Autologous atrial appendage micrografts transplanted during coronary artery bypass surgery: design of the AAMS2 randomized, double-blinded, and placebo-controlled trial

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Abstract

**Background**—The AAMS open-label clinical study demonstrated safety and feasibility of epicardial transplantation of autologous right atrial appendage micrografts (AAMs) during coronary artery bypass grafting (CABG) surgery. Delivered in an extracellular matrix patch, the study also provided first indications of reduced ischemic scar and increased live ventricular wall thickness associated with AAMs therapy. To further evaluate the initial beneficial effects observed in the AAMS study, we designed the randomized, double-blinded, and placebo-controlled AAMS2 trial. Focusing on patients with ischemic heart failure with reduced ejection fraction (iHFrEF), the AAMS2 trial aims to generate state-of-the-art structural and functional imaging data on the myocardium treated with an AAMs-patch during CABG.

**Methods**—The AAMS2 trial recruits iHFrEF patients who are set to undergo non-urgent CABG and present with a myocardial scar in preoperative cardiac magnetic resonance (CMR) with late gadolinium enhancement. Patients are randomized evenly (1:1) to receive an collagen-based matrix patch (Hemopatch®), with or without AAMs, epicardially onto the scar border. The primary endpoints at 6 months post-operatively are the effect change on the myocardial scar size by the AAMs-patch transplantation site, as assessed by CMR with late gadolinium enhancement and change in blood N-terminal-pro-BNP in the timeframe. The trial's secondary endpoints address feasibility, safety, echocardiography, quality of life, symptom scaling, and 6-minute walk test.

**Discussion**—Data from the AAMS2 trial provide the first randomized, blinded, and placebo-controlled evaluation of efficacy on epicardial AAMs transplantation for iHFrEF. This data then enables the rational design of larger AAMs therapeutic efficacy-addressing trial(s).

**Trial Registration:** ClinicalTrials.gov, NCT05632432, registered 30 November 2022, https://clinicaltrials.gov/study/NCT05632432

**INTRODUCTION**

During myocardial infarction (MI), timely revascularization limits the injury and recover the stunned myocardium (1). However, the activation of innate responses to damage, i.e. scarring and imbalanced compensatory mechanisms, still unfortunately often drive the disease towards heart failure (HF) (2),(3). Disturbingly, on top of its rising prevalence in the elderly, ischemic HF is increasingly affecting the obese and peoples on low-income countries (4, 5).

Unfortunately, no therapy, excluding heart transplantation, capable to cure the ischemic myocardial damages exists. These damages and adverse remodelling uphold the ischemic HF in many of those who resist even the best contemporary care, i.e. robust revascularization and modern pharmacotherapy (6). To bridge this gap in therapy, reactivation of myocardial regenerative processes has been explored for several decades (7). This rational stem from findings that zebrafishes and axolotls regenerate myocardium throughout their lifespan (8), while such endogenous healing in mice is limited to the first postnatal week (9). Notably, however, by inhibiting fatty acid oxidation in cardiomyocyte mitochondria,
the dormant regenerative healing program was recently reactivated for a marked repair of adult mice heart (10). Pertinent evidence on existing cardiac regenerative processes has been reported also in humans. Bergmann et al. measured the incorporation of $^{14}$C, derived from the Cold War nuclear tests, into human myocardia to show the cardiomyocytes to undergo a lifelong, minute turnover (11). Later, in 2016, Haubner et al. reported a full recovery of a human neonate suffering a massive perinatal MI and cardiogenic shock (12). These data highlight the human myocardial regeneration to be possible, but to be endogenously tightly restricted.

Massive efforts have aimed to translate the myocardial regeneration phenomenon from the bench to the bedside. Unfortunately, the success has been limited (7). The causes for such dim results span the hardships to fully model the clinical disease (8), pharmacokinetic challenges of injection-based delivery of therapies (13) and likely the allogenicity of the many such investigated cells (7). The modest results stress the evaluation of alternative approaches. To this end, straightforward protocols with beneficial pharmacokinetics are of value (14).

Cells from the atrial appendages harbour paracrine potential and can induce cardiac regeneration (15–18). In addition, the appendages can be readily harvested for cell therapies during coronary artery bypass grafting (CABG) (19, 20). Recently, we reported the intraoperative grinding of right atrial appendage biopsy to micrografts (AAMs) and their epicardial transplantation safe and feasible during CABG in patients with ischemic HF with reduced ejection fraction (iHFrEF) and myocardial scar visible in cardiac magnetic resonance (CMR) with late gadolinium enhancement (n = 6, Fig. 1) (19, 20). Based on our clinical intraoperative processing of right atrial appendage (RAA) biopsy to AAMs and subsequent micrograft analysis, the method yielded > 90% cellular viability (n = 7) and a cell yield of $9.76 \times 10^6 \pm 0.53$ / g of tissue (n = 11) (20). Further, culturing of the human AAMs for days revealed their viability (20). In mice, histology two months post-MI-transplantation also demonstrated AAMs' viable epicardial persistence (15). In pigs, their safety and immunosuppressive potency were noted (21). Interestingly, as measured six months after CABG by CMR with late gadolinium enhancement, the clinical open-label study demonstrated a significant increase in the live myocardium thickness by the documented AAMs-patch transplantation site (+ 1.0 mm [0.2–1.3 mm]) as compared to controls (− 1.4 mm [− 1.7 to 0.0 mm]).(19, 20) Notably, by this time, the AAMs-patch was intractable. An indicative trend for scar mass reduction was also obtained. Together, these results support the postulation that similar cardioreparative processes, as identified in our mice model of ischemic HF (15), are active when the AAMs-patch is delivered in the clinical setting.

This AAMS2 randomized, double-blinded, and placebo-controlled trial extends the perioperative AAMs-patch method evaluation to its surrogate therapeutic efficacy. The trial recruits 50 iHFrEF patients with a myocardial scar in preoperative CMR and set to undergo non-urgent CABG. The patients are randomized to AAMS2 (collagen-based patch + AAMs) and control (only patch) groups. To standardize the pathophysiology, the patients with a MI in the last 30 days prior CABG will be excluded. The primary endpoints are the changes in the myocardial infarct scar area and mass, as determined by CMR with late
gadolinium enhancement prior and six months after CABG, and the change in N-terminal pro-brain natriuretic peptide (NT-pro-BNP).

**METHODS**

1.1. **Endpoints**—The trial primary and secondary endpoints are listed in Table 1. The primary endpoints, as supported by the power calculations based on our previous open-label study (19), are (1) the change in myocardial scar tissue mass (g) and area (%) at the AAMs-patch transplantation site across preoperative and 6-month-postoperative CMR recordings and (2) change in NT-proBNP circulatory levels across the timeframe. Secondary endpoints focus on cardiac structural and functional parameters, feasibility, and safety.
Table 1
The AAMS2 trial endpoints

<table>
<thead>
<tr>
<th>Primary</th>
<th>- Change in the myocardial scar tissue by the patch transplantation site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative vs 6-month-follow-up 5SD CMR for mass (g) and area (%)</td>
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<tr>
<td></td>
<td>- Change in the plasma NT-proBNP levels across the trial period</td>
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<tr>
<td>Secondary</td>
<td></td>
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<tr>
<td>Efficacy</td>
<td>- Measured by CMR preoperatively and at 6-month-follow-up by the patch site</td>
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<tr>
<td></td>
<td>• Change in the live left ventricular wall thickness</td>
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<tr>
<td></td>
<td>• Change in viable left ventricular myocardium</td>
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<tr>
<td></td>
<td>• Change in the local ventricular wall systolic and diastolic function</td>
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<td></td>
<td>• Change in left ventricular ejection fraction</td>
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<tr>
<td></td>
<td>- Measured by TTE preoperatively and at 3-month-follow-up by the patch site</td>
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<tr>
<td></td>
<td>• Changes in systolic and diastolic function of the ventricular wall</td>
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<tr>
<td></td>
<td>• Changes in local myocardial strains</td>
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<tr>
<td></td>
<td>- Change in NYHA class at the 3- and 6-month vs. preoperative NYHA</td>
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<tr>
<td></td>
<td>- Change in 6-minute walk test (preoperative vs. 6-month-follow-up)</td>
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<td></td>
<td>- Changes in the quality of life (RAND36 preoperative vs. 6-month-follow-up)</td>
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<tr>
<td>Safety</td>
<td>- Telemetric rhythm postoperatively</td>
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<td></td>
<td>• Incidence of VT, VF, atrial fibrillation / flutter, or other arrhythmias</td>
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<td></td>
<td>- Days in need for invasive vasoactive medication postoperatively</td>
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<tr>
<td></td>
<td>- In-hospital infections (Transplant-related = 1; non-related = 2; no = 0)</td>
</tr>
<tr>
<td></td>
<td>- Days in hospital</td>
</tr>
<tr>
<td></td>
<td>- MACCE* during the whole trial period</td>
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<tr>
<td></td>
<td>- Anticipated SADE* (serious adverse device effects) during the trial</td>
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<tr>
<td></td>
<td>- Other serious adverse events* and unanticipated SADE* during the trial</td>
</tr>
<tr>
<td></td>
<td>- Deaths and deaths due to primary cardiovascular cause</td>
</tr>
</tbody>
</table>

* See section 1.6 for the adverse event definitions. **CMR**, cardiac magnetic resonance imaging with late gadolinium enhancement; **LVEF**, left ventricular ejection fraction; **MACCE**, major adverse cardiac and cerebrovascular events; **RAA**, right atrial appendage; **RAND36**, questionnaire for quality of life; **TTE**, transthoracic echocardiography; **VT/VF**, ventricular tachycardia/fibrillation; **5SD**, 5-standard deviation.
### Primary
- **Change in the myocardial scar tissue by the patch transplantation site**

<table>
<thead>
<tr>
<th>Feasibility</th>
<th>- Success in completing the delivery of the patch to the epicardium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• 0 = success, 1 = no success</td>
</tr>
<tr>
<td></td>
<td>• Waiting time for the ready micrograft transplant</td>
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<tr>
<td></td>
<td>• Time from the ready AAMSs-gel to the AAMSs-patch transplantation</td>
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<tr>
<td></td>
<td>• Waiting time in minutes for the heart</td>
</tr>
<tr>
<td></td>
<td>• After all the anastomoses finished and before the patch transplanted</td>
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<tr>
<td></td>
<td>• Closing the right atrial appendage</td>
</tr>
<tr>
<td></td>
<td>• Closing the RAA after biopsy for preparing the AAMSs-patch.</td>
</tr>
</tbody>
</table>

0 = no additional suturing needed, 1 = additional suturing needed

* See section 1.6 for the adverse event definitions. CMR, cardiac magnetic resonance imaging with late gadolinium enhancement; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events; RAA, right atrial appendage; RAND36, questionnaire for quality of life; TTE, transthoracic echocardiography; VT/VF, ventricular tachycardia/fibrillation; 5SD, 5-standard deviation.

The power analysis was carried out using SAS 9.4 TS Level 1M4 software (SAS Institute Inc., Cary, NC, USA), the POWER Procedure Wilcoxon-Mann-Whitney Test with the fixed scenario elements O’Brien-Castelloe approximation method and two-sided statistical evaluation. With a total sample size of 50 (two groups, group size 25, distribution 1:1) these parameters yield a power greater than 80% at an α of 0.05. Figure 3 presents the power analysis output graphs. A detailed consideration on the nature of the power analysis is provided at the end of Discussion.

#### 1.2. Patient selection and enrolment

The AAMS2 trial will recruit patients with chronic ischemic HFrEF requiring surgical revascularization, and with a visible myocardial scar in preoperative CMR. Patients with an AMI within last 30 days will be excluded.

Similar to our AAMSs-patch feasibility pilot (20), and the AAMSs-patch open-label study (19), patients regardless of their gender will be first evaluated by an academic hospital cardiologist in Finland at either Helsinki University Central Hospital or Oulu University Hospital. The patients are then listed on the hospital’s elective surgery list. The usual waiting time on the list ranges between 2–8 weeks, thus allowing any changes to medication, as made by cardiologist on clinical grounds unrelated to the trial, to take effect before surgery. After CABG, the recruited patients are called for a clinical control visit (denoted as the 3-month follow-up) and a dedicated trial control visit (at 6–8 months postoperatively, denoted as the 6-month follow-up).
Patients meeting both the inclusion and exclusion criteria (Table 2) will be provided a consent form describing the trial and are provided with sufficient time and information to make an informed decision on participation. Before a subject undergoes any study procedure, an informed consent discussion will be conducted and written informed consent will be obtained. If either one of the following, i.e. the systolic impairment (LVEF < 50%) or ischemic scar, are not present in the preoperative CMR, the recruited patients are excluded from the trial due to screening failure (Table 2). This also applies if logistical issues prevent the CMR, typically done just days prior planned CABG, to be performed before the CABG in adequate time. The trial will be conducted following the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (22). The data collected in the trial will fulfil EU regulations for personal health data protection, including General Data Protection Regulation (GDPR).
# Table 2
The AAMS2 trial criteria

<table>
<thead>
<tr>
<th>Eligibility</th>
<th>Age: 18–75 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sexes: All</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers: not accepted</td>
</tr>
</tbody>
</table>

### Inclusion
- Informed consent obtained
- Ischemic heart disease requiring CABG for revascularization
- LVEF between $\geq 15\%$ and $\leq 40\%$ at recruitment (echocardiography)
- NYHA Class II-IV heart failure symptoms

### Exclusion
- Heart failure due to left ventricular outflow tract obstruction
- Expected life expectancy $<$ 1 year
- Acute myocardial infarction (AMI) within last 30 days
- History of life-threatening and likely repeating ventricular arrhythmia or resuscitation, or an implantable cardioverter defibrillator
- Stroke or other disabling condition in 3 months before screening
- Severe valve disease or scheduled valve surgery
- Renal dysfunction (GFR $<$ 45 ml/min/1.73m)
- Other major disease limiting life expectancy
- Contraindications for coronary angiogram or CMR
- Allergy or hypersensitivity to the fibrin glue or to the Hemopatch®
- A part of any special patient group*  
- Participation in some other clinical trial

### Screening
- No visible scar or LVEF $\geq 50\%$ in preoperative CMR
- Preoperative CMR has not been performed prior scheduled CABG

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* Pregnant, nursing, handicapped, persons in an especially vulnerable position, or those in emergency situations.  
  
*CABG*, coronary artery bypass grafting;  
*CMR*, cardiac magnetic resonance imaging with late gadolinium enhancement;  
*LVEF*, left ventricular ejection fraction.

### 1.3 Baseline morbidity
A general baseline morbidity assessment will be carried out and involves a patient information system search for significant cardiovascular and non-cardiovascular comorbidities, electrocardiogram, structured patient interview and a case report form fill-out comprising dyspnea and *angina pectoris* symptom scaling, numeration of quality of life (section 1.10), traditional Framingham cardiovascular risk factors (23), supplemented with body mass index (BMI), personal history of MI, or...
family history of IHD, and medication list review. Data on patient medication will be curated and analyzed based on anatomical therapeutic chemical classification using the defined daily dose values enabling dosage comparisons. The CRF and trial checklists are provided as online-only supplementary files (Supplementary Files S1-9).

1.4. Randomization and blinding—Participants passing the screening failure are randomized to receive either standard CABG and collagen-based patch without the AAMs (control group) or CABG with an epicardial transplantation of an AAMs-patch (collagen-based patch + AAMs) after all the anastomoses have been completed (the AAMs-patch group). Patients are randomized to the groups using sex-stratified block randomization via openly available online tool at www.sealedenvelope.com with block sizes 2 and 4, and stratification according to sex (female, male). Randomization is carried out by the study nurse. In this trial, we randomize 50 participants that are treated during CABG either with Hemopatch® or the AAMs-patch. Specifically, the research nurse texts the Sealed Envelope service phone number 'AAMS2' (the trial abbreviation), followed by an execution command 'randomise', and completed by the detailing patient pseudonym. Then, the research nurse receives the allocation information by a text message. This is done a day prior each patient's surgery to grant adequate time to organize practicalities required for the perioperative AAMs-patch assembly. The study nurse oversees the allocation in a double-blind manner, where the patient and the evaluating cardiologist(s), radiologist(s) and academic researchers remain blinded to the allocation. Given the nature of the treatment intervention (intraoperative AAMs preparation vs. no preparation) the operating cardiac surgeon or the study nurse are not blinded intraoperatively. However, the operating cardiac surgeon is blinded during the time of planning the anastomoses prior CABG. Moreover, all CMR and transthoracic echocardiography (TTE) measurements as well as laboratory analyses are done by persons blinded to the patients’ study group allocations. In this trial, if considered acutely mandatory to remove the epicardial patch material, it is done without consideration of the randomization. Hence, no emergency unblinding is required in this trial.

1.5. Preparation and administration of atrial appendage micrografts—A piece of the RAA is harvested at the beginning of cardiac surgery upon right atrial cannulation as a part of the heart-and-lung-machine setup. For the AAMs-patch group, the RAA tissue is then weighed and mechanically grinded to micrografts in the operating room by using the Rigeneracon blade (Rigenera-system, HBW s.r.l., Turin, Italy) as previously described (15, 19, 20). As in the open-label safety and feasibility study (19), the targeted weight of the sample tissue for AAMs preparation is around 0.6–1.0 grams. For the CABG control group, the tissue piece is stored as a sample (section 1.7). Dedicated CE-marked instrumentation kits to support tissue processing in the operating room are purchased from EpiHeart Oy (EpiHeart CMT Core kit, Helsinki, Finland).

After grinding the RAA with Rigeneracon blade and subsequent centrifugation (5 min, 400 rcf) to pellet the AAMs, the cold cardioplegia supernatant is removed. The AAMs-pellet is collected with 0,4mL of Tisseel (Baxter AG, Vienna, Austria) fibrinogen solution (diluted in 0.9% NaCl with 1:1 relation). Then, the AAMs in fibrinogen are spread onto a cooled sterile metallic dish. The spread AAMs in fibrinogen are mixed in situ with 0,2mL of Tisseel thrombin solution (diluted in 0.9% NaCl with 1:30 relation). The
AAMs–fibrinogen–thrombin mixture is allowed to undergo spontaneous gelling for at least 10–15 minutes. Then, the gelled AAMs–fibrin mixture is maintained cooled (+6 – +8°C), covered, and sterile when waiting for transplantation. When all the anastomoses are ready, the AAMs–fibrin gel is gently lifted with spatula and transplanted onto the dry matrix sheet (Hemopatch® Sealing Hemostat 45 mm × 45 mm; catalog ref. 1506256; Baxter International Inc., Illinois, USA), thus forming the AAMs-patch (Supplementary Video S1), just prior to transplantation by the surgeon. Then, the Hemopatch® edges are moistened with sodium bicarbonate solution (4.2–8.4%) to activate the polyethylene glycol-coating of the patch. Finally, to achieve proper epicardial adherence, the AAMs-patch with the moistened patch edges is transplanted onto the epicardium of the scarred border zone of the myocardium by using a dry gauze with uniform pressure for 2 minutes. The exact transplantation site is preoperatively assessed with CMR to the ischemia-induced scar. In this trial, the AAMs patch shall not wait longer than 6 hours prior transplantation.

To standardize the effects of the patch material, the control group receives the patch without the AAMs. The place of transplanted patch is documented with a photo. The patients are carefully monitored according to clinical routine after the operation in the intensive care unit (Table 2, Supplementary File S4). Tissue processing-supporting instrumentation (EpiHeart Oy, Helsinki, Finland) usability is recorded in detail by the research nurse, and the usability outcome is included as one of the AAMS2 trial’s secondary outcomes (Table 1).

1.6. Data collection—Clinical, laboratory and drug treatment data are collected to the hospital electronic health records. Any visit related to the operation or their cardiovascular system condition as well as drug treatment changes are collected by the study investigators. This data is stored pseudonymised with the other data from the patient. In addition, the cardiovascular-related changes in medication with Anatomical Therapeutic Classification (ATC) codes and defined daily doses (DDDs) will be recorded for analysis to serve as a source data for surrogate modelling of an improved (i.e., reduced medication or dosage) or worsened (added medication or increased dosage) disease state.

1.7 Concomitant care, possible harm, and trial adherence—No concomitant care or interventions are prohibited. In this trial, based on regulatory body and company evaluations, the use of Hemopatch® is on-label (CE-marked) in the AAMs-patch-treated cohort, whilst an off-label use in the control group, where no AAMs are given. Hence, any product liability on possible participant harm(s), which can be causally verified to the use of Hemopatch® sealing the AAMs micrografts epicardially, is on the manufacturer, Baxter International Inc., Illinois, USA. Also in the control cohort, where no AAMs are being used, the liability for compensation for any participant harm(s) that can be causally linked to the Hemopatch® product itself is on the manufacturer. If the possible participant harms cannot be causally linked to the product, the liability is on the respective hospital trial investigators, all of which have an extensive insurance covering compensation for any damages in accordance with the national Patient Injury Act of Finland during the treatment and examination of patients. In Finland, these processes are centrally governed and handled by the Patient Insurance Centre.
The participant adherence is monitored by the research nurse by continuously updating an anonymized stepwise trial progression le. In case of participant- or investigator-derived deviation from the trial protocol, the participant is followed and examined per the trial protocol as extensively as possible after the deviation. In case of participant discontinuation, the study data accumulated until the date of discontinuation is included in the trial datasets and reported separately.

1.8. Adverse events—In this trial, adverse events are divided to (1) major adverse cardiac and cerebrovascular events (MACCE), (2) anticipated SADE (serious adverse device effects), (3) other SAE (serious adverse events), (4) unanticipated SADE. All the events are continuously monitored and reported to regulatory bodies. In this trial, the MACCE definition comprise death (all-cause), MI, any acute coronary revascularization, or stroke. MI is defined according to fourth universal definition on MI as either (1) Perioperative myocardial injury (i.e., type 5 MI ≤ 48 hours post-CABG, creatinine kinase muscle-brain isoenzymes [CK-MB] ≥ 10 times the upper reference) or (2) Postoperative MI (i.e., an increase in the CK-MB or troponin concentration above the upper reference limit with ischemic symptoms or signs). Stroke is indicated by neurological deficits and confirmed by a neurologist on the basis of imaging modalities with lesion(s) concordant to the clinical presentation. New revascularizations span any postoperative coronary intervention.

As a part of our overall comprehensive safety evaluation of the method, anticipated SADE are events identified by us with possible causal relationship to the AAMs-patch therapy. These include: (1) mediastinitis, (2) postoperative pericardial effusion requiring subxiphoidal drainage or resternotomy, (3) major bleeding (BARC classes 4–5)(24) from the RAA biopsy site, or (4) major postoperative arrhythmia (ventricular fibrillation, or ventricular tachycardia over 30 seconds). Mediastinitis is diagnosed according to Centers for Disease Control and Prevention (CDC) guidelines (25). The accumulated published data in mice (15), pigs (21), and human (19) on the AAMs-patch method does not indicate heightened risk for these events.

Other SAE, comprise any adverse event that has led to either death, life-threatening illness, (prolongation of) hospitalization, medical intervention to prevent life-threatening illness, or chronic disease. According to our risk assessment of the AAMs-patch therapy, these could include: myocarditis, pericardial effusion, HF exacerbation, resternotomy, atrial tachycardia or fibrillation, atrial flutter, transient ischemic attack, major bleeding (BARC 3–5) (24), acute kidney injury, or other hospitalization due to ischemic cause.

1.9. Blood samples—The AAMS2 trial assesses blood, plasma, and RAA tissue (section 1.7) samples for their RNA with a focus on their contained post-transcriptional, epitranscriptomic, modifications as described in the IHD-EPITRAN study design (www.ihd-epitran.com) (26). These modifications, as recently reviewed comprehensively by us in the field of cardiovascular diseases (27), are emerging regulators of both cardiac disease and regeneration. The schedule of sample collection is summarized in Fig. 2.

Briefly, the study blood sample set contains tubes for RNA-stabilized TEMPUS™ whole blood (3 mL × 8) as well as EDTA-stabilized blood (9 mL × 3) from which RNA-stabilized plasma (900 µL × 10) and standard plasma (500 µL × 4–10) are separated and aliquoted for storage (26). In addition, a sample for...
N-terminal pro-brain natriuretic peptide (NT-pro-BNP) measurement by the clinical laboratory (HUSLAB, Helsinki, Finland; NordLab, Oulu, Finland) is collected in the trial preoperatively and at both 3-month and 6-month clinical and study follow-ups, respectively.

1.10. RAA tissue samples—Since the RAA tissue sample removed during CABG in control group is not used for producing AAMs, it is collected for analyses. The initial piece of RAA tissue will be divided into two pieces and stored as previously described (Fig. 2) (26).

The RNA-stabilized RAA piece is used to profile a m⁶A-epitranscriptome as in IHD-EPITRAN study (26). Briefly, the RNAlater-stored (AM7021, ThermoFisher Scientific Inc., Waltham, MA, USA) RAA piece will be subjected for RNA extraction, fractionation and m⁶A-targeted sequencing, and bioinformatics. Overall, these produced epitranscriptomes are correlated for changes in repeated CMR, TTE, and blood sampling results.

1.11. Echocardiography—For functional and anatomical insight of the participants’ cardiac status, the participants are assessed with TTE both pre- and postoperatively (Fig. 2). Postoperative TTE recordings are done at hospital discharge (approximately one week postoperatively) and at 3-months of follow-up. The recordings are performed with prespecified acquisition methods and imaging windows by few designated cardiologists and include both anatomical and functional assessments of atria, valves, and ventricles. The presence or absence of pericardial effusion, thrombus and aneurysm is recorded. The detailed protocol is provided as a Supplementary File S10. Also, a perfusion anesthesiologist will perform transesophageal echocardiography in the operating room during anesthesia to evaluate both left and right atrial appendages and atria for blood flow velocities, possible sludge, thrombus, and anatomy before CABG. The raw data will also be exported and stored for further state-of-the-art functional analyses (myocardial imaging), such as strain and strain rate measurements.

1.12. Late gadolinium enhancement cardiac magnetic resonance imaging (CMR)—CMR is performed preoperatively to evaluate cardiac function, and myocardial anatomy, including ischemic and fibrotic areas. The preoperative CMR excludes patients without any fibrotic scar or systolic impairment (Table 2) and guides the AAMs-patch transplantation site at the end of CABG surgery (section 1.4). Its repetition at a 6-month of follow-up enables assessment of any change in either scar or vital myocardial tissue mass by the epicardial AAMs-patch transplantation site. Specifically, a whole body 1.5-T MRI scanner (In Helsinki, Siemens Sola or Avanto-fit, Siemens AG, Erlangen, Germany; in Oulu, Siemens Sola or Sola-fit, Siemens AG, Erlangen, Germany) is used for CMR image acquisition. Cardiac structure and function are evaluated with a standardized CMR protocol using electrocardiogram and respiratory gating. Short-axis cine images are used for left and right ventricular volumetric measurements. Myocardial contractility is evaluated using longitudinal, circumferential, and radial strain measurements from short- and long-axis cine images. Late gadolinium enhancement is used to measure infarction volume and mass using 5-SD semiautomatic gain estimate, as previously suggested for semiautomatic thresholding for infarction detection (28). Image post-processing is performed with Medis Suite software (Medis Medical Imaging Systems, Leiden, The Netherlands) with QMass and QStrain applications.

1.13. Quality of life assessment—Health-related quality of life (HRQoL) is measured using the RAND36
short form questionnaire (SF36) (29). The questionnaire is standardized with specified mean and standard deviation values for eight dimensions that range from physical functioning and subjective feeling of vitality and health to bodily pain. The obtained scores are compared for a Finnish cohort with any chronic disease. Also, a subjective symptom-evaluation is performed for the two cardinal symptoms of IHD and HF, *angina pectoris* and exertional dyspnea, with standardized classification systems develop by Canadian Cardiovascular Society (CCS) and New York Heart Association (NYHA), respectively (30, 31).

1.14. Six-minute walking test (6MWT)—To measure the general physical capacity, a 6MWT is performed for all participants preoperatively and postoperatively at the 6-month follow-up (32). The parameters included are the following: the actualized walking distance in meters, the predicted walking distance, oxygen saturation (baseline at rest and lowest during the test), and subjective related symptoms according to Borg's scale (0–10). The test is scheduled just before CABG (Fig. 2) to minimize the effects of preoperatively initiated pharmacotherapies on exertional capacity prior CABG and AAMs-patch transplantation.

1.15. Data confidentiality—In this trial, all personal or other study information collected is done by healthcare professionals committed in verbatim to the utmost standards of data safety and confidentiality. The produced data are stored in the research registry in the University Hospitals' and University of Helsinki safe network hard drives and on the servers of the Finnish IT Center for Science designed for sensitive data storage, all with an automated backup. The tailored data storage services—SD-Connect, SD-Desktop and SD-Publish—provided by Finnish CSC–IT Center for Science (https://research.csc.fi/sensitive-data) are financially supported by the Finland's Ministry of Education and Culture. These services are designed to comply with the EU General Data Protection Regulation (GDPR), which is followed throughout the trial lifetime. The accession to these registries is controlled via role-based accession rights. Only those specified in the registry description approved by the HUS Ethics Committee can access the data therein.

The participants' TTE and CMR data is stored and accessed via software fulfilling the Hospitals' data security guidelines. Case report formats are both physically stored in the safe Hospital premises with an access control and electronically in the research registry. The pseudonymized sequencing datasets with metadata will be made available upon publication principally via the long-term sensitive data storage service SD-Publish, which appoints an independent data monitoring committee to evaluate and process all applications (in writing) by other scientists interested to re-use the deposited pseudonymized research data. The principal investigators of the trial will have access to the final trial dataset. Contractual agreements will not limit this right. Theidentificatory data and pseudonymized data will be stored 15 years after the trial completion. Then, the pseudonymization codes and keys will be erased and the anonymized data will be principally stored in SD-Apply, or in another public repository. The anonymized group-level datasets of the trial results will be made available by submission(s) for publication(s) in esteemed journals of the field of cardiology and cardiac surgery.

1.16. Trial monitoring—The AAMS2 trial is externally monitored by the Clinical Research Institute HUCH to ensure trial subjects’ rights, safety, and well-being. A detailed monitoring plan has been formulated
and is in effect with the monitor and the research group. The trial monitor regularly audits the trial data registries for appropriateness and completeness. In addition, the AAMS2 trial has a Trial Monitoring Committee with national medical professionals from the field of cardiology, cardiac surgery, and nursing to evaluate the appropriateness of the trial in a periodical manner, as each 10 patients are recruited, from a focused medical viewpoint. As per local standards and the trial size, this trial does not have a separate data monitoring committee.

1.17. Interim analysis—If considered appropriate, an interim analysis can be performed when a significant proportion of the total recruited participants (i.e., 20–30 patients) have undergone the full protocol. This consideration is undertaken jointly with the Trial Monitoring Committee. All the trial investigators can access the interim analysis results. The principal investigator makes the final decision to terminate the trial.

1.18. Statistical analyses—Comparisons between groups will be performed with the Mann Whitney U test. Ordinal variables are tested with the Chi Square test. Multiple comparisons are corrected with the Bonferroni method, significant findings are further tested groupwise using the Mann Whitney U test. Quality of life data is presented as mean and analysed with the independent samples t-test (two-sided). Analyses are performed with the IBM SPSS Statistics 27 program (IBM Corp., Armonk, NY) or equivalent. Only those participants are included in the main endpoint analyses (Table 1) that have both undergone the treatment (AAMs-patch or Hemopatch®-only) and completed the 6-month follow-up CMR imaging with late gadolinium enhancement.

1.19. Protocol amendments—Any important protocol modifications (i.e., changes to eligibility criteria, endpoints, or analyses) are communicated to trial investigators, participants, registries, and regulators as soon as practically possible by the trial coordinators. In a such unlikely case, the protocol modification is coordinated in a close mutual communication with the trial external regulatory bodies (e.g. HUS Ethical Committee, Trial Monitor, Trial Monitoring Committee, and FIMEA).

DISCUSSION

Each year, 8.9 million lives are prematurely lost to IHD (33). The enlarging and lipid-laden coronary plaques, the IHD hallmarks, are prone to rupture (2). This event, by sparking a hyperacute local thrombotic occlusion and downstream ischemic anoxia, may acutely kill even a billion cardiomyocytes (34). Fortunately, carefully designed revascularization protocols and pharmacotherapy often limit the damage, given they are timely initiated (35). Unfortunately, whether through recurrent insults or missed timing, the inappropriately propagating adverse myocardial remodelling, sustained cardiomyocyte apoptosis, chronic fibrotic build-up, and loss of elasticity (36) still often later exhaust the heart’s functional reserve to manifest as ischemic HF (2).

Globally, 64.3 million people are estimated to suffer from HF (5). Largely due to major global phenomena of ageing, westernization of life habits, and increases in comorbidities, HF prevalence has been consistently estimated to increase, and to exceed 8.0 million people in USA by 2030 (37). Global predictions corroborate this estimation (5). Although HF is associated with a near 50% overall and ~ 25%
cardiovascular-related mortality within 5-years of diagnosis, its dismal prognosis has failed to show trend for marked improvement (38). This stresses the need for novel therapies.

For the ischemic failing human heart, a large body of research over the last decades have evaluated the injections of stem and more differentiated cells as myocardial regeneration-inducing strategies. Overall, as recently reviewed, these studies have emerged with mixed results (7). While all the larger-scale trials have failed to demonstrate increase in LVEF (7), three have reported benefit on MACE endpoints in iHFrEF by utilizing ixmyeloceT cells or bone-marrow-derived mesenchymal stem cells (BM-MSCs) (39–41), both of which are known for their immunomodulatory properties (42). Since 1990, kickstarting from the discovery of elevated tumor necrosis factor-α (TNF-α) levels in patients with cachectic HFrEF (43), investigations has verified that also inflammation within myocardium, alongside deranged angiogenesis, ischemia, and maladaptive remodelling, plays a pivotal role in the progression of ischemic HFrEF (Fig. 4). Indeed, several cytokines have shown to be crucial regulators between the phenotypes of early adaptive remodelling, and ultimately irreversible maladaptive myocardial remodelling in ischemic HFrEF (44).

Clinically, this 'cytokine hypothesis' of the HF progression has been further confirmed by the phase III CANTOS trial recently, which reported improved HF outcomes after MI for those with elevated high sensitivity complement reactive protein (hsCRP > 2 mg/mL) and were treated with a suppressing monoclonal antibody for IL-1β (45). Analogously, despite failing to meet its primary endpoint of recurrent HF hospitalizations or secondary endpoints on its congestive complications, the recent DREAM-HF trial, the largest HF cell therapy trial so far with 537 patients treated by transendocardial BM-MSC injections (TEi-BM-MSCs), found reduction in the 3-point MACE (MI, stroke, cardiovascular death), and an increase LVEF during its median ~ 30 months follow-up in those with hsCRP > 2 mg/mL (46). While the further study of injection-based methods to instigate reparation of the ischemic and failing myocardium are warranted, the need for novel avenues is evident.

In our approach, we cover the epicardially transplanted AAMs with a matrix sheet to concentrate and direct the transplant's paracrine effects specifically towards the myocardium. Mechanistically, the revisited view on the cardiac cell therapy mechanisms posits an integral role for paracrine factors released by the transplanted cells in the activation of myocardial responses towards structural and functional restoration (17)(16, 18). As the atrial tissue is endogenously highly active in secretion of regulatory factors, such as natriuretic peptides and extracellular miRNA-containing vesicles with reported reparative myocardial effects (47), we hypothesize that epicardial AAMs provides a targeted, lasting, and local supply of factors for reparation-inducing myocardial therapy. The migration of atrial macrophages has suggested to contribute to the beneficial remodelling after epicardial atrial transplantation (48). Also other cells of the appendages demonstrate roles on cardiac healing after ischemic damage (16–18).

Preclinically, the epicardial AAMs-patch transplantation during LAD-ligation-induced MI and HF demonstrated myocardial tissue protection, attenuated scarring, and retained cardiac function (Fig. 4) (15). Mass-spectrometry-based anatomical site-targeted proteomics revealed widespread cardioprotective effects right under the AAMs-patch, which also reached the remote areas, as assessed
from the samples of interventricular septum (15). After treatment with AAMs, this subtransplant myocardium demonstrated significant negative associations for oxidative stress and mitochondrial electron transport chain, while positive association for increase in antioxidant glutathione metabolism was also noted in tandem (15). These changes suggested AAMs-induced coordinated changes in myocardial metabolism towards glycolysis, a process pervasively linked to increased regenerative capacity of myocardium (49–52). As such, in this experimental ischemic HF model, epicardially transplanted AAMs appeared to induce via paracrine effects a beneficial microenvironment for the surviving cardiomyocytes, and thus setting the stage for functional recovery (15). Together, our preclinical results indicate topical epicardial AAMs-patch with beneficial effects on the key pathophysiologic processes that drive the progression of iHFpEF (Fig. 5).

Clinically, the surgical procedure of epicardial administration of AAMs (20) was feasible and safe during CABG (Fig. 1) (19). Intraoperative processing time of 7–8 minutes for micrograft isolation together with the total time of approximately 30 minutes for the full perioperative AAMs-patch assembly provided both confidence and the clinical proof-of-concept for the utilisation and further development of the AAMs-patch therapy (19).

The AAMS2 trial has notable advantages to the field of cardiac cell therapy. First, the straightforward perioperative protocol with minimal mechanical grinding avoids many of the hindrances arising from the integration of most of the currently investigated cardiac cell therapy protocols to the everyday clinical practice. Most of the approaches are costly, time-requiring, depend on specialized infrastructure, and span cell harvesting, sorting, expansion, storage, and transplantation (14). Furthermore, the transplantation of cells often necessitates an invasive procedure. Second, the autologous AAMs-patch circumvents the issues of immunoreactivity arising from allogenic cells. Third, the on-site transplantation of the AAMs, encased in an ECM sheet, is inherently powered to concentrate the cells and paracrine effects to the site—a major hardship in previous trials (53, 54). Owing this aspect, the tissue-engineering based approaches has been advocated for cardiac cell therapies (14).

Although this researcher-initiated AAMS2 trial is limited in size to assess the secondary endpoints exhaustively, the power calculations support the evaluation of its primary endpoints (Fig. 3) (19). Several trials of the field have proven the general scale of recruitment goal rational, including the phase II trial assessing TEi-BM-MSCs in iHFpEF patients (n = 60), which achieved its primary endpoint of improved left ventricular end systolic volume and increased LVEF (55). However, the qualitative aspects of the power calculations (Fig. 3) warrant discussion. The power analysis source data, as derived from the only available applicable data, is from our open-label safety and feasibility study (19), which is both non-randomized and unblinded. Also, the controls did not receive any study intervention (i.e., an empty patch) during CABG. In this trial, the patch material has been adapted to the routinely clinically used product (i.e., Hemopatch®) in both participating national clinical centres and the control arm receives an empty patch. With these decisions, while the evaluation of the AAMs’ effects becomes possible, the direct applicability of the power analysis is reduced. Hence, we consider the power analysis to be indicative, while we consider the AAMS2 trial’s major strengths to lie in its randomized, double-blinded, and
placebo-controlled design, an imperative first step on the road to properly evaluate the method's therapeutic efficacy in a stepwise manner, first at the smaller scale and surrogate level. Further, therapeutically, the trial's primary surrogate endpoint on change in local scar mass is both sound and relevant. Furthermore, the primary endpoint is evaluated by a state-of-the-art imaging modality that is objective and feasible to record. Moreover, by considering its intraoperative and autologous nature against the major gap in the IHD therapeutics lacking myocardium-repair-inducing remedies, the method can be envisioned to harbour a particularly clear complementary applicability to the clinic. On this road, the results from this researcher-initiated trial will enable the rational design of larger AAMs therapeutic efficacy-addressing trial(s).

TRIAL STATUS

At the time of submitting this manuscript no patient has yet been recruited, but the recruitment started on 1.4.2024 and is ongoing. The completion of patient recruitment is estimated to be finished by the early 2026. The full completion date of the trial is set for the end of 2026. The participants are recruited in two national academic hospital centers in Finland: Helsinki University Hospital in Helsinki (estimated recruitment of 25–30 participants), and Oulu University Hospital in Oulu (estimated recruitment of 20–25 participants). Together, these hospitals perform yearly more than 500 isolated CABG surgeries.

PROTOCOL DATE & VERSION

11.4.2024, version 1.

Abbreviations

AAMs, atrial appendage micrografts

ATC, Anatomical Therapeutic Classification

BARC, Bleeding Academic Research Consortium

BMI, body mass index

CABG, coronary artery bypass grafting

CCS, Canadian Cardiovascular Society (classification of angina pectoris)

CK-MB, creatinine kinase muscle-brain isoenzyme

CMR, cardiac magnetic resonance (imaging)

DDD, defined daily dose

ECM, extracellular matrix
GDPR, General Data Protection Regulation

IHD, ischemic heart disease

HF, heart failure

HRQoL, health-related quality of life

iHFrEF, ischemic heart failure with reduced ejection fraction

hsCRP, high sensitivity complement reactive protein

LAD, left anterior descending (coronary artery)

LLMs, large language models

LVEF, left ventricular ejection fraction

MACE, major adverse cardiovascular event

MACCE, major adverse cardiac and cerebrovascular event

MI, myocardial infarction

NT-pro-BNP, N-terminal pro-brain natriuretic peptide

NYHA, New York Heart Association (classification of dyspnea)

RAA, right atrial appendage

SADE, serious adverse device effects

SF36, short form questionnaire RAND36 (for HRQoL)

TEi-BM-MSCs, transendocardial injection of bone-marrow-derived mesenchymal stem cells

TNF-α, tumor necrosis factor-α

TTE, transthoracic echocardiography

6MWT, 6-minute walking test

Declarations

Ethics approval and consent to participate—The trial protocol has been approved by the ethics review board at Helsinki University Hospital (HUS; Dnr. HUS/12322/2022), and the Finnish Medicines Agency
The trial will be conducted following the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (22). The cardiac surgeon part of the trial obtains the informed consent from the participants after sufficient information orally and in verbatim has been provided with adequate time for making this consent. No trial procedure is performed prior obtaining the consent from the participant.

**Consent for publication**—Not applicable.

**Availability of data and materials**—The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**—E.K. and A.Nu. are stakeholders in EpiHeart Oy. A.K. is the Chief Engineer at EpiHeart Oy, which provides dedicated CE-marked instrumentation kits to support tissue processing in the operating room for AAMs-patch assembly. The other authors have no competing interests to disclose. Large language models (LLMs) or other artificial intelligence (AI)-based tools were not utilized in the preparation of this manuscript.

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**Authors' contributions**—A.Ny., A.V., E.K., K.O., R.K., S.K., V.S. conceived the study design and coordination. V.S. wrote the manuscript, collected the literature, and designed the illustrations; E.K. and A.Ny., wrote and revised the manuscript as well as provided supervision. All authors read, provided comments, and approved the final manuscript. A.K. and E.K. recorded the Supplementary Video S1. Authorship requirements for the trial result publication(s) will follow the International Committee of Medical Journal Editors (ICMJE) guidelines.

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**References**


Supplementary Video S1
Supplementary Video S1 is not available with this version

**Supplementary Video S1** — Transfer of The AAMs–Fibrin Gel onto The Hemopatch®

## Figures

**CMR**
- iHFrEF patient
- Elective CABG
- LVEF 35–54%
- Scar present

**CABG / CABG + AAMs**
- Median assembly time 33 min
- Grinding
- Gelling
- Biopsy
- AAMs
- Transfer
- Ischemic scar
- Hemopatch
- AAMs-patch

**6-MONTHS**
- Patch invisible
- No calcification
- Wall thickness
- Scar mass

**BELOW THE TRANSPLANT**
- CMR preoperative vs. 6-month postoperative results
- Scar mass: $p = 0.089$
- Thickness: $p = 0.0085$
- CABG CABG AAMs-patch

### Figure 1

The results of the clinical AAMs-patch open-label pilot study.

The preclinical results of an epicardial AAMs-patch transplantation are presented in Figure 4. It prompted an open-label study, focusing on ischemic HF patients with a myocardial scar in preoperative CMR, which supported the feasibility and safety of AAMs-patch transplantation as an intraoperative therapy adjuvant to CABG in a clinical setting (19). This study revealed the median time for the AAMs-patch setup to be 33 minutes, while only minutes were needed for the attachment onto the epicardium. The 6-month follow-up found good tolerability of the AAMs-patch. Moreover, in the 6-month-follow-up CMR records, the AAMs-patch was intractable, but, interestingly, the transplant area showed statistically significant increase in the live ventricular wall thickness as compared to the baseline (19). An indicative trend for reduction in scar mass by the site was also observed. The results support a larger randomized, double-blinded, and placebo-controlled clinical trial, as described herein. AAMs, atrial appendage micrografts; AAMs-patch, decellularized extracellular matrix patch encasing atrial appendage micrografts; CABG, coronary artery bypass grafting; CMR, cardiac magnetic resonance imaging with late gadolinium enhancement; LVEF, left ventricular ejection fraction. The CMR results are reprinted as minutely modified from Frontiers in Cardiovascular Medicine, 2021 Nummi A, Mulari S, Stewart JA, et al. Epicardial Transplantation of Autologous Cardiac Micrografts During Coronary Artery Bypass Surgery (2021) (19). with permission from the publisher under the Creative Commons Attribution License (CC BY 4.0).
Figure 2

Outline of the AAMS2 trial.

The key inclusion criteria ('ECHO'), screening failure criteria ('CMR'), and the primary endpoints ('CMR' and 'NT-proBNP') of the trial are shown in red. In addition to transthoracic echocardiography ('ECHO'), which is performed: (i) at recruitment, (ii) preoperatively, and postoperatively at (iii) hospital discharge
and (iv) at 3-month follow-up, also transesophageal echocardiography is done by perfusion anesthesiologist at the beginning of CABG surgery to assess RAA anatomy and any presence of sludge. Clinical metadata ('OTHER') include recording of Framingham cardiovascular risk factors, electrocardiogram, medication with changes, MACCE, overall quality-of-life assessment with SF-36 questionnaire, and survey of dyspnea and angina pectoris symptoms with NYHA and CCS classifications. The RNA-stabilized study blood samples, focusing on adenosine-based epitranscriptomic profile characterization, are collected as previously published (26). Abbreviations: AAMS, atrial appendage micrografts; AAMSs-patch, decellularized extracellular matrix patch encasing atrial appendage micrografts; CABG, coronary artery bypass grafting; CCS, Canadian Cardiovascular Society (angina pectoris grading classification); CMR, cardiac magnetic resonance imaging with late gadolinium enhancement; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association (dyspnea grading classification); MACCE, major adverse cardiac and cerebrovascular events; RAA, right atrial appendage; SF-36, 36-item short form survey (standardized questionnaire for assessment of overall quality of life); 6MWT, 6-minute walking test.

**Figure 3**

**Power analysis for the AAMS2 trial.**

The power analysis is based on the unblinded and non-randomized data from the open-label safety and feasibility study of the perioperative AAMS-patch method (n=6, AAMS-patch-treated; n=5 controls without patch) (19). As calculated by the POWER Procedure Wilcoxon-Mann-Whitney Test with fixed scenario elements O’Brien-Castelloe approximation method and two-sided statistical evaluation, the change (Δ) in infarction scar area (%), and mass (g), as evaluated from the 5SD CMR imaging data preoperatively and 6-months postoperatively at the AAMS-patch transplantation site, the total sample size of 50 (two groups, group size 25, distribution 1:1), yield a power greater than 80% at an α of 0.05. Analysis was carried out with SAS 9.4 TS Level 1M4 software (SAS Institute Inc., Cary, NC, USA). The indicative effect size on fibrosis is shown in Figure 1. (19) A trend for decrease in the change of NT-proBNP levels were found in those patients receiving an AAMS-patch (19). (A), Evaluation using change in infarction area percentage (%). (B), Evaluation using change in infarction area mass (g). (C), Evaluation using change in NT-pro-BNP circulatory concentrations (plasma sample analysis preoperative vs. 6-month postoperatively). Red line represents 80% power at a total sample size of 50. A detailed consideration on the nature of the power analysis is provided at the end of Discussion. Alpha, level of type I error (alpha-
error level representing the proportional level for false-positive result assessment); **CMR**, cardiac magnetic resonance imaging with late gadolinium enhancement; **5SD**, 5-standard deviation.

**Figure 4**

**Preclinical AAMs-patch therapy effects in a myocardial ischemia model.**

(A), In our preclinical mouse study with LAD-ligation-induced MI and ischemic HFrEF, (B) even transplantation of only an epicardial patch without the AAMs attenuated scarring and persistently salvaged myocardial function, which was further enhanced when AAMs were included (AAMs-patch) (15). (C), The site-specific untargeted proteomics revealed the molecular-level blueprints and putative mechanisms of action for the AAMs. This analysis revealed that below the AAMs-patch transplantation, in comparison with the patch-only transplantation, more than 200 proteins were expressed differentially in the injured heart, which associated to upregulated cell viability, protein synthesis, muscle formation, angiogenesis, and glycolysis (a metabolic shift associated with an enhanced regenerative ability (49-52), while attenuation of inflammation, oxidative stress, and cell death were noted in tandem (15). The interventricular septal areas also showed significant associations for cell viability. **AAMs**, atrial appendage micrografts; **CABG**, coronary artery bypass grafting; **HFrEF**, heart failure with reduced ejection fraction; **LAD**, left anterior descending (coronary artery); **MI**, myocardial infarction. Subplot (B) is reprinted as minutely modified from the *Journal of Heart and Lung Transplantation*, 39/7,
Proposed mechanisms of epicardial AAMs-patch transplantation in iHFrEF. (A), Progression of iHFrEF relies on four pathophysiologic mechanisms. First, the activation failure of cardioreparative pathways...
after ischemic insults sets the stage for HF development. Second and third, the vascular disturbances (e.g., endothelial dysfunction) and local proinflammatory pathway activation aggravate the cardiomyocyte microenvironment. Fourth, the above processes and the ischemic primary structural damage (i.e., scarring) all synergistically promote adverse secondary remodelling, which is hallmarked by eccentric hypertrophy and dilatation, which, if left unchecked, can spark a self-sustaining spiral of deteriorating ventricular geometry further accelerating cardiomyocyte apoptosis, wall thinning, and ultimately worsening geometry. For a major part, this terminal sequence is driven by the Laplace's law, which estimates increase in wall pressure, the key determinant of cardiomyocyte oxygen demand and survival, given the ventricular wall thins, luminal radius increases, or the pressure conditions worsen (56).

The preclinical data (Figure 4) (15), clinical first indications of increased live myocardium and trend for reduced scar mass by the AAMs-patch site (Figure 1) (19), support together a model of AAMs-patch transplantation to synergistically target these processes to tackle iHFrEF progression. AAMs, atrial appendage micrografts; iHFrEF, ischemic heart failure with reduced ejection fraction.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- completedSPIRITchecklistrevised.pdf
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