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Protocol for the Stimulating β_3-Adrenergic Receptors for Peripheral Artery Disease (STAR-PAD) trial: a double-blinded, randomised, placebo-controlled study evaluating the effects of mirabegron on functional performance in patients with peripheral arterial disease

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ABSTRACT

Introduction There is currently only one approved medication effective at improving walking distance in people with intermittent claudication. Preclinical data suggest that the β_3-adrenergic receptor agonist (mirabegron) could be repurposed to treat intermittent claudication associated with peripheral artery disease. The aim of the Stimulating β_3-Adrenergic Receptors for Peripheral Artery Disease (STAR-PAD) trial is to test whether mirabegron improves walking distance in people with intermittent claudication.

Methods and analysis The STAR-PAD trial is a Phase II, multicentre, double-blind, randomised, placebo-controlled trial of mirabegron versus placebo on walking distance in patients with PAD. A total of 120 patients aged ≥40 years with stable PAD and intermittent claudication will be randomly assigned (1:1 ratio) to receive either mirabegron (50 mg orally once a day) or matched placebo, for 12 weeks. The primary endpoint is change in peak walking distance as assessed by a graded treadmill test. Secondary endpoints will include: (i) initial claudication distance; (ii) average daily step count and total step count and (iii) functional status and quality of life assessment. Mechanistic substudies will examine potential effects of mirabegron on vascular function, including brachial artery flow-mediate dilatation; MRI assessment of lower limb blood flow, tissue perfusion and arterial stiffness and numbers and angiogenesis potential of endothelial progenitor cells. Given that mirabegron is safe and clinically available for alternative purposes, a positive study is positioned to immediately impact patient care.

Ethics and dissemination The STAR-PAD trial is approved by the Northern Sydney Local Health District Human Research Ethics Committee (HREC/18/HAWKE/50). The study results will be published in peer-reviewed medical or scientific journals and presented at scientific meetings, regardless of the study outcomes.

Strengths and limitations of this study

- Stimulating β_3-Adrenergic Receptors for Peripheral Artery Disease builds on strong cellular and preclinical data that stimulating the β_3-adrenergic receptor promotes angiogenesis and improved endothelial function and may be of benefit for patients with intermittent claudication secondary to PAD.
- This is the first randomised, double-blinded, placebo-controlled study of repurposing the clinically approved β_3-adrenergic receptor agonist mirabegron for patients with PAD where there is a major unmet need for drugs effective at improving functional walking distance.
- The primary endpoint of the study, peak walking distance, is clinically meaningful and the standard for approval of new drugs for treatment of PAD by regulatory authorities.
- The study includes secondary endpoints of average daily step count and quality of life as well as mechanistic endpoints of endothelial function and angiogenesis potential.
- While the study is powered for patient-focussed endpoints, a larger study size would be required to examine effects on revascularisation, hospitalisation and mortality.

Trial registration number ACTRN12619000423112; Results.

INTRODUCTION

Atherosclerotic peripheral arterial disease (PAD) is estimated to affect more than 200 million people worldwide[1] (10% of men aged ≥65 years; 20% of men and women ≥75 years[2]) and is a strong predictor of cardiovascular (CV) mortality.[3] During exercise, peripheral limb arterial stenoses limit the ability to increase blood flow, leading to an oxygen supply/demand mismatch and claudication. Thus, the primary pathophysiology of PAD is related to the limitation in blood flow and abnormal haemodynamics during exercise. Severe clinical disease is associated with leg pain at rest and ulceration and gangrene, driving hospitalisation, revascularisation procedures and eventual amputation. The costs of PAD are both direct (medical and surgical intervention and rehabilitation) as well as indirect (related to impaired ability of patients to partake in the workforce), with the total annual costs associated with hospitalisation of patients with PAD in the USA in excess of $21 billion.[4] This is expected to continue to increase with the ageing of the population and increasing incidence of diabetes.

Current guidelines focus on risk factor management to minimise atherosclerosis progression, including lifestyle modifications (weight loss, smoking cessation), lipid-lowering therapy, antihypertensive therapy[5] and the use of antiplatelet agents and exercise programmes based on excellent randomised controlled trials.[6 7] However, the uptake and availability of exercise programmes is low.[8 9] As a result, in some developing countries, the only available treatments for intermittent claudication are endovascular revascularisation but these are costly and associated with complications and poor durability.[10] Only one medication is recommended for treating intermittent claudication by guideline bodies based on some modest benefit in randomised trials: cilostazol.[11 12] This, however, has not been shown to reduce cardiovascular events,[13] has a limited effect on walking ability and is contraindicated or not tolerated by up to 50% of patients.[14 15] It is also not available on publicly funded benefit schemes in many countries, such as Australia, and therefore not accessible to many patients. There is therefore an unmet need for alternative medical treatment options for patients with PAD.

Given the high attrition rates, substantial costs and slow pace of new drug discovery and development, repurposing of currently approved drugs is an increasingly attractive proposition using derisked compounds, with potentially lower overall development costs and shorter development timelines.[16] Our study is an excellent example of such drug repurposing which is also featured in the Australian Medical Research and Innovation Priorities (2018–2020), 'decreasing development costs and decreasing the time needed to deliver new therapies to the patient'. We have made the initial molecular and cellular discoveries and performed the essential cellular and preclinical animal studies required to support this clinical study as the logical next step. Thus, this short and cost-effective study, powered to the major functional endpoint, endorsed by international guideline and approving committees, may have immediate impact for a patient population in grave need.

The generation of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS), in a healthy endothelium, is essential for normal physiological regulation of blood flow and nutrient delivery to tissues[17] and protects against the longer-term development of atherosclerosis. Endothelium-derived NO production is reduced in patients with PAD[18] and this is independently associated with adverse long-term outcomes.[19] β_3ARs are expressed in endothelial cells[20] where they are coupled to eNOS activation.[21 22] We have recently demonstrated that selective β_3AR agonist CL-316,243 strongly protects against endothelial dysfunction and redox/NO imbalance.[23] We also showed a novel role of β_3AR stimulation in promoting angiogenesis in endothelial cells in vitro. Delivery of CL-316,243 in a mouse model of diabetic PAD improved reperfusion following hind limb ischaemia.[24]

The recent approval of an orally available β_3AR agonist, mirabegron, for clinical use in overactive bladder syndrome, and the demonstrated safety and tolerability of this agent in humans makes a phase II clinical trial repurposing a β_3AR agonist to target PAD immediately feasible. Thus, we now present the protocol for STimulating β_3 Adrenergic Receptor in PAD, a novel and low budget, double-blind, randomised clinical trial examining the hypothesis that the oral β_3AR agonist, mirabegron is safe and effective in improving lower limb perfusion and function on a treadmill in patients with PAD.

Objectives

The primary objective of the trial will be to investigate in a double-blinded, randomised controlled method whether β_3AR agonism with mirabegron (50 mg/day) for 12 weeks will improve lower-limb perfusion and intermittent claudication in participants with PAD.

The secondary objectives of the study include: (i) to examine the mechanisms of action of β_3AR agonism on human arterial function, including its effect on lower-limb blood flow, endothelial function, circulating markers of arterial oxidative stress and NO bioavailability and circulating endothelial progenitor cells (EPCs) and (ii) to validate novel, cutting-edge MRI-derived measurements of tissue perfusion and lower-limb arterial flow dynamics as quantitative physiological indices of outcome and as predictors of risk in patients with PAD.

METHODS AND ANALYSIS

Study design

The Stimulating β_3-Adrenergic Receptors for Peripheral Artery Disease (STAR-PAD) trial is a phase II, double-blind, randomised, placebo-controlled trial investigating whether β_3-adrenergic receptor stimulation

with mirabegron for 12 weeks is safe and effective in improving lower limb perfusion and intermittent claudication in patients with stable atherosclerotic PAD. After initial screening and assessment for eligibility, including the ability to walk on a treadmill with <25% variability in peak walk time (PWT) between two consecutive screening treadmill tests, participants will be randomised to receive mirabegron (50 mg/day) or matching-placebo for 12 weeks (table 1). Participants will receive a follow-up phone evaluation 4 weeks after they have completed the intervention. The trial is being conducted under the TGA CTN scheme (approved in February 2019) and was registered with the Australian New Zealand Clinical Trials

Table 1 Schedule of evaluations for participants who will NOT be undertaking advanced arterial studies

Study periods	Screening	Baseline and randomisation	Treatment		
Study week	Week −1	Week 0	Week 4	Week 12 (end of treatment)	Week 16 follow-up phone call
Study visit window from randomisation	±7 days	N/A	±7 days	±7 days	±7 days
Study visit number (as per patient consent form)	1	2	5	6	
Evaluation					
Informed consent	X				
Inclusion/exclusion criteria	X	X			
Demographics (DOB, sex, ethnicity)	X				
Physical examination (abdomen, cardiovascular, mental status, respiratory, peripheral oedema)	X		X	X	
Medical history	X				
Vital signs (height, weight, BMI, heart rate, BP*3)	X		X	X	
12-lead ECG	X		X	X	
Concomitant medications		X	X	X	X
Randomisation		X			
Prescription		X	X		
Study drug or placebo dispensed		X	X		
Study drug returns and pill counts			X	X	
Patient diary		X	X	X	
Quality of life questionnaires (SF-36, EQ-5D-5L, Intermittent Claudication Questionnaire)		X		X	
Wearable technology (measure of daily activities for 4 days)	X			X	
Cardiovascular-related adverse events reporting			X	X	X
Other AE/AESI/SAEs			X	X	X
Pathology blood tests (FBC, EUC, LFT, HbA1c, fasting lipids-TC, LDL, HDL,TG)	X		X[1]	X	
Research blood sample collection (Laboratory assessments)		X		X	
ABI	X			X	
PWT	X	X		X	
Phone follow-up (general health condition)					X

ABI, ankle-brachial index; AE, adverse event; AESI, adverse events of special interest; BMI, body mass index; DOB, date of birth; ECG, electrocardiogram; EQ-5D-5L, EuroQol-5 Dimension-5 Level; EUC, electrolytes urea creatinine (renal function tests); FBC, full blood count; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFT, liver function tests; PWT, peak walk time; SAE, serious adverse event; SF-36, 36-Item Short Form Survey; TC, total cholesterol; TG, triglycerides.

Registry prior to the commencement of recruitment (ACTRN12619000423112).

Endpoints

The primary endpoint of this study will be the change in exercise performance reflecting the peak exercise capacity of the patient and quantified by PWT at t=12 week vs t=0) on a graded treadmill test performed according to the Skinner-Gardner protocol.[25] Change in exercise performance is reproducible and sensitive to treatment effect and is the standard for approval of new drugs by the FDA and European regulatory authorities for management of PAD.

The secondary study endpoints are: (i) change in initial claudication distance; (ii) change in average daily step count and total step count measured over four consecutive days using a wearable device (Misfit Ray) and (iii) patient-based assessments of daily function and health-related quality of life (QoL) using the validated Intermittent Claudication Questionnaire (ICQ)[26] and the Health Status Survey SF-36 questionnaire (SF-36)[27] at Weeks 0 and 12.

Secondary safety endpoints include (i) severe adverse event (SAE); (ii) cardiovascular-related events including need for revascularisation or hospitalisation for cardiovascular condition; (iii) potentially relevant side effects given the known physiological effects of the signalling pathway and (iv) biochemistry and renal function.

Mechanistic substudies: To examine the mechanisms of action of β_3AR stimulation on human arterial function, including its effect on blood flow, we will conduct a number of subgroup analyses. Endothelial function will be assessed by measuring brachial artery reactivity by flow-mediated brachial artery vasodilation. Circulating markers of arterial oxidative stress will be measured including plasma glutathione peroxidase, catalase, superoxide dismutase, F_2-isoprostane 8-iso-prostaglandin $F_{2\alpha}$ and thiobarbituric acid reactive substances. NO bioavailability will be assessed by measuring cyclic GMP, nitrate to nitrite conversion and eNOS activity. Circulating endothelial colony forming cells will be selectively cultured.

Patient and public involvement

Patient input into the study design was made through ongoing consumer engagement sessions. The research question and outcome measures have been developed to address patient priorities, experience and preferences. Patient involvement extends to recruitment to this study. At the completion of the study, patients will be allowed access to the summary results at their request. If incidental findings are uncovered during this research, participants will have a choice as to whether or not they wish to be informed of results that might be of significance to their health. The burden of the intervention is assessed by patient-based assessments of daily function and health-related quality-of-life questionnaires including the Intermittent Claudication Questionnaire (ICQ) and the Health Status Survey SF-36 questionnaire (SF-36).

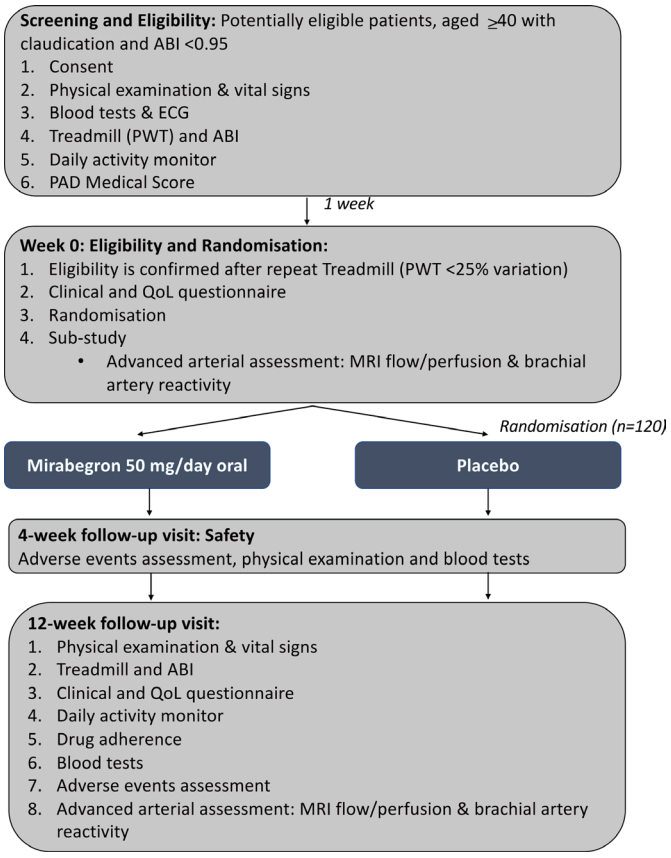

Figure 1 Study design and flow. ABI, ankle-brachial index; PAD, Peripheral Artery Disease; PWT, peak walk time; QOL, quality of life.

Study participants and recruitment

The STAR-PAD trial is recruiting participants from clinics within four hospitals in Sydney, Australia, in which they receive care for PAD. A total of 120 participants will be included in this trial. Participants meeting the initial selection criteria will be referred by a clinician at their hospital to a single testing site, which is based at Royal North Shore Hospital, Sydney. Potential participants will be reviewed by trained research staff and provided with a written study information sheet and a copy of the consent form. Those willing to participate in the study will be invited to attend a screening visit in which a physical examination will be performed, and the participant's relevant anthropometric, physical activity, demographic and medical information will be recorded. Additional assessment of eligibility will be determined via a series of tests, including blood biochemistry, ankle-brachial index (ABI) measurement, PWT and ECG (figure 1). Participant eligibility will be assessed according to the inclusion and exclusion criteria as follows (and in online supplemental file):

Inclusion criteria

1. Aged ≥40 years.
2. Atherosclerotic PAD (unilateral or bilateral) with a resting ABI <0.95

3. Stable intermittent claudication for the previous 3 months not requiring revascularisation.
4. Ability to walk on a treadmill with a variability in PWT <25% between two consecutive treadmill tests 1 week apart.
5. Adherence to a stable medical regimen for 3 months.

Exclusion criteria

1. Ischaemic leg pain at rest, ulceration or gangrene or previous major amputation
2. Acute coronary syndrome or revascularisation of coronary/peripheral arteries in last 3 months
3. Uncontrolled hypertension (SBP >180 or DBP>100 mm Hg).
4. Active inflammatory, infectious or autoimmune diseases.
5. Significant renal impairment (GFR <45 mL/min/1.73 m^2).
6. Concomitant illness, physical impairment or mental condition that could interfere with effective conduct of the study for its duration, including life expectancy <3 months.
7. Contraindication to mirabegron—including severe hepatic impairment (Child-Pugh Class C), moderate hepatic impairment (Child-Pugh Class B) concurrently receiving strong CYP3A inhibitors and pregnant, breastfeeding or fertile female patients without appropriate contraception.

Consent

Written informed consent will be obtained at the start of the screening visit for all participants willing to proceed with the study. Screening visits will be conducted by trained research staff and written informed consent will be obtained by a study subinvestigator who is a clinician. As a part of the discussion for obtaining consent, potential participants will be informed that the screening process will determine eligibility for the study and that signing the consent form does not guarantee enrolment into the study. A signed copy of the participant information sheet and the consent form will be given to the patient along with a copy of the withdrawal of consent form.

Randomisation and allocation concealment

After screening and confirmation of eligibility, participants will be randomised (1:1) to receive either mirabegron (50 mg) or matching placebo once daily in oral tablet form, for 12 weeks. Randomisation will be performed using randomly permuted blocks produced by the NHMRC Clinical Trials Centre, Camperdown, Australia and supplied directly to the distributing clinical trials pharmacist. All study medications will be re-encapsulated and concealed by identical packaging.

Blinding

The study participants and the study investigators will be blinded to treatment allocation, with the exception of the clinical trials pharmacist at the study test site and the unblinded statistician reporting to the Independent Data & Safety Monitoring Committee (IDSMC). Statistical analyses will be performed and validated by statisticians and programmers blinded to group allocation.

Interventions

Participants will be randomised to receive mirabegron (50 mg) or matching placebo once a day in oral form for 12 weeks. Mirabegron will be purchased from Astellas Pharmaceuticals and blinded via re-encapsulation by a cGMP licenced manufacturing facility (PCI Pharma Services). The comparator placebo formulation is a gelatin capsule packed with microcrystalline cellulose and appears identical to the interventional product on the exterior due to overencapsulation. The active study drug (mirabegron) and matching placebo will be shipped with pull-off identification tags and clinical trial pharmacy at the testing site will manage the dispensary of study drugs to participants after randomisation.

Study outcomes

Participant characteristics, anthropometry and hemodynamic parameters

Baseline participant characteristics, such as age, sex, ethnicity, clinical history (including comorbidities, tobacco smoking history, alcohol use or other substance use) and current medication history, will be collected. A study subinvestigator who is a clinician will also perform a physical examination (to assess the cardiovascular, respiratory and gastrointestinal systems and to assess mental status and for peripheral oedema) and a 12-lead ECG at baseline, 4 and 12 weeks. Basic anthropometry (height, weight and body mass index) and haemodynamic parameters (heart rate and blood pressure, after 10 min of rest) will be measured at baseline, 4 and 12 weeks.

Blood biochemistry

Venous blood samples (20 mL) will be taken at baseline, 4 and 12 weeks. At baseline and 12 weeks, blood will be taken after fasting for >8 hours to assess fasting lipids and lipoproteins (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), fasting glucose, HbA1c, a full blood count and renal and liver biochemistry. In addition, a full blood count and renal and liver function will be assessed at 4 weeks (non-fasting). Additional blood samples will be obtained for exploratory and mechanistic studies (described in detail below).

Primary study outcome

The primary study outcome is change in exercise performance assessed by a graded treadmill test, reflecting the peak exercise capacity of the patient. It will be quantified as the PWT in minutes, at 12 weeks compared with baseline, using the *Skinner-Gardner protocol*.[25] Change in exercise performance is reproducible and sensitive to treatment effect and is the standard for approval of new drugs by the FDA and European regulatory authorities for management of PAD.

Secondary study outcomes

Additional treadmill endpoints.

Initial claudication distance will be measured as the distance walked before the onset of ischaemic leg pain.

Average daily step count and total count measured over four consecutive days using the Misfit Ray wearable device or equivalent.

Functional status and QoL) assessment.

Functional status will also be assessed by the validated Intermittent Claudication Questionnaire (ICQ)[26] and the Health Status Survey SF-36 questionnaire (SF-36)[27] at both time points. The ICQ is a self-administered, condition-specific, validated measure of QoL in intermittent claudication. A 5-point scale is used to rate the limitation or frequency of claudication on 16 items, resulting in a score ranging from 0 (best) to 100 (worst). International consensus statements have stressed that QoL instruments should be used in all claudication trials and, ultimately, that QoL measurements may become the primary endpoints.[28]

Ankle-brachial index (ABI)

ABI, a validated and clinically used tool, will be measured after 10 min of supine rest with a hand-held Doppler. Systolic pressures are measured at the dorsalis pedis, posterior tibial and brachial arteries bilaterally.[29] The right and left ABI values will be calculated by taking the higher pressure of the two arteries at each ankle, divided by the higher of the two brachial pressures. The index ABI is defined as the ABI of the extremity with the lowest value.

Mechanistic endpoints

Brachial artery flow-mediated dilatation and glyceryl trinitrate responsiveness

Flow-mediated brachial artery vasodilatation will be used to assess endothelial function with the use of an Acuson Sequoia C256 high-resolution ultrasound unit with a 14-mHz probe (Siemens).[30] Vascular studies will be performed in a quiet, darkened room with the patient fasting. After measurement of the brachial artery diameter, a blood pressure cuff on the forearm will be inflated to a pressure of 50 mm Hg above systolic pressure for 5 min. After deflation, measurements of the brachial artery diameter are performed at 30, 45 and 60 s during reactive hyperaemia. Electronic callipers will be used for the measurement of artery diameter. Flow-mediated vasodilation will be expressed as the maximal per cent change in diameter from the resting condition during the period of reactive hyperaemia (table 2).

(i) Measurement of levels of nitric oxide, markers of oxidative stress and NO signalling

Arterial NO/redox balance will be measured using blood markers of oxidative stress and NO signalling established in our laboratories. Well-characterised markers of redox status will be measured. These include plasma glutathione peroxidase, catalase, superoxide dismutase, F_2-isoprostane 8-iso-prostaglandin $F_{2\alpha}$ and thiobarbituric acid reactive substances. NO bioavailability will be assessed by measuring plasma and urinary nitrogen oxides and cyclic GMP. Peripheral blood mononuclear cells will be extracted and used for selective cell culturing as outlined below (ii and iii). From this, we will measure cellular eNOS activity and NADPH oxidase 2 activity as well as macrophage myeloperoxidase activity. Expression levels of protective enzymes glutaredoxin, thioredoxin 1 and superoxide dismutase will also be measured in erythrocytes and microparticles.

(ii) Circulating endothelial colony forming cells (ECFCs) by flow cytometry

Circulating ECFCs have the ability to repair endothelial damage and are involved in angiogenesis in ischaemic limbs. We will use flow cytometry to quantify the number of ECFCs circulating in patients receiving mirabegron vs placebo. Briefly, fresh, whole blood will be assessed and circulating EPCs will be detected as CD45-/CD31+/CD133+ as described previously.[31]

(iii) ECFC growth kinetics in vitro

The rates of spontaneous growth of ECFCs will be assessed from the peripheral blood mononuclear cell fractions of whole blood, obtained by Ficoll gradient separation and plated in 0.1% gelatin-coated wells of 24-well plates in endothelial cell conditioned culture media.[24 32] Cell morphology, eNOS expression, number and rate of development and CD31/CD133 expression will be correlated to patient profile and treatment group. Measures of oxidative stress and NO bioavailability will be performed as outlined above (i).

(iv) ECFC-derived angiogenesis in ex vivo models

Well-characterised in vitro assays of angiogenesis that are standard in our laboratory will be used to compare behaviour of cells from mirabegron vs placebo subjects including (a) tubule formation assay[33] where 5×10^4 cells are plated onto reduced-growth factor basement membrane extract (Cultrex) in wells of 96-well plates using endothelial cell growth medium with reduced serum content (Lonza) and tubule formation is monitored by time lapse video microscopy over 16 hours; (b) cell migration assays assessing re-endothelialisation of 'wounds' on confluent endothelial cell monolayers and migration/invasion assays using 5 µm Boyden chambers (Merck Millipore); (c) cell proliferation rate using manual trypan blue exclusion counting and/or BrDu incorporation in serum-starved cells plated at low density with serum-stimulation of proliferation.

Adverse events

An Independent Data and Safety Monitoring Committee (IDSMC) will be established and provide final recommendations to the regarding interim analysis and stopping rules for safety, efficacy and futility. All SAEs and cardiovascular-related adverse events experienced after randomisation and until 16 weeks (ie, 4 weeks after

Table 2 Schedule of evaluations for participants who *will be* undertaking advanced arterial studies

Study periods	Screening	Baseline	Randomisation	Treatment		
Study week	Week –3	Week –2	Week 0	Week 4	Week 12 (end of treatment)	Week 16 (end of study)
Study visit window from randomisation	±21 days	±14 days	N/A	±7 days	±7 days	±7 days
Study visit number (as per patient consent form)	1	2	3 and 4	5	6, 7 and 8	
Evaluation						
Informed consent	X					
Inclusion/exclusion criteria	X	X	X			
Demographics (DOB, sex, ethnicity)	X					
Physical examination (abdomen, cardiovascular, mental status, respiratory, peripheral oedema)	X			X	X	
Medical History	X					
Vital signs (height, weight, BMI, heart rate, BP*3)	X			X	X	
12-lead ECG	X			X	X	
Concomitant medications		X		X	X	X
Randomisation			X			
Prescription			X	X		
Study drug or placebo dispensed			X	X		
Study drug returns and pill counts				X	X	
Patient diary			X	X	X	
Quality of life questionnaires (SF-36, EQ-5D-5L, Intermittent Claudication Questionnaire)		X			X	
Wearable technology (measure of daily activities for 4 days)	X				X	
Cardiovascular-related adverse events reporting				X	X	X
Other AE/AESI/SAEs				X	X	X
Pathology blood tests (FBC, EUC, LFT, HbA1c, fasting lipids-TC, LDL, HDL,TG)*	X			X[1]	X	
Research blood sample collection (Laboratory assessments)		X			X	
ABI	X				X	
PWT	X	X			X	
Advanced arterial studies (MRI flow and perfusion; brachial arterial reactivity)†			X		X	
Phone follow-up (general health condition)						X

*Only FBC, EUC, LFT will be tested at the week 4 visit.
†Advanced arterial studies will be conducted in a subset of patients enrolled at participating sites.
ABI, ankle-brachial index; AE, adverse event; AESI, adverse events of special interest; BMI, body mass index; DOB, date of birth; ECG, electrocardiogram; EQ-5D-5L, EuroQol-5 Dimension-5 Level; EUC, electrolytes urea creatinine (renal function tests); FBC, full blood count; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFT, liver function tests; MRI, magnetic resonance imaging; PWT, peak walk time; SAE, serious adverse event; SF-36, 36-Item Short Form Survey; TC, total cholesterol; TG, triglycerides.

completion of the intervention phase of the trial) will be collected and reported to the Trial Steering Committee and IDSMC. A study clinician will assess the causality and expectedness of any events thought to be related to the study drug. An adverse event is deemed serious if it results in death, is life threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, a medically important event or drug reaction or any event that requires medical or surgical intervention to prevent a serious outcome. Serious and cardiovascular-related adverse events will be reported without delay and will be followed up until resolved or resulting in the patients' death. Adverse events that do not fall into these categories are defined as non-serious. A process has been developed to facilitate emergency unblinding when essential to protect and individual participant's safety. If an adverse event occurs that is suspected by treating physician to be related to the investigational product, the study treatment will be discontinued and the participant referred back to

primary care physician. Compensation may be available for participants if injury or complication is caused by the study drug or procedures.

All adverse events will be reviewed by the IDSMC on a 3-monthly basis and serious adverse events (SAE) will be immediately referred to the chair. The study will be stopped in the event of a serious adverse effect that is deemed to be caused by the investigational product. The IDSMC and Trial Management Committee will finalise the interim analysis and study stopping rules prior to data unblinding. This will include an interim analysis when 50% of the study has been completed. Stopping will occur if efficacy has been demonstrated in the primary endpoint and biological mechanism, brachial artery reactivity. Futility will not be assessed due to modest study size.

Statistical analysis

All analyses of study outcomes will be conducted according to the principle of intention-to-treat. The primary analysis of change in PWT at 12 weeks will be performed using an analysis of covariance (ANCOVA) with adjustment for baseline PWT. Continuous secondary outcomes will be analysed similarly. Because of skewed distributions for QoL measures and brachial artery flow-mediated dilation, differences for these outcomes will be compared using Kruskal-Wallis analysis of variance and Wilcoxon rank sum tests. In the case of binary endpoints, log-binomial or logistic regression will be used. There will also be predefined subgroup analyses (eg, by age and sex). A detailed statistical analysis plan will be finalised prior to database lock and unblinding.

Sample size calculation

Power calculations were performed using results from the recently published CLEVER study (of similar patients with PAD) and our clinical experience regarding functionally relevant difference in treadmill performance. We assumed treatment with mirabegron will result in a more conservative improvement in PWT of 2 min, compared with the ~4.6 min achieved by the supervised exercise intervention of CLEVER.[34] We took into consideration the increase in SD seen at 6-month time point and ran calculations assuming that the SD for change from baseline will be 2 in the placebo arm and 4 in the mirabegron arm. A sample size of 54 participants per group will provide 90% power (type-I error rate=5%) to detect a 2.0 min difference between intervention and control arms. Thus, to allow for ~10% out of patients lost to follow-up, 120 patients will be randomised (n=60 per group).

ETHICS AND DISSEMINATION

Ethics, protocol amendments, dissemination and confidentiality

This study is approved by the Northern Sydney Local Health District Human Research Ethics Committee (HREC/18/HAWKE/50) and will be conducted in agreement with the Declaration of Helsinki. Written informed consent will be obtain from all participants in the study prior to the collection of study data. Any significant protocol amendments will be reported to the local ethics committee and the Australian New Zealand Clinical Trials Registry and will be communicated in the primary RCT publication. The conduct of this trial will be overseen by the Trial Steering Committee (authors). The study results will be published in peer-reviewed medical or scientific journals and presented at scientific meetings by the study investigators, regardless of the study outcomes. Neither the sponsor nor any funders will be involved in the analysis and interpretation of data, nor any resulting publications or presentations. No identifiable information will be published.

All data arising from this study will be stored in a confidential manner and within a secure location. Clinical site principal investigators will have direct access to their own site's data set. All principal investigators will be given access to the de-identified, cleaned data set as requested.

Safety and adverse event reporting

An IDSMC will be established. Standard clinical trial protocol for adverse event reporting for Investigational Medicinal Products (safety monitoring and reporting in clinical trials involving therapeutic goods, NHMRC November 2016) will be followed. Professor Figtree will be responsible for reporting to regulatory bodies. Patients who have side effects from treatment sufficient to require a change of therapy will have study treatment stopped (without the need to un-blind or alter follow-up measurements) and will be referred back to the referring clinician to be managed according to standard clinical practice. Given the demonstrated tolerability in trials and clinical practice in cohorts of similar age being treated for bladder instability, and the relatively short time frame of the study, we do not expect significant patient loss due to side effects or tolerability, although for purposes of power calculations, we will allow for 20% dropout.

- *Severe adverse event* (SAE): Percentage of interventional or control group with any SAE including major adverse cardiac events.
- *Potentially relevant side effects:* Percentage with potentially relevant side effects (dizziness, blurred vision, syncope/collapse/fall, chest pain/angina, shortness of breath, cough, wheeze, ankle oedema, skin rash, itching, gout, other), in addition to bladder specific questions (exclude increased incidence of atonic bladder).
- *Biochemistry and renal function:* change in mean potassium, uric acid, blood glucose, cholesterol and subfractions, ALT, AST, UACR (urine albumin-to-creatinine ratio) and creatinine levels.

DISCUSSION

The STAR-PAD Study, derived from our own team's biomedical discoveries, is the first clinical trial to examine the efficacy of β_3AR agonists in PAD. Confirmation that a clinically safe and clinically available β_3AR agonist

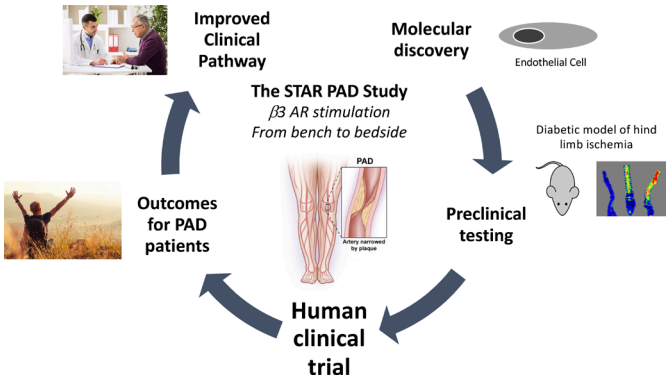

Figure 2 Schematic summary of the path from discovery at a cellular and molecular level, through to in vivo animal studies, and now to a clinical trial repurposing Mirabegron to benefit patients with PAD and intermittent claudication. This has potential for immediate translation to clinical practice and guidelines given the unmet need, and the proven safety record of the agent. PAD, peripheral artery disease.

improves lower limb perfusion in patients with PAD has the potential for immediate benefit to a large patient group with limited medical options, many of whom are currently undergoing recurrent expensive surgical procedures with poor long-term results. This bench to bedside programme is summarised in the schematic figure 2. The trial is powered to determine impact of mirabegron on one of the key primary endpoints (PWT) that has been endorsed by international guideline bodies as well as both the FDA and European regulatory authorities in approving new therapies for PAD. However, in addition to influencing these bodies and practice in itself, the study's primary and secondary mechanistic endpoints will guide further large international clinical studies that include endpoints of hospitalisation, revascularisation, cardiovascular death, in addition to wound healing.

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