Clinical Trial Protocol

First-line cryoablation for early treatment of Persistent Atrial Fibrillation – a randomized study comparing early trigger isolation using the Cryoballoon versus antiarrhythmic medication
(Study Code: CryoStopPersAF)

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1. Synopsis

Objectives: A comparison of early cryoballoon ablation for pulmonary vein isolation (PVI) as first line therapy versus antiarrhythmic drugs (AAD) for reduction of atrial tachyarrhythmia (atrial fibrillation (AF) / atrial tachycardia (AT)) recurrences as primary outcome at 12 months in patients with symptomatic and recurrent persistent AF.

Study design Multicentre, prospective, 1:1 randomized, open, blinded for evaluation of end point (PROBE) controlled parallel-group superiority trial, that compares AAD therapy to cryoballoon PVI regarding freedom from AF/AT (%) assessed by an implantable cardiac monitor (ICM), ECG tracing or Holter at 12 months in patients with persistent AF.

Sample size 220 patients with persistent AF.

Background: AAD therapy is first line treatment before catheter ablation in all types of AF.[1] Three recent randomized trials found that cryoballoon ablation for PVI was superior to AAD as first-line rhythm control in paroxysmal AF patients,[2],[3],[4] reinforcing the results from three previous trials.[5],[6],[7] Shorter times from AF diagnosis to AF ablation have been associated with lower risk for AF recurrences than longer times.[8],[9],[10] Given the progressive nature of AF and the observation that AF recurrences after PVI occur more frequently in persistent versus paroxysmal AF[11], we hypothesized that first-line cryoablation can more effectively halt and reverse further AF progression, as measured by freedom from atrial tachyarrhythmias in persistent AF, versus AAD. Moreover, first-line ablation trials are lacking for persistent AF.

Objective and hypothesis: We hypothesized that first-line cryoablation, compared to AAD, will result in 25 % higher freedom from atrial tachyarrhythmias at 12 months (primary outcome) excluding three months initial blanking, in patients with symptomatic and recurrent persistent AF. Further a superior improvement in QoL, more reverse atrial remodeling, less health care use and less costs, at 12-24 months as compared with drug use.

Inclusion criteria: Non-longstanding symptomatic persistent AF with at least two episodes within the last 24 months, one documented on a 12 lead ECG or Holter monitor, one occurring within the last 6 months; and classified as either a) Persistent AF continuously sustained beyond 7 days and $\leq$12 months in duration[12] OR b) Persistent AF which has progressed from paroxysmal AF (patients cardioverted within 7 days of onset provided a history of spontaneous conversion to sinus rhythm is lacking the past 24 months), in patients aged 18 – 75 years eligible for AF ablation and antiarrhythmic drugs.

Main exclusion criteria; Previous regular (daily) use of AAD (excluding beta-blockers) or PVI, severe heart failure, tachycardiomyopathy, LVEF $\leq$40%, left atrial volume indexed (LAVI, ml/m^2) > 48, significant valvular disease requiring treatment or severe pulmonary disease, planned cardiac intervention within next 12 months, dependent on VVI pacing.

Primary endpoint: Freedom from AF/AT recurrences (%) at 12 months excluding 3 months blanking.

Secondary endpoints (main): AF/AT burden, AF/AT progression and reversion, quality of life, cognitive function, healthcare utilization with associated costs, indirect signs of reverse atrial remodeling by ECG, biomarkers, effects on blood pressure (BP), and echocardiographic LA measures after 12, 24 and 36 month. Predictors for freedom from AF/AT recurrence.

Statistical analysis: The main statistical analysis of the primary endpoint will be based on the intention-to-treat (ITT) population. The binary primary variable will be analysed using a logistic regression with a few predefined covariates. UCR will be responsible for the Statistical Analysis Plan and the statistical report. Sample size based on previous trials measuring AF recurrences.

Trial duration: 36 months.

Expected results: 1st line cryoablation will reduce AF/AT recurrences and stop progression, improve symptoms and reverse remodeling.

2. Abbreviations and definitions

AEs	Adverse Events
BNP	Brain natriuretic phactor
bpm	beats per minute
BSA	Body Surface Area(m^2) = 0.007184 x Height(cm)0.725 x Weight(kg)0.425
CABG	Coronary artery bypass grafting
CRT	Cardiac resynchronization therapy
CT	Computer Tomography
eCRF	electronic CRF
ECG	Electrocardiogram
EF	Ejection fraction
EHRA	European Heart Rhythm Association
LA	Left atrial
LV	Left ventricular
mo	Month
ms	milliseconds
NYHA	New York Heart Association
PET	Positron emission tomography
PV	Pulmonary Vein
PVI	Pulmonary vein isolation
QoL	Quality of life
RA	Right atrial
RF	Radiofrequency
SAEs	Serious adverse events
SD	Standard Deviation
SVT	Supraventricular tachycardia
TEE	Transesophageal echocardiogram

Definitions

Paroxysmal; Recurrent AF episodes, self-terminating or terminated with intervention within 7 days

Persistent, not long standing; AF lasting beyond 7 days including episodes terminated by cardioversion after 7 days or more. Duration of ongoing AF less than 1 year

Long standing; continuous AF $\geq$ 1-year duration, rhythm control adopted.

Permanent AF; is accepted by the patient (and physician).

3. Introduction

3.1. Background

Atrial fibrillation (AF), affecting 3% of the population, is associated with decreased quality of life, increased risk for stroke, morbidity with escalating hospitalisation rates[13] and death.[14] The main indication for rhythm control is to improve quality of life and reduce symptoms. Current AF guidelines recommend antiarrhythmic drugs (AADs) as first line therapy before AF ablation for both paroxysmal and persistent AF,[1],[12] although AADs have poor efficacy for rhythm control on long term.[15],[16],[17],[18],[19] Catheter ablation for pulmonary vein isolation (PVI)[20],[21] show, in randomized trials, significantly greater freedom from AF and symptomatic improvement than drugs,[22],[23] although significantly lower figures are observed for persistent AF.[24],[25] Cryoballoon ablation was in three recent randomized trials superior to antiarrhythmic drugs, as first-line rhythm control in paroxysmal AF patients,[2],[3] ,[4] reinforcing the results from three earlier trials reporting equal or superior AF reduction by ablation versus antiarrhythmic medication.[5],[6],[7] In our recently published randomized CAPTAF trial, in which 70% of patients had only tested beta-blocking agents, the greater improvement in quality of life was for the first time shown to be related to the greater reduction in AF burden achieved by catheter ablation as

compared with AAD, underlining the superiority of intervention at an early stage of the AF disease.[22]
Observations from AF registries show that 15-35 % of AF patients annually progress to more sustainable forms of AF,[26],[27],[28] with time leading to structural remodelling and enlarged left atrium (LA). Increased left atrial volume index (LAVI) and structural changes on MRI has been associated with a poorer outcome and higher AF recurrence rates after ablation in persistent AF patients.[24],[29],[30],[31] These observation may have important implications for the timing of ablation.[32] The "diagnosis to ablation time" was early recognized to be the strongest association with outcome of ablation,[10] which is supported by later reports that shorter "diagnosis to ablation times" are associated with better outcomes than those with longer times.[8],[9] Observations from registries of gradually declining success rates with increasing time from diagnosis to ablation time, with the highest clinical success achieved for PVI performed within the first year of AF diagnosis further confirms the concept of early ablation[8]

3.2. Rationale for performing the study

Given the above background and the universal superiority of ablation over AAD therapy observed in other trials,[2],[3]we hypothesized that cryoablation performed as first-line therapy at an early stage of the disease in persistent AF offers an opportunity to halt or reverse the progressive atrial changes associated with AF, and impart other important clinical outcomes, such as improvements in symptoms and quality of life, reduction in healthcare use such as hospitalisations, emergency room admissions, unplanned out-patient clinics and cardioversions. There are currently no published trials that have explored the effects of early pulmonary vein isolation as first-line rhythm control intervention in patients with persistent AF, compared with antiarrhythmic drugs. There is no information from recent first-line ablation trials in paroxysmal AF regarding effects on AF progression and remodelling and conflicting reports on cardiovascular health care utilization.[2],[3]

Even though freedom from symptoms and improved quality of life may be the most important clinical endpoint, the choice of rhythm (freedom from AF) as primary endpoint was preferred for the important mechanistic comparison with recently published first-line trials in patients with paroxysmal AF.[2],[3] This is important since even though isolation of pulmonary vein triggers is the best choice in the vast majority of paroxysmal AF patients, it is yet unclear whether the lower success rates of PVI reported for persistent AF patients relates to the timing of the ablation or the procedure itself.
Uncertainties also exists whether the isolation of the PV's is the most crucial for maintenance of sinus rhythm or whether the degree of damage to the intrinsic cardiac nervous system (ICNS) is equally or more important. Recent trials have shown that the degree of intrinsic cardiac nervous system (ICNS)[33] injury, as assessed by the release of s100b, is related to the outcome after AF ablation. The degree of intrinsic cardiac nervous system[33] injury will thus be evaluated by the release of s100b and by the blood pressure and heart rate response whereas the cardiac injury by release of troponin T.[34] Secondary aim was therefore to assess s100b before and after ablation.
The demonstrated excellent perioperative success rates with the second generation cryoballoon ablation catheter as measured as PVI efficacy, low complication rates, short procedure times[35],[36],[2],[3] and less operator dependent outcomes than other ablation tools,[37] are properties that favour a more widespread use, particularly in cardiac units in need for increased capacity to meet the rising demand for AF ablation, which had a great impact on the choice of ablation catheter in our planned randomized multicentre trial.

The novelty with the present trial is the concept that first-line catheter ablation may halt further AF progression by eliminating PV trigger at an early stage of the AF disease and beyond arrhythmia recurrence, including a comprehensive quality of life and patient reported outcome assessment, incorporating a mechanistic analysis of possible different outcomes, as well as assessment of health care use and expenditure.
The result will have important implications for health care payers and guidelines committees in their recommendations regarding which first-line therapy (PVI or AAD) should be chosen for the general AF population. It will also have implications for AF patients, since AF ablation is currently only available for a minority of these patients. Further, the observed large hospitalisation rates are expected to decline with significant reduction of health care costs.

4. INVESTIGATIONAL PLAN

4.1. Objectives and Endpoints

4.1.1. Objectives

The main focus is to evaluate the impact of early interventional management of persistent AF.

The **primary** goal is to evaluate if early PVI performed with the Arctic Front cryoballoon as first-line therapy is superior to AAD in preventing atrial arrhythmia recurrences.

The **secondary goal** is to evaluate the impact of early invasive intervention on health related quality of life (HRQOL) and symptoms, and on safety in comparison to primary AAD therapy, using generic and disease-specific HRQOL questionnaires and also assess Quality Adjusted Life Years (QALYs) score and EHRA classification of symptoms.

The **third goal** is to assess the impact of an early intervention on cardiovascular health care use (hospitalisations and other health care utilization) and its relation to AF/AT burden and to assess treatment burden and cost-effectiveness compared to AAD.

4.1.2. P.I.C.O. Model for Clinical Questions:

Patient How would I describe a group of patients similar to mine?	Symptomatic AF patients, aged 18 – 75 years, with non-longstanding persistent AF and at least two AF episodes last 24 months, the latest within previous 6 months and one documented on 12 lead ECG or Holter monitor. At least one AF cardioversion, even one performed within 7 days of AF onset provided a history of spontaneous conversion of AF episodes to sinus rhythm is lacking in near time. Excluding those with regular daily use of AAD, prior ablation or surgery, severe heart failure, reduced ventricular function (≤40%), severely enlarged left atrium, significant valvular disease, severe comorbidity, and expected survival <3 years.
Intervention Which main intervention am I considering?	Pulmonary vein isolation with a cryoballoon.
Comparison	Antiarrhythmic drug dronedarone or flecainide/ propafenon.

What is the main alternative to compare with the intervention?	
Outcome What can I hope to improve?	25 % reduction in any atrial tachyarrhythmia recurrence at 12 months (primary outcome) with ablation versus AAD, and • Superior improvement in symptom, QoL, cognitive function by ablation vs AAD. • Reduced healthcare utilization and cost for cardiovascular reasons (number of cardioversions, ablations, AAD initiations, hospitalizations, emergency department visits and unplanned outpatient visits after 3 months blanking) compared to drug use. • Superior reverse atrial remodeling by ablation (AF/AT burden, P waves; biomarkers NT pro-BNP, IL6, D-dimer; left atrial size and function) compared to AAD use. • Superior safety (less serious adverse events) with ablation versus drugs.
What **T**ype of question are you asking?	Is treatment with ablation superior to drugs for prevention of AF recurrences at 12 months? (primary outcome) Will ablation improve quality of life and symptoms better than drugs at 12 to 36 months? Will ablation reduce healthcare utilization and cost better than drugs? Will ablation be superior in reversing atrial remodeling versus drugs?
Type of **S**tudy you want to find What would be the best study design/methodology?	An open label, prospective, randomized multicentre trial comparing ablation versus drug regarding freedom from AF as primary endpoint at 12 months, using continuous rhythm monitoring blinded to patients and investigators, with evaluations of secondary endpoints up to 36 months.

4.1.3. Hypothesis

We hypothesized that first-line PVI using the cryoballon, at an early stage of the AF disease, will result in:

- A 25 % reduction in any atrial tachyarrhythmia recurrence at 12 months compared to the AAD group.
- Superior improvement in symptoms and QoL with equal or better safety compared to AAD.
- Reduced healthcare utilization and cost for cardiovascular reasons (number of cardioversions, ablations, AAD initiations, hospitalizations, emergency department visits and unplanned outpatient visits after 3 months blanking) compared to AAD use.
- Superior reverse atrial remodeling (P waves; biomarkers NT pro-BNP, IL6, D-dimer; left atrial size and function) compared to AAD use.

4.1.4. Primary and secondary endpoint

4.1.4.1. Primary endpoint

The primary endpoint is freedom from atrial tachyarrhythmia recurrence lasting $\geq$ 6 minutes (in the absence of AAD in ablation group) as documented by 12-lead ECG, ECG rhythm strip, Holter, or an ICM, from initiation of treatment excluding the first 3 months (blanking period) to 12 months post after initiation of allocated treatment.

4.1.4.2. Secondary endpoints

Secondary endpoints are to compare the effect of the two first-line treatments with respect to the following secondary endpoints during the total study period of 36 months in patients with symptomatic persistent AF at 12, 24, and 36 months (if not otherwise specified):

1. Total atrial arrhythmia burden (% time in AF/AT).
2. AF progression and reversion as measured by combination of reduced number of AF /AT progressions or increased number of AF/AT reversions after 3 months blanking. Progression or transition to more severe AF/AT forms such as longstanding persistent or permanent AF/AT and AF/AT regression as going in the opposite direction from persistent to paroxysmal to sinus rhythm at 12, 24 and 36 months.
3. Quality of life assessed by; i) EuroQol Five Dimensions Questionnaire (EQ-5D), ii) University of Toronto Atrial Fibrillation Severity Scale (AFSS) and iii) Short Form Health Survey (SF36). The rhythm and pulse at the time of the evaluation will be recorded.
4. European Heart Rhythm Association (EHRA) Symptom Classification assessing the severity of AF related symptoms.
5. Cognitive function as measured by Trail Making Test A and B (TMT A and B). The rhythm and pulse at the time of the evaluation will be recorded.
6. Blood pressure, systolic and diastolic (mmHg) after 10 minutes rest at 12, 24 and 36 months. The rhythm and pulse at the time of the evaluation will be recorded.
7. Healthcare utilization for cardiovascular reasons (number of cardioversions, ablations, AAD initiations, cardiovascular hospitalizations, emergency department visits and unplanned outpatient visits after 3 months blanking) and its relation to AF/AT burden. Cardiovascular means related to atrial fibrillation including any treatment or diagnostic procedure for AF/AT such as cardioversions, medication, further AF ablations after AF/AT recurrence, or any adverse events related to AF/AT or its treatment such as thromboembolic complications (acute stroke), heart failure, myocardial ischemic events; adverse events (eg. pacemaker implantation).
8. Health care costs will be assessed at 36 months. Quality-adjusted life years (QALYs) using the EQ-5D over 3 years will be estimated from serial utility measurements from the Swedish population valuation of the EuroQoL EQ-5D at randomization. Health care use and cost-effectiveness, including CV hospitalization, outpatient visits, treatment costs, with corrections for background variables regarding social economic status. The time spent on sick-leave, duration and cost for not being at work will be retrieved from the sickness insurance institution, by informed consent from the patient, if necessary.
9. Reverse atrial remodeling assessed by P wave variables from ECG (P-wave duration), biomarkers (NT pro-BNP, IL6, D-dimer), and left atrial size and function (LAVI, ejection fraction, atrial strain) by echocardiography, corrected for BSA, at 12, 24 and 36 months, as described in appendix.
10. Single and multiple procedure success (freedom from ECG documented atrial tachyarrhythmia after the 1st and last ablation procedure respectively).
11. Safety outcome parameters. Type and frequency of adverse events will be recorded continuously and classified whether related to treatment, and whether serious.
12. Frequency of withdrawals / ‘cross-overs’ over time.
13. Covariate adjusted primary endpoint (analysis using following covariates at baseline: coronary artery disease, hypertension, LAVI).
    Predictors of non-responders at 12 and 24 months. A responder is arbitrarily defined as one that has maintained sinus rhythm at 12 months without AF/AT recurrences after the first 3 months (blanking period).

The aim is further in a substudy to evaluate novel predictors of responders/non-responders to rhythm control strategy in comparison to conventional risk factors by using;

1. The novel and recently published *4S-AF scheme domains for AF characterization, calculating sum of scores*;
   a. Stroke risk from CHA_2DS_2-VASc score (No OAC/OAC= 0 or 1 point)
   b. Symptom severity (0= EHRA score 1-2a; 1=-EHRA score 2b; 2= EHRA score 3-4) European Heart Rhythm Association (EHRA) Symptom Classification; class 1, 2a-b, 3 and 4 indicating no, mild/moderate, severe and disabling symptoms.
   c. Severity of AF/AT burden (0=short and infrequent, 1= intermediate and/or frequent; 2=long or very frequent)
   d. Substrate: Risk factors-Comorbidity/LA enlargement (0=none; 1=single/mild moderate; 2=multiple/severe). Add +1 point if age >75 years.
2. Conventional risk factors for AF/AT recurrence:
   a. AF duration by history, months
   b. P wave duration on 12 lead ECG (ms)
   c. LAVI (mL/m2) (normal 16-34 ml/m2, mildly enlarged 35-41 ml/m2, moderately enlarged 42-48 ml/ m2, severely enlarged >48 ml/ m2) by echocardiography and atrial strain assessed by echocardiography
   d. Biomarker promotors of AF progression (NTpro-BNP)
   e. BMI
   f. CHA_2DS_2vasc score

An explorative substudy will assess signs of intra cardiac nervous system injury and its relation to AF recurrences after PVI patients by analysing levels of s100b prior and after PVI (see appendix).

Quality of life will be assessed as described below. The rhythm at the time of the evaluation will be documented.

- EuroQol Five Dimensions Questionnaire (EQ-5D), which essentially consists of 2 pages - the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). It comprises of the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, some problems, extreme problems.
- University of Toronto Atrial Fibrillation Severity Scale (AFSS), is disease specific, and quantifies 3 domains of AF-related symptoms: frequency, duration, and severity.
- Medical Outcomes Study Short Form-36 (SF-36) questionnaire. It contains 8 subscales: physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. Scores range from 0 to 100, with lower scores representing a poorer QoL

4.2. Study Design

This is a multicentre, prospective 1:1 randomized open blinded for evaluation of end point (PROBE) controlled parallel-group superiority trial comparing first-line pulmonary vein isolation using the cryoballoon and first-line antiarrhythmic drug therapy. The study schedule is shown in Figure 1 and Table 1.

4.2.1. Randomization

Patients will be randomized 1:1 to first-line pulmonary vein isolation using the cryoballoon or to first-line antiarrhythmic drug therapy. A 1:1 block randomization will be performed immediately after ICM implantation before run-in using Viedoc's randomization system avoiding a delay in pre-ablation oral anticoagulation therapy to be initiated 3-4 weeks prior allocated ablation. The study will utilize a block randomization that is stratified per center. The randomization will also be stratified by type of persistent AF (classical persistent AF as defined by ESC guidelines versus persistent AF which progressed from paroxysmal and thus not fulfilling the 7 day duration requirement). The date at which randomized allocation will be initiated (ablation or start of AAD) should be scheduled immediately after randomization to a date maximum one week after end of run-in ensuring an initiation of treatment within maximum 2 weeks after run-in for protocol adherence.

4.2.2. Run-in

After fulfilling inclusion criteria and no exclusion criteria, an ICM (Reveal Linq ™, **Medtronic,** Inc., Minneapolis, MN) will be injected subcutaneously for continuous rhythm monitoring during a 2 months "run-in" period, i.e. 2 months prior initiation of allocated treatment, and for monitoring during follow up.
Antithrombotic treatment, preferably NOACS, will be initiated at baseline in patients who have risk factors for thrombo-embolism, or 3 weeks prior to allocated PVI, according to latest ESC guidelines.

4.2.3. Monitoring of AF episodes

Remote monitoring and retrieval of AF episodes from the ICM will be performed with standardised intervals (Table 1). A 6 minutes detection cut-off for AF will be used. The device programming will be standardized and programmed to "AF ablation setting" (see appendix). The heart rhythm will continuously be monitored and all atrial tachyarrhythmias (AF) automatically detected by the ICM during the 3 years of follow-up. The patients and the physicians will be blinded to the ICM result. The ICM is thereafter explanted at the discretion of the physician.
Recordings from ICM will start immediately after ICM implantation and continue for 2 months run-in until allocated treatment is initiated. Recordings will restart at discharge after initiation of allocated therapy but the three month blanking period after treatment will not be included in the analysis. The data should be transferred regularly by the patient every 3rd month to the remote monitoring station via Care Linq at the centre.
SAEs detected via ICM will be reported/managed independently from the blinding of AF.
If the ICM has to be explanted (infection, severe discomfort) the patient should continue in the study and the steering committee should be contacted for advice regarding alternative monitoring tool. For the continuous evaluation of AF after ICM explantation, the patients will carry either a 7 day (continuous) ECG recorder or a 14 day event recorder (with daily transmissions of 6 minutes) at 6, 12, 18, 24, 30 and 36 months.

4.2.4. Study duration

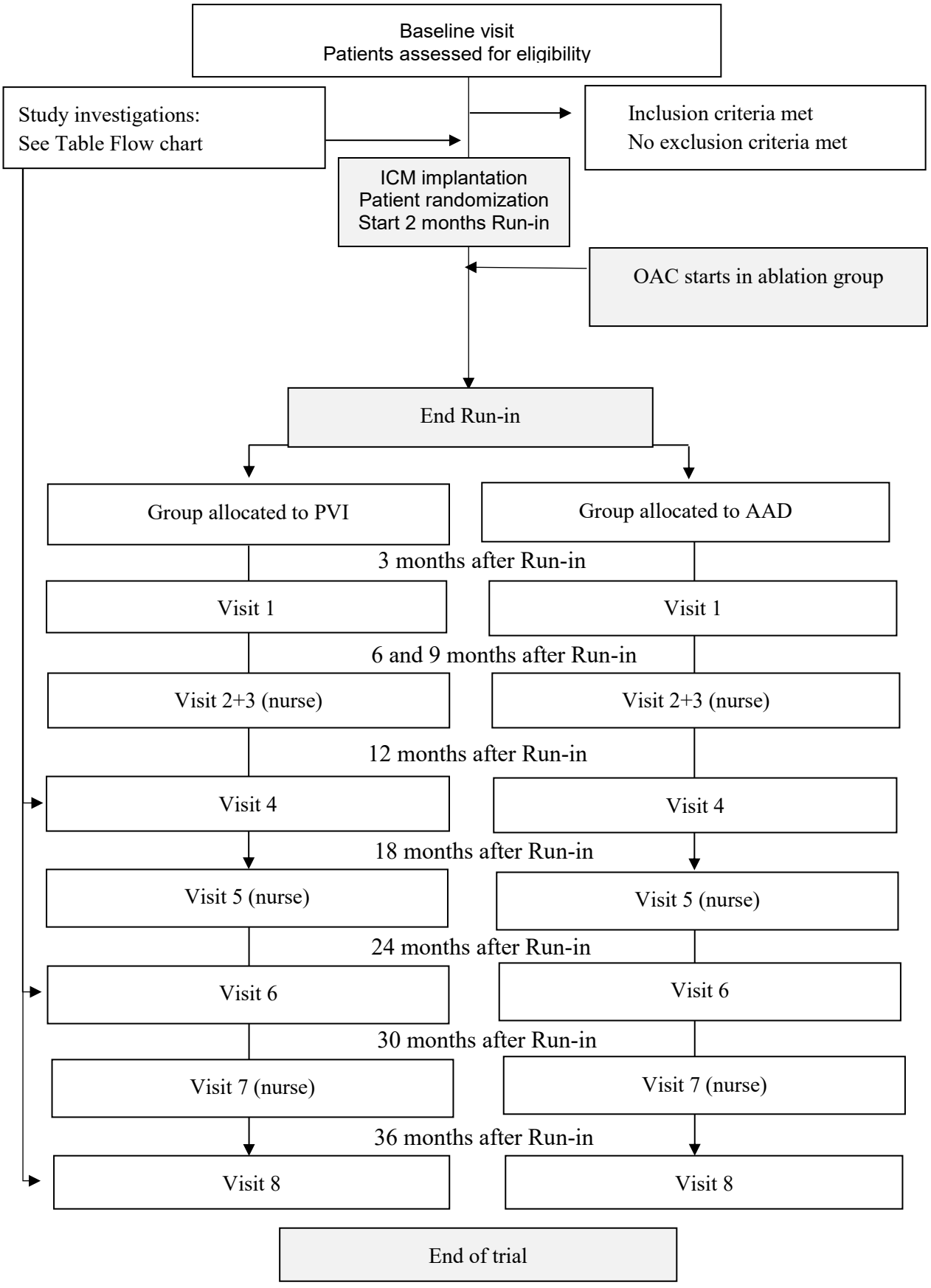
The study duration is 3 years with 18 months' enrolment period. The last follow-up will be at 36 months for evaluation of AF progression and health economy.
Patients will be followed at three, six, nine, 12, 18, 24, 30 and 36 months after the ablation procedure or start of AAD therapy. The intervals with which each visit and various investigations will be performed are outlined in Table 1.
Definition of the end of the study (End of Trial) is when the last patient has attended the last visit.

The patients will then be referred to either a general practitioner or a cardiologist at their local hospital depending on their disease state or, if deemed medically necessary, continued follow-up within the department, after the end of the trial.

4.2.5. Centers

The trial will be performed at 4-5 university centres in Sweden, at 1-2 centres in UK and at 4-5 centres in other parts of Europe, all with experience in Cryoballoon AF ablation. Each centre will register at least 30 patients for a total of 220 patients.

Figure 1. Patient Enrollment, Randomization, and Follow-up



4.2.6. Table 1. Flow chart of visits

Schedule Visits	Visit -2	Visit 0	Visit 1 Centre	Visit 2 Nurse[1]	Visit 3 Tel Nurse	Visit 4 Centre	Visit 5 Nurse[1]	Visit 6 Centre	Visit 7 Tel Nurse	Visit 8 Centre
Investigations	Base-line	After 2 mo run-in Ablation/AAD	3 m ± 2 w	6 m ± 2 w	9 m ± 2 w	12 m ± 2 w	18 m ± 2 w	24 m ± 2 w	30 m ± 2 w	36 m ± 2 w
Medical history – New clinical events; comorbidities (HF type), risk factors, interventions, NYHA Fc, AE, SAE	X X	X	X	X	X	X	X	X	X	X
AF evaluation - AF type, CHA_2DS_2VASC[3], 4 S scheme	X X		X	X	X	X X	X	X X	X	X X
Vital signs (BP, weight) Physical exam	X X		BP	BP	BP[2]	X X	BP	X X	BP[2]	X X
AF medication & Concomitant medication	X	X	X	X	X	X	X	X	X	X
Informed Consent	X									
Inclusion/Exclusion	X									
AF biomarkers (NTproBNP, GFR, IL6, D-dimer): Troponin T, NTproBNP, S100b[4]:	X -	 3 times	X			X		X		X
12 lead ECG	X	X	X	X		X	X	X		X
Echocardiography (TTE)	X					X		X		X
Randomization at time of ICM implant	R									
QoL assessed (SF36, AFSS, EQ5D), EHRA score	X					X		X		X
ICM implant[5] ICM check /interrogation[6]	Op X	X	X	X	X	X	X	X	X	X
Cognitive function (TMT A,B)	X					X		X		X
Health care use (visits, hospitalizations, AF therapy)	X		X	X	X	X	X	X	X	X

Abbreviations: AAD = antiarrhythmic drugs; AF = atrial fibrillation; 1= may be visit off-site, 2= BP recorded at home or general practitioner; 3 = CHA_2DS_2VASC updates by nurse confirmed by cardiologists, 4 = 3 times in ablation group; 5 = within 2 weeks after baseline, 6 = Patients should transmit ICM data every third month to Care Link (i.e. apart from above also at 15, 21, 27 and 33 months); EHRA SC=EHRA symptom classification, Fc = function class; HF = Heart Failure; EF =ejection fraction; m=month; QoL = quality of Life; TMT A,B = Trail Making Test A och B; w = week; Electrical cardioversion within 3 days after start of AF for all patients.

4.3. Study population

The target population are patients with symptomatic persistent AF episodes aged 18 – 75 years who have not been treated with antiarrhythmic drugs class I or III on a daily basis nor undergone any AF ablation intervention.

4.3.1. Inclusion criteria;

1. Non-longstanding persistent symptomatic AF with at least 2 episodes within last 24 months, the latest episode within the previous 6 months and one documented on a 12 lead ECG or Holter monitor, that is classified as either
    a. Classical persistent AF (continuously sustained beyond 7 days and ≤12 months in duration) as defined by ESC guidelines[12] OR
    b. Persistent AF which has progressed from paroxysmal AF (patients who have been cardioverted within 7 days of onset provided a history of spontaneous conversion to sinus rhythm is lacking during the past 24 months).
2. Age 18 – 75 years,
3. Candidate for rhythm control therapy; AF ablation or AAD based on symptomatic AF. As an example, BMI ≥35 would not according to clinical praxis be a candidate for AF ablation and thereby not suitable for participation in the study.

4.3.2. Exclusion criteria;

1. Regular daily use of AAD class I or III at adequate therapeutic dosages (pill-in-the-pocket permitted, beta-blockers permitted).
2. Previous AF ablation or surgery.
3. Severe heart failure (NYHA III-IV).
4. Reduced left ventricular ejection fraction (LVEF ≤40 % during sinus rhythm).
5. Hypertrophic cardiomyopathy (septal or posterior wall thickness >1.5 cm)
6. Severely enlarged LA with left atrial volume indexed to body surface area (LAVI, ml/m^2) > 48.
7. Significant valvular disease requiring treatment or valve protesis.
8. Severe COPD stage III or chronic kidney disease (eGFR< 30 umol/l)).
9. Planned cardiac intervention within the next 12 months or cardiac surgery last 6 months.
10. Myocardial infarction, revascularisation previous 6 months.
11. Stroke or TIA within previous 6 months.
12. Tachycardiomyopathy.
13. Dependent on VVI pacing.
14. Conventional contraindications for AF ablation including AF due to reversible causes and contraindications for both class IC and class III antiarrhythmic drugs.
15. Expected survival less than 3 years, alcohol or drug abuse.
16. Participation in another trial or absence of consent.

4.4. Conduct of study

4.4.1. Initial clinical evaluation

Each patient will be evaluated by a cardiologist. Prior to inclusion a search for correctable or primary causes of AF should be performed.
Demographic variables, prior and present medical history, current medications, CHA_2DS_2 Vasc score, and comorbidities will be recorded at baseline.
Patients with heart failure should be evaluated at an adequate rate control of AF or during sinus rhythm to exclude tachycardiomyopathy and to assess whether symptoms are related to the

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heart failure or the atrial fibrillation. Patients should be subject to optimization of medical therapy for comorbidities, particularly hypertension.
All screened patients who sign an informed consent and satisfy inclusion and exclusion criteria will undergo a *clinical evaluation* at baseline;

4.4.1.1. Structured characterization of AF according to 4S

1. Stroke risk by **CHA_2DS_2-VASc score**
2. Symptom severity by **EHRA symptom score** and QoL questionnaires;
3. Severity of AF/AT burden by
   - **AF type (**paroxysmal, persistent, longstanding persistent, permanent**),**
   - Patient estimate of frequency and duration of symptomatic episodes during the last 12 months.
   - Duration of AF history since first detection
   - Number of previous cardioversions for AF previous 12 months
4. Substrate **severity;** presence of comorbidities/cardiovascular risk factors, atrial CMP (atrial enlargement/dysfunction), incident AF risk scores, AF progression risk scores.

4.4.1.2. Clinical evaluation and search for possible correctable or primary causes of AF

- Physical examination including height, weight, BMI, blood pressure after 10 minutes rest.
- Evaluation of heart failure; assessment of left ventricular function.
- Current antithrombotic, anticoagulation and cardiovascular medication.
- 12-lead electrocardiogram,
- Transthoracic echocardiography,
- Clinical laboratory tests; Hb, white blood cell count, platelets, serum-creatinine, eGFR, CRP, S-potassium, S-calcium, S-albumin, liver enzymes (ASAT, ALAT), fB-glucose, NT pro-BNP, thyroid tests unless previously tested (TSH, freeT4).
- Questionnaires related to the trial (HRQoL, Cognitive tests, EHRA score)– see Table 1
- Blood sampling for biomarkers related to the trial - see Table 1

4.4.1.3. Medical history;

History of thromboembolism
  - Ischemic stroke (acute loss of neurological function caused by an ischemic or hemorrhagic event with residual symptoms at least 24 hours after onset).
  - Transient ischemic attack (TIA) (acute loss of neurological function caused by an ischemic event with resolution of symptoms by 24 hours after onset).
  - Systemic peripheral embolism (abrupt vascular insufficiency with clinical and radiological or pathological evidence of arterial occlusion of a vascular bed other than cerebrovascular system in absence of other mechanisms (e.g. atherosclerosis).
- History of hemorrhage
  - Intracranial hemorrhage (hemorrhagic conversion of a primary ischemic stroke, subarachnoid hemorrhage, intracerebral hemorrhage, subdural and epidural hematomas or unknown)
  - Other major bleeding
- Concomitant cardiovascular disease; vascular disease defined as coronary artery disease (defined as prior acute coronary syndrome, coronary artery revascularization, stenosis on angiography $\geq$ 50%, significant stenosis on CT, myocardial ischemia on exercise test or PET) or PAD (peripheral artery disease) or complex aortic plaque; hypertension defined as $\geq$135 and/or $\geq$85 mmHg at home or $\geq$140 and/or $\geq$90 mmHg in office; heart failure (HFrEF defined as HF with reduced left ventricular EF (LVEF) of $\leq$40%; HFmrEF, HF with mildly reduced

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LVEF of 41-49% and HFpEF, HF with preserved LVEF of ≥50%; see Appendix 8:7); valvular heart disease (documented moderate or severe stenosis or regurgitation); dilated cardiomyopathy (left ventricular systolic dysfunction, LVEF< 0.45); and supraventricular tachycardia.

- Other diseases; diabetes mellitus; chronic lung disease; thyroid disorder; chronic kidney dysfunction; alcohol consumption and smoking.

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4.4.2. Allocated treatment

The allocated therapy should be initiated or performed after the two months run-in period within maximum 2 weeks after its' ending in all patients.

4.4.2.1. Antiarrhythmic drug treatment group

The AAD therapy should be prescribed by the local investigators and collected by the subjects at a pharmacy as in normal clinical practice. They should be initiated within maximum 2 weeks after the end of the 2 months run-in period with either dronedarone or flecainide as first options, considering possible contraindications and side-effects in the individual patient, with dosages outlined in AF guidelines[12] and in separate Summary of Product Characteristics (SmPC) Appendix 1-4:

Dronedarone: - 400 mg twice daily.

Flecainide: - (50-)100 (-200) mg twice daily or slow release (100-)200 mg once daily.

The 3-month "blanking period" will allow for drug titration and optimization. A change to the other of the two first option AADs (unless contraindicated) should be guided by a lack of efficacy as defined by the patient's AF related symptoms or presence of intolerable side effects. The AAD should be titrated to maximum tolerated dose in case of clinical inefficacy. Thereafter, other AADs may be tested (unless contraindicated) in order; propafenone and sotalol as long as the medication changes is within the 3 months blanking period:

Propafenone: - 150 mg three times daily increasing to 300 mg twice daily, if necessary max 300 mg three times daily. Dose reduction for patients <70 kg bodyweight.

Sotalol: - 80 mg twice daily up to 160 mg twice daily. Dose reduction to half dosage if a creatinine clearance is 30-60 ml/min.

Once the blanking period has ended, a change in medication will be defined as clinical endpoint, i.e. treatment failure.

Surveillance of proarrhythmia risk should be undertaken by analysing ECG for prolonged corrected QT interval (QTc), widened QRS, and prolonged PR interval at each follow-up. Specifically, ECG monitoring on days 1-3 in patients prescribed flecainide, propafenone, or sotalol is recommended since proarrhythmic events tend to cluster shortly after drug initiation, especially if a loading dose or a change in usual dosage is prescribed.

Cardioversion should be performed as soon as possible with adequate protection against thromboembolism if at risk.

Physicians are advised to keep patients in the same treatment arm during the advocated follow-up period. If symptoms worsen despite having tested and failed at least one AAD therapy due to limited efficacy at adequate dosages, the patient may, if requested, undergo AF ablation with PVI following preferably a minimum of 12 months AAD therapy. If AF ablation is requested by the patient, all end-points will be evaluated prior to requested AF ablation. If a patient requires AF ablation it will be classified as treatment failure. Atrioventricular-node ablation and implantation of a pacemaker is an option if preferred by the patient.

Patients developing intolerable adverse reactions will be treated individually with dose reduction or change of drug according to defined recommendations, as outlined in separate Summary of Product Characteristics (SmPC) Appendix 1-4 and in Section 4.5.3.2-3 Criteria for temporary and permanent termination of study intervention. Temporary discontinuation of the allocated drug therapy is permitted for a period of 10 days. Indications for permanent discontinuation of study drug are outlined in section 4.5.3.2-3.

4.4.2.2. Catheter ablation strategy

The allocated PVI procedure must be performed within maximum 2 weeks after the end of the 2 months run-in period.

Pre-ablation management.

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Patients randomized to ablation who are not already on oral anticoagulation (OAC) will be initiated on NOACs as first choice at least 3 weeks prior to scheduled ablation or earlier if indicated. If warfarin is preferred, it should be within therapeutic range INR 2-3 at least 3 weeks prior and during the ablation procedure. Continuous oral anticoagulation (the morning NOACs dose may be omitted on the ablation day and given some hours after ablation according to local routine) is advocated during the procedure with addition of heparin during the ablation procedure. Pre-procedure transesophageal echocardiograms (TEE) will be performed 1 day prior to the procedure according to local practice and published AF ablation guidelines, to exclude atrial thrombus or other structural contraindications to the procedure. Contrast Tomography (CT) or Magnetic Resonance imaging (MRI) will be performed at the discretion of the investigator.

Pulmonary vein isolation

A multipolar 5-6 F catheter is placed in the coronary sinus (cs). Transseptal puncture will be performed according to local tradition. Following transseptal puncture, intravenous heparin is administered and adjusted to maintain an activated clotting time (ACT) 250-350 sec for the duration of the procedure. ACT levels should be checked every 20-30 minutes.

PVI alone will be performed using a 28 mm cryoballoon (Arctic Front Advance^{R}, Medtronic) equipped with an Achieve circular mapping (ACM) catheter for recording of pulmonary vein potentials, and advanced to the left atrium through a 15 Fr steerable sheath (Flexcath^{R}, Medtronic). A smaller 23 mm balloon may be used based on physicians judgement after failure of the 28 mm balloon or if exceptionally small PV diameters.

The ACM will be positioned in the PV for recording of PV potentials and may be advanced further if required for stability. The cryoballoon will be positioned in the PV ostium and the degree of occlusion will be judged by visual evaluation of diluted contrast injection or PV pressure monitoring. Optimal occlusion should always be the goal.

The integrity of the phrenic nerve should be checked during each cryo application in right PV with a technique routinely used by the centre (phrenic nerve pacing or regular deep breaths). Ablation should be stopped immediately upon any perceived reduction in the strength of diaphragmatic contraction.

Cryoballoon application for 4 minutes duration in each vein will be used and should be performed guided by the disappearance of PV potentials from the Achieve catheter or by reduction of the temperature to at least minus 40 C degrees within the first 120 seconds after start of freezing if no PV potentials can be visualized. If the PV potentials are lost from the Achieve catheter or the temperature is reduced to at least - 40 C degrees within 60 sec or if the temperature is exceptionally low, at least < -60 C, the total duration may be shortened to 3 minutes at the discretion of the investigator. If the stipulated goal cannot be reached the ablation should be terminated and balloon repositioned and a new lesion given.

Once PVI is achieved, a bonus application is recommended if outlined criteria above are borderline or could not be reached within stipulated time. A 2nd cryoapplication may thus be given if indicated by delayed disappearance of PV potentials or borderline freeze temperature. No safety cryoapplication is needed if PVI is achieved as stated above. The stipulated cut-offs for times and temperatures does not apply to PV with common os.

If the operator fails to isolate the PV, new attempts with the 23 mm cryoballoon or with an 8 mm Cryocath catheter (Freezor Max) may be used to target the PV. If that fails – the patient is defined as a failure but will still be followed.

Entrance block

After each application, PV entrance block should be assessed by recording from the Achieve catheter.

After ablation of all pulmonary veins, entrance block during sinus rhythm should be assessed with PV entrance block reconfirmed and *documented* from each PV using either the Achieve

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catheter or a circular mapping catheter. If the patient is in atrial fibrillation, a cardioversion with either an AAD iv or using electrical cardioversion is required, for evaluation of entrance block. Entrance block should be assessed during distal CS pacing for left PV and during SR/right atrial pacing for right PV showing absence of conduction into PV from LA. Exit block is optional and may be added by pacing from the ACM catheter at high output 5mA from 4 PV quadrants with absence of conduction from PV to LA/CS. A waiting period to confirm electrical isolation of all pulmonary vein is not required. The endpoint for ablation is complete entrance block for all pulmonary veins during sinus rhythm. No other testing of PVI such as adenosine testing should be performed as they are of limited value. No other LA ablation lesions are permitted.
For patients in AF from the start of the procedure or if AF is initiated during the procedure electrical or pharmacological cardioversion should be performed ensuring sinus rhythm at the time for confirming entrance block in all veins.

Cavotricuspid isthmus (CTI) ablation

May be performed in addition to PVI provided the patient has had a CTI dependant flutter documented on 12 lead ECG. The timing for the CTI ablation will be recorded separately, as will the radiation dosages, starting from the introduction of flutter catheter.

Fluoroscopy exposure

The cumulative dose-area product (DAP, expressed in mGy.cm^2 or Gy.cm^2) (defined at each centre) will be recorded for each plane in case a biplane lab is used. The extra exposure during an additive tricuspid isthmus ablation will be recorded separately.

Blood sampling

Biomarkers s100b, troponin T (TnT) and NT pro-BNP will be obtained from peripheral venous blood in the lab on the day of the procedure (baseline) before placing the catheters in the heart and again after all ablation applications after the transseptal catheter has been withdrawn. All three markers are thereafter sampled a third time from peripheral blood after 6-18 hours, and NT pro-BNP at 3, 12, 24, 36 months follow up.

4.4.2.3. Postablation management.

Continuation of systemic anticoagulation postablation is recommended for 2 months but should be continued if indicated based on the patient's stroke risk. It should not be based on the perceived success or failure of the ablation procedure.
Cardioversions should be performed as early as clinically possible if AF recurs after an ablation procedure during the hospital stay and at latest before discharge.
A blanking period of 3 month post ablation is used according to published recommendations.[1]

4.4.2.4. Repeat AF ablations

Re-ablations may be performed at earliest 3 months after the prior AF ablation procedure if symptoms persists or recurs provided an AF/AT is ECG documented. The repeat PVI should be performed without delay within the next 4 weeks once the decision has been made. PVI alone will be performed using a cryoballoon following re-mapping of previously treated PV with a circular mapping catheter for assessing entrance conduction block as described above.
In case the patient suffers from symptomatic AF recurrences after a 2nd PVI procedure, patients may be offered a 3rd AF ablation procedure provided there is documentation of AF recurrence and recurrence of symptoms. In case the patient declines a repeat intervention despite symptomatic recurrence of AF, the patient may be offered an AAD, and will be defined as failed ablation unless a beta-blocking agent alone is prescribed.
If all PVs are found to be isolated at a repeat ablation procedure, other lesions may be performed at the discretion of the investigator, except for posterior wall isolation unless pulsed

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field ablation is used recognizing the increased risk for gastric hypomotility[38] and other complications, but treatment will be defined as failed PVI.

4.4.3. Follow-up

Patients will be followed at three, six, nine, 12, 18, 24, 30 and 36 months after the ablation procedure or start of AAD therapy. The intervals for visit and investigations are outlined in Table 1. A nurse telephone visit will be scheduled at 6, 9, 18 and 30 months for recording of clinical events, medication and adverse events.
A clinical event is any event related to a cardiovascular disease or AF/AT; such as acquisition of a new comorbidity, new risk factor that results in increased bleeding risk, new cardiovascular intervention (ablation and new AAD will be recorded in separate files) such as cardioversion, angiography, bypass surgery etc but which is not defined as an AE.
Recurrence of AF / AT should be documented on ECG but are not defined as clinical failures within the three month blanking period after an ablation procedure or start of AAD strategy.
Patients who require coronary artery bypass surgery (CAGB) or pacemaker (PM), or treatments for other comorbidities will be treated as deemed necessary and remain in the study.
All endpoints will be evaluated at last visit before cross-over. All side effects will be carefully monitored at each visit.

4.4.3.1. Cardioversion

Cardioversion of AF should be performed without delay within 48 hours after AF onset according to ESC AF Guidelines in order to prevent atrial remodeling, provided there is adequate anticoagulation therapy if needed. Cardioversion may be attempted either electrically or pharmacologically using recommended drugs at the discretion of treating physician. Electrical direct-current cardioversion should be conducted using biphasic shocks with adequate energy levels.
Definition of successful cardioversion; Cardioversion is successful when AF has been terminated and at least one beat of an atrial rhythm has been recorded.
Cardioversions should be documented with established rhythm on ECG strip.

4.4.4. Definitions of withdrawals from study / study termination

- A supraventricular reentry tachycardia or an atrial tachycardia as the primary trigger mechanism of AF, not detected prior inclusion.
- Withdrawal of consent.
- Patient lost to follow-up before 12 months.
- Any patient who refuses to undergo follow ups.
- Patients developing serious disease states or conditions that impose a discontinuation of the study prior to 12 months follow-up, as judged by the investigator. The decision of withdrawal should be discussed with the Steering committee.
- Violation of the protocol if a continued participation is medically unjustified.
- An investigator may withdraw a patient from the study anyetime if it is in the patient's best interest.

4.4.5. Criteria for stop of study intervention and stop of trial

4.4.5.1. Criteria for temporary/permanent stop of study intervention

Individual subjects and specific sub-groups allocated to study AAD arm
If the PR-interval prolongs with > 50%, QTc interval reaches > 500 ms, or the QT prolongation exceeds 50 ms after AAD initiation, the study drugs will be titrated downward or temporarily stopped. Inability to bring the QTc interval in a range < 500 ms will lead to withdrawal of the drug, and recorded as adverse event.

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Patients developing intolerable adverse reactions on study AAD will be managed with dose reduction, temporary stop or permanent termination with change to another AAD, at the discretion of the investigator. Temporary discontinuation of allocated AAD therapy is permitted for a period of 10 days.
Permanent termination of study drug is imposed if Torsade de Pointes/sustained ventricular tachycardia/ ventricular fibrillation, AV block III (excluding transient vagal episodes), heart failure (NYHA class III and IV), persistent severe liver or kidney dysfunction (depending on drug), or any other serious adverse event attributable to study drug necessitating intervention of, develop in any patient.
Individual subjects and specific sub-groups allocated to AF ablation arm
Any acute serious adverse event related to the ablation procedure necessitating intervention should lead to termination of the procedure. Such event include the development of Torsade de Pointes/sustained ventricular tachycardia/ ventricular fibrillation, AV block III (excluding transient vagal episodes), severe heart failure/pulmonary oedema, bronchospasm, tamponade, acute myocardial infarction or unstable angina pectoris, cerebrovascular accident, major bleeding, injury to a cardiac valve, or any other serious adverse event attributable to the procedure. Whether a repeat procedure at a later stage can be undertaken will follow clinical routines at the discretion of the investigator.

Patients who require coronary artery bypass surgery (CAGB) or pacemaker (PM), or patients who develop reduced thyroid function during the study, will be treated as deemed necessary and not withdrawn from the study.

4.4.5.2. Criteria for termination of the whole clinical trial.

The trial may be prematurely terminated due to slow recruitment rates despite serious attempts to increase inclusion rate of more centers and patients for at least 2 years. The decision will be discussed with the Steering Committee, CEC and DCMB. Other unpredicted reasons for study termination should be discussed with the Steering Committee, CEC and DCMB.

4.5. Safety

All “Serious Adverse Events” will be documented on the "Adverse events Form" AND "Serious adverse events Form" and reported to an independent Clinical Events Committee. All deaths events will be documented on “Death report forms”.
All-cause death will be classified in the following groups:
1. Non-cardiovascular, including unknown, excluding sudden death.
2. Cardiovascular death
    a. Cardiac (sudden (including arrhythmic, myocardial infarction) vs non-sudden)
    b. Vascular (e.g. embolic, stroke, other): sudden vs non-sudden

SAE and deaths will be classified if related to allocated therapy, i.e. antiarrhythmic drug treatment- or procedure-related

4.5.1. Serious Advers Events

Defined as events that results in i) permanent injury or congenital anomaly/birth defect, ii) death, iii) is life-threatening, iv) medically important event that requires interventions for treatment to prevent other serious outcomes listed here, or v) prolongs or requires hospitalization,
Description of SAE (diagnosis rather than symptoms)
1. Sustained VT/ TdP /VF,
2. 1:1 AV conducting atrial flutter causing syncope or hemodynamic compromise
3. Asystoli requiring treatment and AV block III (excluding transient vagal episodes).
4. Heart Failure of NYHA class III and IV, pulmonary oedema,

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5. Acute myocardial infarction or unstable angina pectoris
6. Stroke, TIA or systemic embolism
7. Pulmonary embolism
8. Major Bleeding (defined as fatal outcome; clinically overt bleeding causing a fall in hemoglobin concentration of ≥ 20 g/l (1.24 mmol/L) or leading to transfusion of ≥ 2 units of whole blood cells; bleeding in areas of concern (retroperitoneal or intracranial hemorrhage, intraspinal or intraocular); or bleeding leading to treatment cessation and/or surgical intervention.)
9. Vascular injury
10. Injury to a cardiac valve that results in a significant change of valve function.
11. Pericardial effusion with or without tamponade, requiring treatment
12. Pulmonary vein stenosis, significant if lumen reduction of ≥ 50 %.
13. Permanent phrenic nerve paralysis (defined as permanent if not resolved by 12 months)
14. Atrio-esophageal fistula
15. Gastroparesis or gastric hypomotility requiring treatment
16. Adverse reactions/complications necessitating intervention of or discontinuation of AAD therapy or interruption or intervention of catheter ablation.
17. Infection resulting in ICM explantation.
18. Other -specify

Interventions related to SAE should be stated and described.

4.5.1.1. Definition of Major AF Ablation Complications

Complications arising during or within 1 month of the procedure, including death, major bleeding, vascular injury, infection, thromboembolism, cerebrovascular accident, pericardial effusion with or without tamponade, cardiac valve injury, pulmonary vein stenosis, atrio-esophageal fistula and oesophageal injury, venous embolism, phrenic nerve palsy that remains at discharge, gastroparesis, and heart block.
Events occurring > 1 month after the procedure defined as related to the procedure are pulmonary vein stenosis, tamponade, retroperitoneal hematoma, and atrio-esofageal fistula (see below).

4.5.1.2. Definition of study AAD related complications

These are side effects known to be related to a specific AAD, including ventricular proarrhythmias and conduction disorders which are specified in separate Appendices for each drug Summary of Product Characteristics (SmPC) 1-4.

4.5.1.3. SAE relation to Device Procedure or Drug

1. **Not related (none):** any adverse event that is not associated with the ablation procedure or antiarrhythmic medication by timing or pathophysiology
2. **Possibly related (possible):** any adverse event determined to be possibly associated with the use of the ablation catheter, mapping system, catheterization, or drug as above.
3. **Related (probable):** any adverse event that is associated with the use of the ablation catheter, mapping system, catheterization, or drug and the investigator believes was caused or contributed to by these procedures.

4.5.1.4. Outcome or consequence of Adverse event;

1. Resolved.
2. Resolved with sequale
3. Not resolved, event ongoing.

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4. Fatal
5. Unknown

All evaluations are blinded regarding: endpoint analysis of 12-lead ECGs, rhythm from ICM, questionnaire for QoL, symptoms and cognitive function, and analysis of echocardiographic measurements in core echo laboratories.

4.5.2. Benefit risk assessment

The risks that the study may entail for patients' health, safety and personal integrity are well balanced related to its scientific value with potential benefits to a larger number of patients who in the future can may receive ablation as first-line treatment. The risk related to randomized treatments does not exceed the risk related to procedures or medication used in clinical routine. Patients may be allocated to an AF ablation procedure earlier in the course of their disease than what is currently the clinical practice, i.e. without having tried an antiarrhythmic drug. This group would be exposed to the risks associated with catheter ablation but is on the other hand not exposed to the risks associated with arrhythmic drugs, which has been recognised to be as high as for catheter ablation in randomized trials. Randomized trials conducted in patients with paroxysmal AF reported no difference complication rates between the two treatment strategies.

5. STUDY MONITORING AND AUDITING

5.1. Source data verification and on-site audits

A monitor will verify that data recorded on the case record forms (CRF) are correct through quarterly communication, review of catheterization reports and medical records.
In accordance with applicable regulations, GCP, a monitor (study nurse from Örebro) will contact the site prior to the start of the study to review with the site staff protocol, study requirements and their responsibilities to satisfy study requirements, if needed. When reviewing data collection procedures the discussion will also include identification, agreement and documentation of data items for which the CRF, patient files, tracings of investigations, and questionnaires, will serve as source document. The investigator and the head of the medical institution allow the monitor direct access to all relevant documents whenever needed and in an event of an audit.
The monitor will monitor the study and site activity to verify the following:

1. Data are authentic, accurate and complete.
2. Safety and rights of subjects are being protected.
3. Studies conducted in accordance with the currently approved protocol and any other study agreements, GCP and all applicable regulatory requirements.

To ensure compliance for the GCP and study protocol the monitor may conduct a quality assurance audit. Such audits/inspections can occur at any time during or after completion of the study. If an auditor inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and relevant issues.

6. DATA MANAGEMENT

The Data Management section at the UCR will be responsible for Data Management including a Data Management Plan (DMP) and a Data Validation Plan (DVP).

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All data will be recorded on CRFs and / or entered directly via eCRF into a Viedoc database, including 12 lead ECGs saved in the MUSE GE database except for electronically available data that will be loaded directly into the study database (ICM transmission). Data from the quality of life questionnaires will be recorded in separate documents attached to the CRFs. Each investigational site will have authorized site personnel responsible for entering the data, as well as for changing and correcting the data according to instructions provided by Uppsala coordinating centre. All changes will be tracked by an audit trail. All inconsistencies detected will be resolved by the monitor or site personnel. The site specific investigator will sign the eCRF electronically when the data have been reviewed and edited, and Source Data Verification has been performed.

6.1. Source Data

Data reported on the CRFs that are transcribed from source documents must be consistent with the source documents. The source documents are filed at each Investigators site.
Source data consists of:

1. Signed copy of patient file (regarding hospitalizations, outpatient visits, health care provisions from other regions, study visits, adverse events etc).
2. Investigation reports: ECG, echocardiography, ablation procedure, but not ICM transmission
3. Patient questionnaires: EHRA symptoms, QoL questionnaires (EQ-5D. Toronto AFSS, and SF-36 (unless filled in directly into Viedoc).
4. Laboratory test results including local reference values.

For all other data the CRFs will constitute the source document.

6.2. Database Closure

The Database Closure will be performed in three steps, the first one when all data from the 12 months are entered, the 2nd when all data from the 24 months period are entered and the 3rd when the complete study period of 36 months are entered.
The procedures below will be followed at all occasions but the randomization code will only be loaded at the first occasion. All datasets used for the 12- and 24 months analyses will be locked separately.
Procedures: When all data are entered into the database and all queries solved, the Database Closure procedures will start. Decisions will be made how to classify patients into analysis populations, and how to handle protocol violations and deviating or missing data. Efforts will be made to maintain the blinding while these decisions are made. All decisions will be dated and documented in a Database Closure document. After that the database will be locked and the randomization code will be loaded.

7. STATISTICAL METHODS

The Biostatistics section at UCR will be responsible for the statistical analysis and will write a study specific Statistical Analysis Plan (SAP) where further details will be specified. The Electronic Data Capture (EDC) system Viedoc will be used for eCRF and randomization.

7.1. Objectives and Endpoints

The objective of the study is to compare the effects of two first line treatments, pulmonary vein isolation using the cryoballoon and antiarrhythmic drug therapy, in patients with non-longstanding persistent atrial fibrillation.

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7.1.1. Primary objective and endpoint

The primary objective of the study is to determine if first-line PVI is superior to first-line antiarrhythmic drug therapy in preventing atrial tachyarrhythmia lasting ≥ 6 minutes in patients with symptomatic recurrent non-longstanding persistent AF.
Primary endpoint: Freedom from atrial tachyarrhythmia as measured by the 12-lead ECG, ECG rhythm strip, Holter, or ICM, from initiation of treatment excluding the first 3 months (blanking period) to 12 months post randomization.

7.1.2. Secondary objectives and endpoints

The secondary objectives are to compare the effect of the two first-line treatment strategies with respect to the following endpoints during the total study period of 36 months in patients with symptomatic non-longstanding persistent AF at 12, 24, and 36 months (if not otherwise specified).

1. Total atrial arrhythmia burden (% time in AF/AT)
2. AF progression and reversion as measured by combination of reduced number of AF/AT progressions or increased number of AF/AT reversions after 3 months blanking. Progression or transition to more severe AF forms such as longstanding persistent or permanent AF and AF regression as going in the opposite direction from persistent to paroxysmal to sinus rhythm at 12, 24 and 36 months.
3. Healthcare utilization for cardiovascular reasons (number of cardioversions, ablations, AAD initiations, cardiovascular hospitalizations, emergency department visits and unplanned outpatient visits after 3 months blanking) and its relation to AF/AT burden. Cardiovascular means related to atrial fibrillation including any treatment or diagnostic procedure for AF/AT such as cardioversions, medication, further AF ablations after AF recurrence, or any adverse events related to AF or its treatment such as thromboembolic complications (acute stroke), heart failure, myocardial ischemic events; adverse events (eg. pacemaker implantation).
4. Health care costs will be assessed at 36 months. Quality-adjusted life years (QALYs) using the EQ-5D over 3 years will be estimated from serial utility measurements from the Swedish population valuation of the EuroQoL EQ-5D at randomization. Health care use and cost-effectiveness, including CV hospitalization, outpatient visits, treatment costs, with corrections for background variables regarding social economic status. The time spent on sick-leave, duration and cost for not being at work will be retrieved from the sickness insurance institution, by informed consent from the patient, if necessary.
5. Single and multiple procedure success (freedom from ECG documented atrial tachyarrhythmia after the 1st and last ablation procedure respectively).
6. Frequency and type of serious adverse events.
7. Frequency of withdrawals / ‘cross-overs’ over time.

The following endpoints are defined as change from baseline to each of months 12, 24, and 36 months if available.

8. Quality of Life measured by SF-36, EQ-5D and AFSS (see Appendix).
9. EHRA Symptom Classification (see Appendix).
10. Cognitive function as measured by Trail Making Test A och B (TMT A och B).
11. Blood pressure, systolic and diastolic (mmHg) after 10 minutes rest.
12. P wave duration from ECG.
13. NT pro-BNP, IL6, D dimer.
14. Proportion of patients with reverse atrial remodelling as defined by ≥ 15 % decrease in left atrial volume index, increase of left atrial strain during reservoir phase (percentage) or left

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atrial strain during contraction phase (percentage) from echocardiography and corrected for BSA at 12, 24 and 36 months.

15. Covariate adjusted primary endpoint (analysis using following covariates at baseline: coronary artery disease, hypertension, LAVI).

Logistic multiple regression analysis to identify baseline predictors of freedom from AF/AT episodes duration > 6 minute after last therapy, respectively (yes/no) including NT-proBNP, LAVI, atrial strain, AF history duration, P wave duration on 12 lead ECG and the AF diagnosis-to-ablation/AAD time (DADT) (time from first diagnosis of AF to first AF ablation/AAD therapy), and in the ablation group only, levels of and increase of s100b after PVI (see appendix) by treatment group at 24 months and 36 months.
All endpoints will be evaluated at last visit before cross-over

7.2. Statistical hypotheses

The null hypothesis (H_0) is that the difference in proportion of primary variable at 12 months between the two treatments is zero;

- Proportion of subjects with the primary endpoint in the catheter ablation group (μ_1)
- Proportion of subjects with the primary endpoint in the optimized conventional pharmacological therapy group (μ_2)

The alternative hypothesis (H_1) is that there is a non-null difference in the proportions for the primary endpoint at 12 months between the two treatments groups:

H_0: $\mu_1 = \mu_2$

H_1: $\mu_1 \neq \mu_2$

The test of the hypotheses will be a logistic regression with pre-specified covariates (see below).

7.3. Statistical analysis

7.3.1. Analysis populations

Safety - All randomized patients will be included in the safety analysis. Only observed observations are used in the safety analysis.
Modified Intention to treat (mITT) - All randomized patients who receive treatment.
Per protocol (PP) – All randomized patients completing the study treatment period of 12 months without any major protocol violation (for example ineligibility, early withdrawals, poor compliance). The PP population will be defined at clean file.

The main analysis will be performed on the m**ITT**-population.

7.3.2. Missing Data

The Primary variable will be imputed using a "worst case imputation" for the Logistic Regression if the magnitude of the missing data is small, say, approximately 5%. The Statistical Analysis Plan will include an outline for the multiple imputation strategy to be used for the situation with a larger amount of missing primary endpoints.

The hypothesis generating analyses of Secondary Variables will use a simplistic Last Observation approach in case the amount of missing data is limited, say, less than 5%. The

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SAP will include an outline for the multiple imputation strategy to be used for the situation with a larger amount of missing secondary endpoints.

7.3.3. Subgroups and explorative analyses

Explorative analyses may be performed to investigate relationships between treatments and endpoints, and for predictive factors for AF recurrences.

7.4. Statistical methods

The Primary variable, freedom from atrial tachyarrhythmia at least 12 months with 90-day initial blanking period, will be analyzed using a Logistic Regression adjusted for the following additional covariates (baseline values):

1. Coronary artery disease
2. Hypertension
3. LAVI

Graphical methods such as Kaplan-Meier plots will be used to visualize the treatment effect in the full study population: “Freedom of tachyarrhythmia up to 12 months with 90-day blanking period”.

The main analysis population will be a modified Intention to Treat population: all treated patients as randomized. The number of randomized subjects who are not treated are assumed to be at most a handful. The Per Protocol population will be defined at the Clean File Meeting and will be used for sensitivity analysis for the analysis of the primary variable using the same methods as for the main analysis.

The set of secondary variables will not be adjusted for multiplicity. All findings among the secondary variables in the main population will be viewed as exploratory and hypothesis generating. The statistical Methods for each of the Secondary Endpoints will be described in detail in the Statistical Analysis Plan.

All continuous variables will be presented per treatment group using descriptive statistics by mean, SD, max and min values, in addition medians, 25th and 75th percentiles will be presented when suitable. The mean difference between treatment groups will be presented with 95% CIs.

7.5. Sample Calculation

The study will be randomized 1:1:
Proportion of freedom from atrial tachyarrhythmia before 12 months with 90-day blanking period is estimated to reach 55% in the Ablation group based on previous cryoballoon AF ablation study of persistent AF patients monitored by ICM[39] versus 30% in the Drug arm, based on the 25% difference in freedom from AF between treatment groups in a first line treatment study of paroxysmal AF patients.[2] The 40% freedom from AF postablation in persist AF patients in Wechselberger[40] trial is judged too pessimistic for a first line trial. Three prior AAD trials evaluating drug efficacy in persistent AF patients post cardioversion, using intermittent recordings, reported freedom from AF ranging between 30 and 42 %.[17],[18],[19] Recognising that ICM would detect more AF episodes, the freedom from AF post cardioversion on AAD at 12 months is therefore estimated to be in the lower range, i.e. 30 %, in this trial.
A 2 minutes AF detection cut-off has limited clinical meaning, is quite rare in patients with exclusive short episodes, and has a lower positive predictive value versus a 6 min cut-off episode duration[40]. A 6 min versus a 2 minute cut-off episode duration will decrease number of total episode count and false positive detection without neglecting relevant clinical

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information[40], keeping AF-burden unaffected[40]. It therefore seems rational to use 6 minutes as AF episode cut-off, which is anticipated to at most increase the freedom from AF by 5%[40] in both treatment groups but without affecting the relative treatment differences in the 2 groups.

To have a 90% chance (i.e. power=90%) at the 5% significance level of detecting an improvement in the primary outcome from 30% in the control group to 55% in the ablation group, a total of 156 patients are needed.
Adjusting for an assumed cross over rate of approximately 10% in the drug arm and 5% in the Ablation arm the total sample size will be 220.

7.6. Randomization and blinding

The treatment allocation sequence is generated in SAS or R by the Biostatistics section at UCR by using permuted blocks and 1:1 allocation, with stratification by center and type of persistent atrial fibrillation (classical persistent AF as defined by guidelines and persistent AF progressed paroxysmal AF). Patients will be randomized using the **Interactive Web Response System** (IWRS) function in Viedoc.

8. Committees and Boards

8.1. Ethics Review Authority

The coordinating centre will apply to the Ethics Committee and the study will be conducted according to the Declaration of Helsinki. Patients will sign a written informed consent.
The trial protocol will be available at European Clinical Trials Database; https://www.clinicaltrialsregister.eu and at http://ClinicalTrials.gov.

8.2. Steering Committee

The trial Steering Committee (SC) consists of a group of expert cardiologists, and an expert biostatistician. The functions of the SC are:

1. Overall responsibility for the execution and scientific reporting of the trial.
2. Advice on the scientific and clinical aspects of the study protocol and related documents.
3. Responsibility for the conduct of the study according to the guidelines of good clinical practice (GCP) including the monitoring of patient recruitment.
4. Reassessment of sample size based on the blind review of the biostatistician if needed.
5. Reassessment of benefit/ risk ratio following the recommendations of the CEC/ DSMB.
6. Decisions on continuation or termination of the study based on the recommendations of the CEC/DSMB.
7. Meeting frequency will be defined by the committee and may vary depending on tasks. Meetings may be conference calls or face-to-face meetings. Minutes of each meeting will be provided.

8.3. Clinical Events Committee (CEC)

The clinical events committee will review and adjudicate all serious adverse events (SAE). It will be blinded to the treatment arm when reviewing the study outcome events. See Charter for CEC in separate Appendix 5

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8.4. Data and Safety Monitoring Board (DSMB)

The DSMB consists of experts that advises, provide expertise and recommendations to the SC and study investigators. It consists of one statistician and two clinicians with expertise in clinical trials and in the management of patients under investigation.
The members of the DSMB regularly monitor SAEs and further AEs selected to their discretion during the course of the trial.
Meetings may be conference calls or face-to-face meetings and may have an open part with guests and a closed part. Minutes of each meeting will be provided. After each meeting, recommendations will be given to the SC in a written form. See Charter for DSMB in separate Appendix 6.

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8. Appendices

8.1. Symptom questionnaires

8.1.1. Modified European Heart Rhythm Association (EHRA) symptom classification

	Symptom severity	**Definition**
EHRA 1	"no symptoms"	AF does not cause any symptoms
EHRA 2a	"mild symptoms"	normal daily activity not affected by symptoms related to AF
EHRA 2b	"moderate symptoms"	normal daily activity not affected by symptoms related to AF, but patient troubled by symptoms
EHRA 3	"severe symptoms"	normal daily activity affected
EHRA 4	"disabling symptoms"	normal daily activity discontinued

The classification is performed by the investigator. EHRA class 2a and 2b can be differentiated by evaluating whether patients are functionally affected by their AF symptoms.
The following items *during presumed arrhythmia episodes* are checked to determine the score: Palpitations, fatigue, dizziness, dyspnea, chest pain, anxiety.

8.1.2. Patient's estimate of frequency and duration of symptomatic episodes:

Frequency: patient estimate of number of symptomatic episodes **per month** (if for example 2 episodes last 6 month – it will be 2/6)

- Duration: patient estimate of duration of most common symptomatic episodes:
  - ≥ 6 minutes – 10 minutes
  - >10 minute to < 1 hour
  - 1 hour to < 6 hours
  - 6 hours to < 12 hours
  - 12 hours to ≤ 23 hours
  - 24 hours – 7 days
  - > 7 days
  - Cardioversion required – never spontaneous conversion within 7 days

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8.2. Quality of life (SF36, AFSS, EQ-5D)

8.2.1. SF36 and Toronto AFSS - see separate pdf files.

8.2.2. EQ-5D-5L

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

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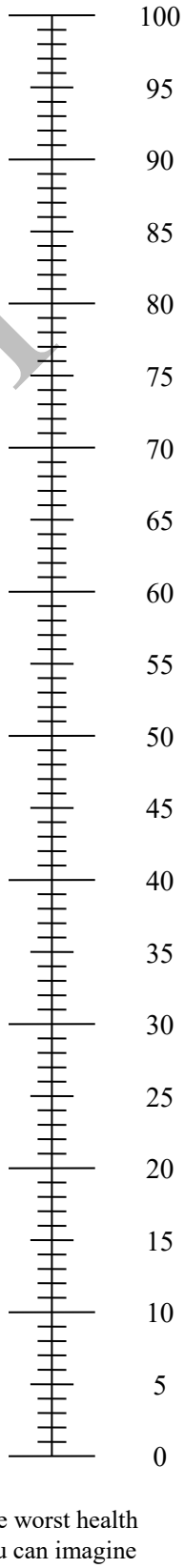
- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Please mark an X on the scale to indicate how your health is TODAY.
- Now, write the number you marked on the scale in the box below.



YOUR HEALTH TODAY = ☐

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8.3. Arrhythmia definitions and analysis

Rhythms are classified as sinus rhythm, atrial fibrillation, tricuspide isthmus (TCI) dependant atrial flutter (defined as typical saw-tooth pattern in inferior leads), other atrial tachycardia (AT), atrial pacing, ventricular pacing, from 12 lead ECG only.
Heart rhythm and rate during atrial tachyarrhythmia (AF / AT) will be assessed on 12 lead ECG and ICM, and any other non-study ECG monitoring.
Atrial flutter / post ablation intra atrial tachycardia should be confirmed on 12 lead ECGs if possible.
All rhythm tracings will be reviewed blindly by an EP physician.

8.3.1. Definitions of arrhythmias

- *Atrial fibrillation*: absence of consistent P waves before each QRS complex and an irregular ventricular rate, lasting ≥ 30 seconds to be classified as sustained AF.
    a. Classifications of AF according to ESC Guidelines 2020 is[12]:
        i. Paroxysmal: AF that terminates spontaneously or with intervention within 7 days of onset.
        ii. Persistent: AF that is continuously sustained beyond 7 days, including episodes terminated by cardioversion (drugs or electrical cardioversion) after ≥ 7 days
        iii. Long-standing persistent: continuous AF of >12 months' duration when decided to adopt a rhythm control strategy.
- *Atrial flutter* a regular atrial activity with a regularly blocked ventricular rate or 1:1 AV conduction. The AA interval may range from ≥200 to 380 ms
- *Cavotricuspid isthmus–dependent flutter circuits are electrocardiographically defined as follows;*
    a. *Counterclockwise atrial flutter:* dominant negative flutter waves in the inferior leads and a positive flutter deflection in lead V1 with transition to a negative deflection in lead V6.
    b. *Clockwise isthmus-dependent flutter will not be defined on ECG.*
- Left atrial tachycardias are characterised by negative P waves in lead aVL.
- *SVT* a narrow regular QRS complex tachycardia, with a heart rate ≥ 100 bpm.
- *Bradycardia* a three-interval heart rate less than 50 bpm during day time.

8.3.2. 12 lead ECG Recording Techniques

A 12 lead ECG should be recorded at every visit and whenever the patient reports AF/AT recurrence at a paper speed 50 mm/s (25 mm/s outside Nordic countries) preferably including rhythm strip at paper speed 25 mm/s.
The parameters to be assessed are:

- Heart rate in beats per minute
- Rhythm: as indicated above
- P wave duration (automatic by MUSE) lead II
- PQ interval (ms)
- QRS duration (ms)
- QT interval (automatic, ms), QTc interval according to automated analysis (ms)

All 12 lead ECG should be saved electronically into MUSE if available.

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8.4. Implantable cardiac monitor

Reveal LINQ (Medtronic Inc., Minneapolis, MN) has an AF detection algorithm that analyses beat-to-beat variability of cardiac cycles over a 2-minute window ECG strip. The algorithm, based on R-R interval stability and P-wave detection, has an overall accuracy of AF detection of 99.4%. For the study the cut-off level is set to 6 min so that the minimum length of an ICM-detected AF/AT is 6 minutes.
The device is capable of determining the timing of occurrence of arrhythmia, quantifying the amount of AF per day - the overall AF burden (percentage of observed time in AF).
The patient is asked to activate the device manually at the time of the first event of AF symptoms after allocated treatment; AAD or ablation intervention.
All ICMs should be inserted over the fourth intercostal space, aiming for R wave amplitude >0.2 mV.

Device programming
Standardized settings of AF/AT detection.
The ICM will be programmed to the "AF ablation" setting, which automatically programs the device to:

- AF detection: on;
- AF detection threshold (Sensitivity) balanced;
- Ectopy rejection - Nominal;
- AF episode storage threshold: all episodes (cut-off detection level for AF at 6 minutes).

Patients will be asked to transmit ICM data every third month to the Medtronic Care Link Network, which should be prior to a study visit.

ICM data analysis: will be done at each site with patients and physician blinded.

1. Lee R, Mittal S. Utility and limitations of long-term monitoring of atrial fibrillation using an implantable loop recorder. Heart Rhythm 2018;15: 287–295.
2. Sanders P, Purerfellner H, Pokushalov E, et al. Performance of a new atrial fibrillation detection algorithm in a miniaturized insertable cardiac monitor: results from the Reveal LINQ Usability Study. Heart Rhythm 2016; 13:1425–1430.

Variables	Unit	Description
Time to first AF recurrence	Days	Primary endpoint- (after ablation or AAD initiation)
AF episodes since last visit	No	Number of total AF episodes since last visit (every 3 months)
AF burden since last visit (% in time)	% of time	% of total time in AF since last visit (every 3 months)
AF episodes > 72 h	No	No of AF episodes that lasted given time since last visit (every 3 months)
AF episodes >48-72 h	No	As above
AF episodes >24 - 48 h	No	As above
AF episodes >12 - 24 h	No	As above
AF episodes >4 - 12 h	No	As above
AF episodes >1 - 4 h	No	As above
AF episodes >10 min - 1 h	No	As above
AF episodes 6 - 10 min	-	As above

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8.5. Biomarkers

Blood samples for cardiac biomarkers, N-terminal pro-brain natriuretic peptide (NT pro-BNP) (ng/L), inflammatory biomarkers, Interleukin-6 (IL6) (ng/L), and coagulation, D-dimer (mg/L), as well as S-creatinine and eGFR, will be obtained from peripheral venous blood at baseline and at 3, 12, 24, and 36 months follow up (Flow chart table 1). Rhythm will be recorded at the time of sampling. Blood samples will be collected by study nurses, marked with *study pat no and date-time*, managed as indicated below and stored in -20 or -70 C freezer equipped with temperature controls and alarms. All blood samples will be analysed by the clinical chemistry lab at local hospital after 12 months and at 24 and 36 months, respectively.
For the ablation group only; samples of s100b ug/L,Troponin T (TnT), ng/L and NT pro-BNP ng/L will be taken from peripheral venous blood before and after ablation in the lab on the day of the procedure;

1. The 1st sample (baseline) should be obtained before placing the catheters in the heart,
2. The 2nd sample after all PVI's are isolated after the Flexcath has been withdrawn.
3. The 3rd sample should be drawn 6-18 hours after the last cryoballoon application.

The samples will be marked with *study pat no and date-time* and *identified by timing* as follows: 1s100b, 2s100b, 3s100b, and 1-TnT, 2-TnT, 3-TnT and 1BNP, 2BNP, 3 BNP, and sent for analysis directly.

Bio-marker	Units	Sample	Sanpling tube	Processing	Centrifugation (swingoutrotor)	Method of analysis
NT pro-BNP	ng/L	Venous or capillary plasma	Li-heparin with gel or capillary sample in microtubes	5-10 tube turns after sampling. Store up-right until room reached temperature.	Centrifugation within 4 hours at 2400 g for 7 min. Separate plasma, freeze –70 ºC. Closed plasma tube may be stored in a cooler 24 h. If longer – plasma should be separated. Store in cooler	Immunochemical Alinity
IL-6	ng/L	Venous or capillary plasma	Li-heparin with gel or capillary sample in microtubes	5-10 tube turns after sampling.	Centrifugation within 4 hours at 2400 g for 7 min. Separate plasma, freeze –70 ºC.	Electrochemiluminiscens detection. Labinstrument Cobas: 1,5 – 5000 ng /L. Samples >5000 ng/L are diluted automatically 1:10 by the instrument.
D-dimer	mg/L FEU	Venous; plasma	Na-citrate 0,11 M vacuum	5 tube turns after sampling. Whole blood preserved 4 h in room temperature.	Centrifugation at 2000 g for 20 min. Separate plasma, freeze –70 ºC.	Immunologic turbidimetric
s100b	ug/L	Venous; plasma		5 tube turns after sampling.	In room temperature for 30 min before centrifugation (within 4 hours at 2400 g for 7 min). Separate plasma, freeze – 70 ºC.	Elektrochemiluminescens
TnT	ng/L	Venous; plasma	Li-heparin with gel	5-10 tube turns after sampling.	Centrifugation within 4 hours at 2400 g for 7 min. Separate plasma, freeze – 70 ºC.	
P-creatinin	µmol/L	Venous; plasma	Li-heparin with gel	5-10 tube turns after sampling.	Centrifugation within 4 hours at 2400 g for 7 min. Separate plasma, freeze – 70 ºC.	
eGFR						

FEU = fibrinogen equivalent units Freezing temperature -70 ´C according to standard temperature monitoring.

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8.6. 2-D echocardiography

Echocardiographic examinations will be made by an experienced technician/physician familiar with the protocol. The results are digitally recorded. Absolute values and values corrected for body surface area will be calculated.
Acquisitions for strain analysis should preferably be performed with GE machines, offline reviews and measurements using EchoPAC, GE software (if this requirement will exclude participation of a centre – another machine will be used). For strain measurements, make sure that frame rate is > 40/min, preferably higher.
The protocol described below will be used as a basis to ensure accurate equipment and expertise. To improve the reproducibility, all the echo examinations should be performed by the same physician/technician using the same ultrasound equipment if possible.
Record at least 5 cardiac cycles for each echocardiographic view, whenever possible without ventricular premature beats or ventricular rates > 100 beats / min. If patient is currently in AF, postpone recording few days if possible or average 5 consecutive beats.

- *Parasternal view: standard long and short axis views, Pulsed-wave (PW) Doppler of the right ventricular outflow tract.*
- *Apical views: (4, 2, apical long-axis views), including dedicated views of LV, RA, and LA.* PW mitral inflow and tricuspid inflow profile, Continuous-wave (CW) of tricuspid regurgitant flow, pulmonary venous flow if possible. CW and PW of transaortic flow, pulsed Doppler of LVOT. Tissue Doppler at basal septum and basal lateral wall of the LV in the 4CV.
    - *Doppler velocity measurements:* Mitral and tricuspid flow velocity curve with pulsed Doppler. Position the sample volume at the tip of the leaflets where velocities are highest, Pulmonary venous velocity using pulsed Doppler, Mitral and tricuspid regurgitation velocity using continuous wave Doppler.
- *Apical 2-chamber view.*
    - *Standard 2D images*; 2 chambers, Only LV,
- *Apical 3-chamber view:* Standard 2D images; 3 chambers, Only LV, Colour visualization of aortic valve regurgitation. *Doppler velocity measurements:* Aortic forward flow velocity curve with pulsed Doppler.

Left Atrial (LA) Volume & LA Volume Index;
Maximal LA volume is assessed by Simpson technique or by the biplane area-length method from apical 4- and 2-chamber views.
Two-dimensional speckle tracking echocardiography (2DSTE);
LA strain analysis by semiautomatic tracing of the endocardial border of the LA in 4-chamber and 2-chamber views. LA divided into 6 segments for each view resulting in total 12 LA segments included in the calculation of the global values. Segments should be excluded if untraceable. Left ventricular (LV) end-diastole is used as a reference point for LA strain assessments. “LA reservoir/peak strain”, max strain during reservoir phase (LASr) in monoplane and, in sinus rhythm, during contraction phase (LASct), are measured.
If AF prevails, the average of three beats should be used.
Variables;

- LVEF (modified Simpsons technique) (%).
- LV end-diastolic diameter (LVEDD) (mm):

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- LV end-diastolic volume indexed (LVEDVi) (ml/m2):
- LV mass index (LVMI) (g/m2)
- Interventricular septal wall thickness (IVS) (mm),
- Posterior Wall thickness (PW) (mm)
- LV Relative wall thickness (RWT); (2 x PW)/ LVEDD
- Mitral deceleration time (ms)
- A-wave, late mitral inflow velocity (cm/s)
- E-wave; early mitral inflow velocity (cm/s)
- E/A ratio
- E/e' ratio at rest
- e'; early peak myocardial diastolic Doppler velocities (cm/s) (measured in septal and lateral wall of mitral annulus in the apical 4-chamber view)
- Septal e' velocity (cm/s)
- V_{TR} peak velocity at rest (m/s)
- Global longitudinal strain (GLS) of the LV in systole (%, as positive value)
- LAV (Left Atrial end-diastolic and end-systolic volume, by uniplane or biplane Simpson or by biplane area-length method from apical 4- and 2-chamber views) (mL);
- LAVI (mL/m2) (left atrial max size)
- LASr, LA reservoir/peak strain (%) (preferably monoplane)
- LASct in sinus rhythm, during contraction phase (%)

Valve *stenosis, regurgitation* measured as standards. Mitral insufficiency graded I, II, III.
Significant valve insufficiency is defined as ≥ moderate insufficiency
All echocardiographical examinations will be performed at each centre but analysed by core centre at XXXX.

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8.7. Heart Failure definitions

HFrEF: Patients with a significant reduction in LV systolic function, reduced LVEF ≤ 40%.

HFmrEF: Patients with LVEF between 41% and 49%, mildly reduced LV systolic function.

HFpEF: Patients with symptoms and signs of HF, with evidence of structural and/or functional cardiac abnormalities and/or raised natriuretic peptides (NPs), and with an LVEF ≥50%

Variables supporting HFpEF are;

- LV mass index (LVMI) ≥ 95 g/m2 (Female), ≥ 115 g/m2 (Male)
- Relative wall thickness (RWT) >0.42
- LA volume index >34 mL/m2 (in presence of AF: > 40 mL/m2)
- E/e' ratio at rest ≥9
- Mitral E velocity >90 cm/s
- Septal e' velocity <9 cm/s
- V_{TR} peak velocity at rest >2.8 m/s
- Global longitudinal strain (GLS) of the LV in systole (%, as positive value) <16
- NT-proBNP >125 (SR) or >365 (AF) pg/mL

E/e' ratio; Peak early diastolic velocity of mitral inflow on transmitral Doppler/early relaxation velocity on tissue Doppler using mean value of e′ recorded at the septal and lateral mitral annulus; TR = tricuspid regurgitationE

For the diagnosis of HFpEF, the greater the number of abnormalities present, the higher the likelihood of HFpEF

Table. Definition of heart failure with reduced ejection fraction, mildly reduced ejection fraction and preserved ejection fraction

Type of HF		HFrEF	HFmrEF	HFpEF
CRITERIA	1	Symptoms ± Signs[a]	Symptoms ± Signs[a]	Symptoms ± Signs[a]
	2	LVEF ≤40%	LVEF 41–49%[b]	LVEF ≥50%
	3	–	–	Objective evidence of cardiac structural and/or functional abnormalities consistent with the presence of LV diastolic dysfunction/raised LV filling pressures, including raised natriuretic peptides[c]

Ref:

Theresa A. et al, 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, European Heart Journal (2021) 42, 3599-3726

Pieske B et al, How to diagnose heart failure with preserved ejection fraction: the HFA–PEFF diagnostic algorithm: European Heart Journal (2019) 40, 3297–3317

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8.8. Summary of Product Characteristics (SmPC)

Separate folder as Appendix I-IV.

8.9. Clinical Events Committee (CEC) and Charters for Data Safety and Monitoring Board (DSMB)

Separate folder as Appendix V and VI.

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10. SVENSK SAMMANFATTNING (Swedish Summary)

Kryoablation som förstahandsval för tidig behandling av persisterande förmaksflimmer - en randomiserad studie som jämför lungvensisolering med rytmstabiliserande läkemedel

Bakgrund: Förmaksflimmer (FF), en folksjukdom, som förutom ökad risk för stroke och dödlighet medför uttalad sjuklighet, nedsatt livskvalitet och frekventa sjukhusvistelser, vilket mer än fyrdubblats senaste 20 åren med höga kostnader som följd. Då rytmstabiliserande läkemedel f.n. är förstahandsbehandling och FF frihet endast uppnås i ca 30% vid persisterande FF är det angeläget att hitta alternativa behandlingar. Detta är särskilt viktigt då FF är en progressiv sjukdom med 15-35 % övergång till mer ihållande svårbehandlade former årligen. Ju tidigare FF ablationer utförts desto högre andel blir fria från återfall enligt registerstudier. Tidig kryoablation som förstahands terapi vid attackvisa (paroxysmalt) FF minskar FF återfall mer effektivt än rytmstabiliserande läkemedel enligt 3 randomiserade studier. Då motsvarande studier saknas för svårare mer ihållande ”persisterande” FF formen, bör detta snarast studeras.

Studieplan: Avsikten är att fastställa om förstahandsbehandling med kryoballongteknik för lungvensisolering, mer effektivt kan minska återfall i FF (primär effektvariabel) jämfört rytmstabiliserande läkemedel vid 12 månader. Patienter i åldern 18-75 år med återkommande, symtomgivande och mer ihållande ”persisterande” FF och som inte provat regelbunden rytmstabiliserande läkemedelsbehandling tillfrågas. Viktigaste exklusions kriterier är genomgången FF ablation eller kontraindikation mot ablation, uttalat förstorat vänster förmak, klaff protes, hjärtsvikt eller förväntad ökad dödlighet inom närmsta 3 åren. Patienter rekryteras från öppenvården, väntelistan för elektrisk konvertering, avdelning, samt från regionen och via annonser i dagstidning, och tillfrågas för lottning 1:1 till antingen lungvensisolering med kryoablation eller konventionell rytm stabiliserande läkemedels behandling. En implanterbar bandspelare injiceras under huden för värdering av rytm basalt och under nästkommande 3 år. Distans avläsning möjliggör nedladdning av FF episoder kontinuerligt, med blindad värdering gentemot patient och uppföljande studieläkare. Härefter ges lottad behandling med ablation eller läkemedel.

Hypotesen är att tidig lungvensisolering med kryoballong som förstahandsbehandling är överlägsen rytmstabiliserande läkemedelsbehandling avseende frihet från FF (primär effektvariabel) vid persisterande FF vid 12 månader och med lika säkerhet. Andra effektvariabler är FF börda (% av tiden i FF), progress till svårare FF eller återgång till lindrigare FF former alt. normal sinus rytm, sjukvårdskonsumtion o kostnad pga av kardiovaskulära händelser (sjukhusinläggningar, akutmottagningsbesök, oplanerade polikliniska besök), symptom, livskvalitet, kognitiv förmåga och komplikationer samt säkerhet. Effekter på vänster förmaksvolym och funktion mäts med ultraljud hjärta för värdering om förändringar är reversibla (sk reverse remodellering). Effekt på blodmarkörer speglande grad av bindvävsbildning, inflammation och muskelskada fastställs. Vidare identifieras faktorer som kan förutspå uteblivet svar på tidig intervention som indikation på att förmaksförändringar blivit irreversibla. Patienterna följs var 3:e månad första året för värdering av primär effektvariabel vid 12 mån, och därefter var 6:e månad för utvärdering av sekundära variabler vid 12, 24 och 36 månader. Data registreras i sk Viedoc CRF datasystem, effekt variabler värderas blindat. Utifrån publicerade läkemedels och ablationsstudier uppskattas ca 220 patienter krävas för att uppvisa en 25% skillnad i primär effektvariabel med hänsyn taget till ca 15 % ”cross over och bortfall. Totalt 5-9 centra beräknas ingå.

Betydelse: Om förstahands ablation kan stoppa återfall i persisterande FF mer effektivt än läkemedel och dessutom ge förbättrad livskvalitet kan sjukvårdskonsumtionen minskas med lägre hälsoekonomiska kostnader som följd. Då ablationsmetoden är lätt att implementera kan den snabbt få allmän spridning och får då omedelbart praktisk betydelse då arytmin är vanlig och svårbehandlad.

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