Statistical Analysis Plan: Prospective, Multicenter, Multidisciplinary, Controlled Clinical Investigation Evaluating the Safety and Efficacy of PerClot® Polysaccharide Hemostatic System

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PerClot® Polysaccharide Hemostatic System

Statistical Analysis Plan
Version 6.0

Clinical Investigation Title: Prospective, Multicenter, Multidisciplinary, Controlled Clinical Investigation Evaluating the Safety and Efficacy of PerClot® Polysaccharide Hemostatic System

The C.L.O.T. Investigation

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Revision History

Version Number	Effective Date	Detail of Changes
1.0	November 24, 2014	Not applicable – new document
2.0	April 27, 2016	Section 1: Remove "Total time to Hemostasis" Add "Hemostasis at 5 and 7 minutes"
2.0	April 27, 2016	Section 1 – Add "The incidence of procedure complications; and
2.0	April 27, 2016	Section 1- remove "procedure complications"
2.0	April 27, 2016	Section 1 – remove "Application to non-target areas"
2.0	April 27, 2016	Section 1 – Delete "Potentiation" Add "Exacerbation"
2.0	April 27, 2016	Section 2 – update investigational site number to "25" and enroll "13 to 40" subjects approximately to each site.
2.0	April 27, 2016	Section 2 – Change to "Follow up to 6 weeks"
2.0	April 27, 2016	Section 2 – Duration of participation for each subject "35 to 49 days".
2.0	April 27, 2016	Section 2 -Update Table 1: Visit Windows – change preoperative timing to 1 month (30 days).
2.0	April 27, 2016	Section 2- Update Table 1: Visit Windows – Update follow up visit timing to 6 weeks, 35 to 49 days postoperatively.
2.0	April 27, 2016	Section 2-Update Table 1: Remove Visit Type - Follow up 3 months.
2.0	April 27, 2016	Section 3: Update double blinded trial to single-blinded trial.
2.0	April 27, 2016	Section 3: Update follow up visit to 6 weeks.
2.0	April 27, 2016	Section 3: Remove "Investigator and study personnel involved with outcome analysis".
2.0	April 27, 2016	Section 3: "so as not to bias the reporting of an adverse events or outcomes".
2.0	April 27, 2016	Sec 4.2.1.1 - Sample size number changed to "154"
2.0	April 27, 2016	Sec 4.2.1.1 – Add the word "approximately"
2.0	April 27, 2016	Sec 4.2.1.1 – Add sentence "See confidence interval at Appendix A"
2.0	April 27, 2016	Sec 4.2.1.1 Add sentence "resulting in a sample size of 162 per arm to ensure >80% power"
2.0	April 27, 2016	Sec 4.2.1.1 Add "assuming an efficacy rate of 90%"
2.0	April 27, 2016	Sec 4.2.1.1 Delete "will be enrolled"
2.0	April 27, 2016	Add Section 4.2.1.2 Blinded Sample Size Re Estimation
2.0	April 27, 2016	Add Appendix A – Sample Size Calculation with PASS
2.0	April 27, 2016	Sec 4.4.4 Safety Analysis – Remove "Application to target area".
2.0	April 27, 2016	Sec 4.4.4 Safety Analysis - Delete "Potentiation" Add "Exacerbation"
2.0	April 27, 2016	Sec 4.6 Covariate Analysis – Add "Complement C3"
2.0	April 27, 2016	Add Appendix B Blinded Sample Size Re estimation Simulation

Version Number	Effective Date	Detail of Changes
2.0	April 27, 2016	Sec 4.7 Modification of alpha to 0.15
2.0	April 27, 2016	Add new References
3.0	June 15, 2016	Sec 4.2.1.2 Add "minimum planned enrollment of 324"
3.0	June 15, 2016	Sec 4.2.1.2 Add "which may be lower or higher than the re-estimated sample size after 20% enrollment"
3.0	June 15, 2016	Sec 4.2.1.2 Add "The loss rate due to technical failures at the interim analysis will be estimated and provided to the DSMB. In making a sample size recommendation, the DSMB may adjust the re-estimated sample size by up to 5% for the loss rate."
3.0	June 15, 2016	Sec 4.2.1.2 Add " Max sample size is at "536(510 with a 5% loss adjustment)"
3.0	June 15, 2016	Sec 4.2.1.2 Modification of Blind Sample Size Re-Estimation
3.0	June 15, 2016	Sec 4.2.1.2 Update Table 1 Title : "Re-estimated Sample Size based on blind efficacy rate before adjusting for loss"
3.0	June 15, 2016	Sec 4.2.1.2 Update Table 1 header "Sample Size" to "Sample Size Re-estimation"
3.0	June 15, 2016	Sec 4.2.1.2 Update Table 1 Sample Size Re-estimation values.
3.0	June 15, 2016	Sec 4.2.1.2 Add "before adjusting for loss"
3.0	June 15, 2016	Sec 4.2.1.2 Update Table 2 Fixed Sample size and Blind Sample Size re-estimation values.
3.0	June 15, 2016	Sec 4.2.1.2 Add "DSMB recommends that….at an interim analysis"
3.0	June 15, 2016	Sec 4.2.1.2 Remove "at the time the study is stopped"
3.0	June 15, 2016	Sec 4.2.1.2 Add "statistical criteria for rejecting the null hypothesis will"
3.0	June 15, 2016	Sec 4.2.1.2 Add "data may not be sufficient ……discussion with the FDA is warranted prior to closure of the trial"
3.0	June 15, 2016	Sec 4.2.1.2 Add "regardless of the observed efficacy and loss rates"
3.0	June 15, 2016	Sec 4.2.1.2 Remove "due to technical failures"
3.0	June 15, 2016	Sec 4.6 Covariate Analysis deleted "Acid Base Status"
3.0	June 15, 2016	Sec 2 Study Subjects Add " For oncology subjects, an additional follow-up will occur at 24 months"
3.0	June 15, 2016	Sec 2 Study Subjects Table 1: Add "Follow-up 24 months 23-25 months (700-760) post-operatively"
3.0	June 15, 2016	Sec 2 Study Subjects Add "*for oncology subjects only"
4.0	July 21, 2016	Sec 4.6 Add "…products known to affect bleeding (including aspirin and colloids)"

Version Number	Effective Date	Detail of Changes
5.0	September 21, 2017	Sec 4.4.2 Add details regarding how Clinical Evaluation Committee image reads will be used to determine primary and secondary endpoint outcomes.
5.0	September 21, 2017	Sec 4.4.3 Add note that adjudicated secondary endpoint outcomes will be determined using the same method as the primary endpoint.
5.0	September 21, 2017	Add Sec 4.9 detailing additional sensitivity analyses planned for primary endpoint.
5.0	September 21, 2017	Correct Revision History table for Version 3.0 effective date, from date of interim draft to date of signature/release
6.0	July 9, 2018	Section 2 – Duration of participation for each subject "28 to 56 days".
6.0	July 9, 2018	Section 2- Update Table 1: Visit Windows – Update follow up visit window for 6 week visit from ±7 days to ±14 days (i.e. 28 to 56 days) postoperatively.
6.0	July 9, 2018	Section 4.4.2 – Change the assessment of the primary endpoint of hemostasis at 7 minutes from being based on the Clinical Evaluation Committee assessment to being based on physician assessment. The Clinical Evaluation Committee assessment will be used as a secondary analysis of this endpoint.
6.0	July 9, 2018	Section 4.4.3 – Add note that the Clinical Evaluation Committee assessment will be used as a secondary analysis of the secondary endpoint of hemostasis at 5 minutes.
6.0	July 9, 2018	Remove Sec 4.9 detailing additional sensitivity analyses planned for primary endpoint since those analyses were planned due to the use of video for primary endpoint assessment.

Signatures

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1 Study Design and Objectives

The device under investigation is PerClot® Polysaccharide Hemostatic System (hereinafter referred to as PerClot). This is a prospective, multicenter, multidisciplinary, controlled clinical investigation evaluating the safety and efficacy of PerClot compared to a similar marketed hemostatic device.

The overall objective of this clinical investigation is to collect clinical data concerning the safety and efficacy of PerClot versus a similar marketed hemostatic device (the control) in multiple surgical disciplines when used as an adjunct to conventional means of achieving hemostasis such as pressure or ligature. Hemostasis is defined as complete cessation of bleeding.

The primary endpoint of this investigation is to demonstrate non-inferiority in the achievement of hemostasis of the treated bleeding site at 7 minutes in subjects receiving PerClot compared to subjects receiving a control hemostatic device. The secondary objective of this investigation is to compare the achievement of hemostasis of the treated bleeding site at 5 minutes in subjects receiving PerClot compared to subjects receiving a control hemostatic device.

The primary endpoint measure is hemostasis of the treated bleeding site at 7 minutes. The secondary efficacy endpoint measure for this investigation is hemostasis of the treated bleeding site at 5 minutes.

Safety data will be summarized by incidence of each adverse event type. Adverse events will be summarized by relatedness, seriousness, and severity. Additional safety endpoints will also consist of:

- Total operative time;
- Hemostasis at 5 and 7 minutes;
- Hemostasis maintenance;
- Total intraoperative estimated blood loss;
- Alternative means required to achieve hemostasis (where applicable);
- Units of blood transfused intraoperatively and postoperatively (where applicable);
- Incidence of reoperation;
- Total hospitalization time;
- The incidence of procedure complications; and
- The incidence of adverse events through final follow-up, including, but not limited to:
    - Adhesions or fibrosis;
    - Anaphylaxis;
    - Blockage of the bladder or ureteral lumen;
    - Blockage of the bypass system;
    - Cancer recurrence and/or progression;
    - Compromised attachment of prosthetic devices to bone or tissue;
    - Death;
    - Embolism;
    - Failure of deep or superficial wound healing;
    - Failure to obtain hemostasis;
    - Fever;

- Hemorrhage;
- Infection;
- Peri-operative hyperglycemia;
- Exacerbation of surgical procedure-associated adverse events;
- Swelling and compression of pressure-sensitive tissues and structures;
- Thromboembolism;
- Thrombosis; and
- Toxicity.

Any procedure complications/adverse events through final follow-up will be reported as per Common Terminology Criteria for Adverse Events (2009): CTCAE-2009.[1]

2 Study Subjects

Three hundred and twenty four subjects across a maximum of 25 investigational sites undergoing open elective cardiac, general, or urological surgical procedures who meet the eligibility criteria will be intraoperatively randomized to receive either PerClot or the control hemostatic device. Subjects will be randomized on a 1:1 basis. Randomization will be stratified by therapeutic area and bleeding severity score within site. Each investigational site is expected to enroll approximately 13-40 subjects. All investigational sites will be located in the U.S. Follow-up will occur at hospital discharge and 6 weeks post-device application.

The expected duration of each subject's participation in this investigation can range from a minimum of 28 to 56 days, based on the visit windows presented in Table 1. For oncology subjects, an additional follow-up will occur at 24 months.

Table 1: Visit Windows for Evaluations

Visit Type	Timing
Preoperative	Within 1 month (30 days) before operative visit
Operative	Day 0
Postoperative	Within 24 hours postoperatively
Follow-Up: Discharge	Within 24 hours of hospital discharge or between 24 hours and 14 days postoperatively
Follow-Up: 6 weeks	28 - 56 days postoperatively
Follow-Up: 24 Months*	23-25 months (700-760 days) postoperatively

*For oncology subjects only.

3 Randomization and Blinding

The randomization schedule will be stratified by site, therapeutic area, and bleeding severity score, and will be generated using random permuted blocks of size two and four. Subjects will be randomized on a 1:1 allocation to either PerClot or the control group. A subject may agree to participate and sign the ICF, but ultimately not be enrolled due to failure to meet the intraoperative eligibility criteria. Only subjects meeting the intraoperative eligibility criteria will be randomized and enrolled.

This will be a single-blinded trial. The subjects will be blinded as to the hemostatic treatment received. Unblinding will be permitted after a subject has completed his or her 6 week follow-up visit evaluations.

4 Statistical Methods

4.1 Study Objectives

The primary objective of this clinical investigation is to demonstrate non-inferiority in the achievement of hemostasis of the treated bleeding site at 7 minutes in subjects receiving PerClot compared to those receiving a control hemostatic device.

The secondary objective of this investigation is to compare the achievement of hemostasis of the treated bleeding site evaluated at 5 minutes for subjects receiving PerClot compared to those receiving a control hemostatic device.

4.2 Study Hypotheses

4.2.1 Primary Efficacy

The primary efficacy hypothesis for this clinical investigation is that the proportion of subjects achieving hemostasis of the treated bleeding site at 7 minutes in the PerClot group is non-inferior to the proportion of subjects achieving hemostasis at 7 minutes in the control group.

H_0: $P_{PerClot} < P_{Control} - \delta$
H_a: $P_{PerClot} \geq P_{Control} - \delta$

where $P_{PerClot}$ is the proportion of PerClot subjects achieving hemostasis at 7 minutes, $P_{Control}$ is the proportion of control subjects achieving hemostasis of the treated bleeding site at 7 minutes, and δ is the non-inferiority margin, with $\delta = 0.10$.

In other words, the null hypothesis is that the proportion of PerClot subjects achieving hemostasis of the treated bleeding site at 7 minutes will be inferior to the proportion of control subjects achieving hemostasis of the treated bleeding site at 7 minutes. The alternative hypothesis is that the proportion of PerClot subjects achieving hemostasis of the treated bleeding site at 7 minutes will be equal to or greater than, or not inferior to, the proportion of control subjects achieving hemostasis of the treated bleeding site at 7 minutes. The null hypothesis will be rejected in favor of the alternative hypothesis if the upper bound of the 97.5% one-sided confidence interval for the difference in treatment proportions ($P_{Control}$-$P_{PerClot}$) is less than 0.10.

4.2.1.1 Sample Size Justification

The sample size was calculated based on the following assumptions:

- One-sided alpha = 0.025;
- Minimum 80% power;
- Non-inferiority margin $\delta = 0.10$;
- Expected 7 minute hemostatic success rate in the PerClot group is 90%; and
- Expected 7 minute hemostatic success rate in the control group is 90%.
- Randomization allocation of 1:1

A sample size of 154 subjects in each group will provide approximately 80% power to reject the hypothesis of the inferiority of PerClot to the composite control by a 10% margin, using a one-sided 97.5% confidence interval (see Appendix A). As the primary endpoint data is collected intraoperatively, no adjustment is made for attrition; however, the sample size was increased to account for the possibility of up to 5% loss within either group due to technical failures (i.e., contraindications, misdiagnosis, or intraoperative death prior to treatment, etc.), and subjects not treated according to randomization assignment, resulting in a sample size of 162 per arm to ensure >80% power. Thus, a total of 324 subjects is the planned sample size to enroll, assuming an efficacy rate of 90%.

A non-inferiority margin of 10% is consistent with the margins used in other controlled clinical trials involving hemostatic agents.[2, 3] A non-inferiority margin $\delta = 0.10$ was chosen for this study because excluding a 10% difference between the treatments would be supportive of non-inferior performance of PerClot compared to the control.

4.2.1.2 Blinded Sample Size Re-estimation

In order to protect against lower than planned power due to misspecification of the assumed success rates, blinded sample size re-estimation (BSSR) will be performed[4]. The initial sample size calculation will be repeated based on the observed pooled efficacy rate (i.e. the proportion of all subjects, regardless of randomization assignment, with 7 minute hemostatic success) rather than the initially assumed rate of 90%.

The re-estimation will be performed using the pooled efficacy results of the first 20% and 50% of the minimum planned enrollment of 324 (i.e. 64 and 162 subjects). The re-estimated sample size after 20% enrollment will provide an initial re-calibration of the sample size. The re-estimated sample size after 50% enrollment will determine the final sample size, which may

be lower or higher than the re-estimated sample size after 20% enrollment.

The sample size calculation will result in an increase in sample size if the observed efficacy rate is less than 90%. The loss rate at the interim analysis will be estimated and provided to the DSMB. In making a sample size recommendation, the DSMB may adjust the re-estimated sample size by up to 5% for the loss rate. The minimum sample size for the trial, regardless of the observed efficacy and loss rates, is the initially planned sample size of 324. A maximum sample size is set at 536 (510 with a 5% loss adjustment) for any observed efficacy rate ≤ 80%. The table below provides examples of the revised sample size before adjusting for loss based on hypothetical results.

Table 2: Re-estimated sample size based on blinded efficacy rate before adjusting for loss

Blinded Efficacy Rate	Re-estimated Sample Size
90%	308
89%	332
88%	352
87%	374
86%	394
85%	414
84%	434
83%	454
82%	474
81%	492
80%	510

Because the sample size re-estimation is blinded and based only on a nuisance parameter, there is no impact on the Type I error rate. Larger sample sizes are required due solely to greater variability in the binomial outcome when the equal PerClot and control efficacy rates are closer to 50%, at which point variability is at a maximum.

The Type I error, average sample size before adjusting for loss, and power were evaluated through a statistical simulation where binomial data was generated under several alternative hypotheses where the efficacy rate is equal between the two arms, but the efficacy rate is varied from 70% through 90%. The simulation results in the table below demonstrate that the Type I error is not inflated when the sample size is increased based on the pooled efficacy estimate and that power is maintained at 80% across all scenarios. The R code for the simulation is provided in Appendix B.

Table 3: Fixed versus BSSR Power and Sample Size Under Various Alternative Hypotheses

	Fixed Sample Size		Blinded Sample Size Reestimation (after 20% and 50% of subjects)	
Alternative hypotheses (equal success rates)	**Sample Size (total after 5% loss)**	**Simulated Power**	**Final Sample Size (mean total after 5% loss)**	**Simulated Power**
90%	308	81.1%	328	83.9%
85%	308	68.2%	414	80.1%
80%	308	59.0%	486	78.6%
75%	308	52.8%	508	73.9%
70%	308	48.3%	510	69.2%
Null hypothesis	**Sample Size (total after 5% loss)**	**Type I Error**	**Final Sample Size (mean total after 5% loss)**	**Type I Error**
80% (PerClot) vs 90% (Control)	308	2.3%	414	2.4%

If the observed efficacy rate is ≤ 60% when the blinded sample size re-estimation is performed at 20% of enrollments or ≤ 64% when the blinded sample size re-estimation is performed at 50% of enrollments, the DSMB will evaluate the overall study data and make a recommendation regarding whether to stop the study prematurely due to an unacceptable efficacy rate. The boundaries of 60% and 64% were selected based on those observed rates have <5% chance of being observed if the population efficacy rate is 70% or higher.

In the event that the DSMB recommends that the study be stopped due to low efficacy at an interim analysis, the two study arms will be compared based on the available data in the As Treated population. There is the possibility that a low pooled success rate is due primarily to one of the two study arms. If the PerClot success rate is sufficiently higher than the control success rate that the non-inferiority Z-value would cross a group sequential boundary based on a Lan-DeMets alpha-spending function with the information fraction based on the planned sample size of 324 (boundaries are Z=4.42 at 20% and Z=2.80 at 50%), then the statistical criteria for rejecting the null hypothesis will be met based on the available data. However, as the data may not be sufficient to adequately assess the overall performance of PerClot in this scenario, discussion with FDA is warranted prior to closure of the trial.

4.2.2 Secondary Efficacy

The secondary efficacy objective will be evaluated at 5 minutes in a manner similar to the primary efficacy analysis. The secondary hypothesis for this clinical investigation is that the proportion of subjects achieving hemostasis of the treated bleeding site at 5 minutes in the PerClot group is non-inferior to the proportion of subjects achieving hemostasis of the treated bleeding site at each evaluation time point in the control group.

H_0: $P_{t,PerClot} < P_{t,Control} - \delta$
H_a: $P_{t,PerClot} \geq P_{t,Control} - \delta$

where $P_{PerClot}$ is the proportion of PerClot subjects achieving hemostasis of the treated bleeding site at time point *t*, $P_{t,Control}$ is the proportion of control subjects achieving hemostasis of the treated bleeding site at time point *t*, and δ is the non-inferiority margin, with $\delta = 0.10$.

In other words, the null hypotheses are that the proportion of PerClot subjects achieving hemostasis of the treated bleeding site at each evaluation time point will be inferior to the proportion of control subjects achieving hemostasis of the treated bleeding site at each evaluation time point. The alternative hypotheses are that the proportion of PerClot subjects achieving hemostasis of the treated bleeding site at each evaluation time point will be equal to or greater than, or not inferior to, the proportion of control subjects achieving hemostasis of the treated bleeding site at each evaluation time point. Each null hypothesis will be rejected in favor of the corresponding alternative hypothesis if the upper bound of the 97.5% one-sided confidence interval for the difference in treatment proportions ($P_{t,Control}$-$P_{t,PerClot}$) is less than 0.10.

4.2.3 Trial Success Criteria

Trial success will be the rejection of the primary efficacy null hypothesis, with the upper bound of the 97.5% one-sided confidence interval for the difference in proportions of subjects achieving hemostasis at 7 minutes between treatment groups ($P_{Control}$-$P_{PerClot}$) being less than 0.10, in the As Treated population.

4.3 Analysis Populations

Analysis populations are defined as follows:

- The Intent-to-Treat (ITT) population includes all randomized subjects, regardless of treatment received;
- The As Treated (AT) population includes all subjects who were randomized and treated with either PerClot or Arista, with subjects assigned to the treatment group for the treatment received (even if this differs from randomization assignment); and

- The Per Protocol (PP) population includes all subjects who were randomized and treated with either PerClot or Arista, and had no major protocol deviations, where major protocol deviations are defined as:
    - Failure to meet any preoperative inclusion/exclusion criteria;
    - Failure to meet any intraoperative inclusion/exclusion criteria; or
    - Any informed consent violation.

All efficacy analyses and summary statistics will be performed on the ITT, AT, and PP populations. Safety analyses will be performed on the ITT population.

Trial success will be evaluated on the AT population. Effectiveness analyses of the ITT and PP populations will be supportive of the AT analysis. Any clinically significant differences in effectiveness results between the analysis populations will be examined relative to protocol deviations and discussed.

4.4 Analysis Methods

4.4.1 General Statistical Considerations

Demographics, baseline characteristics, and the comparability of the PerClot and control group subjects will be assessed by tabulations of mean, median, range, standard deviation and analyses using t-tests of means or Wilcoxon tests for continuous factors. An appropriate test will be chosen by first testing for normality of two samples with Shapiro-Wilk test at alpha = 0.1. If normality is rejected for either sample, then the Wilcoxon test will be conducted. If normality is not rejected for either sample, then variances of two samples will be tested at alpha=0.1. If the equality of variance is rejected, then the t-test with Satterthwaite approximation will be conducted. If the equality of variance is not rejected, then a t-test will be conducted. For categorical factors, frequency, percent and Fisher's exact tests of proportions will be carried out. Achievement of hemostasis for all treated bleeding sites will be assessed by tabulating frequency of success as a percentage and analyses using Chi-square test.

4.4.2 Primary Efficacy

Primary efficacy will be measured by the proportion of subjects achieving hemostasis of the treated bleeding site at 7 minutes. The hemostasis status at 7 minutes will be used to define this endpoint. If a subject achieves hemostasis at an earlier time assessment (i.e., 5 minutes), but begins to re-bleed and the bleeding has not ceased by the 7 minute assessment, the subject will not be counted as achieving hemostasis at 7 minutes. The proportion of subjects achieving hemostasis will be summarized by treatment group. The difference between groups will be reported, along with the 95% confidence interval of the difference. Non-inferiority will be evaluated by comparing the upper bound of the confidence interval for the difference in treatment proportions ($P_{Control}$-$P_{PerClot}$) to the non-inferiority margin of 0.10. If the upper bound is found to be less than 0.1, the null hypothesis will be rejected and treatment will be considered non-inferior to control.

This analysis will be performed on the AT, ITT, and PP populations. The AT population will serve as the primary analysis population for the evaluation of the primary efficacy endpoint. Analyses based on the ITT and PP populations will be presented as supportive analyses. Additionally, the number of subjects in whom hemostasis is achieved at 7 minutes, but have a re-bleed during the 5 minute observation period following the 7 minute assessment will be summarized. A sensitivity analysis of the endpoint will be conducted where all subjects that achieved hemostasis at 7 minutes, but did not maintain it for the 5 minutes following the assessment or any time after the observation period, will be counted as 'failures' in the analysis.

Of note is that only subjects who have achieved hemostasis at 7 minutes will be observed for the additional 5 minutes. In cases where the wound has not achieved hemostasis at 7 minutes, it would be considered a failure and the surgeon is instructed to treat using alternate methods. Likewise, if the subject achieved hemostasis at 7 minutes and there is a breakthrough or re-bleeding at any point between the 7 and 12 minutes of assessment or any time after the observation period, the surgeon will immediately treat with alternate methods.

Success of the primary and secondary endpoints is determined by the treating physician's assessment. A supplementary secondary analysis of hemostasis endpoints will be performed based on the overall majority rule CEC assessment of hemostasis. The use of the CEC adjudications for analysis applies to all randomizations under protocol version 11 amendment 4 through version 11 amendment 7, as video capture was added to the protocol under version 11 amendment 4 and version 11 amendment 7 was the last amendment to require video capture. This analysis, which is based on CEC assessment of hemostasis rather than treating physician assessment, will exclude subjects randomized under a version of the protocol that does not require capture of video.

The CEC members assess a video of the bleeding region captured during the procedure. If the first two readers to assess a video agree about the outcome, a third reader is not required. A majority of two out of three members in agreement with each other is required to determine the overall CEC assessment. The table below indicates how the reader assessments are used to determine the overall CEC assessment of 7 and 5 minute hemostasis by a majority vote.

Table 4: Clinical Evaluation Committee Individual Assessments and Overall Endpoint Outcome

Clinical Evaluation Committee Individual Assessments			Overall CEC Outcome
Hemostasis	Hemostasis	3rd reader not required	Hemostasis
No Hemostasis	No Hemostasis	3rd reader not required	No Hemostasis

Not Evaluable	Not Evaluable	3rd reader not required	Indeterminate/missing
Hemostasis	No Hemostasis	Hemostasis	Hemostasis
Hemostasis	Not Evaluable	Hemostasis	Hemostasis
Hemostasis	No Hemostasis	No Hemostasis	No Hemostasis
No Hemostasis	Not Evaluable	No Hemostasis	No Hemostasis
Hemostasis	No Hemostasis	Not Evaluable	Indeterminate/missing
Hemostasis	Not Evaluable	Not Evaluable	Indeterminate/missing
No Hemostasis	Not Evaluable	Not Evaluable	Indeterminate/missing

4.4.3 Secondary Efficacy

Secondary efficacy will be measured by the proportion of subjects achieving hemostasis of the treated bleeding site at 5 minutes. The hemostasis status at 5 minutes will be used to define this endpoint. The proportion of subjects achieving hemostasis will be summarized by treatment group. The difference between groups will be reported, along with the 95% confidence interval of the difference. Non-inferiority will be evaluated by comparing the upper bound of the confidence interval for the difference in treatment proportions ($P_{Control}$-$P_{PerClot}$) to the non-inferiority margin of 0.10. If the upper bound is found to be less than 0.1, the null hypothesis will be rejected and treatment will be considered non-inferior to control.

This analysis will be performed on the AT, ITT, and PP populations. The AT population will serve as the primary analysis population for the evaluation of the secondary efficacy endpoint. Analyses based on the ITT and PP populations will be presented as supportive analyses. Additionally, the number of subjects in whom hemostasis is achieved at 5 minutes, but has a re-bleed after the 5 minute assessment will be summarized. A sensitivity analysis of the endpoint will be conducted where all subjects that achieved hemostasis at 5 minutes, but did not maintain it after the assessment will be counted as 'failures' in the analysis. The use of Clinical Evaluation Committee adjudications for this endpoint will be the same as for the primary efficacy endpoint, to provide a supplementary secondary analysis of this secondary endpoint.

4.4.4 Safety Analyses

Safety data will be summarized by incidence of each adverse event type along with 95% exact confidence intervals. Adverse event rates will be reported as the proportion of subjects who experience an event and the total number of each event type occurring.

Adverse events will be summarized by relatedness, seriousness, and severity. The comparison of safety between treatment arms will be based on Fisher's exact tests for the proportion of subjects with serious device-related adverse events, unanticipated adverse device effects, and frequent adverse events (≥ 5% overall incidence) in the two treatment groups.

The other following safety endpoints will also be summarized using descriptive statistics:

- Total operative time;
- Hemostasis at 5 to 7 minutes;
- Hemostasis maintenance;
- Total intraoperative estimated blood loss;
- Alternative means required to achieve hemostasis (where applicable);
- Units of blood transfused intraoperatively and postoperatively (where applicable);
- Incidence of reoperation;
- Total hospitalization time;
- Incidence of procedure complications;
- Incidence of adverse events through final follow-up, including, but not limited to:
    - Adhesions or fibrosis;
    - Anaphylaxis;
    - Blockage of the bladder or ureteral lumen;
    - Blockage of the bypass system;
    - Cancer recurrence and/or progression;
    - Compromised attachment of prosthetic devices to bone or tissue;
    - Death;
    - Embolism;
    - Failure of deep or superficial wound healing;
    - Failure to obtain hemostasis;
    - Fever;
    - Hemorrhage;
    - Infection;
    - Peri-operative hyperglycemia;
    - Exacerbation of surgical procedure-associated adverse events;
    - Swelling and compression of pressure-sensitive tissues and structures;
    - Thromboembolism;
    - Thrombosis; and
    - Toxicity.

Safety analyses are descriptive in nature. There are no formal hypothesis tests associated with these outcomes. All safety analyses will be performed on the ITT population.

4.5 Subgroup Analysis

Analyses will be conducted to determine the consistency of study results across the following subgroups: gender, race, and bleeding severity score. These analyses will be descriptive in nature, and as such there are no formal hypothesis tests. There are no labeling claims being sought out for these subgroups analyses.

Subgroup analyses will be performed on the primary efficacy measure (proportion achieving hemostasis at 7 minutes), secondary efficacy measure (proportion achieving hemostasis at 5 minutes), and safety measures (proportion with device related events and serious adverse events). For efficacy measures, analyses will be performed on the AT population. Safety subgroup analyses will be based on the ITT population.

For each endpoint measure the proportion of subjects will be reported by subgroup. The difference in proportions and its associated 95% confidence interval will be reported by subgroup. To test for a differential treatment effect across subgroups a logistic regression model will be fit, testing for the interaction of treatment group by subgroup. In the case that poolability across a subgroup is questionable, as indicated by statistical significance of the interaction effect at alpha ≤ 0.05, the estimated treatment difference and 95% confidence interval from the subgroup adjusted logistic regression model will be reported.

4.6 Covariate Analysis

Covariate analyses will be performed to assess the association with certain study parameters on the primary efficacy endpoint. These models will be fit using logistic regression, measuring the odds of primary efficacy success. All models will include treatment group as a covariate. Covariates found to be significant at a 0.1 alpha level in the univariate assessment will be included in a multivariable model. After the multivariable model is fit, covariates will remain in the final multivariable model if they maintain significance at a 0.1 alpha level. For both univariate and multivariate models, the model derived estimates (i.e. beta estimates) for the association with primary efficacy success will be displayed, along with the Type III p-values. An adjusted estimate of the difference between proportions and associated 95% confidence interval (treatment versus control) will be obtained from the multivariable model.

Covariate assessments may consist of, but are not limited to, the following variables:

- Demographic measures such as age, gender, ethnicity, and BMI;
- Medical history;
- Therapeutic area;

- Use of blood modifiers or products known to affect bleeding (including aspirin and colloids);;
- Administration of blood products;
- Fluids administered (including colloids and crystalloids);
- Surface area of the anatomic site;
- Initial bleeding severity (as continuous measure);
- Indication for surgery;
- Complete blood count and coagulation status;
- Complement C3;
- Smoking history;
- Use of oral contraceptives;
- Diabetes;
- Presence of any malignancies;
- Amount of hemostat applied;
- Core body temperature;
- Level of glycosylated hemoglobin;
- Other hemostatic efforts made;
- Procedure type;
- NNIS risk index;
- Blood glucose at randomization;
- Blood glucose within 1 hour of device application;
- American Society of Anesthesiologists Classification;
- Total operative time;
- Placement of wound drain;
- Drainage volume within 24 hours postoperatively;
- Total drainage volume;
- Total drainage duration; and
- Volume of fluid used to rinse away excess study device.

In the event that a covariate is significant at the 0.05 alpha level in the univariate assessment, an additional model will be fit to test for an interaction effect of treatment and that covariate.

4.7 Poolability Analysis

It is expected that data will be poolable across trial sites, as all sites and Investigators will follow a common protocol with identical inclusion/exclusion criteria and defined objective efficacy parameters.

Treatment effect will be evaluated by the proportion of subjects achieving hemostasis at 7 minutes by site. Trial site will be tested as a covariate in a logistic regression model, with the primary endpoint measure as the outcome. An additional logistic regression model will be fit to test for a Treatment by site interaction effect. In the case that poolability across site is confirmed, as indicated by statistical significance at alpha > 0.15, the assumption of homogeneity will be accepted. In the case that poolability across site is questionable, as indicated by statistical significance at alpha ≤ 0.15, the assumption of homogeneity will be rejected. An additional primary

efficacy analysis will be fit in order to ascertain the treatment effect after adjustment for site.

Additionally, it is expected that data will be poolable across therapeutic areas. Potential variability in time to hemostasis between therapeutic areas is mitigated by limiting the types of procedures and bleeding severities for which subjects can be enrolled into the investigation.

The proportion of subjects achieving hemostasis at 7 minutes will be summarized by therapeutic area. The difference in proportions and its associated 95% confidence interval will also be reported by therapeutic area. Therapeutic area will be tested as a covariate in a logistic regression model, with the primary endpoint measure as the outcome. To test for a differential treatment effect across therapeutic area a logistic regression model will be fit, testing for the interaction of treatment group by therapeutic area. In the case that poolability across a therapeutic area is questionable, as indicated by statistical significance of the interaction effect at alpha ≤ 0.15, the estimated treatment difference and 95% confidence interval from the subgroup adjusted logistic regression model will be reported.

The reasons for any differences in outcome across site and/or therapeutic area will be investigated and reported. Poolability analyses will be conducted on the AT population.

4.8 Missing Data

Every effort will be made to collect all data points in the study. The amount of missing data will be minimized by appropriate management of the clinical study, training of participating investigators and study coordinators, and sufficient monitoring of study sites. As the primary efficacy endpoint data are collected intraoperatively, missing data is expected to be minimal, and subject withdrawal and loss to follow-up during the follow-up period is not expected to impact the efficacy analysis.

To assess the impact of missing data on the primary endpoint, a sensitivity analysis will be conducted. The primary endpoint analysis will be repeated under two scenarios: one in which all missing endpoints are assumed to be successes for the PerClot group and failures for the control group, and another in which all missing endpoints are assumed to be failures for the PerClot group and successes for the control group. If these scenarios do not impact the conclusion of the analysis, then the impact of missing data will be considered inconsequential. The reason for any significant differences between scenarios will be investigated and reported.

5 Analysis Software

All analyses will be performed using SAS statistical software (SAS Institute; Cary, NC) or a comparable statistical software.

Appendix A – Sample Size Calculation with PASS

PASS sample size calculation software (version 13) was used to calculate the power for the primary endpoint (shown below).

Analysis of Non-Inferiority Tests of Two Independent Proportions

Numeric Results for Non-Inferiority Tests Based on the Difference: P1 - P2
H0: P1 - P2 ≤ D0. H1: P1 - P2 = D1 > D0. Test Statistic: Score test (Farrington & Manning)

Diff Power	Diff N1	Target N2	Actual N	Ref. P2	P1|H0 P1.0	P1|H1 P1.1	NI D0	D1	Alp ha
	Alpha								
0.79885	154	154	308	0.9000	0.8000	0.9000	-		
0.1000	0.0000	0.0250							

Note: Direct Binomial distribution calculations for alpha and power were only used when both N1 and N2 were less
than 100. In all other cases, Normal approximation was used.

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Report Definitions

Power is the probability of rejecting a false null hypothesis.
N1 and N2 are the number of items sampled from each population.
N is the total sample size, N1 + N2.
P2 is the proportion for Group 2. This is the standard, reference, or control group.
P1 is the treatment or experimental group proportion. P1.0 is the smallest treatment-group response rate that still yields a non-inferiority conclusion. P1.1 is the proportion for Group 1 at which power and

sample
size calculations are made.
D0 is the non-inferiority margin. It is the difference P1 - P2, assuming H0. D1 is the difference P1 - P2
assumed for power and sample size calcualtions.
Target Alpha is the input probability of rejecting a true null hypothesis. Actual Alpha is the value of alpha
that is actually achieved.

Summary Statements
Sample sizes of 154 in group one and 154 in group two achieve 80% power to detect a non-inferiority margin difference between the group proportions of -0.1000. The reference group proportion is 0.9000. The treatment group proportion is assumed to be 0.8000 under the null hypothesis of inferiority. The power was computed for the case when the actual treatment group proportion is 0.9000. The test statistic used is the one-sided Score test (Farrington & Manning). The significance level of the test was targeted at 0.0250. The significance level actually achieved by this design is NA.

Appendix B – Blinded Sample Size Re-estimation Simulation

```
library(gsDesign) # for nBinomial Farrington-Manning sample size

set.seed(843112)

ssFMnoninf <- function(pA=0.9, pB=0.9, delta=-0.10, alpha=0.025, beta=0.20)
{
        out <- ceiling(nBinomial(p1=pA, p2=pB, alpha=alpha, beta=beta, delta=delta,
ratio=1, sided=1)/2)
}

# Re-estimated Sample Size Table
# original sample size of 154 per arm based on approximately 80% power
# efficacy rate of 0.901 used to match sample size of 154 per arm after 5% loss
effrates <- c(0.901, 0.89, 0.88, 0.87, 0.86, 0.85, 0.84, 0.83, 0.82, 0.81, 0.80)
cbind(round(effrates, 2), sapply(effrates, function(x) { 2*ssFMnoninf(pA=x, pB=x)}))

ssFMnoninfeq <- function(pooled.estimate, tol=10**-3)
{
 # If any estimates are 0 or 1 then replace with something very close
 pooled.estimate[pooled.estimate == 0] <- tol
 pooled.estimate[pooled.estimate == 1] <- (1-tol)

 pooled.estimate.unique <- unique(pooled.estimate)

 sample.sizes.unique <- ssFMnoninf(pA=pooled.estimate.unique,
pB=pooled.estimate.unique)
 sample.sizes <- rep(NA, length(pooled.estimate))

 for (i in 1:length(pooled.estimate.unique)) {
  sample.sizes[pooled.estimate == pooled.estimate.unique[i]] <- sample.sizes.unique[i]
 }

 return(sample.sizes)
}

binarynoninfsim <- function(n.sim=10**5,
                    n.init=154, n.interim1max=154, n.interim2max=255,
                    prop.interim1=0.20, prop.interim2=0.5,
                    pA=0.9, pB=0.9, margin=-0.1,
                    alphaBSS=0.025, alphaFIX=0.025,
                    tol=10**-3)
{

# n.init - initial planned sample size
# n.interim1max - maximum final sample size allowed at interim 1 re-estimation
# (setting n.interim1max to n.init results in 2nd interim at n.init*prop.interim2)
# n.interim2max - maximum final sample size allowed at interim 2 re-estimation
```

```
# Correct sample size if population proportions were known
correct.ss <- ssFMnoninf(pA=pA, pB=pB)

# n.interim1 is the expected effective sample size at the 1st interim, after 5% loss
n.interim1 <- round(n.init * prop.interim1)

a.interim1 <- rbinom(n=n.sim, size=n.interim1, prob=pA)
b.interim1 <- rbinom(n=n.sim, size=n.interim1, prob=pB)

pooled.estimate.interim1 <- (a.interim1 + b.interim1) / (2*n.interim1)
sample.sizes.interim1 <- ssFMnoninfeq(pooled.estimate.interim1, tol)
n.total.interim1 <- pmin(n.interim1max, pmax(n.init, sample.sizes.interim1))

# Metrics to assess performance of interim sample size re-assessment
prob.ss.10pct.interim1 <- sum(abs(n.total.interim1 - correct.ss)/correct.ss < 0.1)/n.sim
prob.ss.5pct.interim1 <- sum(abs(n.total.interim1 - correct.ss)/correct.ss < 0.05)/n.sim

# n.interim2 is the expected effective sample size at the 2nd interim, after 5% loss
n.interim2 <- round(n.total.interim1 * prop.interim2)

# set n.interim1max = n.init if
#   2nd interim analysis is at 50% of n.init regardless of n.total.interim1
#   and n.total.interim2 takes precedence over n.total.interim1
# code to handle if 2nd interim is at a larger n than n.init (only possible if n.interim1max
> n.init)
n.tofix <- rep(NA, n.sim)
a.fix <- rep(NA, n.sim)
b.fix <- rep(NA, n.sim)
n.fix <- rep(NA, n.sim)
n.tointerim2 <- rep(NA, n.sim)
a.interim2 <- rep(NA, n.sim)
b.interim2 <- rep(NA, n.sim)

interim2GEinit <- (n.interim2 >= n.init)
n.interim2GEinit <- sum(interim2GEinit)
n.interim2LTinit <- sum(!interim2GEinit)

# when interim2GEinit
  # simulate up to n.init...
  # Fixed sample size analysis - done here if occurs before reaching n.interim2
  n.tofix[interim2GEinit] <- n.init - n.interim1
  a.fix[interim2GEinit] <- a.interim1[interim2GEinit] + rbinom(n=n.interim2GEinit,
size=n.tofix[interim2GEinit], prob=pA)
  b.fix[interim2GEinit] <- b.interim1[interim2GEinit] + rbinom(n=n.interim2GEinit,
size=n.tofix[interim2GEinit], prob=pB)
  n.fix[interim2GEinit] <- n.interim1 + n.tofix[interim2GEinit]
  # ...and then the rest up to n.tointerim2
  n.tointerim2[interim2GEinit] <- n.interim2[interim2GEinit] - n.init
  a.interim2[interim2GEinit] <- a.fix[interim2GEinit] + rbinom(n=n.interim2GEinit,
size=n.tointerim2[interim2GEinit], prob=pA)
```

```
  b.interim2[interim2GEinit] <- b.fix[interim2GEinit] + rbinom(n=n.interim2GEinit,
size=n.tointerim2[interim2GEinit], prob=pB)

# when !interim2GEinit
  # or simulate all n.tointerim2 at once
  n.tointerim2[!interim2GEinit] <- n.interim2[!interim2GEinit] - n.interim1
  a.interim2[!interim2GEinit] <- a.interim1[!interim2GEinit] +
rbinom(n=n.interim2LTinit, size=n.tointerim2[!interim2GEinit], prob=pA)
  b.interim2[!interim2GEinit] <- b.interim1[!interim2GEinit] +
rbinom(n=n.interim2LTinit, size=n.tointerim2[!interim2GEinit], prob=pB)

pooled.estimate.interim2 <- (a.interim2 + b.interim2) / (2*n.interim2)
sample.sizes.interim2 <- ssFMnoninfeq(pooled.estimate.interim2, tol)
n.total.interim2 <- pmin(n.interim2max, pmax(n.total.interim1, sample.sizes.interim2))

prob.ss.10pct.interim2 <- sum(abs(n.total.interim2 - correct.ss)/correct.ss < 0.1)/n.sim
prob.ss.5pct.interim2 <- sum(abs(n.total.interim2 - correct.ss)/correct.ss < 0.05)/n.sim

n.tofinal <- n.total.interim2 - n.interim2

a.final <- a.interim2 + rbinom(n=n.sim, size=n.tofinal, prob=pA)
b.final <- b.interim2 + rbinom(n=n.sim, size=n.tofinal, prob=pB)

n.final <- n.total.interim2

pvals <- 1-pnorm(testBinomial(a.final, b.final, n.final, n.final, delta0=-0.1)) # one-sided
by default

n.tofix[!interim2GEinit] <- n.init - n.interim2[!interim2GEinit]
a.fix[!interim2GEinit] <- a.interim2[!interim2GEinit] + rbinom(n=n.interim2LTinit,
size=n.tofix[!interim2GEinit], prob=pA)
b.fix[!interim2GEinit] <- b.interim2[!interim2GEinit] + rbinom(n=n.interim2LTinit,
size=n.tofix[!interim2GEinit], prob=pB)
n.fix[!interim2GEinit] <- n.interim2[!interim2GEinit] + n.tofix[!interim2GEinit]

pvalsfix <- 1-pnorm(testBinomial(a.fix, b.fix, n.fix, n.fix, delta0=-0.1)) # one-sided by
default

return(list(n.interim1=n.interim1, n.interim2.mean=mean(n.interim2),
          fix.power=sum(pvalsfix<alphaFIX)/n.sim,
          bssr.power=sum(pvals<alphaBSS)/n.sim,
          ss.mean=mean(n.final), ss.sd=sd(n.final),
          ss.min=min(n.final), ss.max=max(n.final),
          ss.probmax=sum(n.final==n.interim2max)/n.sim,
          ss.wi10pct=prob.ss.10pct.interim2, ss.wi5pct=prob.ss.5pct.interim2))

}

# Type 1 Error
binarynoninfsim(n.sim=10**5, n.init=154, n.interim1max=154, n.interim2max=255,
              prop.interim1=0.2, prop.interim2=0.5,
```

```
pA=0.8-10**-3, pB=0.9, margin=-0.1, alphaBSS=0.025, alphaFIX=0.025)


effrates <- c(0.901, 0.85, 0.80, 0.75, 0.70)
interim.n.equal <- sapply(effrates, function(a) { binarynoninfsim(n.sim=10**5,
n.init=154, n.interim1max=154, n.interim2max=255,
                                                    prop.interim1=0.2, prop.interim2=0.5,
                                                    pA=a, pB=a, margin=-0.1,
alphaBSS=0.025, alphaFIX=0.025) })
interim.n.equal

# Table of Operating Characteristics
oc <- cbind(round(effrates,2), t(interim.n.equal[c(3,5,4),]))
for(i in 1:nrow(oc)) { oc[[i,2]] <- round(oc[[i,2]], 3) }
for(i in 1:nrow(oc)) { oc[[i,3]] <- round(oc[[i,3]])*2 }
for(i in 1:nrow(oc)) { oc[[i,4]] <- round(oc[[i,4]], 3) }
oc

# Thresholds for low pooled efficacy rate
pstop <- function(threshold, size, prob) { pbinom(floor(threshold*size), p=prob,
size=size) }

pstop64 <- sapply(seq(0.9, 0.7, by=-0.05), function(x) { pstop(threshold=0.60, size=64,
prob=x)})
round(100*pstop64, 5)
pstop162 <- sapply(seq(0.9, 0.7, by=-0.05), function(x) { pstop(threshold=0.64,
size=162, prob=x)})
round(100*pstop162, 5)
```

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