PEER REVIEW HISTORY

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ARTICLE DETAILS

| TITLE (PROVISIONAL) | Protocol for the Stimulating β3-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 |
| AUTHORS | Bubb, Kristen; Harmer, Jason A.; Finemore, Meghan; Aitken, Sarah Joy; Ali, Zara; Billot, Laurent; Chow, Clara; Golledge, Jonathan; Mister, Rebecca; Gray, Michael; Grieve, Stuart; Hamburg, Naomi; Keech, Anthony; Patel, Sanjay; Puttaswamy, Vikram; Figtree, Gemma |

VERSION 1 – REVIEW

| REVIEWER | Belletti, Alessandro |
| REVIEW RETURNED | 05-Apr-2021 |
| GENERAL COMMENTS | In this manuscript, Prof. Figtree and colleagues present the protocol for a RCT investigating the role mirabegron on patients with PAD. Overall, the protocol is well written and the topic is of clinical relevance. I have few comments for the Authors: 1. It is unclear from the manuscript whether major clinically relevant events (need for revascularization, hospitalization, mortality) will be part of the secondary/safety outcomes. 2. Please describe the subgroup analyses already planned 3. Does the study have planned interim analyses or stopping rules for safety/efficacy/futility? |

| REVIEWER | Shetty, Suchith |
| REVIEW RETURNED | 15-Apr-2021 |
| GENERAL COMMENTS | Reviewer comments In the current study protocol “Protocol for the Stimulating β3-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” the authors are proposing evaluation of mirabegron a beta-3 adrenergic receptor agonist on the functional performance in patients with peripheral arterial disease (PAD). |
In the current double blind, randomized, placebo control trial the authors will primarily evaluate the effect of mirabegron on peak exercise capacity at baseline and compare it to placebo after 12 weeks of treatment. Claudication distance, step counts and quality of life measurements are compared before and after treatment to assess the treatment effect as secondary outcomes. In addition, safety endpoints and mechanistic sub-studies are performed to further strengthen the hypothesis of treatment efficacy. The proposed study protocol has immense clinical significance and is based on findings from animal studies previously performed by the same group. Overall, this is a well proposed study protocol. The research question is clear and appropriate. The methodology described is acceptable. There are a few points that need further attention.

Reviewer’s query:
1. Author must consider providing details about exclusion criteria #7. Since this is a study protocol, inclusion and exclusion criteria must be comprehensive and exhaustive. If the list of illness is lengthy consider including it as a supplement.

Minor corrections
- Line 17 - Grammatical correction, “…drugs for treatment of PAD…..”

VERSION 1 – AUTHOR RESPONSE

Reviewer Reports:

Reviewer: 1. Dr. Alessandro Belletti, IRCCS San Raffaele Scientific Institute

Comments to the Author:

In this manuscript, Prof. Figtree and colleagues present the protocol for a RCT investigating the role of mirabegron on patients with PAD.

Overall, the protocol is well written and the topic is of clinical relevance.

I have a few comments for the Authors:
1. It is unclear from the manuscript whether major clinically relevant events (need for revascularization, hospitalization, mortality) will be part of the secondary/safety outcomes.
All cardiovascular-related adverse events and serious adverse events will be included in analysis of safety outcomes, but these will not form part of the secondary outcomes analysis. This has been made clearer at lines 186-188.

“ii) Cardiovascular-related events including need for revascularisation or hospitalisation for cardiovascular condition”

2. Please describe the subgroup analyses already planned
We will analyse the mechanisms of action of β3AR stimulation by measuring circulating markers of oxidative stress and nitric oxide bioavailability in blood serum or plasma and in peripheral blood monocytes and colony forming endothelial cells. This has now been included in further detail as outlined below. Please note that during editing of this section, we have also updated the nomenclature of the circulating endothelial cells that we will drive, in line with current accepted cell type, from endothelial progenitor cells to endothelial colony forming cells. We have also updated a reference from a conference abstract to full manuscript that is now published (ref 24, Bubb et al, 2021).
Mechanistic sub-studies: To examine the mechanisms of action of β3AR stimulation on human arterial function, including its effect on blood flow we will conduct a number of sub-group 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, F2-isoprostane 8-iso-prostaglandin F2α 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.

Well-characterised markers of redox status will be measured. These include plasma glutathione peroxidase, catalase, superoxide dismutase, F2-isoprostane 8-iso-prostaglandin F2α 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.

3. Does the study have planned interim analyses or stopping rules for safety/efficacy/futility? We have amended the adverse events section to clarify the stopping rules and safety assessment process as follows:

Lines 419-420

“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.”

Lines 430-440

“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. 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.”

Reviewer: 2 Dr. Suchith Shetty, The University of Iowa Healthcare

Comments to the Author:

In the current study protocol “Protocol for the Stimulating β3-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” the authors are proposing evaluation of mirabegron a beta-3 adrenergic receptor agonist on the functional performance in patients with peripheral arterial disease (PAD).

In the current double blind, randomized, placebo control trial the authors will primarily evaluate the effect of mirabegron on peak exercise capacity at baseline and compare it to placebo after 12 weeks
of treatment. Claudication distance, step counts and quality of life measurements are compared before and after treatment to assess the treatment effect as secondary outcomes. In addition, safety endpoints and mechanistic sub-studies are performed to further strengthen the hypothesis of treatment efficacy.

The proposed study protocol has immense clinical significance and is based on findings from animal studies previously performed by the same group. Overall, this is a well proposed study protocol. The research question is clear and appropriate. The methodology described is acceptable. There are a few points that need further attention.

Reviewer’s query:
1. Author must consider providing details about exclusion criteria #7. Since this is a study protocol, inclusion and exclusion criteria must be comprehensive and exhaustive. If the list of illness is lengthy consider including it as a supplement.

The investigators thank the reviewer for noting the opportunity to provide further detail regarding the inclusion and exclusion criteria. This has been submitted as a supplementary document, and for immediate reference it is attached below. Please note that in regards to exclusion criteria #7 (now #6 due to protocol change to exclusion criteria to remove peak walk time variability), due to the small sample size of this study we defer all cases of illness, physical impairment or mental condition that may affect study adherence, or life expectancy, to the consenting physician for their expert opinion on whether the participant has the physical and mental capacity to strictly adhere to the study regimen. Participants are excluded if the opinion of the study physician is that they are unlikely to adhere to the protocol. The wording used in the manuscript is verbatim from the approved protocol and therefore we are unable to change any specific wording in the manuscript.

The STARPAD trial exclusion criteria include the following:
1. Ischaemic rest pain, ulceration or gangrene, or previous major amputation;
2. Acute coronary syndrome or revascularisation of coronary/peripheral arteries in the last 3 months;
3. Uncontrolled hypertension (>180/100mmHg)
4. Active inflammatory, infectious, or autoimmune diseases
5. Significant renal impairment (eGFR<45mL/min/1.73m2)
6. Concomitant illness, physical impairment or mental condition that could interfere with effective conduct of the study during its duration, including life expectancy <3 months
7. Contraindication to Mirabegron
   a. Mirabegron is not recommended in patients with:
      i. End-stage renal disease eGFR <15mL/min/1.73m2);
      ii. Severe hepatic impairment (Child-Pugh Class C);
      iii. Moderate hepatic impairment (Child-Pugh Class B) concomitantly receiving strong CYP3A inhibitors; and
   iv. Pregnant, breastfeeding, or fertile patients without appropriate contraception.
   b. Cautions:
      i. A dose reduction to 25 mg is recommended in patients with severe renal impairment (GFR 15 to 29 mL/min/1.73m2) concomitantly receiving strong CYP3A inhibitors (although these patients will already fail to meet eligibility for this study due to renal impairment);
      ii. Mirabegron can increase blood pressure. Blood pressure should be measured at baseline and periodically during treatment with Mirabegron, especially in hypertensive patients;
      iii. Since patients with a known history of QT prolongation or patients who are taking medicinal products known to prolong the QT interval were not included in tolerance studies, the effects of mirabegron in these patients is unknown. Caution should be exercised when administering mirabegron in these patients;
   iv. Urinary retention in patients with bladder outlet obstruction (BOO) and in patients taking antimuscarinic medicinal products for the treatment of overactive bladder (OAB) has been reported in
post-marketing experience in patients taking mirabegron. Mirabegron should be administered with caution to patients with clinically significant BOO. Mirabegron should also be administered with caution to patients taking antimuscarinic medicinal products for the treatment of OAB; v. In patients with mild to moderate renal impairment (GFR 30 to 89 mL/min/1.73m2) or mild hepatic impairment (Child-Pugh Class A) concomitantly receiving strong CYP3A inhibitors, such as itraconazole, ketoconazole, ritonavir and clarithromycin, the recommended dose of Mirabegron is 25 mg once daily; and vi. Caution is advised if mirabegron is co-administered with medicinal products with a narrow therapeutic index and significantly metabolised by CYP2D6, such as thioridazine, Type 1C antiarrhythmics (e.g., flecainide, propafenone) and tricyclic antidepressants (e.g., imipramine, desipramine). Caution is also advised if mirabegron is co-administered with CYP2D6 substrates that are individually dose titrated.

c. Specific Drug-Drug Interactions:

i. Thioridazine (a medicine for mental illness), propafenone or flecainide (medicines for abnormal heart rhythm), metoprolol (a medicine for high blood pressure or heart problems), imipramine or desipramine (medicines used for depression). These specific medicines may require dose adjustment when taking mirabegron;

ii. Some anti-fungal medicines (e.g. itraconazole, ketoconazole);

iii. Ritonavir (a medicine used to treat HIV/AIDS);

iv. Digoxin (a medicine for heart failure or abnormal heart rhythm). Blood levels of digoxin are carefully titrated and mirabegron use may interfere with digoxin effectiveness, requiring dose adjustment of digoxin;

v. Medicines used for the management of overactive bladder (e.g. solifenacin, tolterodine, oxybutynin, darifenacin, trospium, fesoterodine) and medicines used for the management of enlarged prostate (e.g. tamsulosin); and

vi. Some medicines that are known to prolong QT interval, such as quinidine, sotalol, amiodarone (medicines used for abnormal heart rhythm), mesoridazine, haloperidol (medicines for mental illness), or erythromycin, clarithromycin (anti-infectives).

8. Participation in a concurrent clinical trial of an investigational medical product. A patient will become eligible if at least 3 months have passed since end of participation in an investigational clinical study.

Minor corrections
- Line 17 - Grammatical correction , “…drugs for treatment of PAD…..”
  Changed (please see line 90).
