Medtronic

Statistical Analysis Plan

Clinical Investigation Plan Title	FIRE AND ICE – Re-Ablations (retrospective data collection)
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1.   Version History

Version	Summary of Changes	Author(s)/Title
1.0	Not Applicable, New Document	Christopher Anderson, Project Statistician

2.   List of Abbreviations and Definitions of Terms

Abbreviation	Definition
AF	Atrial Fibrillation
AVNRT	Atrioventricular Node Reentrant Tachycardia
Blanking period	The 90-day period after the index ablation
CFAE	Complex fractionated atrial electrogram
CIP	Clinical investigation Protocol
CTA	Clinical Trial Agreement
CTI	Cavotricuspid Isthmus
DMP	Data Management Plan
DRF	Data Release Form
EA	Electroanatomical
ERC	Endpoint Review Committee
LA	Left Atrium
MCAR	Missing Completely At Random
PV	Pulmonary Vein
PVI	Pulmonary Vein Isolation
RA	Right Atrium
RF	Radio Frequency
SAP	Statistical Analysis Plan
SD	Standard Deviation

3.   Introduction

The purpose of the Statistical Analysis Plan (SAP) for the Fire and Ice Re-Ablations retrospective data collection is to provide pre-analysis documentation and rationale for the statistical procedures that will be employed in the planned analyses that are performed throughout this study. Specifically, this plan outlines methods used in the study's final report, as well as in any planned publications. It does not limit the analysis that will be completed, as further analysis beyond what is specified in this document is likely.

This SAP was developed based on version 1 of the Fire and Ice Re-Ablations Clinical Investigation Protocol (referred to as the CIP in this SAP), dated 20-SEP-2017. It, along with the study's Data Management Plan (DMP) defines the procedures for data review, database cleaning and issuing and resolving data queries as well as the procedures for verification, validation and securing of electronic clinical data systems.

The purpose of the retrospective data collection done for the Fire and Ice Re-Ablations is to gain insight into re-occurrence of atrial arrhythmias resulting in a re-ablation after a performed index ablation procedure. Data to be collected from subject charts includes pulmonary vein anatomy, documented atrial arrhythmias prior tore-ablation, pulmonary vein reconnection, pulmonary vein ablation lesion gaps and gap location, ablation lesions performed during the re-ablation, procedure parameters of the Radio Frequency (RF) or cryo ablation catheter utilized during the re-ablation, and success of re-ablations performed. The resulting data may provide insight into the possible causes for re-occurrence of atrial arrhythmias requiring re-ablation.

4.   Study Objectives

Objective: Atrial arrhythmias prior to re-ablation
Summarize the documented atrial arrhythmias prior to re-ablation

Endpoint: Each documented atrial arrhythmia will be classified into one of the following categories:
- Atrial Fibrillation (AF)
    - Paroxysmal
    - Persistent
- Atrial Tachycardia
- Atrial Flutter
    - Typical
    - Atypical

Objective: Reconnected pulmonary veins
Summarize the number of reconnected pulmonary veins

Endpoint: Each pulmonary vein will be classified as electrically isolated or not prior to re-ablation.
Electrically isolated is defined as bi-directional block, entrance and exit block of PV potentials.

Objective: Number and location of gaps in pulmonary vein ablation lesions
Summarize the number of gaps and location of gaps present in pulmonary vein ablation lesions

Endpoint: For a redo conducted with a focal catheter and 3D EA mapping, a gap within the pulmonary vein ablation lesion is defined as an electrically reconnected pulmonary vein which after a single spot reablation the response to re-ablation is either PV re-isolation or PV activation-sequence change. OngoingPV conduction after a single spot re-ablation is attributed to an additional gap. Additional gaps are
defined similarly.
For a redo conducted with no 3D EA Mapping, gap location may have been estimated utilizing a mapping catheter.
Each gap location will be identified by the classifications provided in **Figure 1.**

Figure 1.
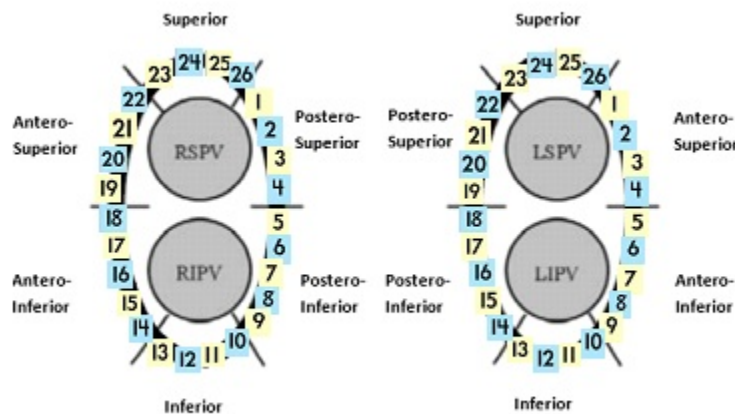
To define the location of a gap, including the differentiation for spot gap or linear gap the ipsilateral LA-PV junction is divided into 6 equally distributed segments; superior, antero-superior, antero-inferior, inferior, postero-inferior, and postero-superior.

Spot gap is defined as millimeter size, requiring only a single application without moving the catheter. Linear gap (larger continuous gap) is defined by requiring multiple applications or drawing of the catheter.

Objective: Ablation lesion sets performed

Summarize ablation lesion sets created during re-ablation procedure.

Endpoint: Each lesion set during re-ablation will be classified into one of the following categories:

- Pulmonary vein isolation (PVI)
- LA AF Trigger
- RA AF Trigger
- Superior vena cava trigger
- Inferior vena cava trigger
- Cavotricuspid Isthmus (CTI)
- Mitral valve isthmus or line
- Left sided roofline
- Complex fractionated atrial electrogram (CFAE)
- Posterior wall
- AVNRT ablation
- other

Objective: Acute procedural success

Summarize acute procedural success as assessed by the sites

Endpoint: Definition of success for each lesion set found below

- Pulmonary vein isolation (PVI)
    - Acute failure is defined as inability to isolate the pulmonary vein (minimally assessed for entrance block and, where assessable, exit block). Acute success is defined as the absence of acute failure.
- LA AF Trigger
    - Acute success is defined as elimination of trigger by ablation procedure.
- RA AF Trigger
    - Acute success is defined as elimination of trigger by ablation procedure.
- Superior vena cava trigger
    - Acute success is defined as elimination of trigger by ablation procedure.
- Inferior vena cava trigger
    - Acute success is defined as elimination of trigger by ablation procedure.
- Cavotricuspid Isthmus (CTI)
    - Acute success is defined as bidirectional conduction block at the CTI line
- Mitral valve isthmus ablation
    - Acute success is defined as bidirectional conduction block at the mitral isthmus line
- Left atrial roofline
    - Acute success is defined as confirmed block at the roof line
- Left atrial posterior wall
    - Acute success is defined as confirmed block at the posterior wall
- Complex fractionated atrial electrogram (CFAE)
    - Acute success is defined when ablation of electrograms resulted in termination of atrial fibrillation to sinus rhythm or until all complex fractionated regions were completely eliminated
- AVNRT ablation
    - Acute success is defined as 1) no AVNRT inducibility; 2) no jump; 3) single echo only.
- Other

Objective: Re-ablation procedure times

Summarize re-ablation procedure times.
Endpoint:
1. *Total procedure time* is defined as time from first venous access to time of last sheath removal.
2. *Left atrial dwell time* is defined as time from transseptal puncture to time of removal of last sheath from the left atrium.
3. *Fluoroscopy time* is defined as total fluoroscopy time used during the procedure.

Objective: Re-ablation hospitalization length of stay

Summarize the length of stay during the hospitalization for re-ablation
Endpoint: Length of stay is defined as the number of days from admission to discharge.

Objective: Anti-arrhythmic drug use at re-ablation hospitalization discharge

Summarize the anti-arrhythmic drug use at time of discharge from the re-ablation procedure.
Endpoint: Anti-arrhythmic drug is defined as a Class I or III antiarrhythmic drug

Objective: Summarize adenosine testing
Summarize adenosine testing
Endpoint: Adenosine testing utilized at the time of re-ablation.

5. Investigation Plan

The Fire and Ice Re-Ablations study is a retrospective chart review on a subset of (at a maximum) 110 subjects from the original FIRE AND ICE randomized trial who had repeat ablation procedures during the trial. The inclusion criteria for the Fire and Ice Re-Ablations study are (1) consent for data collection as required per local law (data release form - DRF) and (2) meeting all entry criteria for the original trial at their time of enrollment in it (e.g., consent to be randomized to cryoballoon ablation or RF ablation for drug-refractory paroxysmal atrial fibrillation, able to comply with study procedure, 18 – 75 years in age, no recent cardiovascular operations, etc.).

One final analysis will be completed. At the completion of the review by the endpoint review committee (ERC), the ERC classifications will be entered into the e-capture.net database (hosted by the contract research organization genae), and the database will be frozen. Analyses comparing randomization arms will not be conducted until after the database is frozen.

The Fire and Ice Re-Ablations study design is a retrospective cohort study. It is noted that while subjects were initially randomized to receive either cryoballoon or RF catheter ablation in the original Fire and Ice trial, non-random consequences of the therapies themselves, as well as potentially non-random decisions affecting subject eligibility (e.g., whether a repeat ablation would be performed) limit the strength of inferences between groups compared to the original randomized trial.

6. Statistical Methods

6.1. Study Subjects

6.1.1. Disposition of Subjects

Subjects are eligible for the Fire and Ice Re-Ablations study if they were enrolled in the original Fire and Ice trial and followed until a repeat ablation procedure was performed. The 110 subjects meeting these criteria are known at the time of this writing. In the final report, a diagram or figure will describe (at a minimum) the following:

- Number of eligible subjects who agreed to the additional data collection

- Subjects found to be ineligible for any reason that was discovered upon chart review

- Subjects who were eligible and signed DRF, but for whom no endpoint data could be obtained

These will be described by therapy arm wherever appropriate.

6.1.2. Clinical Investigation Plan (CIP) Deviations

This study is retrospective, and deviations are expected to be uncommon. Any deviations from the CIP or Clinical Trial Agreement (CTA) observed over the course of the retrospective data collection will be displayed in a listing appended to the final report.

6.1.3. Analysis Sets

The full analysis set consists of all subjects who agreed to the additional data collection. The full analysis set will be used for all study objectives.

6.2. General Methodology

6.2.1. Objective #1: Atrial arrhythmias prior to re-ablation

Summarize the documented atrial arrhythmias prior to re-ablation

Endpoint Definition

Each documented atrial arrhythmia will be classified into one of the following categories:
- Atrial Fibrillation
  - Paroxysmal
  - Persistent

- Atrial Tachycardia
- Atrial Flutter
  - Typical
  - Atypical
- Other

Analysis methods

The first re-ablation for each subject will be used for analysis. Each first re-ablation will be grouped into the following categories:
1. atrial fibrillation (paroxysmal)
2. atrial fibrillation (persistent)
3. atrial flutter or atrial tachycardia
4. other

The percent of subjects by indication for re-ablation will be summarized in a frequency table. The distribution of type of arrhythmia will be compared between randomization arms using exact methods.

6.2.2. Objective #2: Reconnected pulmonary veins

Summarize the number of reconnected pulmonary veins

Endpoint Definition

Each pulmonary vein will be classified as electrically isolated or not prior to re-ablation. Electrically isolated is defined as bi-directional block, entrance and exit block of PV potentials.

Analysis Methods

The first re-ablation for each subject will be used for analysis. The number of electrically isolated pulmonary veins will be assessed for each subject and the number of PVs isolated will be compared between randomization arms using a two-sample t-test. It is anticipated that the majority of subjects will have four major pulmonary veins; LIPV, LSPV, RIPV, and RSPV. If a subject has an anatomy in which electrical isolation of more or less than the four major PVs are tested, the number of electrically isolated pulmonary veins for that subject will be normalized to 4.

In addition, a logistic regression model will be fit, to test whether cryo vs RF therapy affect the odds of pulmonary vein reconnection. In the example code below, observations are successes and trials at the subject level, and SAS proc logistic (using trials events syntax) is used. This model assumes similar probabilities of reconnection for all veins within a subject (e.g., reconnection probability is not significantly different between LSPV, LIPV, RSPV, RIPV). This assumption will be tested using frequency tables and Fisher's exact test to assess whether reconnection rates are independent of treatment group by each anatomic location of ablation. This testing will only be performed for locations with 5 or more subjects treated per arm. Odds ratios and corresponding confidence intervals will be back-transformed into probabilities for presentation purposes.

```
proc logistic;
class rand  (param = ref ref = 'RF') <other covariates>;
model N_reconnected/N_possible = rand <other covariates>;
run;
```

6.2.3.   Objective #3: Number and location of gaps in pulmonary vein ablation lesions

Summarize the number and location of gaps in pulmonary vein ablation lesions

Endpoint: For a redo conducted with a focal catheter and 3D EA mapping, a gap within the pulmonary vein ablation lesion is defined as an electrically reconnected pulmonary vein which after a single spot reablation the response to re-ablation is either PV re-isolation or PV activation-sequence change. OngoingPV conduction after a single spot re-ablation is attributed to an additional gap. Additional gaps are defined similarly.

For a redo conducted with no 3D EA Mapping, gap location may have been estimated utilizing a mapping catheter. Each gap location will be identified by the classifications provided in **Figure 1**. To define the location of a gap, including the differentiation for spot gap or linear gap, the ipsilateral LA-PV junction is divided into 6 equally distributed segments; superior, antero-superior, antero-inferior, inferior, postero-inferior, and postero-superior. Spot gap is defined as millimeter size, requiring only a single application without moving the catheter. Linear gap (larger continuous gap) is defined by requiring multiple applications or drawing of the catheter.

Analysis methods

The number and location of gaps will be presented using summary statistics. Summary statistics are, in this case, frequencies and proportions. Additionally, a contour or density plot may be presented, with higher densities indicating greater numbers of observed reconnections.

6.2.4. Objective #4: Ablation lesion sets performed

Summarize ablation lesion sets created during re-ablation procedure.

Endpoint Definition

Each lesion set during re-ablation will be classified into one of the following categories:

• Pulmonary vein isolation (PVI)

• LA AF Trigger

• RA AF Trigger

• Superior vena cava trigger

• Inferior vena cava trigger

• Cavotricuspid Isthmus (CTI)

• Mitral valve isthmus or line

• Left sided roofline

• Complex fractionated atrial electrogram (CFAE)

• Posterior wall

• AVNRT ablation

• Other

Analysis methods

The first re-ablation for each subject will be used for analysis. The number of lesion sets for each subject will be counted. If a subject has multiple PVs ablated, each PV will count as a lesion set towards the subjects total count. The number of lesion sets will be compared between randomization arms using a two-sample t-test.

6.2.5. Objective #5: Acute procedural success

Summarize acute procedural success as assessed by the sites.

Endpoint Definition:

Definition of success for each lesion set found below

• Pulmonary vein isolation (PVI)

o Acute failure is defined as inability to isolate the pulmonary vein (minimally assessed for entrance block and, where assessable, exit block). Acute success is defined as the absence of acute failure.

• LA AF Trigger

o Acute success is defined as elimination of trigger by ablation procedure.

• RA AF Trigger

o Acute success is defined as elimination of trigger by ablation procedure.

• Superior vena cava trigger

o   Acute success is defined as elimination of trigger by ablation procedure.
- Inferior vena cava trigger
o   Acute success is defined as elimination of trigger by ablation procedure.
- Cavotricuspid Isthmus (CTI)
o   Acute success is defined as bidirectional conduction block at the CTI line
- Mitral valve isthmus ablation
o   Acute success is defined as bidirectional conduction block at the mitral isthmus line

- Left atrial roofline

o   Acute success is defined as confirmed block at the roof line

- Left atrial posterior wall
o   Acute success is defined as confirmed block at the posterior wall
- Complex fractionated atrial electrogram (CFAE)
o   Acute success is defined when ablation of electrograms resulted in termination of atrial fibrillation to sinus rhythm or until all complex fractionated regions were completely eliminated
- AVNRT ablationo   Acute success is defined as 1) no AVNRT inducibility; 2) no jump; 3) single echo only.

Other lesions sets may be observed on rare occasions, but acute procedural success will not be assessed for lesion sets not listed above. The denominator of the acute procedural success metric will only include subjects with a 'yes' or a 'no' listed in response to the case report form question as to whether acute procedural success was attained for all lesions.

Analysis methods
The first re-ablation for each subject will be used for analysis. For subjects receiving only re-isolation of pulmonary veins, acute procedural success is defined as minimally assessed for entrance block, and where assessable, exit block. For subjects receiving additional lesions beyond PVI, acute success is defined as successful PVI plus successful ablations as defined in the endpoint definition for all ablations. Each subject will be classified as an acute procedural success or not. Acute procedural success will be compared between randomization arms using exact methods.

6.2.6.   Objective #6: Re-ablation procedure times
Summarize re-ablation procedure times.

Endpoint:
1. *Total procedure time* is defined as time from first venous access to time of last sheath removal.
2. *Left atrial dwell time* is defined as time from transseptal puncture to time of removal of last sheath from the left atrium.
3. *Fluoroscopy time* is defined as total fluoroscopy time used during the procedure.

Ablation methods
The first re-ablation for each subject will be used for analysis. Procedural times will be compared

between randomization arms with a two-sample t-test. Separate t-tests will be used for each procedure time metric.

6.2.7. Objective #7: Hospitalization length of stay

Summarize the length of stay during the hospitalization for re-ablation

Endpoint Definition

Length of stay is defined as the number of days from admission to discharge.

Analysis Methods

The first re-ablation for each subject will be used for analysis. Hospital length of stay will be compared between randomization arms with a two-sample t-test. If the distribution of length of stay is skewed non-normal, a non-parametric Wilcoxon test will be utilized.

6.2.8. Objective #8: Anti-arrhythmic drug use at discharge

Summarize the anti-arrhythmic drug use at time of discharge from the re-ablation procedure.

Endpoint Definition

Anti-arrhythmic drug is defined as a Class I or III antiarrhythmic drug prescribed at discharge.

Analysis Methods

The first re-ablation for each subject will be used for analysis. The percent of subjects discharged from re-ablation on anti-arrhythmic drugs will be compared between randomization arms using exact methods.

6.2.9. Objective #9: Adenosine testing

Summarize adenosine testing utilized during re-ablation procedure.

Endpoint Definition

Use of adenosine testing during re-ablation. Subjects for whom it is unknown whether adenosine testing was performed will not be included in the denominator.

Analysis Methods

The first re-ablation for each subject will be used for analysis. The percent of subjects with adenosine testing will be compared between randomization arms using binomial exact methods.

6.3. Center Pooling

Data from all sites will be combined without regard to center location for the analysis of the study's endpoints. No assessment of site-level heterogeneity is planned.

## 6.4. Handling of Missing, Unused, and Spurious Data and Dropouts

It is anticipated that approximately 90% of the eligible subjects will be re-consented (DRF) and have available data from charts with which to perform an abstraction. Of these, some subjects' records will not contain data for one or more endpoints. Also, in some instances, charts from re-consenting subjects may contain complete data, but it will contain values that cannot be used in analysis of one or more objectives (i.e., when the CRF value of 'unknown' is selected instead of 'yes' or 'no'). If, for any of these reasons, 22 or more subjects do not have data for the reconnection of pulmonary veins endpoint (Objective #2) or the acute procedural success endpoint (Objective #5), further analyses relating to missingness of data may be performed.

Demographics of re-do cohorts will be compared. If no major differences in variables collected at baseline in the original Fire and Ice trial are observed between cryo and RF ablation arms, then data will be assumed "Missing Completely At Random" (MCAR) for all objectives, and only complete cases analysis will be performed. However, if differences are present and deemed significant, and <88 subjects have complete data for objectives #2 (reconnection of PV's) and #5 (acute procedural success), then up to two additional sensitivity analyses will be performed.

The sensitivity of between-group differences in reconnected pulmonary veins as a function of possible values in missing subjects will be illustrated graphically in a density plot like the one shown below in **Figure 2**. Regions of the plot that represent the combinations of reconnected PV's per subject observed among missing cryo and RF subjects which produce significant p-values in the between-groups comparison will be highlighted, and summary statistics (mean and SD of # reconnected PV's) for all significant combinations and all non-significant combinations will be displayed by group. The sensitivity of acute procedural success rates to missing data will be assessed for two additional scenarios: the "best case", and the "worst case" scenarios. The best case scenario will be calculated by assuming all subjects from the cryo arm were acute procedural successes compared to none of the RF subjects; the worst case scenario assumes that all cryo arm subjects were acute procedural failures and all RF arm subjects were acute procedural successes. Fisher's exact methods, as described above in **section 6.2.5**, will be used to assess between-therapy arm differences under these two sets of assumptions, and will be presented alongside the complete case scenario.

Figure 2.

Conclusion of analysis by composition of missing data

2-sample t-test; one-sided alpha = 0.025 (Cryo < RF)
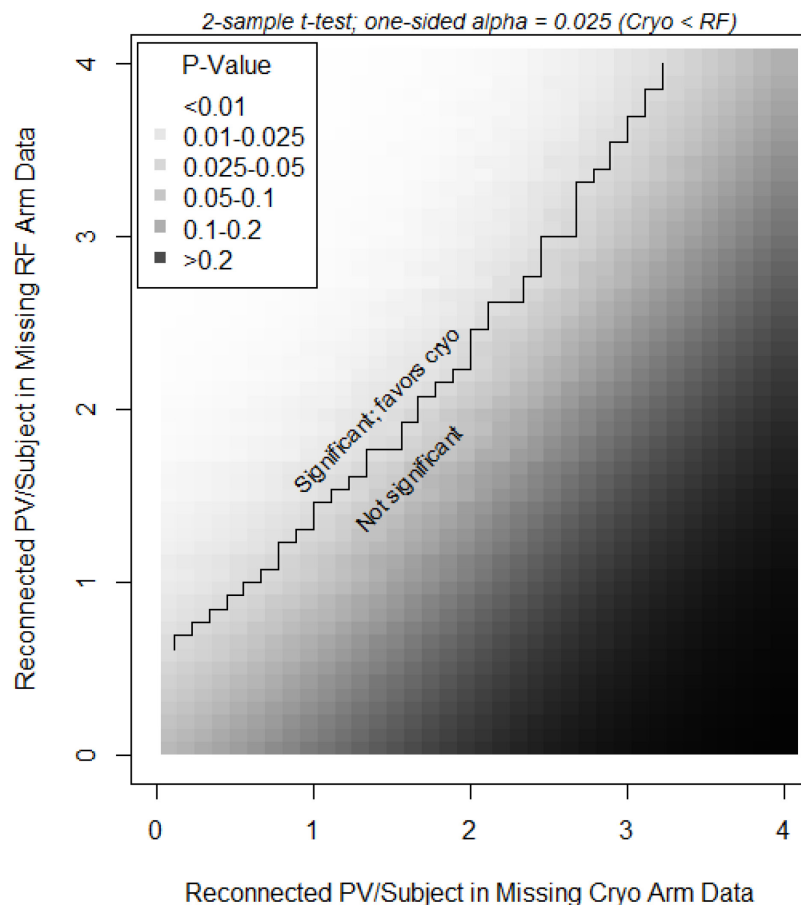
6.5. Adjustments for Multiple Comparisons

No adjustments for multiple comparisons will be made. All hypothesis tests will be conducted at the nominal 0.05 level of alpha.

6.6. Interim Analyses

No interim analyses are planned.

6.7. Subgroup Analysis

No subgroup analyses are planned.

6.8. Changes to Planned Analysis

Additional details on analysis methods have been added to this statistical analysis plan, but no changes to the methods defined in CIP are noted. Any deviation(s) from this SAP will be described and justified in the final report, as appropriate.

7.   Determination of Sample Size

The sample size of this study is limited by the number of subjects from the original Fire and Ice trial with repeat ablations who consent to additional data collection. The study objectives are generally descriptive rather than comparative; however, there are hypotheses tests of interest between treatment arms that have an associated type II error and power based on the number of data points included (rate of re-consenting subjects) and effect size. Of particular interest is the hypothesis test of whether the mean number of reconnected pulmonary veins is greater in the RF vs. cryoablation arms (Objective #2; see **section 6.2.2**). To demonstrate the ranges of values at which the hypothesis test will be sensitive to between-treatment differences, power for the two-sample t-test was assessed by Monte Carlo simulations (10^6 iterations), assuming a 90% subject reconsent rate, and assuming PV reconnection was binomial with rates of 36.8% for cryo-treated subjects and 58.1% in subjects initially treated with RF ablation (based on published data from Buist et al.). Results are shown below in Figure 3 and in Table 1.

Figure 3.

Power for 2-Sample t-test
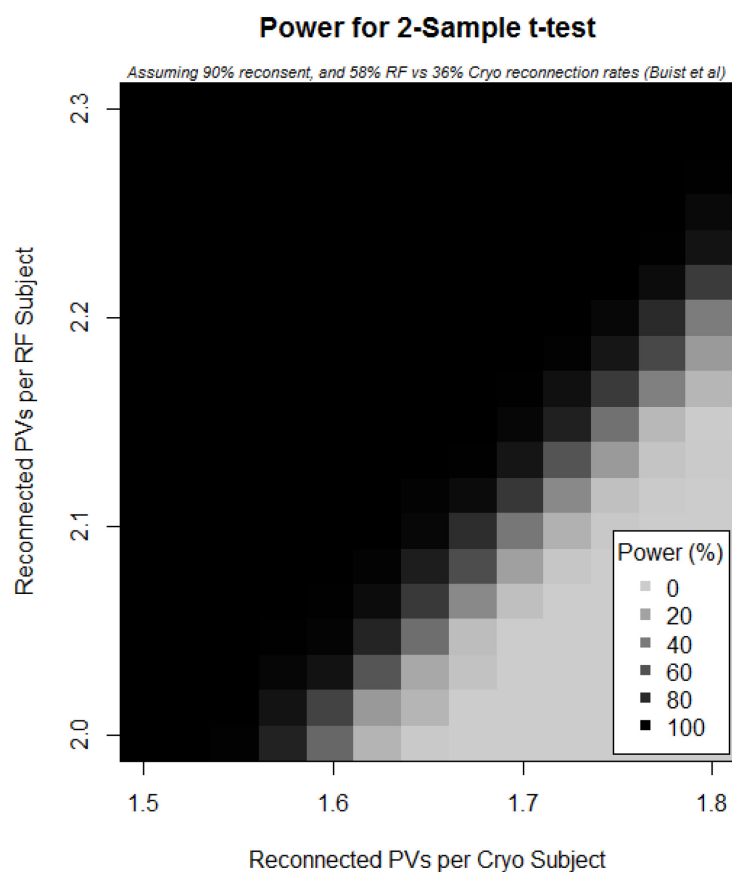
Table 1.

	RF – Cryo Difference (Reconnected PV's/Subject)				
	0.34	*0.37*	*0.40*	*0.43*	*0.46*
Statistical Power[1]	0.3%	7.1%	40.5%	87.5%	99.0%

[1]Test parameters: two-sample t-test with pooled variance, 5% alpha, average group means = 0.368*4 = 1.47 per subject in the Cryo arm, average standard deviation (Cryo group) = 0.93.

In other words, between-group differences of >0.34 reconnected pulmonary veins per subject will have at least an 87.5% chance of being declared statistically significant by the two-sample t-test with alpha = 0.05 described in section 6.2.2.

8.   Validation Requirements

Statistical programming in pursuance of all study objectives (see section 6.2) will be subject to a minimum of level II validation (code review by a statistician who did not author the statistical program). Any analyses that are not related to objectives 1 – 9 will be subject to a minimum of level II validation if presented externally in an abstract or publication.

9.   References

Buist T, Adiyaman A, Smit J, Misier A, Elvan A. Arrhythmia-free survival and pulmonary vein reconnection patterns after second-generation cryoballoon and contact-force radiofrequency pulmonary vein isolation. Clin Res Cardiol 2018 Feb 6. doi: 10.1007/s00392-018-1211-9. [Epub ahead of print]