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Clinical development and proof of principle testing of new regenerative vascular endothelial growth factor-D therapy for refractory angina: rationale and design of the phase 2 ReGenHeart trial

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ABSTRACT

Background Despite tremendous therapeutic advancements, a significant proportion of coronary artery disease patients suffer from refractory angina pectoris, that is, quality-of-life-compromising angina that is non-manageable with established pharmacological and interventional treatment options. Adenoviral vascular endothelial growth factor-D$^{\Delta N\Delta C}$ (AdVEGF-D)-encoding gene therapy (GT) holds promise for the treatment of refractory angina.

Methods ReGenHeart is an investigator-initiated, multicentre, randomised, placebo-controlled and double-blinded phase 2 clinical trial that aims to study the safety and efficacy of intramyocardially administered angiogenic AdVEGF-D GT for refractory angina. Patients will be randomised in a 2:1 ratio and blocks of six to receive either AdVEGF-D or placebo. Primary endpoints are improvements in functional capacity assessed with the 6 min walking test and angina symptoms with Canadian Cardiovascular Society class after 6 month follow-up. Secondary endpoints are improvements in myocardial perfusion assessed with either positron emission tomography or single-photon emission CT after 6 month follow-up and functional capacity and angina symptoms after 12 months. In addition, changes in the quality of life, the use of angina medication and the incidence of major adverse cardiac and cerebrovascular events will be evaluated.

Conclusions The phase 2 ReGenHeart trial will provide knowledge of the safety and efficacy of AdVEGF-D GT to ameliorate symptoms in refractory angina patients, extending and further testing positive results from the preceding phase 1/2a trial.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Refractory angina is highly prevalent among patients with coronary artery disease, affecting up to 15% of this patient population based on some estimates. In the phase 1/2a Kuopio Angiogenesis Trial, intramyocardially delivered adenoviral vascular endothelial growth factor-D$^{\Delta N\Delta C}$ (AdVEGF-D) gene therapy alleviated angina symptoms and increased myocardial perfusion in patients with refractory angina.

WHAT THIS STUDY ADDS

⇒ The randomised, placebo-controlled and double-blinded phase 2 ReGenHeart trial will test the safety and efficacy of AdVEGF-D gene therapy for refractory angina by recruiting patients suffering from ischaemic chest pain despite established optimal medical or interventional therapies in six European countries. Compared to previous phase 2 angiogenic gene therapy trials, ReGenHeart investigates the efficacy of VEGF-D, the most angiogenic growth factor in the VEGF family.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ If found effective, the future testing of AdVEGF-D gene therapy will take place in the phase 3 setting to face the unmet clinical needs of this rapidly increasing patient group.

INTRODUCTION

Despite the enormous advancements in the treatment of coronary artery disease (CAD), a significant number of patients experience disabling angina refractory to optimal medical therapy or established interventional coronary procedures.[1] Depending on the used database and classification, refractory angina (RA) affects 5–15% of all CAD patients.[2 3]

Gene therapy (GT) based on vascular endothelial growth factor-D (VEGF-D) encoding adenoviruses is a promising addition to the current treatment options for RA.[4] In this method, ischaemic but still viable myocardium is targeted with angiogenic adenoviral vectors that induce angiogenesis and arteriogenesis in areas with impaired perfusion.[5 6] VEGFs are naturally occurring growth factors whose translation is caused by various physiological and pathological stimuli, such as hypoxia and inflammation.[7] VEGF-D$^{\Delta N\Delta C}$ is a proteolytically processed short form of its precursor VEGF-D with both angiogenic and lymphangiogenic properties.[8]

Phase 1/2a Kuopio Angiogenesis Trial 301 (KAT301) was the first randomised, placebo-controlled and single-blinded trial ever to investigate the safety and feasibility of intramyocardial adenoviral VEGF-D$^{\Delta N\Delta C}$ (AdVEGF-D) GT for the treatment of RA.[9] This treatment was well-tolerated, resulting in a significant decrease in angina symptoms and a significant increase in myocardial perfusion during the 1 year follow-up. GT was also found to be safe in the long term.[10 11]

As a continuation of KAT301, the ongoing European Union-funded phase 2 ReGenHeart trial aims to solidify the evidence regarding the safety and efficacy of intramyocardial AdVEGF-D GT and evaluate whether the encouraging results of the phase 1/2a trial can be repeated in a multinational, multicentre study in a larger study population.

METHODS

Trial design

ReGenHeart is a randomised, placebo-controlled and double-blinded phase 2 trial that will compare the safety and efficacy of intramyocardially administered AdVEGF-D GT to placebo by recruiting RA patients in the European Union. The trial is registered on ClinicalTrials.gov (NCT03039751). The study flow and the list of the ReGenHeart investigators and study centres are presented in figure 1 and online supplemental table 1, respectively. ReGenHeart will be conducted in accordance with the Declaration of Helsinki.

Target population

Patients with CAD and Canadian Cardiovascular Society (CCS) class 2–3 angina symptoms despite the optimal medical therapy and for whom revascularisation procedures are not feasible will be enrolled. The full list of inclusion and exclusion criteria is presented in box 1. Patients will be invited to the screening visit based on their contact with the investigators or a referral from their treating cardiologist. If deemed eligible, informed consent will be obtained from the patient before any study procedures are undertaken. Drug therapy for symptom relief, blood pressure, lipids and glucose control will be optimised during the screening and follow-up visits.

Randomisation

Patients will be randomised in a 2:1 ratio and blocks of six to receive either intramyocardially administered AdVEGF-D or placebo in a double-blinded fashion. Stratification by site will be used. Randomisation will be conducted in connection with the study drug preparation and before the GT procedure using a statistical SAS Proc/PLAN software (SAS Institute Inc., Cary, North Carolina, USA) and implemented in the Electronic Data Capture tool Viedoc (Viedoc Technologies AB, Uppsala, Sweden) provided by Biocomputing Platforms Ltd (Espoo, Finland).

Investigational medicinal product

The first-generation, replication-deficient, serotype 5-based AdVEGF-D and the matching placebo diluent were produced according to Good Manufacturing Practice methods by FinVector Ltd (Kuopio, Finland). The formulation of the placebo diluent is identical to AdVEGF-D apart from the omitted adenovirus vectors, consisting of 5 mM HEPES adjusted to pH 7.8 with NaOH and 20% v/v glycerol in water.

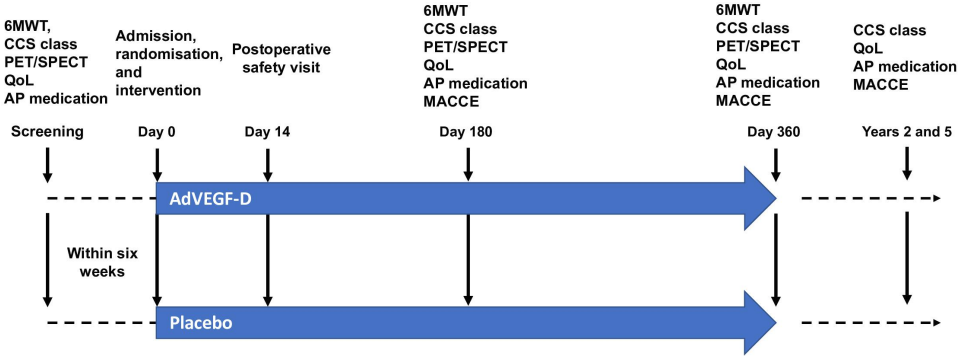

Figure 1 Study flowchart. 6MWT, 6 min walking test; AdVEGF-D, adenoviral vascular endothelial growth factor-D$^{\Delta N\Delta C}$; AP, angina pectoris; CCS, Canadian Cardiovascular Society; MACCE, major adverse cardiac and cerebrovascular events; PET, positron emission tomography; QoL, quality of life; SPECT, single-photon emission CT.

Box 1 Inclusion and exclusion criteria

Inclusion criteria:

1. Informed consent signed.
2. Age 30–85 years.
3. Significant angina pectoris (CCS 2–3) despite optimal medication.
4. Significant stenosis (>60%) in coronary angiography (<12 months), which is not amenable to revascularisation.
5. Contraindication to CABG or PCI due to diffuse or distal stenosis, chronic total occlusion (>3 months after previous chronic total occlusion procedure attempt), vessels with difficult anatomy, stenosis with severe calcifications or stenosis in small vessels (<2.5 mm).
6. Angina pectoris or equivalent symptoms in the 6MWT.
7. Left ventricle wall of <8 mm in the treatment area detected by transthoracic echocardiogram.
8. Reversible myocardial ischaemia in myocardial perfusion imaging.

Exclusion criteria:

1. Women of childbearing potential.
2. Diabetes with severe complications such as proliferative diabetic retinopathy or nephropathy with renal insufficiency.
3. Clinically significant anaemia (haemoglobin, <120 g/L in male, <110 g/L in female; hematocrit, <0.36), leucopenia (leucocyte count, $<3.0\times10^9$/L), leucocytosis (leucocyte count, $>12.0\times10^9$/L) or thrombocytopenia (platelet count, $<100\times10^9$/L).
4. Renal insufficiency (creatinine, >160 µmol/L).
5. Liver insufficiency (alanine aminotransferase or alkaline phosphatase over 2×normal).
6. Haematuria of unknown origin.
7. Severe hypertension (systolic blood pressure, >200 mmHg or diastolic blood pressure, >110 mmHg) or significant hypotension (systolic blood pressure, <90 mmHg).
8. Significant obesity (body mass index, >35 kg/m^2).
9. Acute infection.
10. Current systemic immunosuppressive medication.
11. Significant impairment of left ventricular function (ejection fraction, <25%).
12. Symptomatic congestive heart failure (New York Heart Association score 3–4).
13. Haemodynamically significant aortic or mitral regurgitation (grade 3–4) or other heart diseases needing surgery.
14. Recent acute coronary syndrome, myocardial infarction, PCI, CABG or stroke.
15. Current or suspected malignancy.
16. Inability to perform a 6MWT.

6MWT, 6 min walking test; CABG, coronary artery bypass grafting; CCS, Canadian Cardiovascular Society; PCI, percutaneous coronary intervention.

Box 2 Trial endpoints.

Primary endpoints:

Exercise capacity in 6MWT after 6 months.
CCS class after 6 months.

Secondary endpoints:

Exercise capacity in 6MWT after 12 months.
CCS class after 12 months.
Myocardial perfusion assessed with PET or SPECT after 6 months.
QoL assessed with SAQ-7, SF-36, and EQ-5D-5L.
Use of angina pectoris medication.
Incidence of major adverse cardiac and cerebrovascular events.

6MWT, 6 min walking test; CCS, Canadian Cardiovascular Society; EQ-5D-5L, 5-level EuroQol; PET, positron emission tomography; QoL, quality of life; SAQ-7, Short Version of Seattle Angina Questionnaire; SF-36, 36-item Short Form Survey; SPECT, single-photon emission CT.

Intervention

AdVEGF-D with a total dose of 1×10^{11} viral particles will be administered as ten 200 µL intramyocardial injections using an endocardial electromechanical mapping and injection catheter (NOGA, Johnson & Johnson, New Brunswick, New Jersey, USA).[12] The injections will be targeted into electrically viable left ventricular myocardium with impaired perfusion and decreased mechanical function. Areas of impaired perfusion will be assessed with coronary angiography and myocardial positron emission tomography (PET) or single-photon emission CT (SPECT) perfusion imaging. Areas of viability and decreased contractility will be identified with the NOGA electromechanical mapping system (Johnson & Johnson, New Brunswick, New Jersey, USA). As the placebo procedure, ten 200 µL boluses of the diluent solution will be injected similarly to the intramyocardial AdVEGF-D GT.

Endpoints

The primary endpoint is to demonstrate the efficacy of the GT in improving exercise capacity as measured by the 6 min walking test (6MWT) and in relieving angina pectoris symptoms as measured by CCS score 6 months after the intervention (box 2).

The secondary endpoints are the efficacy of the GT to improve functional capacity using the 6MWT and to relieve symptoms assessed by CCS score after 12 months and the improvement in myocardial perfusion either in PET or SPECT after 6 months. In addition, after 6 and 12 months follow-up, quality of life, the use of short-acting angina pectoris medication and the incidence of major adverse cardiac and cerebrovascular events, a composite endpoint of cardiovascular death, myocardial infarction, stroke, revascularisation and hospital admission due to CAD, will be evaluated.

Assessments

The trial assessments and their schedule are summarised in online supplemental table 2. For the principal clinical trial data, visit date, concomitant medication and symptoms will be recorded during each visit. In addition, adverse events will be recorded at each time point after the screening.

6MWT will be performed to evaluate the efficacy of the AdVEGF-D on functional capacity at the baseline and after 6 and 12 month follow-ups according to the guidelines of the American Thoracic Society.[13]

Symptoms related to angina pectoris will be assessed by CCS score. The effect of AdVEGF-D on the quality of life will be assessed with the disease-specific Short Version of Seattle Angina Questionnaire (SAQ-7), the generic 36-item Short Form Survey (SF-36) and the 5-level EuroQol (EQ-5D-5L).

Myocardial perfusion will be assessed with PET or SPECT depending on the centre's preference using the standard clinical protocols. The image analysis will be performed blinded in a central core laboratory at Turku PET Centre (Turku, Finland). The PET data will be analysed with Carimas software using quantitative perfusion models specific to each PET tracer.[14] The SPECT analysis is based on visual reading using commercial 4DM software with standard perfusion image scoring. The analyses are performed paired with the pre- and post-therapy images analysed in the same analysis session.

In addition to the 12month follow-up, long-term outcomes of the patients will be assessed by telephone 2 and 5 years after the intervention. The long-term safety assessments will be organised similarly to the earlier long-term safety studies.[10]

Laboratory assessments will be conducted at each time point apart from 2 and 5 years. Immunoassays and quantitative PCR for the vector DNA will be analysed at A.I. Virtanen Institute for Molecular Sciences. The complete list of the planned laboratory tests and their schedule is provided in online supplemental table 3.

STATISTICAL ANALYSIS

The power calculations were performed assuming at least a 20% improvement in the 6min walking distance in AdVEGF-D-treated patients compared with the control group. With an alpha level of 0.05 and a power of 80% (two-sided test), a significant change in the independent-samples t-test was assumed to be achieved by at least 22 patients in the GT arm and 10 in the control arm with a mean between-group difference of 56 m (SD 50 m).

A formal statistical analysis plan will be prepared before data unblinding. In brief, statistical analysis will be carried out both with the per-protocol and intention-to-treat principles. Linear mixed model will be used for within and between-group analysis of repeated measurements. If the assumption of normality does not hold for a particular variable, common transformations, such as Lg10, can be used. If the prerequisite for normality is still not met, generalised estimating equation will be used instead of linear mixed model. Two-sided Fisher's exact test or generalised estimating equation will be used for dichotomous variables. Analysis of cumulative incidence will be conducted using a Log-rank test with the Cox regression method to calculate HRs. $p \leq 0.05$ will be considered statistically significant.

Data management

The trial data management will be provided by Biocomputing Platforms Ltd. Information from each study visit will be recorded in a validated and fully Good Clinical Practice-compliant Electronic Data Capture system Viedoc, also provided by Biocomputing Platforms Ltd. Adherence to pharmacovigilance and Good Clinical Practice will be monitored by Medfiles Ltd (Kuopio, Finland) through participation in the site initiation visits, regular on-site visits, telecommunication with the investigators and source data verification. An independent Data and Safety Monitoring Board consisting of two individual experts in the field will be appointed to ensure the compliance of the study protocol to Good Clinical Practice and to provide bias-free evaluation and adjudication of adverse events.

DISCUSSION

ReGenHeart is the first phase 2 trial to investigate the efficacy and safety of intramyocardially administered AdVEGF-D for the treatment of RA. The study protocol has been designed to address the limitations and combine the best methods of the earlier larger-scale trials.

Although definitive evidence to support the clinical use of AdVEGF GT is still lacking, previous trials have yielded positive signals. In 2003, the phase 2 Kuopio Angiogenesis Trial showed as its secondary endpoint that intracoronary infusion of AdVEGF-A resulted in improved myocardial perfusion assessed with SPECT.[15] However, the primary endpoint, in-stent restenosis rate, and other secondary endpoints, functional capacity and anginal symptoms, remained unchanged. Conversely, in the REVASC trial, AdVEGF-A GT via minithoracotomy led to increased functional capacity and a lower CCS class but no change in SPECT perfusion was observed.[16] Paradoxically, myocardial perfusion results were in favour of the control group. The NOVA trial was terminated prematurely due to a slow recruitment rate, and the results from the 17 already enrolled patients demonstrated no improvement in exercise capacity, time to the ischaemic threshold or myocardial perfusion.[17]

ReGenHeart has several advantages over the above-discussed phase 2 trials. First, its vector is based on adenoviruses, which are superior to plasmid vectors in their gene transfer efficacy.[18] The negative results in trials that have used the latter might be accounted for, at least to some extent, by the vector choice.[19 20] Second, the translated protein, VEGF-D$^{\Delta N \Delta C}$, has many attractive properties over other members of the VEGF family, such as VEGF-A. In contrast to the other VEGFs, VEGF-D is more angiogenic and produces diffuse angiogenesis extending well beyond the local injection sites.[8 21] Owing to its affinity to VEGF receptor 3, VEGF-D is also lymphangiogenic, theoretically reducing the accumulation of pericardial effusion observed with higher AdVEGF doses.

The administration with NOGA mapping and injection catheter allows direct intramyocardial targeting of the myocardium, achieving higher vector concentration in the target tissue with less biodistribution to the periphery than with the intracoronary infusion.[22] The gene transfer efficacy also remains better.[23 24] Electroanatomical mapping provides essential support for reaching the designed target area,[12] and the risk of complications is most likely reduced as well. A robust double-blinded setup can be maintained because the endovascular procedure is identical to the control group.[19]

In many previous studies, patient selection has allowed recruitment of participants with anginal symptoms as severe as CCS class 4.[16 17 19 20] CCS class 4 is associated with a higher cumulative hazard of revascularisation

procedures and all-cause mortality as compared with the lower angina grades, including CCS class 3.[25] Based on our clinical experience, the most severely symptomatic patients who are non-eligible for revascularisation are often affected by complex comorbidity and difficult overall situation and thus likely exhibit an insufficient regenerative capacity to benefit from the intervention. Therefore, the patient selection in ReGenHeart is optimised by including only patients with CCS class 2–3 symptoms.

6MWT was chosen as the method for evaluating functional capacity based on the recommendation from the Finnish Medicines Agency and international regulatory bodies and because it is practical and cost-effective to perform in each site without prior experience with ergometry or a treadmill. 6MWT has an excellent correlation to other types of exercise tests; however, it is considered more acceptable by the patients. It also reflects better daily activity of cardiac patients[13 26]

We prefer PET instead of SPECT to assess the change in myocardial perfusion after GT. PET renders it possible to detect more subtle perfusion defects compared with SPECT due to its superior sensitivity and accuracy.[27 28] In addition, the more favourable tracer kinetics allow dynamic and reliable imaging of myocardial tracer uptake through the series of acquired time frames.[29] Thus, the perfusion can be assessed in absolute terms and, consequently, also diffuse myocardial ischaemia can be detected, for example, in patients with left-main or three-vessel disease and those with a history of CABG.[12] In contrast to PET, ischaemia detection in SPECT depends on a reference zone with normal blood flow; thus, it may underestimate the degree of myocardium under jeopardy in patients with balanced ischaemia patterns.[30] This is important because all but one patient in KAT301 had CABG in their history,[9] in addition to our general observation that RA patients often present with advanced and complex CAD phenotype.

Limitations

Despite its aim to employ the best designs from previous studies, ReGenHeart still has some limitations. First, the patient recruitment was significantly affected by the COVID-19 pandemic, leading to lockdowns and closure of trial-related operations in the invasive cardiology units for almost 2 years. Furthermore, because the availability of PET is still limited in some countries, SPECT is allowed to be used as a substitute. As discussed, PET is the preferred imaging modality, as it is superior to SPECT in accuracy and can quantify myocardial perfusion in absolute terms. However, results from both scans can still be processed comparably. As an added benefit, incorporating both imaging modalities allows us to compare the performance of PET and SPECT in assessing changes in myocardial perfusion after AdVEGF-D GT.

CONCLUSIONS

In conclusion, the phase 2 ReGenHeart trial will investigate the safety and efficacy of intramyocardially administered AdVEGF-D GT for the treatment of RA. As there is still a shortage of randomised, placebo-controlled and double-blinded trials in the field, its results will provide important knowledge not only of the most promising vector candidate for the treatment of RA but of myocardial GT in general.

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Contributors AJL wrote the manuscript. JEKH and SY-H conceptualised and designed the study and secured the trial funding. AJL, JEKH, JK, AM, MG, FF-A, RS-R, WW, AG, AAQ, RL, MN, MK, DAJ, MEF-S, KH, AS, MJ, AS and NV will perform the data acquisition and practical conduction of the trial. AJL, JK and AS will analyse and interpret the data. AJL, JEKH and SY-H are the guarantors of the paper. All the authors have approved the submitted version of the manuscript.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by TUKIJA dnro 50/06.00.01/2017NVK 1703450. Participants gave informed consent to participate in the study before taking part.

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