Clinical Study Protocol

Effect of Amplifi™ Vein Dilation System Treatment on Radiocephalic AVF Maturation-3

Short Title:

FIH-3

Study Sponsor:

Amplifi Vascular, Inc.
3965 W 83rd Street, #300
Prairie Village, KS 66208-5308

Study Contact:

Erdie De Peralta, VP Regulatory and Clinical Affairs
Amplifi Vascular
telephone: 978.408.0949
email: edeperalta@amplifivascular.com

RELEASED

Table of Contents

List of Appendices

List of Tables

List of Figures

List of Abbreviations

ACT .....Activated Clotting Time
ADE .....Adverse Device Effect
AE.....Adverse Event
AVF.....Arteriovenous Fistula
AVG.....Arteriovenous Graft
CBC.....Complete Blood Count
CKD.....Chronic Kidney Disease
CRF .....Case Report Form
CSA .....Clinical Study Agreement
ECMO.....Extracorporeal Membrane Oxygenation
EDC.....Electronic Data Capture
eNOS.....Endothelial Nitric Oxide Synthase
ESRD.....End Stage Kidney Disease
FIH.....First-in-Human
FDA .....US Food and Drug Administration
FMEA.....Failure Mode and Effect Analysis
GCP.....Good Clinical Practice
GUI .....Graphical User Interface
ICF.....Informed Consent Form
IFU.....Instructions For Use
EC.....Independent Ethics Committee
KDOQI .....Kidney Disease Outcomes Quality Initiative
LDH .....Lactate Dehydrogenase
PICC.....Peripherally Inserted Central Catheter
pSLOT .....Piggyback Straight-Line On-Lay Technique
RPM.....Revolutions Per Minute
SADE.....Serious Adverse Device Effect
SAE .....Serious Adverse Event
USADE .....Unanticipated Serious Adverse Device Effect
VNH.....Venous Neointimal Hyperplasia
WSS .....Wall Shear Stress

Revision History

Revision	Date	Description of Changes	Originator
A	01/24/2023	Initial Release	E. De Peralta
B	12/19/2023	Updated study design for nonrandomized inpatient cohort	E. De Peralta
C	01/28/25	Updated study design for reduced treatment duration; change in vein diameter inclusion criteria to 1.7 mm-3.3 mm; update on criteria for AVF creation; additional safety outcome measures; clarification on Definition of terms; change the Title from Amplifi-1 to FIH-3; Identified CRO and Investigation Site; overall update for consistency	E. De Peralta
D	02/06/25	Clarification on the outcome measures to categorize some safety outcome measures into technical performance measures. Also, added definition of treatment/device success in the Definition of Terms section.	E. De Peralta

Protocol Signature Page

I, the undersigned Investigator(s), have read and understood the terms of the Protocol. I agree to perform and conduct the Study as described in the Protocol and in accordance with the ICH/GCP Guidelines and 21 CFR 812.

Principal Investigator's Signature	Date
Co-investigator's Signature	Date
Co-investigator's Signature	Date
Co-investigator's Signature	Date
Sponsor's Signature	Date

Contact Information

The sponsor of this Clinical Study is as follows:

Sponsor Information

Erdie De Peralta
Amplifi Vascular, Inc.
3965 W 83rd Street, #300
Prairie Village, KS 66208-5308
978-408-0949
edeperalta@amplifivascular.com

Data Management:

MMC Medical International Services 430 Forest Ave.
Laguna Beach, CA 92651

Investigational Site

Sanatorio Italiano El Dorado 284 Casa Zanotti
Asuncion, Paraguay 1849

1.0 Protocol Synopsis

INVESTIGATIONAL DEVICE	Amplifi™ Vein Dilation System ("Amplifi System")
STUDY TITLE	Effect of Amplifi™ Vein Dilation System Treatment on Radiocephalic AVF Maturation- (FIH3)
STUDY DESCRIPTION	Prospective, nonrandomized, single-arm, open-label clinical study (the "Study") in patients with end-stage renal disease (ESRD) receiving maintenance hemodialysis using a catheter for vascular access ("Subjects"). Patients who are indicated for creation of a forearm radiocephalic AVF based on an Investigator's standard practice and who have a forearm cephalic vein diameter of ≥ 1.7 mm and ≤ 3.2 mm at the proposed Study AVF creation site will be enrolled in the study. The Subjects will be treated in an inpatient (hospital) setting and will receive Amplifi System treatment and undergo subsequent AVF creation (a "Study AVF") immediately after treatment. Amplifi System removal immediately followed by AVF creation will be performed when Subject had: • Amplifi treatment for ≥ 48 hours duration and that cephalic vein is suitable for AVF creation determined by physician (e.g., vein diameter ≥ 3.3 mm as defined in Dageforde[17] as Quartile 3 and baseline diameter increased ≥ 50%); or • Amplifi treatment could be extended up to 60 hours (± 12hrs) if the cephalic vein was not determined to be adequate by the investigator. Data will be collected at screening, baseline, during Amplifi System implantation, during Amplifi System treatment, during Amplifi System removal and AVF creation, and follow-up patient visits at 1, 2 and 6 weeks after AVF creation.
OBJECTIVES	The Amplifi System will be assessed and analyzed for effectiveness to demonstrate similar outcome from the earlier First-in-Human Studies with shorter treatment duration. Safety analysis will be performed to confirm that major adverse events will be in an acceptable range.
INDICATIONS FOR USE	The Amplifi Vein Dilation System is a temporary, ambulatory, extracorporeal blood pump system designed to dilate upper extremity veins in patients with a forearm cephalic vein diameter of ≥ 1.7 mm and ≤ 3.2 mm prior to forearm arteriovenous fistula creation to increase vein diameter and improve forearm arteriovenous fistula maturation.

BACKGROUND	Arteriovenous fistula (AVF) is the preferred form of vascular access for hemodialysis. AVF use has demonstrated lower rates of associated morbidity, mortality, contributing to lower overall cost of care[1,4]. Forearm AVF is widely viewed as the preferred location. The National Kidney Foundation recommends a distal to proximal approach to AVF creation citing ease of creation, low complication rates, and preservation of more proximal access options, yet only 32% of hemodialysis patients in the US use a forearm AVF for vascular access[2-4] and more than 80% of forearm AVFs fail to mature within 6 weeks of creation, with many abandoned prior to routine use[4-7]. In studies performed to evaluate likelihood of AVF maturation, initial vein diameter was identified as one of the strongest predictors of AVF maturation success[11-13]. In the HFM study[14], 92% of forearm AVFs failed to mature 2 weeks (84% @ 6 weeks) after AVF creation based on KDQOI criteria. The HFM study suggested that the probability of AVF maturation increases with increasing initial vein diameter at AVF creation. A retrospective study of 158 subjects undergoing brachiobasilic or brachiocephalic AVF showed increased minimum vein diameter was associated with an increased likelihood of fistula maturation and secondary patency[15]. In this study, the authors reported that hazard ratios (HRs) indicate that for each 1.0-mm increase in Minimum Vein Diameter MVD, there was approximately a 45% reduction in the risk for failure of maturation (HR, 0.555; P = .005) and a 36% reduction in the risk of fistula failure (HR, 0.639; P = .001). The authors stated that vein diameter is the only clinical or demographic factor associated with both AVF maturation and long-term patency in their study. In a first-in-human (FIH) clinical study with a total of n=10 Subjects, Amplifi Vascular, Inc. (“Sponsor”) demonstrated that treatment with the Amplifi System increased the diameter of the mid forearm cephalic vein by an average of 150% prior to AVF creation, and that AVFs created with treated forearm cephalic veins matured rapidly and demonstrated high maturation rates.
PRODUCT RATIONALE	The Amplifi System is designed to deliver continuous, non-pulsatile, venous blood to upper extremity veins to stimulate vein wall remodeling and persistent dilation. The Amplifi System capable of increasing vein diameter prior to fistula creation would increase the prevalence of forearm AVFs in patients who are not suitable to receive AVF due to small vein diameter. The Amplifi system would also improve AVF maturation through AVF creation with larger veins that mature rapidly, reduce catheter contact time and prolong AVF patency.

PATIENT POPULATION	Patients with end-stage renal disease (ESRD) and are currently receiving maintenance hemodialysis via a tunneled, cuffed hemodialysis catheter indicated for forearm radiocephalic AVF creation based on an Investigator's standard practice and have a wrist or distal forearm cephalic vein diameter of ≥ 1.7 mm and ≤ 3.2 mm at the proposed Study AVF creation.
EFFECTIVENESS MEASURES	**Percent change in cephalic vein diameter and blood flow** in the middle third of the arm as measured by ultrasonography [Timeframe: from baseline, during Amplifi System treatment (minimum daily), and prior to Amplifi System removal and AVF creation] **Mean vein diameter of the cephalic vein and blood flow of brachial artery** in the middle third of the forearm as measured by ultrasonography [Timeframe: 1, 2and 6 weeks post AVF creation] **Forearm AVF maturation success at 2 and 6 weeks post-AVF creation** [Timeframe: 2, 6 WK post AVF creation] **Percentage of subjects who experience an increase in the cephalic vein diameter of ≥2.7 mm from their baseline measurement, or an increase of >1 mm from baseline, following treatment with the Amplifi System.** [Timeframe: prior to removal of the Amplifi System and creation of the AVF].
SAFETY MEASURES	**Rate of Occurrence of Major Device-Related Adverse Events defined as:** [Timeframe: from device implantation and up to 30 days from device removal and AVF creation] • Amplifi System-related death; • Amplifi System-related major bleeding, major pneumothorax, major air embolism, major thrombosis, major thromboembolism, major hemolysis, or major thrombocytopenia; localized infection • Amplifi System-related re-hospitalization or prolonged hospitalization; • Amplifi System-related major surgery or major interventional procedure; or • any Amplifi System-related complication determined to be an Amplifi System-related major complication by both an Investigator and the Medical Monitor.

	Rate of Occurrence of Cannulation-related Complications [Timeframe: from AVF creation through end of follow-up period]
EXPLORATORY TECHNICAL PERFORMANCE MEASURES	**Rate of Occurrence of Amplifi System Treatment Failure** [Timeframe: from device implantation through less than required minimum treatment hours of duration (e.g., 48 hours] **Rate of Occurrence of Amplifi System component replacement** [Timeframe: from device implantation through device removal and AVF creation] **Rate of Occurrence of Pump Stoppages and Device Malfunction** [Timeframe: from device implantation through device removal and AVF creation]
NUMBER OF SUBJECTS	Up to n=10 Subjects, all Treatment Subjects
STUDY DURATION	All Subjects will be followed for 1, 2 and 6-weeks post AVF creation or to a censoring event (including return of kidney function, kidney transplant, death, loss to follow-up, or Study end) whichever occurs first.
INCLUSION CRITERIA	1. Age ≥ 18 years 2. Indicated for wrist or distal forearm radiocephalic AVF creation based on Investigator's standard practice 3. ESRD currently receiving maintenance hemodialysis via a tunneled, cuffed hemodialysis catheter 4. Baseline wrist or distal forearm cephalic vein diameter of $\geq$ 1.7 mm and $\leq$ 3.2 mm at the proposed Study AVF creation site, wherein the wrist or forearm cephalic vein at the proposed Study AVF creation site is suitable for Amplifi Distal Outflow Catheter placement and Amplifi System treatment, including:

	1. ≥ 1.7 mm to ≤ 3.2 mm diameter of the wrist or forearm cephalic vein at the proposed Study AVF creation 2. patency and continuity of blood flow from the proposed Study AVF creation site centrally to the upper arm at the elbow through to at least one or more of: 1) the upper arm cephalic vein; 2) the median cubital vein to the upper arm basilic system; or 3) the perforator vein to the deep venous system; and 3. no occlusion or stenosis ≥ 50% along the course of the forearm cephalic vein and at least one upper arm venous outflow up to the subclavian vein, as determined by duplex ultrasonography 5. Estimated forearm hemodialysis needle cannulation zone length ≥ 18 cm after creation of the proposed Study AVF 6. At least one patent internal jugular vein suitable for Amplifi Inflow Catheter insertion 7. Subject has voluntarily signed written informed consent
EXCLUSION CRITERIA	1. Known allergy to Amplifi System components (polyurethane, nitinol), iodinated contrast agents, heparin, or apixaban 2. Known or suspected active infection at the proposed time of Amplifi System placement 3. Known bleeding diathesis, including from uncorrected coagulopathy 4. Known thrombophilia, requiring treatment 5. Hb <10 gm/dL 6. Platelet level < 100,000 /mm^3 7. Need for continued treatment with anti-platelet agents or other anticoagulants (other than apixaban) during the Amplifi System treatment period 8. Known history of patent foramen ovale ≥ 2 mm 9. History of recent intracranial or gastrointestinal bleeding 10. Documented recent central venous or right atrial thrombus 11. Known presence or recent history of intracranial or gastrointestinal bleeding or presence of underlying conditions that would predispose to intracranial or internal bleeding. 12. History of ipsilateral central venous occlusion, stenosis ≥ 50%, angioplasty, or stent placement 13. Pregnancy, lactation, or plans to become pregnant during the Study

	14. Unwillingness or inability to comply with procedures specified in the Protocol, or difficulty or inability to return for follow-up visits as specified by the Protocol 15. Participation in any other investigational drug or medical device study that has not completed primary endpoint evaluation or clinically interferes with the endpoints from the Study, or planned future participation in such studies prior to the completion of the Study
STUDY FOLLOW-UP INTERVALS	• Screening • Baseline • Amplifi System placement • Amplifi System treatment • Amplifi System removal and creation of Study AVF • Discharge after Amplifi System removal and creation of Study AVF • 1, 2 and 6 weeks after creation of Study AVF
STATISTICAL CONSIDERATION	General descriptive statistics will be used for the analysis of data

2.0 Introduction to Amplifi System

2.1 Background: End Stage Kidney Disease, Hemodialysis, and Vascular Access

Kidney function is essential to sustain life. Patients with end-stage kidney disease (ESRD) have an irreversible loss of kidney function and need some form of kidney replacement therapy for survival. The preferred form of kidney replacement therapy is kidney transplantation; however, the availability of transplant organs is limited. Hemodialysis remains the most prevalent kidney replacement therapy in the US[1] and worldwide.

There are more than 550,000 patients in the US on hemodialysis[1], a population projected to surpass one million by 2030[2]. During hemodialysis, blood is removed from a patient's body, filtered through a synthetic dialyzer membrane, and returned to the body. Prior to initiating hemodialysis, physicians must create a vascular access site to provide access to a patient's circulatory system for blood removal and return. The main types of vascular access sites, includes each requiring a surgery or interventional procedure for site creation:

- Hemodialysis catheter
- Arteriovenous graft (AVG)
- Arteriovenous fistula (AVF)

An AVF is the preferred type of access and is created by directly connecting an artery to a vein. AVFs are usually created surgically, but percutaneous AVFs are an option for some patients. Common locations for AVF placement include the wrist/forearm, and upper arm. The radial and brachial arteries and the cephalic and basilic veins are most often used to make AVFs. After creation, the inflow arteries and outflow veins of AVFs are subjected to marked changes in hemodynamic stress that induce dilation and increases in blood flow, a process known as "maturation".

There are several widely recognized thresholds for AVF outflow vein diameter and blood flow that are required to enable routine needle cannulation and to ensure adequate delivery of blood to a hemodialysis machine and back to the patient for hemodialysis treatments. AVF maturation usually requires several weeks and may take several months to a year, often resulting in long catheter contact times for patients and increasing their risk of catheter-related blood stream infections, which can lead to sepsis and death. A depiction of a distal forearm radiocephalic AVF is shown in **Figure 1** , with needle cannulation of the outflow vein to remove and return blood for hemodialysis.

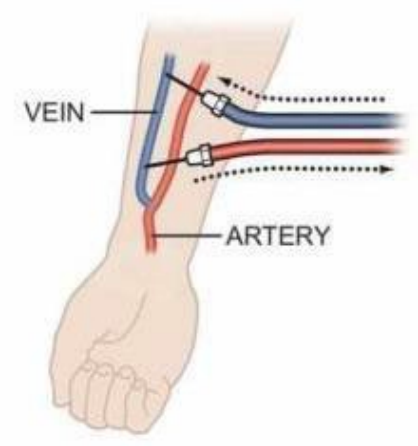


Figure 1: Example of a Distal Forearm Radiocephalic AVF

Survival of hemodialysis patients is directly related to adequacy of their hemodialysis which in turn, is dependent on the availability and function of their vascular access. Vascular access failure is common, resulting in interruptions in hemodialysis care and leading to more than two million surgeries and interventional procedures each year in the US alone, with an estimated $2.8 billion in annual Medicare costs[3]. Vascular access complications are also common, often resulting in hospitalization and contributing to the high mortality rates seen in the ESRD population.

Patients using an AVF for hemodialysis have lower rates of morbidity, mortality, and the overall cost of care[4] when compared with other forms of vascular access for hemodialysis. The National Kidney Foundation recommends a distal to proximal approach to AVF creation for preservation of more proximal access options for later use . However, prevalence of wrist and forearm AVFs is low, especially in the US. Many ESRD patients are unsuitable for AVF surgery [5,6] (especially in the forearm) primarily due to inadequate vein diameter. For those patients that are suitable, maturation failure rates are high, up to 84% at 6 weeks in some studies[6-9]. The high rate of maturation failure of forearm AVFs has led to a practice of creating upper arm AVF as an earlier option for many patients[10-12].

In studies performed to evaluate likelihood of AVF maturation, initial vein diameter was identified as one of the strongest predictors of AVF maturation[13-15]. These findings were corroborated by the Hemodialysis Fistula Maturation Study conducted by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health which showed the probability of AVF maturation increases with increasing initial vein diameter and blood flow[16]. As shown below, **Figure 2** from the Hemodialysis Fistula Maturation Study shows the estimated probability that patients will meet KDOQI anatomic and physiologic AVF maturation criteria based on 1-day (dashed curves) and 2-week (solid curves) AVF outflow vein diameter and blood flow. Shown are the predicted probabilities that AVF maturation will be reached at 6 weeks for different 1-day (dashed curves) or 2-week (solid curves) outflow vein diameters (horizontal axis) and AVF blood flow with the black, blue, and red curves representing AVF blood flows of 250, 500, and 1000 mL/min, respectively. The curves were fit by using logistic regression with cubic splines for both

AVF blood flow and outflow vein diameters. As shown, larger AVF outflow vein diameter and higher initial AVF blood flow are positively correlated with a greater probability of achieving AVF maturation.

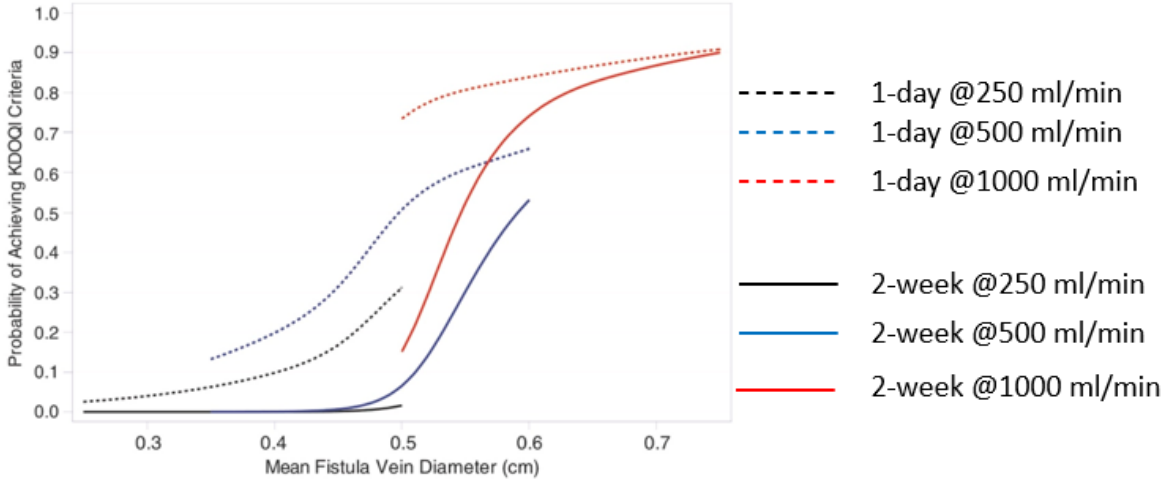


Figure 2: Human Fistula Maturation Study Data

A retrospective study of 158 subjects undergoing brachiobasilic or brachiocephalic AVF showed increased minimum vein diameter was associated with decreased risk of failure to mature, which is synonymous with an increased likelihood of fistula maturation and secondary patency[17]. The authors assigned 25th, 50th, and 75th percentile rank values to statistically define minimum vein diameter (MVD) quartiles to the population. As reported by the authors, each patient's MVD was classified as being within the first, second, third, or fourth quartile. Univariate Kaplan-Meier survival analysis with log-rank tests was used to determine whether preoperative MVD quartile was associated with AVF maturation and long-term patency.

The results indicated that vein diameter data were normally distributed with a mean of 3.4 ± 1.1 mm (see **Figure 3**). MVD quartiles were defined as:

- Quartile 1: < 2.7 mm
- Quartile 2: 2.7 to 3.2 mm
- Quartile 3: 3.3 to 4.1 mm
- Quartile 4: > 4.1 mm

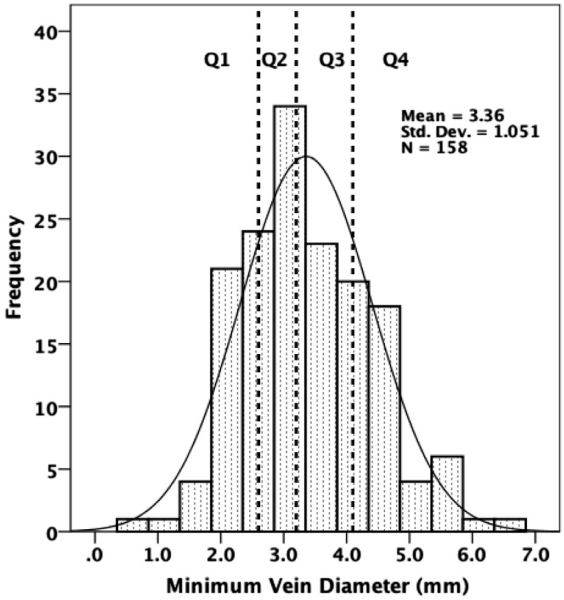


Figure 3: Histogram reproduced from Dageforde, et al

The Kaplan-Meier estimates of fistula maturation were reported as 63%, 79%, 90%, and 90% for the quartiles.

The authors report that hazard ratios (HRs) indicate that for each 1.0-mm increase in MVD, there was approximately a 45% reduction in the risk for failure of maturation (HR, 0.555; P = .005) and a 36% reduction in the risk of fistula failure (HR, 0.639; P = .001). Quartile 3 had significantly greater likelihood of fistula maturation than Quartile 1 (P = 0.028), with Quartile 3 and Quartile 4 both having fistula maturation of 90%. The authors stated that vein diameter is the only clinical or demographic factor associated with both AVF maturation and long-term patency in their study.

2.2 Amplifi System and Intended Use

The Amplifi System is designed to rapidly dilate and remodel upper extremity veins prior to AVF creation by delivering continuous, non-pulsatile venous blood flow to upper extremity veins and providing sustained increases in vein wall shear stress (WSS) during the treatment period.

The Amplifi System comprises of the following main components:

- an extracorporeal blood pump with an integrated cable (“Pump”) to pump blood;
- a catheter to convey blood from the central venous circulation to the Pump (“Inflow Catheter”)
- a catheter assembly to convey blood from the Pump to upper extremity veins) (comprising a “Proximal Outflow Catheter” joined to a “Distal Outflow Catheter”);

- two access ports to enable flushing of the Amplifi System and the injection of radiographic contrast (“Access Ports”);
- a blood flow sensor with an integrated cable (“Blood Flow Sensor”); and
- a controller (“Controller”) to provide power to the Pump and control Pump function.

A complete version of the Amplifi System on a patient is shown in **Figure 4** with the front flap of the Patient Vest open. Patients can carry the Controller from place-to-place, allowing them to carry on with activities of daily living during treatment while in the hospital.

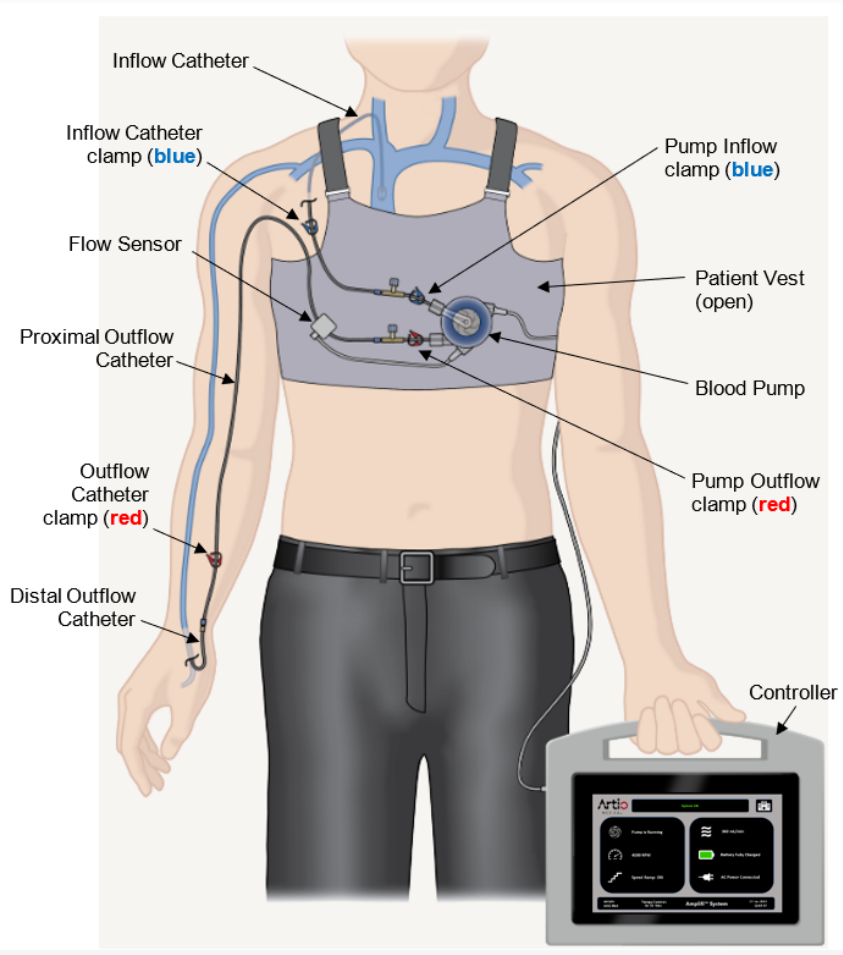


Figure 4: Amplifi System in Place During Treatment

The Amplifi System is designed for placement by trained physicians using standard percutaneous, minimally invasive techniques. The Inflow Catheter tip is inserted into an internal jugular vein and advanced under fluoroscopic guidance to the right atrium. The proximal portion of the Inflow Catheter is tunneled under the skin of the chest for a short distance to provide resistance to infection using a custom Inflow Catheter Tunneler provided with the Inflow Catheter. The proximal end of the Inflow Catheter can be trimmed to the appropriate length and

attached to the Inflow Access Port via a quick-connect ("Screw Lock") fitting. The Distal Outflow Catheter tip is inserted into a vein in the upper extremity, with a taper to facilitate easy vein insertion. The Distal Outflow Catheter is joined to the Proximal Outflow Catheter using a connector ("Inline Connector") and Screw Lock fittings. The proximal end of the Proximal Outflow Catheter can be trimmed to the appropriate length and attached to the Outflow Access Port via a Screw Lock fitting. During placement of the Amplifi System, the Pump is connected to the Controller via the integrated Pump cable. A control algorithm in the Controller automatically increases the speed of the Pump to maintain an elevated WSS in the cephalic vein as the treatment progresses and the cephalic vein dilates. During placement of the Amplifi System, the Blood Flow Sensor is connected to the Pump via the integrated Blood Flow Sensor cable.

Additional details of the Amplifi System can be found in the Investigator's Brochure.

2.3 Product Rationale

KDOQI guidelines suggest wrist and distal forearm AVF is the best first option for vascular access, if a patient is expected to need hemodialysis for at least a year. However, the prevalence of forearm AVF is low in the US and the failure of forearm AVFs to mature often results in patients using hemodialysis catheters, AVGs, and upper arm AVF.

The Amplifi System dilates veins by persistently increasing WSS in treated veins and inducing vein wall remodeling through the well understood process of flow-mediated dilation where rapid blood flow results in elevated WSS and nitric oxide (eNOS) release from endothelial cells, leading to vascular smooth muscle cell relaxation. If rapid blood flow is sustained for a period of days to weeks, there is remodeling of the vein wall that is characterized by elastin and collagen fragmentation, endothelial and smooth muscle cell proliferation, and a persistent increase in diameter[18].
The use of the Amplifi System to dilate veins prior to AVF creation could provide the following benefits for patients:

- **Rapid, robust, and reliable vein dilation** – due to the controlled delivery of high flow, non-pulsatile venous blood to the treated vein, and better control of WSS during the vein maturation period when compared with conventional AVF creation.
- **Improved suitability for AVF creation** – enabling AVF creation in patients who are not suitable to receive an AVF due to small vein diameter, especially in the forearm.
- **Improved AVF maturation** – through AVF creation with larger veins, which has been shown to correlate with improved AVF maturation, and also from high WSS in the AVF inflow artery after connection to a large, low resistance, Amplifi System-treated vein, which could stimulate faster and greater outward remodeling of the AVF inflow artery and result in higher AVF blood flows.
- **Reduced time to AVF maturation and reduced catheter contact time** – by creating AVFs with large veins that mature rapidly and allow for early catheter removal.
- **Prolonged AVF patency** – by creating AVFs with higher blood flows (that are resistant to thrombosis) and larger outflow veins (that are more resistant to

stenosis formation).

- **Early identification and treatment or exclusion of vein segments with pre-existing disease** – by identifying vein segments with hypertrophic valves or fibrosis that do not dilate in response to elevated WSS and treating them with angioplasty immediately prior to AVF creation or excluding them from the AVF circuit. This could provide an opportunity to address pre-existing outflow vein problems that would have otherwise gone undetected at the time of AVF creation and could impair AVF maturation.

2.4 Proposed Indication for Use

The Amplifi Vein Dilation System is a temporary, ambulatory, extracorporeal blood pump system designed to dilate upper extremity veins in patients with a wrist or forearm cephalic vein diameter of ≥ 1.7 and ≤ 3.2 mm prior to wrist or forearm arteriovenous fistula creation to improve wrist or forearm arteriovenous fistula maturation.

3.0 Prior Bench Testing and First-in-Human Clinical Study

3.1 Performance and Safety Testing of Amplifi System

Verification and validation testing for the Amplifi System was conducted to ensure design outputs meet defined design inputs. A high-level summary of Sponsor's testing for the Amplifi System is presented in **Table 1**: Performance and Safety Testing.

Table 1: Performance and Safety Testing

Item	Evidence/Meets Standard	Pass/Fail
Design Validation	First-in-human (10 subjects) – Vein diameter increased by over 100%; no serious adverse events	Pass
Biocompatibility	ISO 10993-1:2018 Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process	Pass
ETO Sterilization	ISO 11135:2014 and A1:2018 Sterilization of health care products – Ethylene oxide - Requirements for the development, validation, and routine control of a sterilization process for medical devices	Pass
	ISO 10993-7:2008/AMD 1:2019 Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals	Pass
	ISO 11138-1:2017 Sterilization of health care products - Biological indicators - Part 1: General requirements	Pass
Packaging	ASTM D4169 Standard Practice for Performance Testing of Shipping Containers and Systems	Pass
Catheter Design	ISO 10555-1:2013 Intravascular catheters - Sterile and single-	Pass

Verification	use intravascular catheters - Part 1: General requirements +AMENDMENT 1 (2017)	
Electrical Safety	IEC/ISO 60601-1:2005 Ed3+C1; C2; A1 Medical Electrical Equipment – Part 1: General Requirements for Basic Safety and Essential Performance	Pass
Software Verification	IEC 62304:2006 Ed.1 +A1 Medical Device Software - Software Life Cycle Processes	Pass
Labeling	ISO 15233-1:2021 Medical devices - Symbols to be used information to be supplied by the manufacturer- Part 1: General requirements	Applied
	ISO 18242:2016 Cardiovascular implants and extracorporeal systems — Centrifugal blood pumps	Applied

3.2 First-In-Human Study

A prospective, nonrandomized, single arm First-in-Human (FIH) clinical study was performed at the Sanatoria Italiano Hospital in Asuncion, Paraguay in 2021-2022 under the direction of Principal Investigator Adrian Ebner, MD. The study sponsor for this research was Artio Medical, Inc., which later transitioned to Amplifi Vascular, Inc. A total of n=10 subjects underwent inpatient treatment with the Amplifi System followed by AVF creation with treated veins. AVF maturation was assessed by duplex ultrasound 2 and 6-weeks after AVF creation.

Treatment of the first cohort (FIH1; n = 5) was concluded in Q2 2021 with a mean treatment time of 8.6 days. All FIH1 subjects had successful AVF creation with treated veins and 4 out of 5 AVFs matured (based on KDOQI criteria). Treatment of the second cohort (FIH2; n = 5) was concluded in Q3 2022 with a mean treatment time of 6.3 days. All FIH2 subjects had successful AVF creation with treated veins and all AVFs were mature at 2 weeks after AVF creation (based on KDOQI criteria).

3.2.1 Device Performance Outcome

For both FIH1 and FIH2 the primary performance endpoint was the percent change in the treated cephalic vein diameter from baseline to the end of Amplifi System treatment. As shown in Figure 5**,** the cephalic vein diameter increased by 105% in FIH1 subjects and 150% in FIH2 subjects during the treatment period. As shown in **Figure 6**, mean imputed cephalic vein blood flow (as measured in the Proximal Outflow Catheter) increased 186% for FIH1 and 134% for FIH2 during the treatment period.

AVFs were successfully created with treated veins in all FIH1 and FIH2 subjects. As shown in Figure 5, for FIH1 subjects, mean AVF outflow vein diameter increased to 6.3 mm and mean AVF blood flow (imputed from brachial artery blood flow in some FIH1 subjects) increased to 851 mL/min six weeks after AVF creation (**Figure 6**). As shown in **Figure 5** and **Figure 6**, for FIH2 subjects, mean AVF outflow vein diameter increased to 7.0 mm and mean AVF blood flow (imputed from brachial artery blood flow in all FIH2 subjects) increased to 1,153 mL/min six

weeks after AVF creation. There were no Amplifi System related or procedure-related serious adverse events.

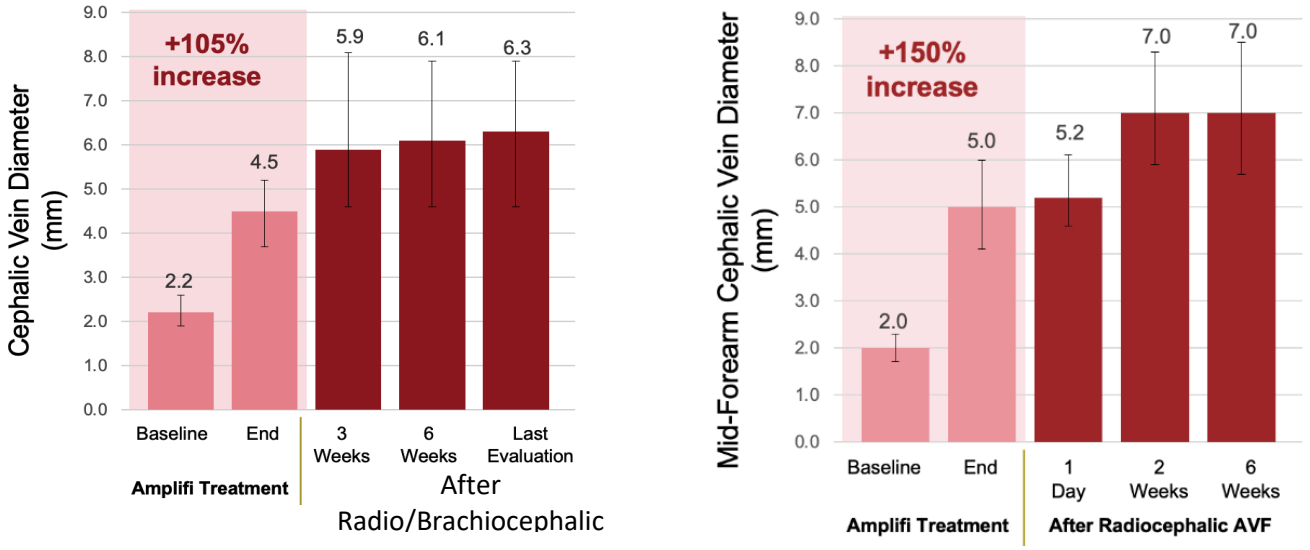


Figure 5: Cephalic Vein Diameter Measurements in FIH1 (left) and FIH2 (right)

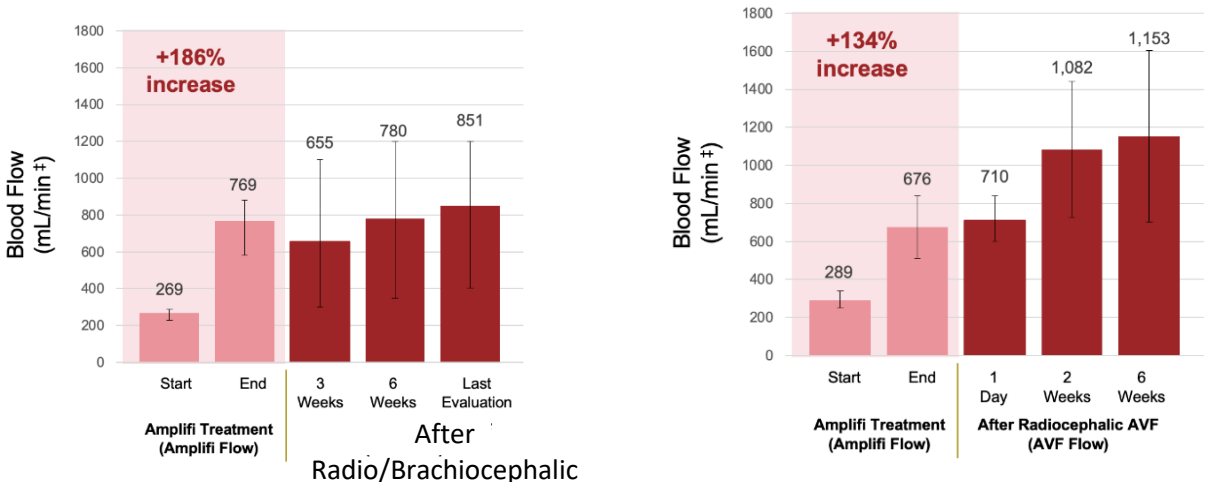


Figure 6: Blood Flow Measurements in FIH1 (left) and FIH2 (right)

3.2.2 Treatment Duration

In the FIH2, vein diameter experienced rapid dilation, started to level off at 48 hours, and remained constant up to 120 hours of treatment duration as shown in **Figure 7**. Immediate dilation of the vein (as early as less than 24 hours of therapy) is indicative treatment effect in the early stage of vein dilation. With controlled increase of non-pulsatile flow, vein dilation plateaus at around 48-72 hours indicative of vein remodeling with sustained effect up to 120 hours. Based on this data, the proposed treatment duration for Amplifi treatment requires a minimum of 60 hours (± 12 hours) of treatment to ensure that optimal vein dilation through remodeling is achieved. The recommended treatment duration of 60 hours (± 12 hours) is based on the data that vein dilation remained constant as shown in the plot.

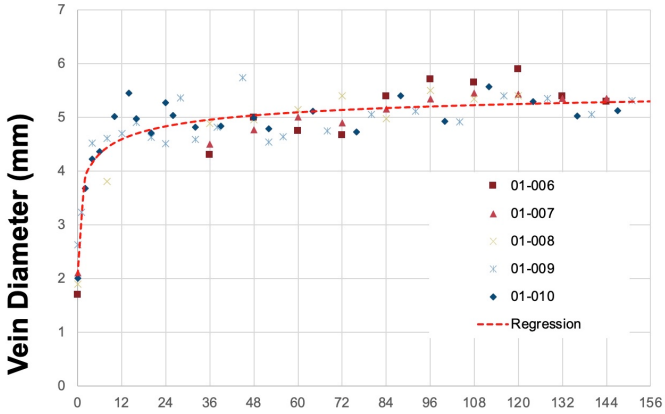


Figure 7: FIH2 Treatment Duration

3.2.3 Safety Outcome

The primary safety endpoint was freedom from the composite of death, re-hospitalization, bacteremia, Amplifi System-related or AVF-related infection, thromboembolic events, or major bleeding from Amplifi System placement to six weeks after AVF creation. There were no serious adverse events (SAEs), deaths, re-hospitalizations, Amplifi System-related or AVF-related infections, major bleeding, or thromboembolic events from Amplifi System placement to six weeks after AVF creation. **Table 2** outlines all adverse events that occurred in the FIH study. Notably, although none of the events were considered more than mild, a minor pump design modification was incorporated to reduce the need for pump replacement.

Table 2: Adverse Events for FIH1 and FIH2

Subject	Adverse Event	Severity	Relation to Amplifi System	Relation to Procedure	SAE or UADE
01	Pulmonary air embolism	Mild	Not related	Definite	No

03	Blood oozing at catheter insertion site	Mild	Definite	Definite	No
	Pump unable to achieve desired speed; Pump replaced	Mild	Definite	Possibly	No
04	Pump unable to achieve desired speed and low blood flow alarm; Pump replaced	Mild	Definite	Possibly	No
05	Low blood flow alarm; Pump replaced	Mild	Definite	Possibly	No
07	Decreasing Pump speed and blood flow with increasing current; Pump replaced	Mild	Definite	Possibly	No
08	Decreasing Pump speed and blood flow with increasing current; Pump replaced	Mild	Definite	Possibly	No
	Decreasing Pump speed and blood flow with increasing current; Pump replaced	Mild	Definite	Possibly	No
	Decreasing Pump speed and blood flow with increasing current; Pump replaced	Mild	Definite	Possibly	No

3.2.3 Summary

The FIH Study met its primary performance endpoint. Cephalic vein diameter increased by >100% in all ten Subjects during treatment, with a mean increase of 105% for the FIH1 cohort and 150% for the FIH2 cohort. 7 of 10 FIH subjects had successful radiocephalic AVF creation and 3 of 10 Subjects had successful brachiocephalic AVF creation. In FIH2, the starting forearm cephalic vein diameters were 1.7, 1.9, 1.9, 2.2, and 2.4 mm, all below the widely recognized suitability threshold of 2.5 mm for AVF creation, suggesting the Amplifi System could be used to increase forearm AVF suitability in patients with small forearm veins. All five FIH2 subjects had fully mature forearm AVFs two weeks after creation, with a mean AVF outflow vein diameter of 7.0 mm and an imputed AVF blood flow of 1153 mL/min, a remarkable result considering that all five FIH2 subjects had pre-treatment forearm cephalic vein diameters < 2.5 mm and forearm AVFs.

There were no serious adverse events, deaths, re-hospitalizations, bacteremia, major Amplifi System-related, or AVF-related infection, thromboembolic events, or major bleeding from Amplifi System placement to six weeks after AVF creation.

In some FIH subjects, replacement of the Pump portion of the Amplifi System was required to complete treatment. Subsequent analysis of replaced Pumps determined lower bearing gaps

were out of specification (low) in Pumps that were replaced, likely resulting in reduced washing under the impeller and thrombus formation in the lower bearing gap. Upon completion of the FIH study, an updated assembly process was developed, implemented, tested, and verified. Analysis has confirmed that the lower bearing gap for all Pumps built with the updated manufacturing process have been within specification to date.

The FIH study demonstrated that treatment of upper extremity veins with the Amplifi System prior to AVF creation was feasible and safe, and the treatment resulted in large increases in cephalic vein diameters. AVFs made with treated veins matured quickly and fully. These highly promising results support using the Amplifi System to increase AVF suitability (especially forearm AVF), improve maturation, and reduce time to maturation.

The data on the treatment duration from the FIH2 study was used to design the FIH3 study to further optimize the treatment duration of the Amplifi System and further confirm safe use of the device.

4.0 Study Design

This nonrandomized study is designed to confirm the safety and effectiveness of using the Amplifi System for dilating upper extremity veins prior to surgical AVF creation as observed during the FIH study. The working hypothesis for the study is that the cephalic vein in hemodialysis patients can be treated safely with the Amplifi System and the treatment will result in dilation of the cephalic vein, and the treated and dilated cephalic vein can be used to make an AVF and improve AVF maturation.

4.1 Study Objectives

The Amplifi System will be assessed and analyzed for effectiveness to demonstrate similar outcome from the earlier First-in-Human Studies with shorter treatment duration. Safety analysis will be performed to confirm that major complications will be in an acceptable range.

4.2 Study Population

Subjects who have ESRD and are currently receiving maintenance hemodialysis via a tunneled, cuffed hemodialysis catheter (“Subjects”) will be enrolled in a prospective, nonrandomized, multicenter, open label clinical study designed to evaluate the safety and effectiveness of the Amplifi System as specified in this protocol (“Protocol”).

4.3 Study Design

The Study is a prospective, nonrandomized, single-arm, open-label clinical study evaluating the use of the Amplifi System.

Up to ten (10) Subjects with a forearm or upper arm cephalic vein with a diameter ≥ 1.7 mm and ≤ 3.2 mm, and radial artery diameter ≥ 2.0 mm who have ESRD and are receiving maintenance hemodialysis using a catheter for vascular access. The Subjects will be treated in an inpatient (hospital) setting and will receive Amplifi System treatment and undergo subsequent AVF creation (a “Study AVF”) immediately after treatment. Amplifi System removal

immediately followed by AVF creation will be performed when Subject had:

- Amplifi treatment for ≥ 48 hours duration and physician determined that cephalic vein is suitable for AVF creation determined by physician (e.g., vein diameter ≥ 3.3 mm as defined in Dageforde[17] as Quartile 3 and baseline diameter increased ≥ 50%); or
- Amplifi treatment could be extended up to 60 hours (± 12hrs) if the cephalic vein was not determined to be adequate by the investigator.

After the Amplifi System treatment period, the device will be removed completely and an AVF will be made using the treated cephalic vein. After AVF creation, Subjects will be discharged from the hospital with outpatient follow up visits 1, 2 and 6-weeks after AVF creation.

4.4 Study Site and EC / EC

Subjects will be enrolled at Sanatorio Italiano, Asuncion, Paraguay Investigation Site.

4.5 Primary Clinical Measures

Effectiveness Measures

- **Percent change in cephalic vein diameter and blood flow** in the middle third of the arm as measured by ultrasonography [Timeframe: from baseline, during Amplifi System treatment (daily), and prior to Amplifi System removal and AVF creation]
- **Mean vein diameter of cephalic vein and blood flow of brachial artery** in the middle third of the forearm as measured by ultrasonography [Timeframe: 1, 2and 6 weeks post AVF creation]
- **Forearm AVF maturation success at 1, 2, 6 weeks post-AVF creation** [Timeframe: 1, 2, 6-WK post AVF creation]
- **Percentage of subjects who experience an increase in the cephalic vein diameter of ≥2.7 mm from their baseline measurement, or an increase of >1 mm from baseline, following treatment with the Amplifi System.** [Timeframe: prior to removal of the Amplifi System and creation of the AVF].

Safety Measures

- **Rate of Occurrence of Major Device-Related Adverse Events (MDRAE)* defined as:** [Timeframe: from device implantation and up to 30 days from device removal and AVF creation]
  - Amplifi System-related death;
  - Amplifi System-related major bleeding, major pneumothorax, major air

embolism, major thrombosis, major thromboembolism, major hemolysis, or major thrombocytopenia; local infection
- Amplifi System-related re-hospitalization or prolonged hospitalization;
- Amplifi System-related major surgery or major interventional procedure; or
- any Amplifi System-related complication determined to be an Amplifi System-related major complication by both an Investigator and the Medical Monitor.

*Detailed definition of Major Device-Related Adverse Events is described in **Attachment 1: Definitions**

- **Rate of Occurrence of Cannulation-related Complications** [Timeframe: from AVF creation through end of follow-up period]

Exploratory Technical Performance Measures

- **Rate of Occurrence of Amplifi System component replacement** [Timeframe: from device implantation through device removal and AVF creation]

- **Rate of Occurrence of Amplifi System treatment failure** [Timeframe: from device implantation through less than required minimum treatment hours of duration (e.g., 48 hours)

*Detailed definition of Treatment Failure is described in **Attachment 1: Definitions**

Rate of Occurrence of pump stoppages and device malfunction [Timeframe: from device implantation through device removal

4.6 Inclusion and Exclusion Criteria

Inclusion Criteria

- Age ≥ 18 years
- Indicated for wrist or distal forearm radiocephalic AVF creation based on Investigator's standard practice
- ESRD currently receiving maintenance hemodialysis via a tunneled, cuffed hemodialysis catheter
- Baseline wrist or distal forearm cephalic vein diameter of ≥ 1.7 mm and ≤ 3.2 mm at the proposed Study AVF creation site, wherein the wrist or forearm cephalic vein at the proposed Study AVF creation site is suitable for Amplifi Distal Outflow Catheter placement and Amplifi System treatment, including:

- ≥ 1.7 mm to ≤ 3.2 mm diameter of the wrist or forearm cephalic vein at the proposed Study AVF creation site;
- patency and continuity of blood flow from the proposed Study AVF creation site centrally to the upper arm at the elbow through at least one or more of: 1) the upper arm cephalic vein; 2) the median cubital vein to the upper arm basilic system; or 3) the perforator vein to the deep venous system; and
- no occlusion or stenosis ≥ 50% along the course of the forearm cephalic vein and at least one upper arm venous outflow up to the subclavian vein, as determined by duplex ultrasonography

- Estimated forearm hemodialysis needle cannulation zone length ≥ 18 cm after creation of the proposed Study AVF
- At least one patent internal jugular vein suitable for Amplifi Inflow Catheter insertion
- Subject has voluntarily signed written informed consent

Exclusion Criteria

1) Known allergy to Amplifi System components (polyurethane, nitinol), iodinated contrast agents, heparin, or apixaban
2) Known or suspected active infection at the proposed time of Amplifi System placement
3) Known bleeding diathesis, including from uncorrected coagulopathy
4) Known thrombophilia, requiring treatment
5) Hb <10 gm/dL
6) Platelet level < 100,000 /mm^3
7) Need for continued treatment with anti-platelet agents or other anticoagulants (other than apixaban) during the Amplifi System treatment period
8) Known history of patent foramen ovale ≥ 2 mm
9) History of recent intracranial or gastrointestinal bleeding
10) Documented recent central venous or right atrial thrombus
11) Known presence or recent history of intracranial or gastrointestinal bleeding or presence of underlying conditions that would predispose to intracranial or internal bleeding. Documented recent central venous or right atrial thrombus
12) History of recent ipsilateral central venous occlusion, stenosis ≥ 50%, angioplasty, or stent placement
13) Pregnancy, lactation, or plans to become pregnant during the Study

14) Unwillingness or inability to comply with procedures specified in the Protocol, or difficulty or inability to return for follow-up visits as specified by the Protocol
15) Participation in any other investigational drug or medical device study that has not completed primary endpoint evaluation or clinically interferes with the endpoints from the Study, or planned future participation in such studies prior to the completion of the Study

6.0 Study Enrollment, Informed Consent and Subject Withdrawal

6.1 Enrollment

6.1.1 Informed Consent

Informed Consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. Investigators or designated research staff will obtain a patient's informed consent before any study procedures or data collection are performed.

Patient or Legal Authorized Representative (LAR) will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The key information about the study will be organized and presented in lay terminology and language that facilitates an understanding of why one might or might not want to participate. Informed Consent form(s) (ICF) will be EC-approved, and patients will be asked to read and review the consent form. Patient (or LAR) must sign the ICF prior to starting any study procedures being done specifically for this trial. Once signed, a copy of the ICF will be given to the patient for their records. The original form shall be maintained in the patient's medical records at the site. Patient who signed informed can refuse to participate and can withdraw at any time without concern for further treatment.

If new information becomes known to the Sponsor that may affect the risks and benefits of a Subject's participation in the Study, Investigators will revise the informed consent and ICF as necessary and re-consented Subjects. Revisions to the ICF will be approved by Sponsor and ECs prior to re-consenting Subjects. Sites will keep the original signed ICFs for each Subject, including any updated ICFs, on file and available for inspection by Sponsor or its designees.

6.1.2 Enrollment and Study Exit

After signing informed consent, potential Subjects will be screened against inclusion and exclusion criteria for entry into the Study as close to the scheduled Amplifi System placement time as possible. If all inclusion criteria and no exclusion criteria are met, the patient can be enrolled into the Study as a Subject.

Subjects will undergo screening duplex ultrasonography prior to enrollment and the results will be documented on the appropriate CRF. The protocol for the baseline screening duplex

ultrasonography is summarized in the **FIH3 Duplex Ultrasonography Protocol.** A complete blood count (CBC) will be evaluated at baseline and will be used to assess eligibility for the study.

A patient is considered enrolled in the Study (and a Subject) after all the following criteria are met:

- patient has provided informed consent;
- Investigator has determined that the patient continues to meet all the inclusion criteria and none of the exclusion criteria; and
- an attempt has been made to treat the patient with the Amplifi System by inserting at least a portion of the Inflow Catheter or Distal Outflow Catheter into the patient's body.

Each subject enrolled in the study will be assigned a unique identification number (see **Section 11.5**). Subjects who receive any amount of Amplifi System treatment and had AVF creation will be followed at 1,2 and 6 weeks after Study AVF creation and will then exit the Study. Subjects who receive any amount of Amplifi System treatment but did not have AVF creation will be followed up to 30 days after Amplifi System removal. Patients in which no portion of the Inflow Catheter or Distal Outflow Catheter was inserted into the body will be considered screening failures and will not be considered enrolled as Subjects. There will be no data entry or follow-up for these patients.

For all consented patients, adverse events shall be collected, and the patient followed until they have resolved, have a stable level of sequelae or, in the Investigator's opinion, are no longer clinically relevant, or study exit (whichever comes first) for safety.

6.2 Subject Withdrawal

Subjects retain the right to withdraw from the Study at any time if they wish to do so, without any consequences. Should a Subject elect to withdraw from the Study, the Investigator or Site Personnel will record the reason for withdrawal in source documentation and on the appropriate CRF. In addition, the Investigator may discontinue the Subject from the study due to any of the following situations:

- Serious Adverse Event (SAE); or
- any other reason determined by the Investigator to be in the best interest of the subject.

Subjects with an ongoing AE at the time of withdrawal should be followed on study until the clinical event has been resolved or is stable if possible.

7.0 Procedures and Surgeries

The Amplifi System placement, operation, and removal will be done in a hospital's interventional suite or operating room. All subjects will be treated in an inpatient hospital setting. Subjects will be discharged only after Amplifi System removal with or without AVF creation.

Investigators, and other Site Personnel involved in placing, operating, monitoring, interacting

with, or removing the Amplifi System, including during Study-related procedures or surgeries, will review the Amplifi System IFU and Operator's Manual prior to these activities and will refer to the Amplifi System IFU during these activities, as needed.

Please refer to Amplifi System IFU and Operator's Manual for the use and operation of the Amplifi System.

7.1 Amplifi System Placement

Prior to placing the Amplifi System, Investigators will review the Amplifi System IFU and Protocol for instructions on how to place the Amplifi System and will refer to the Amplifi System IFU during the placement procedure, as needed.

The Inflow Catheter will not be placed in fully occluded internal jugular veins, even after recanalization. The Inflow Catheter will not be placed in other veins such as the external jugular, subclavian, and femoral veins.

7.2 Amplifi System Treatment

Prior to treating Subjects with the Amplifi System, Investigators will review the Amplifi System IFU and Protocol, and will refer to the Amplifi System IFU, Operator's Manual and Protocol during treatment, as needed.

If, during the Amplifi System treatment period, a Subject has an *Amplifi System-related major hemolysis event*, the Investigator will discontinue Amplifi System treatment, provide treatment for the hemolysis, and create an AVF with the treated cephalic vein as soon as possible, if clinically indicated. Subject(s) will continue their post AVF follow-up, if applicable.

If, during Amplifi System treatment period, a Subject has experienced pump early stoppage, malfunction or deficiencies prior to the scheduled end of treatment, please refer to **Section 7.5 Pump Malfunction and Stoppage.**

7.3 Amplifi System Removal

Prior to removing the Amplifi System, Investigators will review the Amplifi System IFU, Operator's Manual and Protocol for instructions on how to remove the Amplifi System and will refer to the Amplifi System IFU during the removal procedure, as needed.

If a suspected thrombus is present in the Amplifi System after removal, high-resolution color photographs will be obtained of all blood-contacting components of the Amplifi System such that any thrombus present is clearly seen and documented. The Pump will be opened, the impeller removed, and photographs taken of the top bottom of the impeller and the blood contacting surfaces of the top and bottom housing.

7.4 Study AVF Creation

Although Study AVF creation in the distal forearm using an anastomosis surgical technique of "artery side to vein end" is encouraged, Investigators will create a Study AVF for each Subject at

the location and using a technique they believe is best for each Subject.

The Protocol specifies that Investigators should create Study AVFs at the location pre-identified by the Investigator prior to enrollment. If, after Amplifi System treatment, in the Investigator's clinical judgment, creation of a Study AVF at the pre-planned location is not in the best interest of the Subject, the Investigator should create an AVF at a location that, in the Investigator's clinical judgment, is in the best interest of the Subject, if possible. If, after Amplifi System treatment, in the Investigator's clinical judgment, creation of an AVF is not in the best interest of the Subject, the Investigator should create a vascular access that, in the Investigator's clinical judgment, is in the best interest of the Subject, if possible.

* If, at any time during Amplifi System treatment, in the Investigator's clinical judgment, creation of a Study AVF is in the best interest of the Subject, the Investigator should create an AVF.
**

If the pump stops prior to the schedule end of treatment duration, please refer to **Section 7.5 Pump Malfunction and Stoppage**.

7.5 Pump Malfunction and Stoppage

If a Pump experiences a deficiency or malfunction and stops prior to the scheduled end of treatment (e.g., < 48 hours of treatment) and cannot be re-started:

- The patient may be scheduled for Blood Pump replacement or possible AVF creation, if clinically indicated as determined by the Investigator
- To reduce the risk of thrombus formation, the patient should continue to take apixaban anticoagulant until advised by physician to discontinue
- The Amplifi System should be removed as specified in the Instruction For Use (IFU); the Blood Pump, Inflow Catheter, and Outflow Catheters should not be flushed; and no angiography should be performed using the Access Ports.
- An ultrasound examination of the treated upper extremity will be performed to determine the amount of thrombus formation in the veins of the treated arm documented in eCRF, if any, as described in **8.1.2 Ultrasonography During Amplifi System Treatment.**
    - If thrombus is present in the treated cephalic vein and the Investigator believes the thrombus can be removed during AVF creation, then the Investigator should proceed to AVF creation, if clinically indicated. Any thrombus in the cephalic vein should be managed appropriately prior to AVF creation (e.g., using a method such as mechanical thrombectomy).
    - High resolution color photographs will be obtained of all blood-contacting components of the Amplifi System such that any thrombus present is clearly seen and documented. An investigation of the pump will be conducted per the Sponsor's Device Return Investigation procedure.
- A venogram of the treated upper extremity, central veins, and right atrium will be performed by the Investigator during Amplifi System removal, as described in **Section 8.5 Contrast Venography.**

All Amplifi System unplanned stoppages, including Pump stoppages, that occur and are observed during the Study shall be documented by the Investigator and other Site Personnel by submitting a completed CRF in the Study EDC System as described in **Section 12.3 Amplifi System Unplanned Stoppages, Malfunctions, and Potential** Device Deficiencies and reporting as described in **Section 12.10.6 Reporting of Amplifi System Unplanned Stoppages and Malfunctions** and data analysis will be conducted as described in **Section 14.2.2 Analysis of Effectiveness Measures.**

8.0 Study Assessments and Follow Up

The total duration of Subject participation in the nonrandomized cohort will be **up to 6 weeks** after AVF creation. **Table 3** summarizes Study assessments and actions and **Table 4** summarizes the assessment window.

Table 3: Study Assessments and Actions

Assessment or Action	*Screening*	*Day 0* - Amplifi System Placement	*Day 1 – 3* - Amplifi System Treatment	Amplifi System Removal and Study AVF Creation	Week 1,2 and Week 6 after Study AVF Creation
Duplex Ultrasonography	X	X	X	X	X
Inclusion and Exclusion Criteria	X				
Informed Consent and HIPAA Authorization	X				
Demographics and Medical History	X				
Clinical Assessment, Physical Exam	X	X	X	X	X
Laboratory Testing - Chemistry, Hematology	X	X	X	X	
Laboratory Testing – ACT		X[2]		X[2]	
Laboratory Testing – PFH, LDH, and CBC	X	X	X[3]	X	X
Amplifi System Blood Flow Measurement (From Recorded Amplifi System Data)		X	X	X	

B-Mode Ultrasonography During Amplifi System Treatment (by Investigator)		X	X[3]		
Contrast Venography (by Investigator)		X	X[1]	X	
Concomitant Medications		X	X	X	X
Adverse Event Logging		X	X	X	X
Determination of Study AVF Maturation (Y/N)					X
Documentation of unplanned stoppages, malfunction and deficiencies		X	X	X	
Documentation of Study AVF Thrombosis Events, Interventional Procedures, and Surgeries					X
Documentation of Access-Related Thrombosis Events, Interventional Procedures, and Surgeries (all types of access)					X

1. For Subjects that experience a prolonged Pump stoppage only
2. ACT measurements during Amplifi System placement and AVF creation per Amplifi System IFU
3. Daily testing

Table 4: Time Windows for Study Assessments

Assessment	Time Window[1]
Baseline Screening Ultrasonography	≤14 days prior to planned Amplifi System placement
Inclusion and Exclusion Criteria, Demographics and Medical History, and Informed Consent and HIPAA Authorization	≤14 days prior to planned Amplifi System placement
Baseline Clinical Assessment and Physical Exam	≤14 days prior to planned Amplifi System placement
Baseline Laboratory Testing	≤14 days prior to planned Amplifi System placement
Daily Ultrasonography During Amplifi System Treatment	Within 12-hour period of each day[2]
Daily Clinical Assessment and Physical Exam during Amplifi System Treatment	Within 12-hour period of each day
Daily Laboratory Testing During Amplifi System Treatment	Within 12-hour period of each day
Documentation of unplanned stoppages, malfunction, and deficiencies	Within 48-hour period of the time of event
Ultrasonography at the End of Amplifi System Treatment	≤4 hours prior to the planned end of treatment
Contrast Venography	During Amplifi System placement

Assessment	Time Window[1]
	During Amplifi System removal and Study AVF creation surgery
Laboratory Testing after Study AVF Creation	±7 days
Clinical Assessment and Physical Exam after Study AVF Creation	±7 days
Study AVF Ultrasonography	±7 days for two (2) week ultrasonography ±2 weeks for six (6) week ultrasonography
Determination of Study AVF Maturation	±7 days for two (2) week ultrasonography ±2 weeks for six (6) week ultrasonography
Documentation of Study AVF Thrombosis Events, Interventional Procedures, and Surgeries	±7 days at 2 weeks ±2 weeks at 6 weeks
Documentation of Vascular Access-Related Thrombosis Events, Interventional Procedures, Surgeries (all types of access)[3]	±7 days at 2 weeks ±2 weeks at 6 weeks
Documentation of Hemodialysis Vascular Access-Related Hospitalizations (all types of access)[4]	±7 days at 2 weeks ±2 weeks at 6 weeks
Adverse Event Logging	Within 24-hour period of each day during Amplifi System treatment ±7 days at 2 weeks ±2 weeks at 6 weeks

1. Unless otherwise specified, times are measured from Study AVF creation date
2. Required
3. Does not include Study AVF
4. Includes Study AVF

8.1 Duplex Ultrasonography

8.1.1 Baseline Screening Ultrasonography

As shown in **Table 3**, Subjects will undergo screening duplex ultrasonography prior to enrollment and the results will be documented on the appropriate CRF. The protocol for the baseline screening duplex ultrasonography is summarized in **FIH3 Duplex Ultrasonography Protocol**. The patency, diameter, and blood flow of the veins of both upper extremities will be assessed, including the cephalic, basilic, median cubital, and perforator veins, to provide Investigators information to help them determine patient eligibility for the Study, choose potential locations for insertion of the Distal Outflow Catheter, and choose a planned location for Study AVF creation. The patency, diameter, and blood flow of the arteries of both upper extremities will be assessed, including the radial artery, to provide Investigators information to help them

determine patient eligibility for the Study, and to choose a planned location for Study AVF creation. The patency, diameter, and blood flow of both internal jugular veins will be also assessed to provide Investigators information to help them choose potential locations for insertion of the Inflow Catheter, if needed.

Vein diameters will be measured in both the vertical and horizontal vessel dimension and artery diameters will be measured in the vertical dimension only, and reported in fraction of millimeters using digital calipers. A tourniquet will be applied at the proximal portion of the upper arm during assessment of upper extremity vein diameters. No tourniquet will be used during assessment of vein blood flow, and artery diameter and blood flow.

8.1.2 Ultrasonography During Amplifi System Treatment

Subjects will have limited ultrasonography at least daily to assess the patency and diameter of the cephalic vein in the middle third of the forearm. The protocol for ultrasonography during Amplifi System treatment is summarized in **FIH3 Duplex Ultrasonography Protocol.**

Vein diameters will be measured in both the vertical and horizontal vessel dimension. Amplifi System treatment will continue during this ultrasound examination and **tourniquet will be placed.** At the time of the first measurement post AVF creation, a mark will be made on the skin at the location of the first measurement using a permanent marker, and this mark will be used to make subsequent measurements.

Subjects that experience a prolonged Pump stoppage will have a duplex ultrasound examination of the treated upper extremity to evaluate patency of the upper extremity arm veins and identify any signs of injury, stenosis, occlusion, aneurysm formation, or intraluminal thrombus using the protocol for ultrasonography at the end of Amplifi System treatment, as summarized in **FIH3 Duplex Ultrasonography Protocol,** Sonographers will identify and record the location and extent of intraluminal thrombus present in the treated upper extremity after a prolonged Pump stoppage, if any, on the appropriate CRF. If no thrombus is identified, that will also be recorded on the CRF.

8.1.3 Ultrasonography at the End of Amplifi System Treatment

Subjects will have duplex ultrasonography at the end of the Amplifi System treatment period prior to Study AVF creation. The protocol for the ultrasonography after Amplifi System Treatment is summarized in **FIH3 Duplex Ultrasonography Protocol.** The patency, diameter, and blood flow of the veins of both upper extremities will be assessed at the same locations as the baseline screening duplex ultrasound examination.

Vein diameters will be measured in both the vertical and horizontal vessel dimension. Amplifi System treatment will continue during this ultrasound examination and **tourniquet will be placed on the treated arm.**

8.1.4 Ultrasonography of AVF

Subjects will have duplex ultrasonography of the Study AVF at 1, 2 and 6 weeks after Study AVF creation. The protocol for Study AVF duplex ultrasonography is summarized in **FIH3 Duplex Ultrasonography Protocol**. The patency, diameter, and blood flow of the arteries and veins of the upper extremity with the Study AVF will be assessed at the same locations as the

baseline screening duplex ultrasound examination. Vein diameters will be measured in both the vertical and horizontal vessel dimension and artery diameters will be measured in the vertical dimension only. **No tourniquet will be placed.**

8.2 Demographics and Medical History

All Subjects will have demographics and a medical history recorded in the Study EDC System.

8.3 Clinical Assessments and Physical Exams

Clinical assessments and physical exams, including vital signs and temperature, will be performed at screening, day 1 (prior to Amplifi System placement), and at the end of the Amplifi System treatment period. Assessments will be recorded in the Study EDC System.

8.4 Laboratory Assessments

- A routine chemistry panel will be evaluated at baseline, at day 1 prior to Amplifi System placement, and at the end of Amplifi System treatment.
- A complete blood count (CBC) will be evaluated at baseline, on day 1 prior to Amplifi System placement, daily during the Amplifi System treatment period, at the end of Amplifi System treatment after Amplifi System removal, and 1, 2 weeks and 6 weeks after Study AVF creation. The baseline CBC results will be used to assess eligibility, the daily testing during the Amplifi System treatment period and the testing 1, 2 weeks and 6 weeks after Study AVF creation will be used to assess for hemolysis and anemia.
- PFH and LDH will be evaluated at baseline, on day 1 prior to Amplifi System placement, daily during the Amplifi System treatment period, at the end of Amplifi System treatment period after Amplifi System removal, and 1, 2 weeks and 6 weeks after Study AVF creation to assess for hemolysis.
- ACT will be evaluated during Amplifi System placement and removal, as per the Amplifi IFU, and during AVF creation, as per Standard of Care.

8.5 Contrast Venography

The protocols for contrast venography are summarized in the **FIH3 Contrast Venography Protocol.** The Investigator performing each fluoroscopic, venographic, or angiographic study will document the findings on the appropriate CRF.

8.5.1 Venography Prior to Amplifi System Placement

Contrast venography of the upper extremity, ipsilateral central veins, superior vena cava, and right atrium using an IV catheter *will* be performed for all Subjects prior to insertion of the Inflow Catheter or Distal Outflow Catheter evaluate patency of the cephalic vein, the draining veins of the upper arm, the ipsilateral central veins, the superior vena cava, and the right atrium.

Evaluating the veins of the arm by ultrasound is generally preferred to contrast venography. Ultrasound is generally inadequate in identifying subtle stenotic issues and flow pattern in the normal low flow state. It is also not adequate to visualize the central veins and right atrium, so contrast venography is needed to evaluate the patency of the central veins and right atrium prior to Amplifi System placement. If ultrasound is not available, or if the information obtained from an

ultrasound examination is incomplete or uncertain, then contrast venography may also be used to evaluate upper extremity vein patency and flow.

Preferably, contrast venography should be performed through a standard IV catheter prior to placement of the Distal Outflow Catheter. If contrast venography cannot be performed through a standard IV catheter, or if the information obtained from this venography is incomplete or uncertain, then contrast venography may be performed through a Distal Outflow Catheter.

Preferably, contrast venography should be performed prior to starting the Amplifi System Pump. If contrast venography cannot be performed prior to starting the Pump, or if the information obtained from this venography is incomplete or uncertain, then contrast venography may be performed after starting the Pump.

Contrast venography will not be used to make vein diameter measurements due to variability in the volume and rate of contrast injections. Contrast venography will not be used to identify signs of vein injury, stenosis, or occlusion during Amplifi System placement due to the potential to induce vasospasm during vein access and contrast injection and cause false positive results.

8.5.2 Angiography of the Inflow Catheter During Amplifi System Placement

Contrast angiography of the right atrium after insertion and positioning of the Inflow Catheter *will* be performed for all Subjects during Amplifi System placement to identify Inflow Catheter tip location and to evaluate for right atrial thrombus.

If contrast angiography of the Inflow Catheter and the right atrium cannot be performed prior to starting the Amplifi System Pump, or if the information obtained from this venography is incomplete or uncertain, then contrast venography of the Inflow Catheter and the right atrium may be performed after starting the Pump.

8.5.3 Venography During Amplifi System Treatment

Contrast venography of the upper extremity, ipsilateral central veins, superior vena cava, and right atrium *will* be performed for Subjects that experience prolonged Pump stoppage to:

1) evaluate patency of the cephalic vein, the draining veins of the upper arm, the ipsilateral central veins, the superior vena cava, and the right atrium;
2) identify any signs of injury, stenosis, occlusion, aneurysm formation, or intraluminal thrombus formation in the cephalic vein, the draining veins of the upper arm, the ipsilateral central veins, and the right atrium;
3) evaluate flow in the forearm cephalic vein, and to and through the draining veins of the upper arm and central veins to the right atrium.

Investigators will identify and record the location and extent of intraluminal thrombus present in the treated upper extremity, ipsilateral central veins, superior vena cava, and right atrium after a prolonged Pump stoppage, if any, on the appropriate CRF. If no thrombus is identified, that will also be recorded on the CRF.

Contrast venography of the upper extremity, ipsilateral central veins, superior vena cava, and right atrium may *optionally* be performed during Amplifi System treatment for Subjects that experience other problems or potential problems, as clinically indicated.

8.5.4 Angiography of the Inflow Catheter During Amplifi System Treatment

Contrast angiography of the right atrium using the Inflow Catheter *will not* be performed for Subjects that experience a prolonged Pump stoppage.

Contrast venography of the right atrium may *optionally* be performed during Amplifi System treatment Subjects that experience other problems or potential problems, as clinically indicated.

8.5.5 Venography at the End of the Amplifi System Treatment Period

Contrast venography of the upper extremity, ipsilateral central veins, superior vena cava, and right atrium *will* be performed at the end of the Amplifi System treatment period, and will be used to:

1) evaluate patency of the cephalic vein, the draining veins of the upper arm, the ipsilateral central veins, the superior vena cava, and the right atrium;
2) identify any signs of injury, stenosis, occlusion, aneurysm formation, or intraluminal thrombus formation in the cephalic vein, the draining veins of the upper arm, the ipsilateral central veins, and the right atrium;
3) evaluate flow in the forearm cephalic vein and to and through the draining veins of the upper arm and central veins to the right atrium.

Contrast venography will not be used to make vein diameter measurements due to variability in the volume and rate of contrast injections. At the end of the Amplifi System treatment period the potential for vasospasm is reduced and therefore contrast venography may be used to identify signs of vein injury, stenosis, or occlusion *if the Outflow Access Port is used to make contrast injections and venography is performed with the Pump running*.

8.5.6 Angiography of the Inflow Catheter at the End of the Amplifi System Treatment Period

Contrast angiography of the right atrium at the end of the Amplifi System treatment period *will* be performed for all Subjects to identify Inflow Catheter tip location and to evaluate for right atrial thrombus or signs of right atrial injury.

8.6 Concomitant Medications

All concomitant medications the Subject is prescribed during the Study Period will be recorded in the Study EDC System.

9.0 Training

9.1 Investigator Training

All Investigators and Site Personnel will undergo training by the Sponsor or its designee on the design, function, storage, placement, use, operation, maintenance, care, cleaning, monitoring, interaction with, removal, and disposal of the Amplifi System and its components. Only trained Site Personnel will be allowed to place, use, monitor, interact with, or remove the Amplifi System and perform Study-related procedures or surgeries. The Amplifi System Instructions for Use (IFU) will be provided to educate and assist Investigators and other appropriate Site Personnel on the proper placement, operation, monitoring, care, maintenance, and removal of the Amplifi System.

10.0 Study Oversight, Monitoring, and Quality Control

10.1 Investigator Selection

Prior to the initiation of the Study, Sponsor will evaluate potential Investigators through a formal review process prior to selecting Investigators for the Study to ensure patient safety and optimize patient outcomes. Investigators will be selected based on:

- qualifications, training, skill, experience, and education;
- perceived commitment to ethics and confidentiality, and
- expected adherence to the Protocol.

Sponsor will maintain an updated list of Investigators.

10.2 Site Selection

Prior to the initiation of the Study, Sponsor will evaluate Sites through a formal review process prior to selecting Sites for the Study. Sites will be selected based on:

- experience with the care of patients with ESRD undergoing AVF creation;
- availability of adequate resources at the Site to conduct the Study in accordance with the Protocol, including the availability of adequate personnel, facilities, equipment, and other research infrastructure to perform the tests, examinations, procedures, surgeries, and interventions specified in the Protocol;
- personnel with experience conducting clinical studies; and
- ability to enroll Subjects in a timely manner.

Sponsor will maintain an updated list of Sites.

10.3 Pre-Enrollment Site Visit

Sponsor will conduct a Site initiation visit prior to Subject enrollment. During the Site initiation visit, Sponsor or its designees will confirm and document that each Investigator and Site and all Site Personnel understands and accepts their Study responsibilities, as specified in **Section 15.** If an Investigator attends a Study-specific Investigator meeting where Sponsor accepts and document this understanding, this Investigator meeting may take the place of a Site initiation visit.

10.4 Monitoring and Periodic Monitoring Visits

Sponsor and its designees will monitor the Study to ensure Investigators, Sites, and Site Personnel are meeting their Study responsibilities, as specified in **Section 15.0 Study Responsibilities**.

Details related to Site monitoring will be documented in the Sponsor's Study-specific monitoring plan. Ongoing communication with Investigators and Site Personnel related to Site monitoring will be performed through written correspondence and telephone conversations.

After the Study has been initiated, Sponsor and its designees will perform additional periodic Site monitoring visits to assess Study progress, perform Amplifi System storage and

accountability reviews, assess the adequacy of records, and to ensure adherence to the Protocol. Monitoring visits will be conducted in accordance with applicable local, regional, and national regulations, the Study-specific monitoring plan, and Sponsor's procedures. A summary of monitoring visits, including documentation of completed previous action items, new or outstanding action items, and significant findings will be provided to the Investigator.

In addition to periodic Site monitoring visits, Sponsor may perform remote monitoring to ensure data is submitted in a timely manner. The Site, Investigators and Site Personnel will, upon request, provide to the Sponsor the necessary Study documents and records necessary for a complete review of the work related to the Study at the Site by Investigators and Site Personnel. These records include, but are not limited to, access to Amplifi System storage and accountability logs and records, CRFs and original source documents and records such as ICFs, clinical charts, examination reports, procedure or operative reports, test results and radiology reports.

To meet its responsibility for monitoring the Study to ensure the Protocol is being appropriately followed, Sponsor and its designee's clinical, regulatory, engineering, and research personnel may be present at Study-related Subject examinations, tests, procedures, interventions, and surgeries. Sponsor may contract with a contract research organization to help Sponsor meet its responsibilities for monitoring the Study.

10.5 Case Report Forms

Appropriate Site Personnel will be trained on the Study EDC System and database and will receive a unique username and password. Sponsor may grant or restrict the right of all Site Personnel to access the Study EDC System and database throughout the duration of the Study in its sole and absolute discretion. Investigators will ensure that Study observations and findings are recorded correctly and completely in CRFs. Data submitted during of the Study will be reviewed by the Sponsor or its designees for accuracy and completion. Source documents will be available for access by the Sponsor to review for monitoring of data accuracy and validity of all data recorded on CRFs.

10.6 Maintenance of Source Documentation

Original documentation supporting data recorded on CRFs and eCRFs will be maintained at Sites, including clinical charts, medical records, laboratory reports, test results, physician referral or consultation letters, and radiology and pathology reports. During Site monitoring visits, Sponsor and its designees may review source documents to ensure the accuracy and validity of data recorded on the CRFs, including eCRFs. Sponsor and its designees will maintain confidentiality of Subject source documents.

10.7 Maintenance of Study Documentation and Retention of Records

Sites will maintain adequate and accurate Study documents, records, and data generated by the Sites during the Study, including electronic and hard copies of documents, with such documents, records, and data subject to inspection by Sponsor and its designees, and local, regional, and national regulatory bodies. Investigators will also maintain Study documents and records in accordance with their local EC requirements, regulations of local, regional, and

national regulatory bodies and agencies, the CSA, and the Protocol. At a minimum, the following Study documents will be maintained by each Site:

- Protocol and amendments, including current Protocol;
- current Amplifi System IFU;
- local EC approval(s);
- local EC correspondence;
- current, local EC-approved ICF(s) including translation certification, if applicable;
- current local EC membership roster(s);
- CSA and amendments, including current CSA;
- Investigator agreement(s), including current Investigator agreement;
- updated Investigator curriculum vitae(s);
- confidentiality agreement(s), if separate from CSAs;
- updated financial disclosures;
- Site Personnel Signature and Delegation of Responsibilities Log;
- current ICF form and signed ICFs;
- source documentation, including Subject clinical charts, medical records, laboratory records, test results, and radiology and pathology reports;
- current laboratory certification(s) and normals, if applicable;
- Sponsor correspondence;
- documentation of Investigator and Site Personnel training;
- Monitoring Visit Log; and
- Amplifi System storage and accountability logs and records.

Investigators will maintain adequate and accurate Study documents, records, and data, including source documents, of all observations and data Investigators generate during the Study, including electronic and hard copy documents, with such documents, records, data, and source documents subject to inspection by Sponsor and its designees, and local, regional, and national regulatory bodies. Investigators will also maintain Study documents and records in accordance with their local EC requirements, regulations of local, regional, and national regulatory bodies and agencies, the CSA, and the Protocol. At a minimum, the following Study documents will be maintained by each Investigator:

- Protocol and amendments;
- Investigator agreement(s);
- Investigator curriculum vitae(s);
- Amplifi System IFU;
- Amplifi System labeling
- Signed ICFs
- CRFs;
- all Study-related correspondence;
- observations and data generated by Investigator during the Study; and
- other documents pertaining to the conduct of the Study.

Investigator shall not dispose of any records relevant to the Study without written permission from Sponsor and until providing an opportunity for Sponsor to collect such records.

10.8 Protocol Amendments

Significant changes to the Protocol during the Study will be made by formal protocol amendment. Sponsor will collect information on the appropriateness and completeness of the Protocol from Investigators and Site Personnel and update the Protocol, as needed, to ensure Subject safety and the integrity of Study data. Sponsor will provide proposed Protocol amendments to Central EC for review and approval.

10.9 Protocol Deviations and Investigator and Site Suspension and Termination

Investigators, Sites, and Site Personnel will conduct the Study in accordance with the Protocol. All deviations from the Protocol will be reported to the Sponsor by Investigators and Site Personnel by submitting a Protocol deviation through the Study EDC System. Sites and Site Personnel will notify their local EC of Protocol deviations in accordance with the Central EC requirements.

If Investigators commit serious or repeated Protocol deviations that may affect the safety of Subjects or the integrity of Study data, or if an Investigator is not complying with the Investigator's Agreement, applicable local, regional, and national regulatory requirements, or any other conditions imposed by the EC or the Sponsor, Sponsor will recommend corrective action, including providing additional training to Investigators. Investigators will notify the EC of any corrective action, as required by the EC and the Sponsor. If there is an inadequate response or action from the Investigator, Sponsor will suspend shipment of Amplifi System components to the Site until an appropriate response or action is obtained. If serious or repeated deviations from the Protocol by an Investigator continue to occur, even after corrective action recommendations and apparently adequate responses and actions, or if Sponsor determines that there is an unacceptable risk to Subject safety if an Investigator were to continue to enroll Subjects or participate in the Study, Sponsor may terminate an Investigator's participation in the Study. After termination of the Investigator's participation, Sponsor may seek another Investigator at the Site or terminate the Study at the Site. In such cases, Sponsor will notify the EC, appropriate regulatory authorities, and other participating Sites, as required by local, regional, and national regulations and the Sponsor.

If Sites and Site Personnel commit serious or repeated Protocol deviations that may affect the safety of Subjects or the integrity of Study data, or if a Site or Site Personnel are not complying with the Investigator's Agreement, applicable local, regional, and national regulatory requirements, or any other conditions imposed by their local EC or the Sponsor, Sponsor will recommend corrective action, including providing additional training to Site Personnel. The Site and Site Personnel will notify their local EC of any corrective action, as required by their local EC and the Sponsor. If there is an inadequate response or action from the Site, Sponsor will suspend shipment of Amplifi System components to the Site until an appropriate response or action is obtained. If serious or repeated deviations from the Protocol by a Site continue to occur, even after corrective action recommendations and apparently adequate responses and actions, or if Sponsor determines that there is an unacceptable risk to Subject safety if a Site were to continue to enroll Subjects or participate in the Study, Sponsor may terminate the Study

at the Site. In such cases, Sponsor will notify their local EC, appropriate regulatory authorities, and other participating Sites, as required by local, regional, and national regulations and the Sponsor.

10.10 Study Termination Activities

A Study termination Site visit will be performed prior to Study termination to ensure all Study data has been transmitted to Sponsor by Site. The following Study termination activities will be performed prior to completion of the Study at a Site in accordance with Sponsor's procedures:

- ensure that all required CRF and eCRFs have been completed and submitted;
- ensure final disposition of Amplifi Systems and components;
- confirm with Investigators and Sites their obligation to retain Study records in accordance with their local EC requirements; and
- prepare a final Study report and submit to the Sponsor and the Central EC.

11.0 Ethical and Regulatory Requirements

11.1 Ethical Requirements

This study will be conducted in accordance with Good Clinical Practices (GCP), an international ethical and scientific quality standard for designing, conducting, recording and reporting clinical studies that involved human subjects.

11.2 EC and Other Regulatory Requirements

Approval of the EC, as well as any other required regulatory body or agency will be obtained prior to initiation of the Study at any Site. Copies of all submissions to, and correspondence with EC (including approvals) and other regulatory bodies or agencies (if any) will be sent to Sponsor and maintained on file at the Site.

11.4 Vulnerable Populations

No patients from vulnerable populations will be recruited for the Study.

11.5 Confidentiality

No individually identifiable or confidential patient or Subject data collected as part of the Study will be released publicly by Sponsor. Unique Study identification codes will be assigned to all Subjects. Sites will use Subject's Study identification codes on all Study-related materials. Subject identifiable data will be de-identified by the Site prior to submission to Sponsor.

12.0 Adverse Events and Adverse Device Effects

All Subjects participating in this study will be assessed for adverse events from the point of enrollment through the follow-up period. Assessments will be performed by the site Principal Investigator. An Independent Safety Board shall monitor the overall safe conduct of the study. The ISB will also make recommendations for or against trial continuation, if required. All serious adverse events (see the definition of serious adverse event, below) occurring after the point of

enrollment through study participation must be reported accordingly.

12.1 Definitions

12.1.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical sign or abnormal lab finding in a Subject, regardless of whether the AE was related to the Amplifi System or Study. Stable, chronic, pre-existing conditions will not be categorized as AEs. However, worsening of these conditions occurring during the Study Period may be categorized as AEs.

Investigators will categorize AEs or suspected AEs as unrelated, possibly related, probably related, or definitely related to the Amplifi System using the relational criteria summarized in **Section 12.2 Classification of Adverse Events.**

Investigators will document all AEs and suspected AEs occurring during the Study Period on the appropriate CRF regardless of whether the AE or suspected AE was related to the Amplifi System or Study. Documentation of the AE or suspected AE will include the characterization of the likelihood the AE was related to the Amplifi System.

Investigators will follow all AEs or suspected AEs until they have resolved, have a stable level of sequelae or, in the Investigator's opinion, are no longer clinically relevant.

12.2.2 Device Adverse Events

For AEs or suspected AEs that are categorized by an Investigator as definitely, possibly, or probably related to the Amplifi System, the Medical Monitor will adjudicate whether the AE or suspected AE is an ADE.

Investigators will review all reports of Amplifi System deficiencies, malfunctions, or unplanned stoppages and determine if an AE occurred during the reported event. If an Investigator determines that an AE or possible AE occurred, they will document the AE or possible AE on the appropriate CRF. The Investigator will also document, in their opinion, the likelihood the AE or possible AE was related to the Amplifi System using the relational criteria summarized in **Section 12.2 Classification of Adverse Events**.

Mild-moderate hand, wrist, arm, neck, or face swelling that resolves within 7 days after the end of the Amplifi System treatment period will not be categorized as an AE.

Investigators will follow all ADEs or suspected ADEs until they have resolved, have a stable level of sequelae or, in the Investigator's opinion, are no longer clinically relevant.

12.2.3 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical sign or abnormal lab finding in a Subject, that:

- causes death;
- causes a life-threatening illness or injury;
- results in permanent disability;
- requires the need for hospitalization or prolongs a hospitalization;

- requires intervention to prevent a life-threatening illness or injury, or permanent disability; or
- is determined to be an SAE by both an Investigator and the Medical Monitor,

regardless of whether the occurrence, disease, injury, or untoward clinical sign was related to the Amplifi System or the Study.

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

Investigators will categorize all SAEs or suspected SAEs as unrelated, possibly related, probably related, or definitely related to the Amplifi System using the relational criteria summarized in **Section 12.2 Classification of Adverse Events**.

Investigators will document all SAEs or suspected SAEs occurring during the Study Period on the appropriate CRF regardless of whether the SAE or suspected SAE was related to the Amplifi System or Study. Documentation of the SAE or suspected SAE will include the characterization of the likelihood the SAE or suspected SAE was related to the Amplifi System.

Mild-moderate hand, wrist, arm, neck, or face swelling that resolves within 7 days after the end of the Amplifi System treatment period will not be categorized as an SAE.

Investigators will follow all ASEs or suspected SAEs until they have resolved, have a stable level of sequelae, or, in the Investigator's opinion, are no longer clinically relevant.

12.2.4 Serious Device Adverse Effects

For SAEs or suspected SAEs that are categorized by an Investigator as definitely, possibly, or probably related to the Amplifi System, the Medical Monitor will adjudicate whether the SAE or suspected SAEs is a SADE.

Investigators will review all reports of Amplifi System deficiencies, malfunctions, or unplanned stoppages and determine if an SAE or suspected SAE occurred. If an Investigator determines that an SAE or suspected SAE occurred, they will document the SAE or suspected SAE on the appropriate CRF. The Investigator will also document, in their opinion, the likelihood the SAE was related to the Amplifi System using the relational criteria summarized in **Section 12.2 Classification of Adverse Events** and the Medical Monitor will adjudicate with PI whether the SAE or suspected SAE is a SADE.

Investigators will follow all SADEs and suspected SADEs until they have resolved, have a stable level of sequelae, or, in the Investigator's opinion, are no longer clinically relevant.

12.2.5 Unanticipated Serious Adverse Device Effect

An unanticipated serious adverse device effect (USADE) is a SADE which, by its nature, incidence, severity, or outcome, has not been identified or fully identified during the Amplifi System risk analysis process and is not included or fully included in the Amplifi System risk analysis documents as a potential SADE.

For SAEs or suspected SAEs that are categorized by an Investigator as possibly, probably, or definitely related to the Amplifi System, Investigators will characterize whether the SAE or suspected SAE is definitely not, possibly, probably, or definitely a SADE. Investigators will document the characterization on the appropriate CRF. The Investigator's characterization of these events in the appropriate CRF(s) along with hospital source records will be reviewed and adjudicated by to the Medical Monitor.

Investigators will follow all USADEs and suspected USADEs until they have resolved, have a stable level of sequelae, or, in the Investigator's opinion, are no longer clinically relevant.

12.2.6 Potential Adverse Events and Serious Adverse Events

AEs and SAEs that may occur during the placement, operation, use, or removal of the Amplifi System, or the subsequent creation and use of an AVF made with a vein treated with the Amplifi System have been identified during the Amplifi System risk analysis process, included in the Amplifi System risk analysis documents, and are summarized here:

- death;
- vascular injury;
- bleeding;
- pneumothorax;
- air embolism;
- vascular thrombosis;
- thromboembolism;
- hemolysis;
- anemia;
- thrombocytopenia;
- Inflow Catheter and Distal Outflow Catheter dislodgement;
- Amplifi System component fracture or laceration;
- Inflow Catheter and Distal Outflow Catheter tunnel or skin exit site infection;
- Amplifi System thrombosis;
- Amplifi System component failure or replacement;
- unplanned re-hospitalization or prolonged hospitalization;
- unplanned surgery or interventional procedure;
- AVF bleeding or thrombosis;
- AVF infection;
- ipsilateral vascular access steal syndrome after creation of an AVF;
- AVF aneurysm or pseudoaneurysm formation;
- allergic reaction; and
- drug toxicity.

AEs and SAEs that may occur during the placement, operation, use, or removal of the Amplifi System, or the subsequent creation and use of an AVF made with a vein treated with the Amplifi System may be related to:

- testing required prior to Amplifi System treatment;
- the Amplifi System placement procedure;
- the operation and use of the Amplifi System;
- procedures where the Amplifi System is evaluated, such as venography;
- the Amplifi System removal procedure;
- surgery to create a Study AVF; and

- cannulation of a Study AVF.

AEs and SAEs that may occur during the placement, operation, use, or removal of the Amplifi System, or the subsequent creation and use of an AVF made with a vein treated with the Amplifi System may or may not be related to the Amplifi System, based on the relational criteria summarized in **Section 12.2 Classification of Adverse Events**.

12.2 Classification of Adverse Events

12.2. Grading of AEs

Investigators will grade adverse events using the following scale.

- *Mild*: easily tolerated
- *Moderate*: sufficiently discomforting to interfere with daily activities
- *Severe*: prevents normal daily activities

12.2.2 Relational Criteria

Investigators will document their opinion of the likelihood of the relationship of all adverse events to the Amplifi System using the criteria summarized below:

- *Unrelated*: a temporal relationship between the adverse event and the placement, use, or removal of the Amplifi System is not clear, or there is evidence of potential alternative causes for the adverse event such as concurrent medication or illness
- *Possibly related*: a temporal relationship between the adverse event and the placement, use, or removal of the Amplifi System is not clear and potential alternative causes for the adverse event such as concurrent medication or illness are possible
- *Probably related*: a temporal relationship between the adverse event and the placement, use, or removal of the Amplifi System is clear and potential alternative causes for the adverse event such as concurrent medication or illness are possible but unlikely
- *Definitely related*: a temporal relationship between the adverse event and the placement, use, or removal of the Amplifi System is clear and no potential alternative causes for the adverse event such as concurrent medication or illness are possible

12.3 Amplifi System Unplanned Stoppages, Malfunctions, and Potential Device Deficiencies

All Amplifi System unplanned stoppages, including Pump stoppages, that occur and are observed during the Study, whether they are associated with an AE, ADE, SAE, SADE, or USADE, shall be documented by the Investigator and other Site Personnel by submitting a completed CRF in the Study EDC System.

All Amplifi System malfunctions and deficiencies that are related to the identity, quality, durability, reliability, safety, or performance of the Amplifi System that occur and are observed during the Study, whether they are associated with an AE, ADE, SAE, SADE, or USADE, shall

be documented by the Investigator and other Site Personnel by submitting a completed CRF in the Study EDC System. The Medical Monitor shall adjudicate device relatedness in accordance with protocol definitions.

12.10 Reporting

12.10.1 Reporting of AEs

Investigators will report AEs or suspected AEs to Sponsor as soon as possible after learning of the event by submitting a completed CRF through the Study EDC System. Investigators will report AEs or suspected AEs to Sponsor regardless of whether they are related to the Amplifi System or Study.

Investigators will characterize AEs or suspected AEs as unrelated, possibly related, probably related, or definitely related to the Amplifi System on the AE CRF, using the relational criteria summarized in **Section 12.2 Classification of Adverse Events**.

Investigators will report AEs or suspected AEs to the Central ECEC, in accordance with the Central ECEC requirements.

12.10.2 Reporting of ADEs

The Medical Monitor will report ADE adjudication decisions to the Investigator and Sponsor or Sponsor delegate in accordance with the charter.

Investigators will report ADEs to the Central EC in accordance with the Central EC requirements.

12.10.3 Reporting of SAEs

Investigators will report SAEs or suspected SAEs to the Sponsor (or Sponsor delegate) as soon as possible, preferably within 24 hours, but no later than two (2) working days of the Investigator or Site Personnel becoming aware of an SAE or suspected SAE, by:

- calling Sponsor;
- emailing Sponsor;
- submitting a completed CRF through the Study EDC System, including documentation of the call and email to Sponsor or Sponsor delegate;

Investigators will report SAEs or suspected SAEs regardless of whether they are related to the Amplifi System or Study.

After notifying Sponsor or (Sponsor delegate) and Medical Monitor of an SAE or suspected SAE, Investigators will provide a written report of the SAE or suspected SAE to Sponsor within five (5) working days of the SAE or suspected SAE through the Study EDC System. Investigators will also transmit the written report to the Medical Monitor in accordance with the charter.

Investigators will report SAEs or suspected SAEs that, in their opinion, possibly related, probably related, or definitely related to the Amplifi System to the Medical Monitor for adjudication as SADEs through the Study EDC System, using the relational criteria summarized in **Section 12.3**.

Investigators will report SAEs or suspected SAEs to the Central EC, in accordance

with their local EC requirements. Sponsor will report all SAEs and communicate relevant SAE information to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements.

12.10.4 Reporting of SADEs

The Medical Monitor will report SADE adjudication decisions to the Investigator and Sponsor or Sponsor delegate in accordance with the charter.

The Medical Monitor will report SADEs to the Central EC in accordance with EC requirements.

Sponsor will report all SADEs and communicate relevant SADE information to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements.

12.10.5 Reporting of USADEs

Investigators will report SAEs or suspected SAEs that are characterized by the Investigator as possibly, probably, or definitely related to the Amplifi System, and also characterized as possibly, probably, or definitely a SADE, to Sponsor (or Sponsor delegate) as soon as possible, preferably within 24 hours, but no later than two (2) working days after Investigator or other Site Personnel becoming aware of a USADE or suspected USADE, by:

- calling Sponsor;
- emailing Sponsor;
- submitting a completed CRF through the Study EDC System, including documentation of the call and email to Sponsor; and

After reporting a USADE or suspected USADE to Sponsor, Investigators will provide a written report about the USADE or suspected USADE to Sponsor within five (5) working days after Investigator or other Site Personnel becoming aware of the USADE or suspected USADE.

With Sponsor communication, Investigators will also report USADEs or suspected USADEs to the Central EC, in accordance with the EC requirements.

Sponsor will report all USADEs and communicate relevant USADE information to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements.

12.10.6 Reporting of Amplifi System Unplanned Stoppages and Malfunctions

Investigators will report all Amplifi System unplanned stoppages or malfunctions (including component malfunctions) on the appropriate CRF and to Sponsor as soon as possible, preferably within 48 hours, but no later than three (3) working days after Investigator or other Site Personnel becoming aware, by:

- calling Sponsor;
- emailing Sponsor; and
- submitting a completed CRF through the Study EDC System, including documentation of the call and email to Sponsor.

After notifying Sponsor (or Sponsor delegate) and Medical Monitor of an Amplifi System unplanned stoppage or malfunction, Investigators will provide a written report of the Amplifi System unplanned stoppage or malfunction to Sponsor and Medical Monitor within five (5) working days of the unplanned stoppage or malfunction through the Study EDC System.

Investigators will also report all Amplifi System unplanned stoppages and malfunctions to the Centrall EC in accordance with the EC requirements.

Sponsor will report all Amplifi System unplanned stoppages and malfunctions to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements.

14.0 Data Analysis and Statistical Considerations

14.1 Study Size Calculation

This study is not powered for a specific outcome, given the small number of Subjects in the study. Rather, the goal of this study is to collect additional data on multiple effectiveness and safety measures as described in **Section 4.1 Study Objectives**.

The effectiveness measure of the Study is to determine percent change in cephalic vein diameter from baseline examination (pre-treatment ultrasound) to end treatment (immediately prior to Amplifi System removal and AVF creation, with Amplifi System running at final speed). The Sponsor reviewed results of Amplifi System treatment during the First-in-Human Study and determined that up to ten (10) Inpatient Subjects would be sufficient to evaluate the safety and effectiveness of the Amplifi System.

14.2 Statistical Methods

14.2.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively using the number of available observations (n), means, standard deviations, medians, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables.

14.2.2 Analysis of Effectiveness Measures

In general, descriptive statistics will be provided. Categorical variables will be summarized using frequencies and percentages of Subjects in each category. Continuous variables will be summarized using descriptive statistics and 95% confidence intervals for means will be computed where appropriate. Statistical modeling will be used to identify potential predictors of treatment success as well as safety events and effectiveness.

A subgroup analysis will be performed for patients who received Amplifi System treatment but have Amplifi System stoppage and AVF creation prior to the specified treatment duration. Patients who received Amplifi treatment at the specified treatment duration (e.g., completed the maximum treatment duration (i.e., 72 hours) without having an AVF created will be reported,

along with the reasons for not having one (e.g., inadequate vein diameter, other factors).

14.2.3 Analysis of Safety Measures

All safety data for the Subjects will be summarized descriptively and the number of AEs, ADEs, SAEs, and SADEs will be reported as a single group. The AEs, ADEs, SAEs, and SADEs will be grouped by type, and the number and percentage of Subjects experiencing each type will be reported as a single group. An assessment of the severity and causative relation to Amplifi System placement, treatment, and removal, and Study AVF creation and use for each AE, ADE, SAE, and SADE will be reported.

14.2.4 Handling of Missing Data

Every effort will be undertaken to ascertain completeness of data collection and limit premature discontinuations. All analyses will utilize only actual Subject data which is collected, and no imputation of missing data will be performed. However, partial dates in medication and adverse events may be imputed. The imputation principles will be described in the Statistical Analysis Plan.

15.0 Study Responsibilities

15.1 Sponsor Responsibilities

The Sponsor is responsible for:

- manufacturing, packaging, and sterilizing Amplifi System components;
- preparing the Protocol and updating the Protocol, as needed, by providing proposed Protocol amendments to Investigators for submission to their local ECs for review and approval;
- selecting Investigators and Sites;
- maintaining an updated list of Investigators and Sites;
- selecting consultants for the Study;
- establishing regulatory standards consistent with local, regional, and national governmental regulations for Sites;
- providing financial support to Investigators and Sites, along with product liability and no-fault clinical study insurance;
- conducting Site initiation visits prior to Subject enrollment;
- providing Investigators with a signed Clinical Study Agreement (CSA), Investigator's Brochure, Protocol, all necessary CRFs, and all other Study-related documents necessary to conduct the Study;
- providing the Amplifi System, including all components necessary for Amplifi System placement, operation, use, and removal, to the Sites;
- providing Study-related accessory devices and materials necessary for Amplifi System placement, operation, use, and removal to the Sites for those Study-related accessory devices that are not reasonably available at the Site or the responsibility of the Investigator or Site to obtain or provide;
- providing instructions and training to Investigators and other Site Personnel

involved in the Study on the proper placement, operation, use, care, and removal of the Amplifi System (including all components), and other Study-related accessory devices, either directly or through consultants;

- providing revisions to the ICF to Investigators, as needed;
- maintaining a Clinical Study Master File;
- granting or restricting the right of Site Personnel to access the Study EDC System along with Data Manager and database;
- providing Study monitoring and performing or overseeing all aspects of data quality assurance, including data collection, data auditing and monitoring at Sites, either directly or through consultants;
- reviewing data submitted by Investigators and Sites for accuracy and completion, and reviewing source documents to ensure accuracy and validity of all data recorded on CRFs, as needed;
- performing Site monitoring, either directly or through consultants;
- recommend corrective actions if serious or repeated deviations from the Protocol by a Site occur, or if a Site is not complying with the Investigator's Agreement, applicable local, regional, and national regulatory requirements, or any other conditions imposed by the local EC or the Sponsor;
- terminate an Investigator or Site's participation in the Study if serious or repeated deviations from the Protocol by an Investigator continue to occur, even after corrective action recommendations and apparently adequate responses and actions, or if Sponsor determines that there is an unacceptable risk to Subject safety if an Investigator were to continue to enroll Subjects or participate in the Study;
- reviewing all reports of known or suspected AEs, SAEs, SADEs, and USADEs;
- reviewing all reports of known or suspected Amplifi System deficiencies, malfunctions, and unplanned stoppages;
- communicating information and reporting all SAEs, SADEs, and USADEs to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements;
- completing the Interim Analysis;
- completing the Final Analysis; and
- performing Study termination visits prior to Study termination; and
- reviewing all publications and presentations related to the Study.

15.2 Investigator Responsibilities

The Investigator is responsible for:

- providing Sponsor with current curriculum vitae;
- signing the Protocol and the Investigator's Agreement in the appropriate locations;
- receiving adequate training on the design, function, storage, placement, use, operation, maintenance, care, cleaning, monitoring, interaction with, removal, and disposal of the Amplifi System and its components before enrolling Subjects;
- understanding the Protocol, carrying out the Study in accordance with the Protocol, and

ensuring the Protocol is being properly followed during the Study;

- obtaining local EC approval of the Study before enrolling Subjects;
- keeping the Sponsor informed of all their local EC actions concerning the Study;
- determining if a patient meets all the inclusion criteria and none of the exclusion criteria prior to enrollment;
- obtaining informed consent from Subjects;
- updating and revising the ICF and re-consenting Subjects, as necessary;
- determining if patients are indicated for creation of a wrist or distal forearm radiocephalic AVF based on their standard practice, prior to enrolling patients as Subjects;
- determining if a Subject's internal jugular vein is suitable for Amplifi Inflow Catheter insertion;
- establishing an Amplifi System treatment time for each Subject prior to initiating treatment;
- ensuring Site Personnel carrying out Study activities have appropriate experience and training;
- performing all tests, assessments, procedures, interventions, and surgeries that are specified in the Protocol to be performed by Investigator, including:
    - placing of the Amplifi System;
    - initiating Amplifi System treatment;
    - obtaining contrast venography during Amplifi System placement, evaluation, and removal procedures;
    - obtaining outflow vein measurements using B-mode ultrasonography during Amplifi System treatment, as needed;
    - removing the Amplifi System; and
    - creating the Study AVF,
    - overseeing the performance of other tests, assessments, procedures, and surgeries specified in the Protocol or needed by Subjects;
- providing patient care for Subjects in accordance with the requirements and guidelines of Sites, federal, state, and local regulatory bodies and agencies, and reasonable standards of care;
- providing or overseeing instruction and training on the monitoring and care of the Amplifi System to Subjects and their homecare providers, as needed;
- ensuring that Study observations and findings are recorded correctly and completely in CRFs;
- maintaining accurate, complete, and current Study documents as specified in **Section 10.7** and ensuring the information recorded and submitted to the Sponsor is representative of the Subject's record and other supporting documentation;
- providing Sponsor with the Study documents and records necessary for a complete review of the work related to the Study at the Site by Investigator;
- meeting all Study reporting obligations, including:
    - reporting actual or suspected Amplifi System deficiencies, malfunctions, or

unplanned stoppages to Sponsor, as specified in **Section 12.10 Reporting**;
  - reporting actual or suspected AEs, SAEs, SADEs, and USADEs to Sponsor, as specified in **Section 12.10 Reporting** and submitting accurate, complete, and timely adverse event reports to Sponsor; and
  - submitting all other reports to the Sponsor that are required,
- grading adverse events using the scale summarized in **Section 12.2 Classification of Adverse Events**;
- determining whether, in the opinion of the Investigator, an adverse event is related to the Amplifi System or Study procedures or surgeries using the criteria summarized in **Section 12.2 Classification of Adverse Events**;
- determining whether, in the opinion of the Investigator, an Amplifi System-related complication is an Amplifi System-related major complication;
- following all known and suspected AEs, ADEs, SADEs, and USADEs until they have resolved, have a stable level of sequelae, or, in the Investigator's opinion, are no longer clinically relevant;
- understanding and accepting the obligations of continuing review by their local EC while conducting the Study, including notifying their local EC of any changes to the Protocol per their local EC requirements, and submitting required reports to their local EC;
- reporting deviations from the Protocol to the Sponsor, as specified in **Section 10.9 Protocol Deviations and Investigator and Site Suspension and Termination**;
- notifying their local EC of Protocol deviations in accordance with their local EC requirements;
- reporting known and suspected AEs, SAEs, SADEs, and USADEs to their local EC in accordance with their local EC requirements;
- understanding and complying with all applicable local, regional, and national regulatory requirements while conducting the Study;
- communicating SAE, SADE, and USADE information and reporting all SAEs, SADEs, and USADEs to the appropriate regulatory agencies and bodies in accordance with relevant local, regional, and national regulatory requirements;
- recording the reason for withdrawal in source documentation and on the appropriate CRF for Subjects that withdraw from the Study;
- understanding the requirements for Amplifi System device and component accountability;
- returning all unused Amplifi System components to Sponsor at the end of the Study or at the end of the participation of a Site in the Study;
- submitting a Study summary to the Sponsor and their local EC promptly after the completion of the Study or at the end of the participation of a Site in the Study;
- providing data for publications and presentations related to the study, as needed by Sponsor and other Investigators; and
- reviewing all publications and presentations related to the study, as needed by Sponsor and other Investigators.

15.3 Site Personnel Responsibilities

Site Personnel are responsible for:

- understanding the Protocol, carrying out the Study in accordance with the Protocol, and ensuring the Protocol is being properly followed during the Study;
- receiving adequate training on the design, function, storage, placement, use, operation, maintenance, care, cleaning, monitoring, interaction with, removal, and disposal of the Amplifi System and its components before participating in the care of Subjects;
- reviewing the Amplifi System IFU prior to participating in the storage, placement, use, operation, maintenance, care, cleaning, monitoring, interaction with, removal, and disposal of the Amplifi System and its components;
- providing instruction and training on the monitoring and care of the Amplifi System to Subjects and their homecare providers, as needed.
- meeting all Study reporting obligations, including:
    - reporting deviations from the Protocol to the Sponsor, as specified in **Section 10.9 Protocol Deviations and Investigator and Site Suspension and Termination**;
    - reporting actual or suspected SAEs, SADEs, and USADEs to Investigators, as specified in **Section 12.10 Reporting**;
    - reporting actual or suspected Amplifi System deficiencies, malfunctions, or unplanned stoppages to Investigators, as specified in **Section 12.10 Reporting**; and
    - submitting all other reports to the Sponsor that are required; and
- providing Study documents and records necessary for a complete review of the work related to the Study at the Site to Sponsor.

15.4 Site Responsibilities

Sites are responsible for:

- providing and maintaining adequate resources to the Investigator at the Site to conduct the Study in accordance with the Protocol;
- ensuring Site Personnel carrying out Study activities have appropriate experience and training;
- maintaining copies of all submissions to, and correspondence with, local ECs (including approvals) and other regulatory bodies or agencies (if any) on file at the Site;
- making Site available for a Site initiation visit prior to Subject enrollment;
- keeping Amplifi System components used in the Study stored in a secure area separate from other medical devices in a location with restricted access that is accessible only by authorized Site Personnel and maintain associated Amplifi System storage and accountability logs and records;
- using a Subject's Study identification codes on all Study-related materials and de-identifying data prior to submission to Sponsor.
- providing Subjects treated with the Amplifi System with 24 hours per day, 7 days per week access for consultation with an Investigator or trained Site Personnel to answer questions from Subjects and their caregivers related to the Amplifi System, assist Subjects in responding to Amplifi System alarms and for other unanticipated events or

Subject concerns, and assisting in the scheduling of care for Subjects;

- maintaining Study documents and original documentation supporting the data recorded on CRFs at the Sites;
- keeping original and updated signed ICFs for each Subject on file and available for inspection by Sponsor or its designees;
- retaining Study records in accordance with their local EC requirements;
- notifying their local ECs of Protocol deviations in accordance with their local EC requirements; and
- upon request, providing to the Sponsor the necessary Study documents and records for a thorough review of the work related to the Study at the Site.

15.5 Independent Safety Board (ISB) and Medical Monitor (MM) Responsibilities

The ISB shall consist of a minimum of 3 physicians who are experts in the fields of nephrology, vascular access surgery, and diagnostic and interventional radiology, who are not participating in the study, and who do not have any investment with the study Sponsor. Membership will be restricted to individuals who have no significant conflict of interest (COI) as described in the COI section. One member of the ISB shall serve as the Chair and one member shall serve as the Medical Monitor with an adjudication role but no voting role.

16.0 Risks and Benefits

16.1 Background

The Amplifi System has not been authorized for commercial distribution in the US by the FDA nor has it been authorized for commercial distribution by any other regulatory agency. Bench, laboratory, and animal testing has been performed on the Amplifi System in studies and a first-in-human clinical study has been completed, as summarized in this Protocol and in the Amplifi System Investigator's Brochure.

A risk analysis has been conducted. The risks associated with the placement, operation, use, and removal of the Amplifi System have been identified by performing a Failure Mode and Effect Analysis (FMEA) and Risk Analysis, as a risk/benefit assessment. Risks have been proven, minimized, or eliminated through appropriate design control, confirmed by bench, laboratory, and animal testing, and a first-in-human clinical study.

The Amplifi System shares many similarities with extracorporeal blood pump systems and catheters that convey blood from patients to pumps and to return blood from pumps to patients, including devices for hemodialysis (including home hemodialysis), extracorporeal membrane oxygenation (ECMO), extracorporeal circulatory support, including the following similarities:

- Amplifi System catheters, hemodialysis catheters, ECMO catheters, and extracorporeal circulatory support catheters are designed for insertion into an internal jugular vein with tip placement in the superior vena cava or right atrium;
- Amplifi System catheters, hemodialysis catheters, ECMO catheters, and extracorporeal

circulatory support catheters are designed to connect to extracorporeal blood pumps;
- the Amplifi System pump and catheters and some hemodialysis pumps and catheters, ECMO pumps and catheters; and extracorporeal circulatory support pumps and catheters are designed to remove and return patient's blood at similar flow rates;
- Amplifi System catheters, hemodialysis catheters, and ECMO catheters are intended for chronic use, with hemodialysis catheters routinely used for many months and even years in some cases; and
- Patients and caregivers can be trained to perform Amplifi System and hemodialysis treatment in an inpatient setting.

The risk profile of the Amplifi System is similar or lower than hemodialysis blood pumps and catheters (including those for home use), and ECMO and extracorporeal circulatory support pumps and catheters.

16.2 Review of Literature

Sponsor reviewed the published safety data associated with hemodialysis blood pumps and catheters, ECMO blood pumps and catheters, and circulatory support blood pumps and catheters. The review of this data is summarized in **Attachment 2: Literature Review for Safety**. The potential safety events reported in these devices used in a similar patient population described in **Attachment 2: Literature Review for Safety** are comparable to the potential safety events identified in the risk analysis for the Amplifi System.

16.3 Benefit Analysis

Enlarging the cephalic vein in hemodialysis patients prior to forearm radiocephalic AVF through Amplifi System treatment has the potential to:

- improve suitability for forearm AVF creation;
- increase the rate of forearm AVF maturation;
- reduce the time required for forearm AVF maturation and use;
- identify pre-existing disease and potentially problematic side branches in veins of the upper extremity prior to AVF creation;
- reduce catheter contact time and catheter-related bloodstream infections;
- provide for easier AVF cannulation;
- provide higher hemodialysis session blood flows, which could allow for shorter hemodialysis sessions and more efficient hemodialysis
- reduce complications and hospitalization rates related to vascular access;
- reduce the number of procedures and surgeries needed to regain or maintain vascular access;
- increase wrist and distal forearm radiocephalic AVF prevalence;
- reduce the overall cost of care; and
- reduce mortality risk.

The risk benefit ratio for using the Amplifi System in the Study has been deemed appropriate for an Investigational Device Exempt clinical study and the risk to Subjects has been appropriately mitigated to protect their safety.

16.4 Risk/Benefit Analysis

The risks associated with the placement, operation, use, and removal of the Amplifi System have been identified, confirmed by bench, laboratory, and animal testing and a first-in-human clinical study, are well understood, and have been eliminated or minimized through appropriate design control. Based on bench, laboratory, and animal testing of the Amplifi System, and results of a first-in-human clinical study, the potential benefits to Subjects from creation of a distal forearm radiocephalic AVF with an enlarged cephalic vein that matures rapidly and fully is clear and balances the risk. A literature review shows the risk/benefit profile for Subjects receiving Amplifi System treatment in the Study is similar to patients currently receiving treatment with widely used blood pump systems, including home hemodialysis, ECMO, and extracorporeal circulatory devices, providing additional support for the assessment that the risk/benefit profile for the use of the Amplifi System in the Study is acceptable.

16.5 Risk Mitigation

The Study has been designed to protect the safety and well-being of all Subjects. During the Study, potential risks will be controlled and mitigated through extensive training of Investigators and other Site Personnel, compliance with the Protocol, monitoring of Subjects during the Amplifi System treatment period, and ensuring the availability of additional treatment and support measures for Subjects, as needed.

17.0 Miscellaneous

17.1 Publication Policy

The first publication or presentation of the clinical results of the Study shall be made as a joint, multi-center publication or presentation by the Investigators at Sites that enrolled Subjects and contributed data and analyses, participated in the review of the data, and contributed to the preparation of the publication or presentation. All publications and presentations of the clinical results of the Study will be reviewed by all Investigators and approved by the Sponsor, and in accordance with the Site-specific CSAs.

18.0 References

1. 2020 USRDS Annual Data Report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2020.
2. McCullough KP, Morgenstern H, Saran R, Herman WH and Robinson BM. Projecting ESRD Incidence and Prevalence in the United States through 2030. Journal of the American Society of Nephrology 2019; 30:127-35, https://doi.org/10.1681/asn.2018050531
3. Thamer M, Lee TC, Wasse H, Glickman MH, Qian J, Gottlieb D, Toner S and Pflederer TA. Medicare Costs Associated with Arteriovenous Fistulas Among US Hemodialysis Patients. American Journal of Kidney Diseases 2018; 72:10-8, https://doi.org/10.1053/j.ajkd.2018.01.034
4. Drew DA, Lok CE, Cohen JT, Wagner M, Tangri N, Weiner DE: Vascular access choice in incident hemodialysis patients: a decision analysis. J Am Soc Nephrol, 26: 183-191, 2015 10.1681/ASN.201311123
5. Mendes RR, Farber MA, Marston WA, Dinwiddie LC, Keagy BA, Burnham SJ: Prediction of wrist arteriovenous fistula maturation with preoperative vein mapping with ultrasonography. J Vasc Surg, 36: 460-463, 2002 10.1067/mva.2002.126544
6. Pirozzi N, Mancianti N, Scrivano J, Fazzari L, Pirozzi R, Tozzi M. Monitoring the Patient Following Radio-Cephalic Arteriovenous Fistula Creatioin: Current Perspectives. Vasc Health and Risk Management 2021:17, 111-121
7. Li H, Jen S, Ramayya T, Gregory Bowers H, Rotem E. Unanticipated Late Maturation of an Arteriovenous Fistula after Creation of Separate Graft Access. Quant Imaging Med Surg, 2018;8(4):444-446
8. Tordoir JHM, Zonnebeld N, Van Loom MM, Gallieni M, Hollenbeck M. Surgical and Endovascular Intervention for Dialysis Access Maturation Failure During and After Arteriovenous Fistula Surgery: Review of the Evidence. Eur J Vasc Endovas Surg, 2018;55:240-248
9. Wilmink T, Hollingworth L, Powers S, Allen C, Dasgupta I. Natural History of Common Autologous Arteriovenous Fistulae: Consequences for Planning of Dialysis Access. Eur J Vasc Endovasc Surg, 2016;51:134-140
10. Pisoni RL, Zepel L, Fluck R, Lok CE, Kawanishi H, Süleymanlar G, et al.: International Differences in the Location and Use of Arteriovenous Accesses Created for Hemodialysis: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). American Journal of Kidney Diseases, 71: 469-478, 2018 https://doi.org/10.1053/j.ajkd.2017.09.012
11. Tan T-W, Farber A: Brachial-Basilic Autogenous Access. Seminars in Vascular Surgery, 24: 63-71, 2011 10.1053/j.semvascsurg.2011.05.004
12. Al-Jaishi AA, Oliver MJ, Thomas SM, Lok CE, Zhang JC, Garg AX, et al.: Patency Rates of the Arteriovenous Fistula for Hemodialysis: A Systematic Review and Meta-analysis. American Journal of Kidney Diseases, 63: 464-478, 2014 10.1053/j.ajkd.2013.08.023
13. Feldman HI, Joffe M, Rosas SE, Burns JE, Knauss J, Brayman K: Predictors of successful arteriovenous fistula maturation. Am J Kidney Dis, 42: 1000-1012, 2003 10.1016/j.ajkd.2003.07.003

14. Browne LD, Bashar K, Griffin P, Kavanagh EG, Walsh SR, Walsh MT: The Role of Shear Stress in Arteriovenous Fistula Maturation and Failure: A Systematic Review. *PloS one,* 10**:** e0145795, 2015 10.1371/journal.pone.0145795
15. Bashar K, Clarke – Moloney M, Burke PE, Kavanagh EG, Walsh SR: The role of venous diameter in predicting arteriovenous fistula maturation: When not to expect an AVF to mature according to pre-operative vein diameter measurements? A best evidence topic. International Journal of Surgery, 15: 95-99, 2015 10.1016/j.ijsu.2015.01.035
16. Robbin ML, Greene T, Cheung AK, Allon M, Berceli SA, Kaufman JS, et al.: Arteriovenous Fistula Development in the First 6 Weeks after Creation. Radiology, 279: 620-629, 2016 10.1148/radiol.2015150385
17. Dageforde, LA, Harms, KA, Feurer, ID and Shaffer, D. Increased minimum vein diameter on preoperative mapping with duplex ultrasound is associated with arteriovenous fistula maturation and secondary patency. J Vasc Surg 2015; 61:170-6.
18. Achneck HE, Sileshi B, Li M, Partington EJ, Peterson DA, Lawson JH: Surgical aspects and biological considerations of arteriovenous fistula placement. Semin Dial, 23: 25-33, 2010 10.1111/j.1525-139X.2009.00651.

Appendix 1: Definitions

- *Abandonment of Study AVF* is defined as the discontinuation of the clinical use of Study AVF because it is no longer functional and considered not to be salvageable, including the discontinuation of the clinical use of Study AVF because it can no longer be used for prescribed hemodialysis, because it is unable to provide adequate blood flow, or because it is deemed unsafe for the patient, and the associated problem(s) cannot be corrected by any medical, procedural, or surgical intervention.
- *Amplifi System and AVF local infection* is defined as: 1) the presence of purulent discharge from the Inflow Catheter skin exit site, the Distal Outflow Catheter skin exit site, or the Study AVF skin incision; or 2) the presence of serous discharge from the Inflow Catheter skin exit site, the Distal Outflow Catheter skin exit site, or the Study AVF skin incision with erythema, induration, or tenderness and a positive culture of the serous discharge.
- *Amplifi System component replacement* is defined as the replacement of one or more components of the Amplifi System during the Amplifi System treatment period, not including replacement or addition of Access Port caps, suture anchoring tools, a Patient Vest, or Patient Vest components.
- *Amplifi System-related major complication* is defined as:
  - Amplifi System-related death;
  - Amplifi System-related major bleeding, major pneumothorax, major air embolism, major thrombosis, major thromboembolism, major hemolysis, or major thrombocytopenia; local infection
  - Amplifi System-related re-hospitalization or prolonged hospitalization;
  - Amplifi System-related major surgery or major interventional procedure; or
  - any Amplifi System-related complication determined to be an Amplifi System-related major complication by both an Investigator and the CEC.
- *Amplifi System-related major bleeding* is defined as:
  - overt bleeding plus a drop in hemoglobin of 3 to 5 g/dL wherein the hemoglobin drop is related to the bleeding;
  - transfusion for overt bleeding;
  - bleeding requiring an interventional procedure or surgery for control (not including the placement or removal of skin sutures or pressure dressings);
  - the administration of intravenous vasoactive agents for overt bleeding; or
  - probable or definite fatal bleeding.
- *Amplifi System-related major pneumothorax* is defined as the introduction of air in the cavity between the lungs and the chest wall during the placement, operation, use, or removal of the Amplifi System that is not due to a cause other than the placement, operation, use, or removal of the Amplifi System, wherein the pneumothorax is documented by imaging and a chest tube is placed.
- *Amplifi System-related major air embolism* is defined as the entry of air into the vascular

system during the placement, operation, use, or removal of the Amplifi System that is not due to a cause other than the placement, operation, use, or removal of the Amplifi System; wherein the air embolism is documented by imaging and:

  - results in air embolism-related hypotension, arrhythmia, hemodynamic instability, paradoxical embolization, myocardial infarction, stroke, mesenteric ischemia, or death;
  - requires a procedure or surgery to remove the air that forms after air embolism; or
  - requires a thrombectomy procedure or surgery, thrombolytic therapy, or long-term anticoagulation to remove thrombus that forms after air embolism.

- *Amplifi System-related major thrombosis* is defined as the formation of thrombus in an artery, vein, or the right atrium due to the placement, operation, use, or removal of the Amplifi System, wherein the thrombosis is documented by imaging and the Subject requires a thrombectomy procedure or surgery, thrombolytic therapy, or long-term anticoagulation; not inclusive of a thrombectomy procedure or surgery that occurs during the creation of the Subject AVF.
- *Amplifi System-related major thromboembolism* is defined as thromboembolism in the arterial or venous system due to the placement, operation, use, or removal of the Amplifi System, wherein the thromboembolism is documented by imaging and the Subject requires a thrombectomy procedure or surgery, thrombolytic therapy, or long-term anticoagulation; not inclusive of a thrombectomy procedure or surgery that occurs during the creation of the Subject AVF.
- *Amplifi System-related major hemolysis* is defined as hemolysis documented by laboratory testing during the Amplifi System treatment period that is not due to a cause other than the placement, operation, use, or removal of the Amplifi System, and requires blood transfusion and/or results in thrombotic events, multiple organ failure and mortality.
- *Amplifi System-related major thrombocytopenia* is defined as a platelet level of $\leq$ 51,000 per microliter during the Amplifi System treatment period that is not due to a cause other than the placement, operation, use, and removal of the Amplifi System, and requires transfusion.
- *Amplifi System-related re-hospitalization* is defined as an admission to a hospital due to an Amplifi System-related complication, not inclusive of a visit to an emergency room, clinic, or outpatient facility that does not result in admission to a hospital.
- *Amplifi System-related prolonged hospitalization* is defined as a prolongation of a hospital admission due to an Amplifi System-related complication.
- *Amplifi System-related major surgery* is defined as an open surgery involving an incision and the cutting of the body to address a life-threatening adverse event, to prevent disability, or to treat or prevent permanent impairment or damage to a vital body structure or function due to an Amplifi System-related complication; not inclusive of the placement of pressure dressings, the placement or removal of skin sutures, the creation of an AVF or AVG, or the placement or removal of a hemodialysis catheter.
- *Amplifi System-related major interventional procedure* is defined as an interventional procedure to address a life-threatening adverse event, to prevent disability, or to treat or prevent permanent impairment or damage to a vital body structure or function due to an

Amplifi System-related complication and the loss or damage to sites for dialysis access or the need to convert an AV fistula to an AV graft or hemodialysis catheter; not inclusive of the placement of pressure dressings, the placement or removal of skin sutures, the creation of an AVF or AVG, or the placement or removal of a hemodialysis catheter.

- *Amplifi System-related complication* is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical sign or abnormal lab finding related to the placement, use, operation, or removal of the Amplifi System; not inclusive of mild-moderate hand, wrist, arm, neck, or face swelling that resolves within 7 days after the end of the Amplifi System treatment period.
- *Amplifi System treatment period* is defined as starting when a needle is used to access the internal jugular vein or cephalic vein at the start of Amplifi System placement and ending when the Inflow Catheter and Distal Outflow Catheter have been completely removed and any related incisions have been closed.
- *Aneurysm of Study AVF* is defined as expansion of the intimal, medial, and adventitial layers of the wall of a segment of outflow vein of a Study AVF in the region between the Study AVF anastomosis and the axilla that requires treatment through an interventional procedure or surgery.
- *Anti-platelet agent* is defined as a pharmaceutical that is approved for use to decrease platelet aggregation and inhibit thrombus formation.
- *AVF circuit* is defined as the region starting with the origin of the inflow artery and continuing to the junction of the superior or inferior vena cava and the right atrium.
- *AVF creation* is defined as the making of an opening between the lumen an artery and vein for the purpose of creating a vascular access for hemodialysis, inclusive of interventional and surgical methods.
- *AVF-related intervention* is defined as an interventional procedure or surgery after the creation of an AVF to evaluate an AVF, promote maturation of an AVF, achieve or maintain patency of an AVF, or reduce AVF blood flow, including thrombectomy, pharmacologic thrombolysis, angioplasty and stenting of an inflow artery, outflow vein, or ipsilateral central vein, embolization or surgical ligation of an AVF tributary vein, surgical revision of an AVF anastomosis, the cutting, removal or otherwise rendering of a valve in an AVF outflow vein incompetent, resection of a segment of outflow vein, unplanned superficialization of a segment of outflow vein, and surgical or interventional treatment of ipsilateral arterial steal.
- *AVF maturation* is defined as an AVF having a mid or distal forearm outflow vein diameter $\geq$ 6 mm and blood flow $\geq$ 500 mL/min, both as measured by ultrasound, with AVF blood flow imputed from distal brachial artery blood flow.
- *AVF thrombosis* is defined as the formation of thrombus in a component of the AVF circuit, inclusive of occlusive and non-occlusive thrombus.
- *Bleeding diathesis* is defined as an unusual susceptibility to bleeding, including due to hypocoagulability.
- *Central venous and right atrial thrombus* is defined as intraluminal thrombus in the axillary, subclavian, brachiocephalic, femoral, external iliac, internal iliac, common iliac, or internal jugular veins, the superior or inferior vena cava or the right atrium, inclusive of occlusive and non-occlusive thrombus.

- *Central venous angioplasty* is defined as an interventional procedure or surgery to enlarge the lumen of the axillary, subclavian, or brachiocephalic veins, or the superior vena cava.
- *Central venous occlusion* is defined as the complete blockage of the axillary, subclavian, or brachiocephalic veins, or the superior vena cava.
- *Central venous stenosis ≥ 50%* is defined as a ≥ 50% narrowing of the lumen of an axillary, subclavian, or brachiocephalic vein, or the superior vena cava, wherein a normal segment of vein adjacent to the narrowed segment is used as a reference vessel for making the determination and the maximum observed diameter reduction is used, inclusive of measurements made with ultrasound imaging, venography, or MRI.
- *Central venous stent placement* is defined as an interventional procedure or surgery wherein a metal tube is permanently placed in the lumen of the axillary, subclavian, or brachiocephalic veins, or the superior vena cava for the purpose of keeping the vessel open.
- *Coagulopathy* is defined as a derangement of hemostasis resulting in either excessive bleeding or clotting.
- *Gastrointestinal bleeding* is defined any form of hemorrhage or bleeding that occurs in the gastrointestinal tract from the mouth to the anus.
- *Hemodialysis catheter placement* is defined as the placement of a new hemodialysis catheter, including the replacement of an existing hemodialysis catheter with a new catheter, and not inclusive of the repositioning of a catheter where the same catheter remains in the Subject before and after the procedure.
- *Hemodialysis catheter-related bloodstream infection* is defined as the growth of the same organism from a hemodialysis catheter and a peripheral venipuncture (or a suitable surrogate such as a hemodialysis blood line) in a patient with clinical signs of a bloodstream infection and no alternative source of bloodstream infection other than the hemodialysis catheter.
- *Indicated for forearm radiocephalic AVF creation based on an Investigator's standard practice* is defined as:
    - indicated for wrist or distal forearm radiocephalic AVF creation based on an Investigator's standard practice;
    - a wrist or distal forearm cephalic vein diameter of ≥ 1.75 mm and ≤ 3.2 mm at the proposed AVF creation site;
    - a forearm cephalic vein demonstrating patency and continuity of blood flow from the proposed AVF creation site to and through the upper arm through at least one of:
        - the upper arm cephalic vein;
        - the median cubital vein to the upper arm cephalic or basilic vein; or
        - the perforator vein to the deep venous system

      with no occlusion or stenosis ≥ 50% along the course of the proposed AVF outflow from the proposed AVF creation site to and through the upper arm to the axilla, as determined by duplex ultrasonography; **and**
    - a wrist or distal forearm radial artery diameter of ≥ 2.0 mm at the proposed AVF

creation site.

- *Internal jugular vein suitable for Amplifi Inflow Catheter insertion* is defined as a widely patent internal jugular vein without thrombus and with a lumen diameter ≥ 8.5 mm (during Valsalva) that does not contain any catheter or intravascular implant and is deemed to be suitable for Inflow Catheter insertion by the Investigator.
- *Intervention-free AVF maturation* is defined as AVF maturation (as defined above) in the absence of any interventional procedure, surgery, or medical treatment to promote maturation of the AVF.
- *Interventional procedure, surgery, or medical treatment to promote maturation of the AVF of Study AVF* is defined as an interventional procedure, surgery, or a medical treatment intended to:
  - increase the diameter of a segment of an artery or vein in Study AVF circuit or increase Study AVF blood flow, including by angioplasty or stent placement, cutting, removing, or otherwise rendering a venous valve incompetent, resecting of a segment of outflow vein, and surgical revision of the Study AVF anastomosis;
  - remove thrombus from the lumen of the artery or vein in Study AVF circuit; and
  - reduce the flow of blood in Study AVF tributaries including by embolization or surgical ligation;

  and excluding planned superficialization of a segment of Study AVF outflow vein for the purpose of enabling easier cannulation.
- *Interventional procedure, surgery, or medical treatment to* achieve or maintain functional patency of Study AVF is defined as an interventional procedure, surgery, or a medical treatment intended to:
  - increase the diameter of a segment of artery or vein in Study AVF circuit or increase Study AVF blood flow, including by angioplasty or stent placement;
  - cut, remove, or otherwise render a venous valve incompetent;
  - surgically revise the Study AVF anastomosis;
  - remove thrombus from the lumen of the artery or vein in Study AVF circuit;
  - resect a segment of Study AVF outflow vein; and
  - reduce the flow of blood in Study AVF tributaries including by embolization or surgical ligation;

  inclusive of interventional procedures, surgeries, and medical treatments intended to promote maturation of Study AVF; and

  exclusive of planned superficialization of a segment of Study AVF outflow vein for the purpose of enabling easier cannulation.
- *Intracranial bleeding* is defined as any bleeding within the intracranial vault, including bleeding in the brain parenchyma and meningeal spaces.
- *Pseudoaneurysm of Study AVF* is defined as a locally contained hematoma of a Study AVF outflow vein with persistent blood flow in the hematoma and a neck between the hematoma and the Study AVF outflow vein that does not close spontaneously that is located between the Study AVF anastomosis and the axilla that requires treatment with an interventional

procedure or surgery, inclusive of percutaneous injection of an agent intended to induce thrombosis.

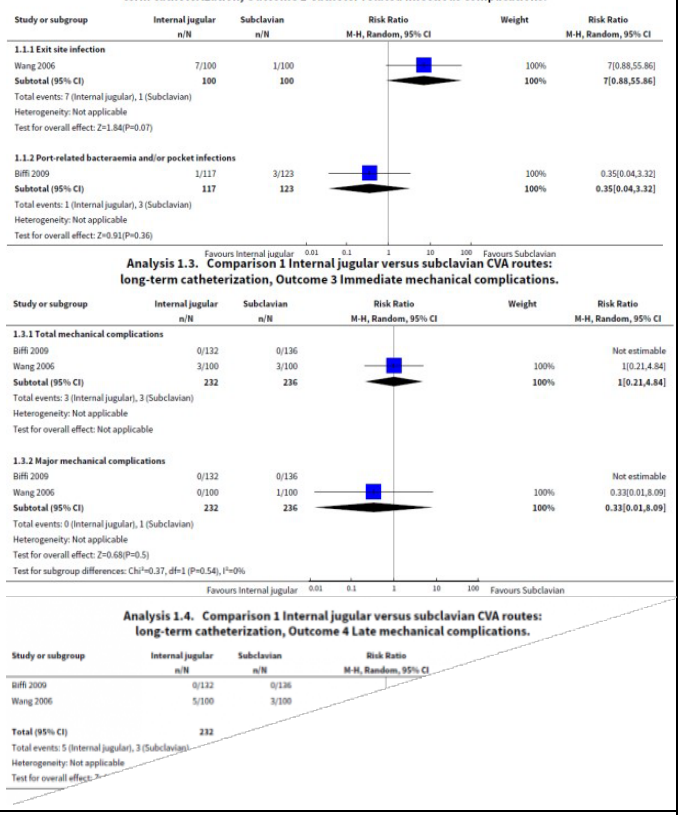
- *Site Personnel* is defined as persons employed by the Site, other than Investigators, that are involved in the storage, placement, use, operation, maintenance, care, cleaning, monitoring, interaction with, removal, and disposal of the Amplifi System and its components.
- *Thrombophilia* is defined as a clinical tendency to thrombosis or molecular abnormalities of hemostasis that predispose to thrombosis.
- Treatment Failure is defined as "a subject will be classified as a treatment failure if they do not achieve the minimum required Amplifi treatment duration of 48 hours. This classification applies regardless of whether the subject has an arteriovenous fistula (AVF) created or not."
  - **Device technical success** is defined as successful operation of the Amplifi system throughout the treatment period (from device implantation through device removal and AVF creation) without unplanned pump stoppages or device malfunctions that require intervention or prevent completion of the intended 48-hour treatment duration.
  - **Treatment success** is achieved when the Amplifi system operates without unplanned pump stoppages or device malfunctions, allowing for the full 48-hour treatment duration, and results in successful creation of an arteriovenous fistula (AVF) or meets the predefined clinical objectives as specified in the study protocol.
- *Vascular access for hemodialysis* is defined as an AVF, AVG, or hemodialysis catheter.
- *Vascular access-related hospitalization* is defined as admission to a hospital for treatment of a vascular access or a vascular access-related complication, not inclusive of a visit to an emergency room, clinic, or outpatient facility that does not result in admission to a hospital.
- *Vascular access steal syndrome* is defined as one or more signs or symptoms of ipsilateral hand ischemia that requires treatment with an interventional procedure or a surgery.
- *Wrist or distal forearm radiocephalic AVF* is defined as an AVF made with the radial artery and the cephalic vein, wherein the anastomosis is made in the hand, wrist, or distal forearm.

Appendix 2: Literature Review for Safety

Table 5: Publications Relevant to Safety and Effectiveness - Catheters

Publication	Overview	Safety Data
Amy Leung, et al. The Incidence of Peripheral Catheter-related infection in surgical patients. Thrombosis: 2016(1):1-6	A prospective observational study determines the incidence of peripheral catheter-related thrombosis in surgical patients. N=54 surgery patients with high risk of venous thrombus studied with ultrasound before placement and at discharge. Catheters were in place between 2-5 days.	9.2% rate of superficial vein thrombus formation – all asymptomatic 1.8% rate of deep vein thrombus formation – all asymptomatic No pulmonary embolism identified this (study) confirmed the asymptomatic nature of peripheral catheter-related thrombosis."
Chick, et al. Significance of Echocardiographically Detected Central Venous Catheter Tip-Associated thrombi. J Vasc Interv Radiol 2016; 27:1872–1877	Review of significance, management, and outcomes of central venous catheter (CVC) tip-associated thrombi incidentally detected on echocardiography. Echocardiogram data of n=170 were screened and n=49 patients with CVC-tip associated thrombi were selected.	29% had catheter-associated thrombus formation "In this sample with CVC tip–associated thrombi but without PFO or other intracardiac shunts, no embolic or other complications were detected, regardless of anticoagulation status. These data suggest a benign course for such thrombi and that anticoagulation, catheter removal, thrombectomy, and thrombolysis may be unnecessary when catheter tip–associated thrombi are incidentally detected on echocardiography."
Miller, et al "Hemodialysis Tunneled Catheter Noninfectious Complications" Canadian Journal of Kidney Health and Disease; Volume 3: 1–10: 2016 sagepub.com/	Review publication on behalf of the Canadian Society of Nephrology Vascular Access Work Group (VAWG)	Noninfectious hemodialysis catheter complications include catheter dysfunction, catheter-related thrombus, and central vein stenosis. Catheter-related thrombus is a less common but serious complication of catheters, requiring catheter removal and systemic anticoagulation.
Bream, et al "Update on Insertion and Complications of Central Venous Catheters for Hemodialysis", Semin Intervent Radiol 2016;33:31–38	Review publication of collaboration with the Accreditation Council for Continuing Medical Education (ACCME) and Tufts University School of Medicine (TUSM)	Acute central venous catheter complications and frequency include the following.

Publication	Overview	Safety Data
		<table><tr><th>Event</th><th>Frequency (%)</th></tr><tr><td>Hemorrhage/hematoma</td><td><2</td></tr><tr><td>Catheter malposition/kinking</td><td><1</td></tr><tr><td>Venous perforation</td><td><1</td></tr><tr><td>Infection</td><td><1</td></tr><tr><td>Arterial puncture</td><td><1</td></tr><tr><td>Pneumothorax</td><td>0–1</td></tr><tr><td>Air embolism</td><td>0–1</td></tr></table> Long-term central venous catheter complications include: infections (overall the rate of catheter-related bacteremia (CRB) are 1.6 – 5.5 episodes/1,000 catheter days; catheter malfunction; thrombosis; catheter tip malposition; and central vein stenosis.
Patel, et al “Central Line Catheters and Associated Complications: A Review” (May 22, 2019) Central Line Catheters and Associated Complications: A Review. Cureus 11(5): e4717. DOI 10.7759/cureus.4717	Joint Review Publication of Kaiser Permanente and the Marshall University School of Medicine	Acute complications of central venous catheters include: arrhythmia; vascular injury (arterial or venous); bleeding hematoma; pneumothorax; air embolism; and chylothorax. Long-term complications of central venous catheters: infection (80 - 189 episodes per 100,000 patient years); and device dysfunction, including fibrin sheath, catheter fracture, venous thrombosis, venous stenosis, or infection.
Hyder, et al “Complications Associated with Permanent Internal Jugular Hemodialysis Catheter: A Retrospective Study” (April 22, 2019) Cureus 11(4): e4521. DOI 10.7759/cureus.4521	Single center experience of n = 212 hemodialysis patients.	Complications were documented in 24% of patients with internal jugular vein hemodialysis catheters.19 patients had acute complications, including: failed puncture (5.1%); cannulation of wrong vessel (0.9%); hematoma (1.9%); and hemothorax (0.9%). 39 patients had long-term complications, including: infection (12.3%); venous thrombosis (2.8%); catheter thrombosis (2.4%); and pneumothorax (0.9%).
Javeri, et al “Indian Society of Critical Care Medicine Position Statement for Central Venous Catheterization and Management 2020” Indian J Crit Care Med. 2020 Jan; 24(Suppl 1): S6–S30.	Clinical practice guidelines developed by Indian Society of Critical Care Medicine	Infection: The clinical practice guidelines include specific recommendations to mitigate infection risks. A systematic review and meta-analysis of 79 studies found that strict adherence to CRBSI (catheter related bloodstream infections) prevention activities reduced its incidence significantly from median 6.4/1000 catheter- days (IQR 3·8 – 10.9) to 2.5/1000 catheter-days (1.4 – 4.8) (IRR 0.44, 95% CI 0.39 – 0.50, p < 0·0001; I = 89%). Mechanical Complications: The clinical practice guidelines include specific recommendations to

Publication	Overview	Safety Data
		mitigate risk of mechanical complications including pneumothorax, hemothorax, arterial puncture, hematoma formation, and malposition of central venous catheter.
Shafique, et al "Hemodialysis Internal Jugular Vein Versus Subclavian Vein Catheters: Complications, Patients' Comfort, Tolerance and Cost-Effectiveness" Pak J Med Sci. 2019;35(1):124- 128.	Single-center experience of n = 66 hemodialysis patients with internal jugular vein and subclavian vein catheters	Table-I: Hemodialysis catheter-related early complications. Variable / Population: SCV catheter / IJV catheter / OR (95% CI) Vein damage; % (f): 6.5 (2) / 13.9 (4) / 2.15 (0.364 - 12.693) Artery rupture: 3.2 (1) / 9.7 (3) / 3.21 (0.316 - 32.741) Pulmonary complications: 3.2 (1) / 3.2 (1) / 1.0 (0.060 - 16.737) Bacterial infection*: 6.5 (2) / 6.5 (2) / 1.0 (0.132 - 7.587) *exit-site infection; p >0.05 (after chi-squared/Fisher's exact test) against all variables. Table-II: Hemodialysis catheter-related late complications. Variable / Population: SCV catheter / IJV catheter / OR (95% CI) Device dysfunctioning; % (f): 3.2 (1) / 9.7 (3) / 3.21 (0.316 - 32.741) Thrombus formation*: 3.2 (1) / 3.2 (1) / 1.0 (0.060 - 16.737) Central vein stenosis: 19.4 (6) / 25.8 (8) / 1.45 (0.436 - 4.814) Bacterial infection: 16.1 (5) / 32.3 (10) / 2.48 (0.733 - 8.369) Infection-based replacement: 12.9 (4) / 25.8 (8) / 2.35 (0.645 - 8.814) *managed through catheter removal and systemic anticoagulation.
Cavallazzi, et al. "Central Venous Access Sites for the Prevention of Venous Thrombosis, Stenosis and Infection (Review)" Cochrane Database of Systematic Reviews 2012, Issue 3. Art. No.: CD004084.DOI: 10.1002 /14651858.CD004084.pub3.	Systematic literature review comparing internal jugular vein and subclavian vein: catheter-related infection rates; thrombotic complications; immediate mechanical complications; and late mechanical complications.	
Ravani, et al, "Associations between Hemodialysis Access Type and Clinical Outcomes: A Systematic Review" J Am Soc Nephrol. 2013 Feb 28; 24(3): 465–	Systematic literature review comparing patient outcomes from 67 articles published 1985 - 2011 reporting data on n = 363 vascular	Catheter versus AVF: Patients using catheters had an increased risk of all-cause mortality (risk ratio [RR] = 1.53, 95% confidence interval [95% CI] = 1.41 – 1.67), fatal (RR = 2.12, 95% CI=1.79–2.52) and nonfatal (RR = 4.66, 95% CI=2.63 – 8.26) infection, major cardiovascular event (RR = 1.38, 95% CI = 1.24 – 1.54), and

Publication	Overview	Safety Data
473.	access type comparisons in 586,337 participants.	hospitalization rates (RR = 1.68, 95% CI=1.33–2.12) compared with patients using AVFs. Catheter versus AVG: Patients using catheters had increased risk of all-cause mortality (RR = 1.38, 95% CI=1.25–1.52), fatal (RR=1.49, 95% CI=1.15 – 1.93) and nonfatal (RR = 2.78, 95% CI = 1.80 – 4.29) infection, cardiovascular event (RR = 1.26, 95% CI = 1.11 – 1.43), and hospitalization (RR = 1.51, 95% CI = 1.30 – 1.75) compared with patients using AVGs.
Leib, et al “Central Line”, NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan	Review paper evaluating central venous catheters	Complications associated with central venous catheters include pneumothorax; pericardial effusion and tamponade; bleeding; arterial puncture with an expanding hematoma; infection; thrombosis; injury to the nerves; losing guidewire inside the vein; air embolism; and arrythmias

Table 6: Publications Relevant to Safety and Effectiveness – Extracorporeal Blood Pumps

Publication	Overview	Safety Data
Thomas, et al. “Bleeding and Thrombotic Complications in the Use of Extracorporeal Membrane Oxygenation Semin Thromb Hemost. 2018 Feb;44(1):20-29. doi: 10.1055/s-0037-1606179. Epub 2017 Sep 12.	Section of Pediatric Critical Care Medicine, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas. Division of Transfusion Medicine and Coagulation, Department of Pathology and Immunology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas.	Extracorporeal membrane oxygenation (ECMO) has been used for >40 years to support lung and heart failure; however, bleeding and thrombosis remain serious complications. The known etiologies of bleeding include heparin effect or overdose, coagulopathy, thrombocytopenia, platelet dysfunction, acquired von Willebrand syndrome, and hyperfibrinolysis. Bleeding sites may include cannula insertion sites, recent surgical incisions, vascular access sites, lung, gastrointestinal tract, mouth, nose, thoracic cavity, abdominal cavity, and brain. Massive bleeding in the brain, the most feared bleeding complication, can be rapidly fatal because it occurs in a rigid closed space, is difficult to drain, and cannot be stopped with direct pressure to the bleeding site. Pulmonary hemorrhage may cause irreversible lung damage. Management should be swift and precise to prevent fatal

Publication	Overview	Safety Data
		bleeding. In contrast, etiologies of thrombosis include high fibrinogen and factor VIII levels, heparin resistance, and platelet activation. Achieving the optimal anticoagulation balance to prevent bleeding and thrombosis in ECMO patients is extremely complex. Experts in hemostasis should be a part of an institutional ECMO team and continuously available for immediate management.
Sniderman J, Monagle P, Annich GM, MacLaren G. "Hematologic concerns in extracorporeal membrane oxygenation." Res Pract Thromb Haemost. 2020 May 15;4(4):455-468. doi: 10.1002/rth2.12346. PMID: 32548547; PMCID: PMC7292669.	Review by International Society of Thrombosis and Haemostasis (ISTH) to consider 'State of the Art' in ECMOs and considerations for choosing outcome measures.	Pre-existing risks to patients for hematologic events may be exacerbated by the ECMO. Blood exposure to artificial surfaces results in fibrinogen, albumin, and other proteins coating the artificial surface, platelet activation, and inflammation. This may be dampened by a heparin-bonded biomimetic surface which reduces cellular activation and inflammation, but systemic anticoagulation is still required. The degree of thrombocytopenia is related to the patient's illness rather than the duration of ECMO treatment. The artificial cannulation surface and the shear stress may contribute to platelet activation and reduced platelet bonding, along with other factors, leading to thrombosis and bleeding. Across multiple pediatric and adult studies, severe hemolysis occurs at a rate of 2-20%. Hemolysis is associated with increased rates of endothelial failure, kidney failure, thrombotic events, transfusions, and mortality. Meta-analysis reports venovenous ECMO hemorrhage rates of 19% with pre-existing respiratory distress syndrome and central cannulation being 2 highly contributing factors. All ECMO configurations carry high risk of infections between 13-40%.
Strunina S, Hozman J, Ostadal P. "The peripheral cannulas in extracorporeal life support." Biomed Tech (Berl). 2019 Apr 24;64(2):127-133. doi:	Review Faculty of Biomedical Engineering, Czech Technical University in Prague, Nám. Sítná 3105, 272 01Kladno, Czech Republic.	When peripheral (rather than central) cannula ECMOs are used, bleeding, thrombosis and hemolysis are the most common complications. The peripheral ECMO cannulas provide greater versatility for cannulation techniques and strive for smaller incisions. Heparin-coated surfaces result in reduced complement and inflammatory

Publication	Overview	Safety Data
10.1515/bmt-2017-0107. PMID: 29648990.	Cardiovascular Center, Na Homolce Hospital, Roentgenova 2/37, 15030 Prague, Czech Republic.	activation due to its anti-inflammatory properties. Nevertheless, heparin causes the development of osteopenia and thrombocytopenia [16], [28]. Heparin-coated surfaces can release cytokines, which exert a damaging effect on the lungs [29]. Furthermore, heparin-based coatings can leach into the blood and result in the exposure of the blood to extremely high heparin doses (>50 U/kg) [30]. This can cause excessive bleeding and can be lethal. The dimensions of the cannula can also play a role in adverse events, and they should be optimized for the patient. High pressure flow can become turbulent and may damage red blood cells. In addition, it can cause microbubbles and induce air emboli. While smaller cannulas reduce bleeding complications, the risk of vascular damage, ischemia, and obstruction of the arteries, it is balanced with using the largest possible to maximize blood flow. Bleeding is the most frequent complication in ECMO therapy. A meta-analysis of 20 studies reported an estimated rate of 41% with the femoral cannula insertion site being the most frequent source. Ischemia is the 2nd most frequent complication of using cannulas. The most concerning is the lower extremity ischemia with VA ECMO at 10-20%. Hemolysis is reported to have an incidence of 5-18%, mostly induced by shear stress when using high flow velocity through small cannulas; consequently, renal insufficiency or multiple organ failure can occur.
Besser MW. “Post-operative of bleeding, haemolysis and coagulation in mechanical circulatory support patients.” Ann Transl Med. 2020 Jul;8(13):832. doi:	Review by Department of Haematology, Addenbrooke's Hospital, Cambridge, UK	Shear force, during ECMO use, can compress blood by deforming the red blood cells and at a molecular level, changing the shape of its platelets. Higher viscosity flow disrupts marginalization of platelets, and a pre-existing thrombocytopenia becomes more symptomatic.

Publication	Overview	Safety Data
10.21037/atm-20-405. PMID: 32793677; PMCID: PMC7396228.		
Vezzani A, Manca T, Vercelli A, Braghieri A, Magnacavallo A. "Ultrasonography as a guide during vascular access procedures and in the diagnosis of complications." J Ultrasound. 2013 Oct 29;16(4):161-70. doi: 10.1007/s40477-013-0046-5. PMID: 24432170; PMCID: PMC3846948.	Review to support clinician ultrasound use. Terapia Intensiva Cardiochirurgica, Dipartimento Cardio Nefro Polmonare, Azienda Ospedaliero, Universitaria di Parma, Parma, Italy. U.O.C. di Pronto Soccorso/OBI/Medicina D'Urgenza, Ospedale G. Da Saliceto, 29100 Piacenza, Italy.	Ultrasound guidance used during catheterization of peripheral veins and for peripheral insertion of central venous catheters increases the catheterization success rate and reduces complications such as catheter-related infections and venous thrombosis. Ultrasound can also access basilic and cephalic veins deeper in the arm that cannot be identified by palpitation. Ultrasound is not indicated for routine catheterization of peripheral veins, but when access is expected to be complicated, it can reduce complications. Insertion of a central venous catheter (CVC) still carries the risk of improper placement and mechanical complications. For this reason, a chest X-ray is advisable to verify the position of the catheter tip and exclude the possibility of iatrogenic pneumothorax. The presence of the catheter tip in the right atrium is reported in 8–47 % of cases; even in expert hands, placement error rates range from 6 to 14 %. FDA recommends the tip of CVC should be placed in the SVC, along the axis of the vessel so as not to slide into the right atrium. Post-procedural pneumothorax occurs in 0.1-0.2% of CVCs inserted into the internal jugular vein. Ultrasound should be used especially with advanced age or complex disease.
Abruzzo A, Gorantla V, Thomas SE. "Venous thromboembolic events in the setting of extracorporeal membrane oxygenation support in adults: A systematic review." Thromb Res. 2022 Apr;212:58-71. doi: 10.1016/j.thromres.2022.02.015. Epub 2022 Feb 23. PMID: 35219933.	Systemic Review of 30 records including n=2049 Department of Anatomical Sciences, St. George's University School of Medicine, Grenada. Department of Internal Medicine, University of Maryland Medical Center MTC, Baltimore, USA. Electronic address: sneha.thomas@umm.edu.	ECMO-supported patients not only experienced high rates of venous thrombosis (VT) and VTE, ECMO was also a positive predictor for VTE. VT and VTE were prevalent in 41.6% of 63 patients on ECMO for ARDS or organ failure with no prior history of thrombosis. There is a positive correlation between incidence of VTE or VT and the length of time patients were supported by ECMO. The longer the patients relied on ECMO therapy, the likelihood of inducing an inflammatory response or undergoing blood clotting factor modification was significantly

Publication	Overview	Safety Data
		increased. Patients on ECMO therapy for prolonged periods of time, advanced age, and those who had developed an infection during treatment are at an increased risk of developing DVT upon weaning. Follow up should be done after ECMO therapy. Low circuit flow, insufficient volume, pump speed, blood flow velocity, kinks, or obstruction in flow, malpositioning, large size of ECMO cannulas, sheer stress and multitude of other factors that activate platelet and coagulation cascade may be contributing to this increased risk of cannula associated DVT and VTE in general. Platelet count less than 100 G/L during ECMO had lower DVT risk.
Gross-Hardt S, Hesselmann F, Arens J, Steinseifer U, Vercaemst L, Windisch W, Brodie D, Karagiannidis C. "Low-flow assessment of current ECMO/ECCO2R rotary blood pumps and the potential effect on hemocompatibility." Crit Care. 2019 Nov 6;23(1):348. doi: 10.1186/s13054-019-2622-3. PMID: 31694688; PMCID:	Department of Cardiovascular Engineering, Medical Faculty, Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany. Department of Perfusion, University Hospital Gasthuisberg, Leuven, Belgium. Center for Acute Respiratory Failure, Columbia University College of Physicians and Surgeons/New	The role of blood pumps in contributing to adverse effects at the lower blood flow rates used during ECCO2R is significant. Current rotary blood pumps should be used with caution if operated at blood flow rates below 2 L/min, because of significant and high recirculation, shear stress, and hemolysis.