether it is considered device related or not. Diseases, signs, symptoms, or laboratory abnormalities already existing at enrollment are not considered AE's unless they worsen (i.e., increase in intensity or frequency). Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an adverse event. Surgical procedures planned prior to enrollment and the conditions leading to these measures are not adverse events.
- An **Adverse Device Effect (ADE)** is any untoward and unintended response to a medical device, including events arising from insufficient or inadequate instructions for the employment of the device or its use.
- A **Serious Adverse Event** (SAE) is any AE that:
    - results in death;
    - is life threatening;
    - results in or prolongs hospitalization;
    - results in permanent impairment of a body function or permanent damage to a body structure;
    - necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure;

Note: For the purposes of this protocol, for a subject who has been treated with standard URS-LL or with Acoustic Enhancer with URS-LL and requires an additional treatment to further fragment or eliminate the stone, this additional procedure will not be considered an adverse event.

- Led to fetal distress, fetal death, or a congenital abnormality or birth defect;
- Other serious medically important event.

Note: Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event

- An **Unanticipated Adverse Device Effects** (UADE):
  The investigational device exemption (IDE) regulations and ISO 14155:2020 (Clinical Investigation of medical devices for human subjects – Good clinical practice) defines an unanticipated adverse device effect (UADE) as any *serious* adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the clinical investigational plan or risk analysis; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects" (21 CFR Part 812.3(s)). UADEs must be reported by the clinical investigator to the sponsor and the reviewing IRB, as described below:
  - Investigators are required to submit a report of a UADE to the sponsor and the reviewing IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event (21 CFR Part 812.150(a)(1)).
  - The Sponsor will immediately conduct an evaluation of a UADE and report the results of the evaluation to the appropriate regulatory agency, all reviewing IRBs and participating investigators within 10 working days after first notice of the effect (21 CFR part 812.46(b), 812.150(b)(1)).

ALL SERIOUS ADVERSE EVENTS AND UNANTICIPATED ADVERSE DEVICE EFFECTS OCCURRING THROUGHOUT THE STUDY MUST BE REPORTED TO THE SPONSOR OR ITS DESIGNEE AND MUST BE RECORDED IN THE EDC SYSTEM WITHIN 24 HOURS OF FIRST LEARNING ABOUT THE EVENT.

The investigator is required to notify the IRB of SAEs in accordance with IRB reporting requirements.

All subjects will be followed at 14 and 30 days post procedure.

Note 1: *Subjects with new and on-going device and/or procedure-related serious adverse events at 30 days post procedure will be followed until the event has resolved or deemed stable by the investigator.*

Note 2: *Subjects with new and on-going hydronephrosis and subjects with pre-existing (prior to treatment) hydronephrosis that has worsened or recurred since baseline at 30-days post procedure will continue to be followed through 90 days.*

Note 3: *At 90-days post procedure, subjects who have on-going device and/or procedure-related serious adverse events (including device and/or procedure related hydronephrosis) and subjects with pre-existing (prior to treatment) hydronephrosis that have worsened or recurred since baseline, will continue to be followed until the event has resolved or until the investigator deems the event is stable prior to study exit. If the events are deemed stable by the investigator, the investigator should refer the subject for further care to the subject's primary provider or urological specialist upon exiting the study.*

11.2. Device Malfunction

For the purposes of this protocol, a device malfunction is defined as the failure of a device to meet its performance specifications or otherwise perform as intended [21CFR803.3(k)]. Examples of a device malfunction include breached packaging, broken Acoustic Enhancer vials.

All device malfunctions must be reported to the Sponsor and entered in the EDC within 24 hours of the knowledge.

12. STUDY MANAGEMENT

12.1. Sponsor Responsibilities

As the Sponsor of this clinical study, Applaud Medical has the overall responsibility for the conduct of the study, including assurance that the study meets US federal and local regulatory requirements appropriate to the conduct of the study. In this study, Applaud will have certain direct responsibilities and might delegate other responsibilities to a Contract Research Organization (CRO). The study sponsor will adhere to sponsor general duties as described in ISO 14155:2020, Clinical investigation of medical devices for human subjects – Good clinical practice, and CFR Part 812, 50, 56, 54 and the World Medical Association Declaration of Helsinki.

General Duties

Applaud will ensure that the application is submitted to the appropriate regulatory authorities, obtaining copies of IRB approvals, and ensuring documentation of IRB approvals prior to the shipment of devices, ensuring proper clinical site monitoring, ensuring subject informed consent is obtained, providing quality data that satisfies regulations and informing the Investigators and IRBs of unanticipated adverse device effects, adverse events, and deviations from the protocol as appropriate.

The investigation must be reviewed and approved by the appropriate IRBs before subject enrollment may begin. All proposed changes to the clinical study must be reviewed and approved by Applaud. Applaud will also obtain any necessary regulatory approval, per local requirements.

Selection of Clinical Sites

The primary requirements of site and Investigator selection and continued participation in the study are: adequate experience, commitment to safety, consistency in adherence to the protocol, and subject volume. Participating sites will be screened to ensure they have sufficient numbers of eligible subjects who are representative of the target population. Each center must have facilities that are capable of processing subjects in the manner prescribed by the protocol.

The study sponsor, Applaud, and its designees will select qualified Investigators, ship or deliver devices only to participating Investigators, obtain signed study agreements, and provide Investigators with the information necessary to conduct the study.

Site Training

The training of appropriate clinical site personnel will be the responsibility of the Sponsor or designee. The Investigator is responsible for ensuring that his/her staff conduct the study according to the protocol. To ensure proper device usage, uniform data collection, and protocol compliance, the Sponsor or designee will present a formal training session to study site personnel which will review the instructions for use of the device, the Clinical Study Protocol, instructions on in-hospital data collection, methods for soliciting data from alternative sources, schedules for follow-up with the study site coordinators, and regulatory

requirements. Detailed feedback regarding completion of forms will be provided by the Sponsor or designee through the regular site monitoring.

Investigator Training

The Sponsor will provide appropriate Investigator training on the technique via the IFU. Each investigator will be trained to the Acoustic Enhancer by the Sponsor prior to treating the first subject. Training will be documented for each investigator as outlined in the IFU.

Subject Confidentiality

Subject confidentiality will be maintained throughout the clinical study in a way that ensures the information can always be tracked back to the source data. For this purpose, a unique subject identification code (ID number and subject name code) will be used that allows identification of all data reported for each subject.

Data relating to the study might be made available to third parties (for example in case of an audit performed by regulatory authorities) provided the data are treated confidentially and that the subject's privacy is guaranteed. As part of the informed consent process, the subject is informed that their medical chart will be reviewed by the Sponsor and its designates, and by government regulatory authorities. Each study site is responsible for ensuring it has in place appropriate policies and procedures to maintain subject confidentiality according to applicable laws, including without limitation, the Health Insurance Portability and Accountability Act (HIPAA) and any applicable state laws. It is also the investigator's responsibility to obtain written authorization from each study subject in compliance with applicable laws allowing the disclosure of all study results described in the protocol before the subject is entered into the study.

Data Management

Applaud or a representative of the Sponsor will be responsible for database creation and validation. Prior to finalizing and locking the database, all decisions concerning the inclusion or exclusion of data from the analysis for each subject will be determined by appropriate clinical and statistical personnel. Any and all exclusions related to either safety or efficacy will be documented in subject listings.

Record Retention

Applaud will maintain copies of correspondence, data, shipment of devices, adverse device effects, Investigator agreements and other records related to the clinical trial. All study records and reports will remain on file at the sites for a minimum of 2 years after completion of the Study, and will further be retained in accordance with local guidelines as identified in the clinical study agreement. Study records are to be discarded only upon notification by the study sponsor. The Investigator must contact the study sponsor before the destruction of any records and reports pertaining to the study to ensure they no longer need to be retained. In addition, the sponsor should be contacted if the Investigator plans to leave the investigational site. All required data for this study will be collected on standardized CRFs or an electronic data capture system. All information and data sent to the Sponsor or Contract Research Organizations (CROs) concerning subjects or their participation in this study will be considered confidential. All data used in the analysis and reporting of this evaluation will be used in a manner without identifiable reference to the subject. The Principal Investigator consents to visits by the staff of the Sponsor and its authorized representatives and the U.S. Food and Drug Administration or any other local governmental body to review the study subjects' medical records including any test or laboratory data that might have been recorded on diagnostic tests media (e.g., CT scans, X-rays, etc.).

12.2. Investigator Responsibilities

The Investigator shall ensure that all work and services described herein, or incidental to those described herein, shall be conducted in accordance with the highest standards of medical and clinical research practice. The Investigator will provide current copies of the study protocol to all Sub- Investigators or other site personnel responsible for study conduct. The investigator should follow the clinical study in accordance with the Acoustic Enhancer Research on Laser Lithotripsy Clinical Study Investigator's Agreement (CLIN-0004).

Upon completion or termination of the study, the Investigator will submit a final written report to the study sponsor and the reviewing IRB. The report should be submitted to the study sponsor within three (3) months of study completion or termination. The Investigator will provide the study sponsor or designee with copies of all IRB actions regarding the study.

IRB Approval and Informed Consent

The investigation must be reviewed and approved by the appropriate IRBs before subject screening may begin. All proposed changes to the investigational plan must be reviewed and approved by the Sponsor. Prior to shipment of study devices, a signed copy of the IRB Committee approval letter identifying the clinical study must be submitted to the Sponsor signifying study approval. Investigators are responsible for obtaining and maintaining approval of the study by the IRB.

Written informed consent is mandatory and must be obtained from all subjects prior to performing any study procedures in this clinical study. Applaud Medical will provide each Investigator site with a Sponsor approved consent template. Each site is expected to modify the template, if necessary, to meet their facilities requirements. Modified ICF templates must be reviewed by the Sponsor prior to submission to their IRB.

Informed consent must be obtained and shall inform the subject as to the objective and procedures of the study and possible risks involved. The subjects must be informed about their right to withdraw from the study at any time and for any reason without sanction, penalty, or loss of benefits to which the subject is otherwise entitled and that withdrawal from the study will not jeopardize their future medical care. The clinical study informed consent must be used in addition to the institution's standard consent form for urological surgery. The institutional standard subject consent form does not replace the study consent form.

It is the responsibility of the Investigator to obtain both an authorization for subject health information and study consent. The IRB approved Informed Consent Forms must be retained at the investigational site along with the other investigational case report forms. A copy of the consent form must be given to each subject enrolled in the study.

Data Collection and Reporting

Electronic Case report forms (eCRFs) will be used to record demographic, procedural, and follow-up data, as well as any unscheduled visits or adverse clinical events which may occur during the study period. The AEs and incidence of morbidity and mortality will be reviewed with the Investigators to assess the safety of the device and the procedure.

Qualified study staff at each clinical site will perform primary data collection drawn from source-document (hospital chart) reviews. The Monitor will perform clinical monitoring, including review of CRFs with verification of study eligibility, informed consent process, scheduled and unscheduled follow-up visits and AEs to the source documentation.

Data Completion and Submission

Investigators, or delegated site personnel, should complete CRFs within the EDC system in a timely fashion, preferably within 10 business days after subject enrollment or follow-up visit. This will enable timely monitoring visits.

Serious adverse events should be reported within 24 hours of first knowledge of the event.

Device Accountability

The Investigator shall maintain adequate records of the receipt and disposition of all study devices. When the enrollment is complete, the Investigator shall return any unused devices to the study sponsor. The Device Accountability Log shall document quantity of device (lot number) received, used and any unused devices that have been returned to the Sponsor, or otherwise disposed of.

Source Documents

The Investigator shall maintain accurate, complete, and current records relating to the Investigator's participation in an investigation including records of each subject's case history and exposure to the device. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. Such records shall include:

- Documents, evidencing the informed consent process and, for any use of a device by the Investigator without informed consent, any written concurrence of a licensed physician and a brief description of the circumstances justifying the failure to obtain informed consent. The case history for each individual shall document that informed consent was obtained prior to participation in the study. All relevant observations, including records concerning adverse device effects (whether anticipated or unanticipated), information and data on the condition of each subject upon entering, and during the course of, the investigation, including information about relevant previous medical history and the results of all diagnostic tests.
- A record of the exposure of each subject to the investigational device, including the date and time of each use, and any other therapy.

Regulatory Filing

The following records must be maintained in designated study administrative files:

- Clinical Protocol and all amendments
- Signed Investigator Agreement
- IRB approval letter(s) and approved Informed Consent(s) (including any revisions)
- Correspondence relating to this study (with Sponsor, Monitors, other Investigators, etc.)
- Correspondence with the IRB
- Instructions for Use
- Curriculum Vitae for all Investigators
- Device log
- Device related paperwork (including shipping and return documentation, including packing slips)
- Site Visit Log
- Delegation of Authority Log
- Blank set of CRFs and instructions for completion
- Reports (including Adverse Event reports, annual reports and final reports from Investigator and Sponsor)

The following records must be maintained for each subject enrolled in the study:

- Signed subject Informed Consent Form
- All completed CRFs
- Record of any side effects/adverse events and treatment failures (with supporting documentation)
- Procedure reports, physician dictations, nursing notes, and subject medical records
- Copies of all subject X-rays, CT Scans and ultrasounds where applicable
- Records of any interventions (procedure reports, physician dictations, nursing notes, etc.)
- Records related to subject deaths during the investigation (including death records, death certificate and autopsy report, if performed)

Investigator files containing all records and reports of the investigation should be retained for a minimum of 2 years after the completion or termination of the investigational study or until 2 years after they are no longer needed to support product approval. They may be discarded upon notification by the Sponsor. To avoid any error, the Investigator should contact the Sponsor before destroying any records and reports pertaining to the study to ensure they no longer need to be retained.

Audits/Inspections

In the event that audits are initiated by the Sponsor (or its designate), or national/ international regulatory authorities, the Investigator shall allow access to the original medical records and provide all requested information.

13. MEDICAL MONITORING

An independent Medical Monitor, with a diverse therapeutic background, both clinical and research expertise, and an active medical license (preferably as a urologist), will be implemented in the study to provide medical and safety oversight as follows:

- Providing input into the study protocol, informed consent forms, risk assessment
- Supporting investigational sites with queries around patient eligibility, safety, etc.
- Reviewing subject safety data to identify trends and risks across the study
- Reviewing safety narratives as requested

14. PROTOCOL DEVIATIONS

A Protocol Deviation (PD) is defined as any alteration or modification to the approved protocol(s). This includes detailed Clinical Study Protocol, protocol summary, and the approved subject Informed Consent Form. A PD is any incident for which the Investigator or site personnel did not conduct the study according to the clinical protocol or the investigator agreement.

Deviations shall be reported to the study sponsor regardless of whether the deviation was medically justifiable, a study oversight, or taken to protect the subject in an emergency. Subject specific deviations will be documented on the Protocol Deviation CRF in the EDC system. Non-subject specific deviations will be reported to the Sponsor in writing (often via a note to file). Investigators will also adhere to procedures for reporting study deviations to their IRB in accordance with their specific IRB reporting policies and procedures.

Good Clinical Practices (GCP) regulations require that Investigators maintain accurate, complete and current records, including documents showing the dates and reasons for each deviation from the protocol. Protocol deviations will be captured in the EDC.

15. STUDY MONITORING PLAN

The monitoring for this study will be conducted by Sponsor or designee. The study will be monitored, in accordance with the Acoustic Enhancer Research on Laser Lithotripsy (AEROLITH) Clinical Study Monitoring Plan (CLIN-0006), to ensure that the protocol, applicable regulations, and Good Clinical Practice Guidelines are followed. Study monitoring will be carried out in compliance with FDA regulations (21CFR Part 812), ISO 14155, and all GCP guidelines. The study monitor will ensure that the rights and well-being of subjects are protected and the clinical trial data are accurate, complete, and verifiable.

Prior to subject enrollment, the Sponsor or designee will obtain the essential regulatory documents required to initiate the study. The sponsor will be responsible for the review and approval of these essential documents. Copies of the documents will be maintained by the Sponsor.

Site Qualification/Initiation Visits

All sites will undergo a qualification process to confirm acceptability for participation into the study.

Study initiation visits are performed at the start of the clinical study to ensure that the study personnel have a complete understanding of the protocol, procedures, responsibilities, and regulations involved with the conduct of a clinical trial. Study personnel will be trained on all essential aspects of the trial prior to the first subject being enrolled in the study. This training will include an in-depth review of the protocol and case report forms, regulatory requirements, serious adverse experience reporting, and other activities as documented in the site initiation plan.

Interim Monitoring Visits

Interim monitoring visits will be made at all active investigational sites throughout enrollment of the clinical study to assure that the Investigator obligations are fulfilled and all applicable regulations and guidelines are being followed. These visits will assure that the facilities are still acceptable; the study protocol and related required protocols are being followed, the IRB has been notified of approved protocol changes as required, complete records are being maintained, appropriate and timely reports have been made to the Sponsor and the IRB, device and device inventory are controlled and the Investigator is carrying out all agreed activities. The monitor will verify accuracy of CRF completion against source documents maintained at the site.

During monitoring visits, the Monitor will perform a review of study eligibility, Inclusion/Exclusion criteria, informed consent, all reports of device malfunction, all events meeting criteria for serious adverse event reporting as well as safety and efficacy endpoints.

The monitor will periodically, at a minimum of annually, monitor the study sites. The investigator and staff are expected to cooperate and provide all relevant study documentation to the monitor upon request, including direct access to the study data, such as electronic medical records. In the event direct access is not provided to the monitor, certified printed copies may be accepted. Corresponding source data and documents are required to verify the accuracy of all data.

Site Close Out Visit

Upon completion of the clinical study (when all subjects enrolled have completed the follow-up visits and the CRFs and queries have been completed), a study closeout visit will be performed. The Monitor will ensure that the Investigator's regulatory files are up to date and complete and that any outstanding issues from previous visits have been resolved. Other topics which may be reviewed at this visit include: discussing retention of study files, possibility of site audits, publication policy, and IRB study closure notification.

Qualifications of Study Monitor

Applaud Medical will select a study monitor qualified by training and experience to monitor the progress of the investigation. The proposed study monitor has suitable academic training, applicable clinical experience, and knowledge of the investigational device.

16. DATA MANAGEMENT

16.1. Data Collection

Study data will be collected by electronic Case Report Forms (eCRFs). Data queries and data updates will also be electronic. Source document verification (SDV) will also be electronic in the database. The investigator signature will also be electronic in the database. Each Patient will be assigned a unique de-identified study identification (ID). All eCRFs for a subject are associated with this de- identified ID.

16.2. Data Storage

The clinical database management system (CDMS) is the electronic data capture (EDC) system Medrio. The Medrio EDC system is developed and hosted by Medrio, Inc. The Medrio EDC system meets CFR 21 Part 11 compliance. Study data will be sent to and securely stored in a central location of Medrio's servers for analysis in the United States (Dallas Texas, Seattle, Washington, and Washington D.C.).

16.3. Confidentiality

Patient confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor, and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to study subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor supplying the study device, representatives of the ethics committee may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by IRB and institutional regulations.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will not include the Patient's contact or identifying information. Rather, individual patients and their research data will be identified by a unique study identification number.

CT images, KUB X-Ray and ultrasound images will be obtained for study evaluation. If published, study subject-identifying information will be removed.

16.4. Study Record Retention

All study records and reports will remain on file at the sites for a minimum of 2 years after completion of the Study and will further be retained in accordance with local guidelines as identified in the clinical study agreement. Study records are to be discarded only upon notification by the study sponsor. The Investigator must contact the study sponsor before the destruction of any records and reports pertaining to the study to ensure they no longer need to be retained. In addition, the sponsor should be contacted if the Investigator plans to leave the investigational site. All required data for this study will be collected on standardized CRFs or an electronic data capture system.

All information and data sent to the Sponsor or Contract Research Organizations (CROs) concerning subjects or their participation in this study will be considered confidential. All data used in the analysis and reporting of this evaluation will be used in a manner without identifiable reference to the subject. The Principal Investigator consents to visits by the staff of the Sponsor and its authorized representatives and the U.S. Food and Drug Administration or any other local governmental body to review the study subjects' medical records including any test or laboratory data that might have been recorded on diagnostic tests media (e.g., CT scans, X-rays, etc.).

17. USE OF DATA, PUBLICATIONS POLICY, REGISTRATION

To safeguard the integrity of the study, reports (summary or interim) will not be submitted for publication without prior agreement from Applaud Medical, Inc.

Anonymized subject data may be used for publication and presentation purposes. Photos and audio/visual recording of the procedure may be performed at the investigator's discretion. It may be used for professional publication or presentation purposes if the information is anonymized and it is covered by the subject's informed consent.

The study will be registered on https://clinicaltrials.gov in compliance with 42 CFR Part 11. Results of the study, including an unanticipated early termination of the trial, will be posted to the Clinicaltrials.gov database at the conclusion of the study.

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REVISION HISTORY TABLE

Revision	DCO	Effective Date	Description and Rationale for Changes
1	DCO-194	28 SEP 2020	Initial Release.
A	DCO-215	20 JAN 2021	• Section 5.1.1: corrected the spelling of "lithotripsy" and "stones" • Section 5.3: Added the word "separate" in the 2nd statement for clarity • Table 2: Schedule of Events, simplified for clarity • Updated ISO 14155 revision from 2011 to 2020 • Section 16: Deleted "Statement of Compliance" because investigator responsibilities are already discussed in section 12.2 • Section 12.2 under Regulatory Filing: added "ultrasounds where applicable" since ultrasounds are required for those subjects who may develop hydronephrosis. • Section 17: added "The study will be registered on https://clinicaltrials.gov in compliance with 42 CFR Part 11. Results of the study, including an unanticipated early termination of the trial, will be posted to the Clinicaltrials.gov database at the conclusion of the study" to comply with 42 CFR Part 11." • Added revision history table following internal procedures for clinical study files
B	DCO-243	15 APR 2021	• Section 5.8 and protocol synopsis section: An exclusion criterion of "Patients who have had prior URS-LL within 3 months on the side to be treated at the time of consent" is added to minimize/prevent confounding clinical outcomes in the analysis of data. • Section 5.8 and protocol synopsis section: Exclusion criterion "If female, last menstrual cycle(s) is >30 days on the study procedure unless patient has a documented history of menstrual cycle(s) that are absent or are >30days if not pregnant" is removed since all females will be required to take a pregnancy test on the day of the procedure. • Protocol Synopsis section: Added Abond CRO, Inc. information. Abond CRO will be responsible for managing the AEROLITH study's data and conducting statistical analysis. • Section 5.5: added the words "and randomized" and "subjects" for clarity. This language, added for clarity, is consistent with the language in the original IDE protocol. • Section 6.3.2: Reworded section to "Re-interventional procedure(s) on stone(s) that were treated as part of the AEROLITH study" for clarity. • Section 6.4.2 "Subjects will be blinded of their treatment allocation through index procedure" was added as a measure to minimize bias. • Section 7.3: Changed the timing of randomization from after subject receives general anesthesia to randomizing a subject the day of the procedure. The rationale for this change is to minimize logistical challenges of unnecessarily transporting temperature sensitive investigational devices to the Operating Room (OR) if it's not needed. Further, this minimizes or prevents an incident of not being able to secure a randomization number from the EDC if the OR does not have a computer or Wifi connectivity. To maintain the scientific soundness of the study, subjects will still be blinded of their treatment allocation through index procedure. • Section 7.5: added subject identification coding system. • Sections 8.1, 8.4, 8.5, 8.6 – Modified the medication language following FDA's request during interactive review (FDA e-mail received on 4/13/21, 10:02am) • Sections 8.5 and 8.6: Added the requirement of conducting a urinalysis and urine culture if subject experiences a UTI adverse event as it was missed in the previous version of the protocol.

			• Table 2: Updated table to be consistent with the protocol required tests and clarified language. • Section 10.3: Added the word "randomized" for clarity. This is consistent with the language in the IDE clinical protocol approved by FDA. • Section 10.8: Clarified language by adding words "subsequent" and "in the AEROLITH study"
C	DCO-288	22 AUG 2021	• Protocol Synopsis_Inclusion Criteria 6 – modified requirement of Urine culture. • Principal Contacts o Updated Study Statistician and Data Management from Abond to ARA o Added Stuart Wolf as Medical Monitor • Sections 5.7.6 - modified requirement of Urinalysis with Urine culture. • Section 8.1 o modified requirement of Urinalysis with Urine culture. o Removed verification of absence of pregnancy as it is covered in section 8.2. • Section 8.2 - modified requirement of Urinalysis with Urine culture. • Section 8.4, 8.5, and 8.6 - modified requirement of Urinalysis with Urine culture. *Rationale for* modifying *requirement of Urine culture – Per AUA Guidelines, Urine culture is conducted only if patient presents with positive urinalysis and/or clinical signs of an infection.*
D	DCO-306	12 NOV 2021	• Section 5.1.1 was updated to reflect the recent approval for full enrollment to a total of 196 subjects per G200183/S007. • Protocol synopsis and Section 5.5, added "each site may enroll no more than 20% of the total sample size of 196 subjects."to address FDA's study consideration detailed in G200183/A001. • Protocol synopsis, Section 5.7.3.2 and 5.8.1, added a note that punctate stones measuring ≤2mm do not count in the 3 stone limit or in the cumulative diameter limit of 20mm for clarity to align with the language in CLIN-0003: Radiographic Evaluation Protocol for the AEROLITH Study, previously approved by FDA (G200183/A001). In Section 4.1.1 of CLIN-0003, it states "Any renal calculi less ≤ 2 mm in diameter (largest diameter in any plane), and/or maximum CT intensity < 100 HU *will not be counted* as stones." • Protocol synopsis and Section 5.8.2, deleted "Patients with >3 stones with a cumulative diameter of > 20mm on the side to be treated". Redundant, noted in 5.8.1 • Protocol synopsis and Sections 5.8.7 and 8.1, added an exception to the rule where if recently tested (within 30 days of consent) urine pH is <5.5 but patient has his/her largest stone measuring a mean stone intensity of ≥500HU and has a historical pH value of ≥ 5.5, patient may qualify for the study. AEROLITH study investigators have advised Applaud that urine pH isn't always predictive of a calcium-based stone (which is the intended stone for treatment by the Acoustic Enhancer) since urine pH can be influenced by diet, diseases, medications and timing of urine pH testing. AEROLITH investigators and Applaud's Advisors are proposing that the largest stone intensity is factored into the determination of calcium-based stones following Spettel, et al 2013 paper on "Using Hounsfield Unit Measurement and Urine Parameters to Predict Uric Acid Stones from Calcium-Based Stones for those patients who fail to meet the pH threshold during the screening process. Applaud's Advisors and AEROLITH investigators propose that these patients may be considered if his/her largest stone intensity ≥500HU and has demonstrated a historical urine pH value of ≥5.5. • Protocol synopsis section and Section 6.1, defined the primary safety endpoint to address FDA's study consideration detailed in G200183/A001. • In protocol synopsis, added the Adverse Events type, over time, severity,

			seriousness, and relatedness to align with the requirement in Section 6.4.3 • Defined and added Study Success in the protocol synopsis and Section 6.6 to address FDA's study consideration detailed in G200183/A001. • Section 6.2, added *"the primary efficacy endpoint will demonstrate that the investigational arm is superior in the fragmentation of stones versus the control arm."* to align with Applaud's proposal of a superiority statistical design for primary efficacy endpoint. • Section 6.3, added "the secondary efficacy endpoint will demonstrate non-inferiority of the mean TRE used between the investigational and control arm to align with Applaud's proposal of a non-inferiority statistical design for the secondary endpoint. It is worthy of note that statistical significance with this secondary efficacy endpoint along with any other effectiveness endpoints are not needed for study success. • Section 6.4.2 changed the language from "re-interventional" to "additional interventional stone procedure" for clarity. • Sections 10.5 and 10.7, modified the secondary hypotheses 1 and 2 to align with the secondary endpoint statistical design.
E	DCO-326	19 JAN 2022	• Inclusion criterion no. 3 of the protocol synopsis and section 5.7.3 are modified to allow patients having multiple stones (>3 stones). The stone cumulative diameter limit does not change and remains 20mm as recommended for URS-LL by the Surgical Management of Stones: AUA/Endourology Society Guideline (2016) as follows: *"21. In symptomatic patients with a total non-lower pole renal stone burden of ≤ 20mm, clinicians may offer SWL or URS. (Index Patient 7) Strong Recommendation; Evidence Level Grade B."* This is being modified to reflect typical kidney stone population that would be eligible for URS-LL. • Inclusion criterion no. 3 of the protocol synopsis and section 5.7.3, added "Note 2: *Some corroborating imaging may be required to exclude a patient if the investigator suspects that the stone has moved to middle/distal ureter (e.g. change in symptoms, an increase in serum creatinine)"* This ensures that those patients whose stones are suspected to have moved (after being found eligible for the study) to middle/distal ureter and may require other form of treatment/management are excluded from the study. and may require other form of treatment/management are excluded from the study. This aligns with the Surgical Management of Stones: AUA/Endourology Society Guideline (2016) as follows: *"8. Clinicians should offer reimaging to patients prior to surgery if passage of stones is suspected or if stone movement will change management. Reimaging should focus on the region of interest and limit radiation exposure to uninvolved regions. Clinical Principle"* • Inclusion criterion no. 4 of the protocol synopsis and section 5.7.4 are modified to extend the baseline CT image cut off from 30 to 60 days. In Rev. D of the protocol, a patient is required to have a repeat CT scan if their CT image is >30days old. The purpose of this change is to reduce the radiation exposure of patients with >30days CT scan having to undergo a repeat CT image. • Exclusion criterion no. 1 of the protocol synopsis and section 5.8.1, deleted ">3 stones" and "the 3 stone limit and in" to align with the proposed criteria of allowing for multiple stones as long as the cumulative diameter limit does not

			exceed 20mm. • Exclusion criterion no. 7 of the protocol synopsis and section 5.8.7, added the words “the patient” for clarity. • Exclusion criterion no. 11 of the protocol synopsis, section 5.8.11, section 8.2 and Table 2, added serum testing as an alternative pregnancy test method. • Section 8.2, added “and prior to randomization” for clarity. • Section 8.2, updated section for clarity and to ensure alignment with the Acoustic Enhancer IFU (LBL-014, Rev. F.), which was approved by FDA under G200183/S005. • Section 10.2, expanded the definition of ITT for clarity and to ensure that in the event a subject develops UTI or has his/her stone moved to the middle/distal ureter or who no longer meet any of the other inclusion/exclusion criteria between the time the subject was initially found eligible and the time they were randomized, are not treated under the protocol and are excluded from the study. • Protocol Synopsis and Section 5.0: Update number of investigational sites to 18 per G200183/S011