PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	PropAngio study protocol: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma A proof of principle study
AUTHORS	Heinhuis, Kimberley; IJzerman, Nikki; Koenen, Anne Miek; van der Graaf, Winette; Haas, Rick; Beijnen, Jos; Huitema, Alwin; van Houdt, Winan; Steeghs, Neeltje

VERSION 1 - REVIEW

REVIEWER	Erin Dickerson University of Minnesota USA
REVIEW RETURNED	21-May-2020

GENERAL COMMENTS	Thank you for the opportunity to review your paper. I enjoyed reading it, and I am pleased to see that a clinical trial is being undertaken to assess the impact of propranolol alone on angiosarcomas. While two cases studies have reported responses in angiosarcoma patients using propranolol alone, a larger study is warranted to address the role of propranolol as a single agent therapy. Overall, the paper appears to adhere to the journal guidelines stipulated by the editors. The paper describes a clinical trial that is already underway (enrollment began in December 2019), presents patient numbers for accrual, and provides endpoints and expected termination dates for the study. There are some minor points that should be addressed before publication. Minor points that must be addressed: 1. In the abstract and throughout the paper, the authors should refer to “hemangioma” as “infantile hemangioma.” Although hemangiomas can occur in adults, then differ in presentation, site, and treatment when compared to infantile hemangioma (IH). Since the gold standard for treatment of IH is now propranolol, the target patient population should be clear. 2. The following statement appears twice in the paper, “Angiosarcoma has several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.” The statement appears both in the abstract (lines 12-15, page 2) and in the Introduction (lines 49-52, page 4). The references provided (#11, 17) do not support this statement as neither

	reference mentions VEGF or β-adrenergic receptor expression anywhere in the text. Hence, other references are needed and should be provided by the authors. For the role of VEGF in angiosarcoma, some of the following references could be used: Itakura E, Yamamoto H, Oda Y, Tsuneyoshi M. Detection and characterization of vascular endothelial growth factors and their receptors in a series of angiosarcomas. J Surg Oncol. 2008;97:74–81. Zietz C, Rossle M, Haas C, Sendelhofert A, Hirschmann A, Sturzl M, Lohrs U. MDM-2 oncoprotein overexpression, p53 gene mutation, and VEGF up-regulation in angiosarcomas. Am J Pathol. 1998;153:1425–1433. Folpe AL, Veikkola T, Valtola R, Weiss SW. Vascular endothelial growth factor receptor-3 (VEGFR-3): a marker of vascular tumors with presumed lymphatic differentiation, including Kaposi's sarcoma, kaposiform and Dabskatype hemangioendotheliomas, and a subset of angiosarcomas. Mod Pathol. 2000;13:180–185. Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, Qin LX, Brennan MF, Singer S, Maki RG. KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. Cancer Res. 2009;69:7175–7179. For IH, a good reference for VEGF and the citations therein is: Greenberger S and Bischoff J. Pathogenesis of infantile haemangioma. Br J Dermatol . 2013 July ; 169(1): 12–19. doi:10.1111/bjd.12435. This reference may also provide a source of references for β-adrenergic receptor expression in IH. For β-adrenergic receptor expression in angiosarcoma, Stiles et 2013, which is already referenced in the paper, can be used. It would also be helpful to replace “supposedly important role of VEGF” with “identified” or “potential” or “suggested” since “supposedly” seems to negate the effort of other investigators to identify a role for VEGF in IH as well and angiosarcoma and put into question the potential role of VEGF. 3. In the abstract, please add that staining for Ki-67 will be used as an indication of proliferation to provide sufficient information regarding the method for a reader. 4. Under strengths and limitations of the study, the authors state: “This proof-of-principle study will help to elucidate the effect of a well-known drug (propranolol) for a new indication (cutaneous angiosarcoma).” Can this statement be rewritten so that it more clearly addresses that this clinical trial will test the use of propranolol as a single-agent treatment? Propranolol is already being used to treat angiosarcoma patients in combination with chemotherapy, so it is not a “new” indication. The authors acknowledge this on page 4, line 54. 5. In the Introduction (line 33) the authors refer to propranolol as nonselective β-adrenergic receptor antagonist. While “nonselective” is often used to describe propranolol, the antagonist is actually highly selective for the β1- and β2-adrenergic receptors and has a

	relatively low affinity for the β3-receptor. Please be more specific. One can also refer to propranolol as β3-AR sparing. 6. On page 5 for the sentence “metastatic angiosarcoma have been treated with propranolol, in combination with various,” it should read “were treated” since the study has been completed and reported. 7. For the following, “These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.” Can another reference be provided as the reference provided was not accessible? Other references listed the dose as being 120-240 mg daily, which is more in line with the dose given to angiosarcoma patients. 8. Under Exploratory Endpoints, p.8, the authors use the gene designation (ADRB1, ADRB2, ADRB3) rather than the protein designation for the receptors (β1-AR, β2-AR, β3-AR). Since the levels of the receptors are being measured by immunohistochemistry, the protein designation should be used. 9. On page 8 (line 26) the authors state, “Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens in vitro.” It is not clear what studies will be done here. Will the authors be using cell lines and performing drug synergy studies? If so, more details should be provided. If this is a separate study, the statement should be removed from the paper.

REVIEWER	Hadrian Schepler Medical Center of the Johannes Gutenberg University, Mainz Department of Dermatology Langenbeckstr.1 55131 Mainz Germany
REVIEW RETURNED	03-Jun-2020

GENERAL COMMENTS	The application of the beta-blocker propranalol as a nedoadjunctive therapy for angiosarcoma seems to be an interesting and realistic challenge to treat this extremely aggressive tumor. Its efficacy has been proven in the past especially in the treatment of infantile hemangiomas. The clinical and histological proximity of the hemangioma to angiosarcoma underlines the potential of propranolol to have a similar effect and may host a new therapeutic strategy. First case reports are encouraging. In the presented study, 14 patients with primary, recurrent and metastatic cutaneous angiosarcoma are to undergo a neoadjunctive therapy with propranolol for a maximum period of 6 weeks. However, it is not clear to what significance radiation - induced angiosarcoma is considered. Since this kind of angiosarcoma will become more and more important in the future due to the breast-conserving therapy with mostly cutaneous manifestations, the question arises to what extent this entity will be considered in the study or rather defined as an exclusion criterion. Here the authors should point out their position. Besides, it would be an important enrichment. Otherwise I am very curious whether patients will benefit from the proposed therapy in future.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

Reviewer Name: Erin Dickerson
Institution and Country: University of Minnesota, USA

Thank you for the opportunity to review your paper. I enjoyed reading it, and I am pleased to see that a clinical trial is being undertaken to assess the impact of propranolol alone on angiosarcomas. While two cases studies have reported responses in angiosarcoma patients using propranolol alone, a larger study is warranted to address the role of propranolol as a single agent therapy.

Overall, the paper appears to adhere to the journal guidelines stipulated by the editors. The paper describes a clinical trial that is already underway (enrollment began in December 2019), presents patient numbers for accrual, and provides endpoints and expected termination dates for the study. There are some minor points that should be addressed before publication.

Reply: Thank you very much for your compliments. We did our best to address your comments. Clearly, it has improved the quality of our manuscript and we hope that you will agree.

Minor points that must be addressed:
1. In the abstract and throughout the paper, the authors should refer to “hemangioma” as “infantile hemangioma.” Although hemangiomas can occur in adults, then differ in presentation, site, and treatment when compared to infantile hemangioma (IH). Since the gold standard for treatment of IH is now propranolol, the target patient population should be clear.

Reply: We have changed ‘hemangioma’ into ‘infantile hemangioma’ throughout the manuscript.

2. The following statement appears twice in the paper, “Angiosarcoma has several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.”
The statement appears both in the abstract (lines 12-15, page 2) and in the Introduction (lines 49-52, page 4). The references provided (#11, 17) do not support this statement as neither reference mentions VEGF or β-adrenergic receptor expression anywhere in the text. Hence, other references are needed and should be provided by the authors.

For the role of VEGF in angiosarcoma, some of the following references could be used:
Itakura E, Yamamoto H, Oda Y, Tsuneyoshi M. Detection and characterization of vascular endothelial growth factors and their receptors in a series of angiosarcomas. J Surg Oncol. 2008;97:74–81.

Zietz C, Rossle M, Haas C, Sendelhofert A, Hirschmann A, Sturzl M, Lohrs U. MDM-2 oncoprotein overexpression, p53 gene mutation, and VEGF up-regulation in angiosarcomas. Am J Pathol. 1998;153:1425–1433.

Folpe AL, Veikkola T, Valtola R, Weiss SW. Vascular endothelial growth factor receptor-3 (VEGFR-3): a marker of vascular tumors with presumed lymphatic differentiation, including Kaposi's sarcoma, kaposiform and Dabskatype hemangioendotheliomas, and a subset of angiosarcomas. Mod Pathol. 2000;13:180–185.

Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, Qin LX, Brennan MF, Singer S, Maki RG. KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. Cancer Res. 2009;69:7175–7179.

For IH, a good reference for VEGF and the citations therein is:
Greenberger S and Bischoff J. Pathogenesis of infantile haemangioma. Br J Dermatol . 2013 July ; 169(1): 12–19. doi:10.1111/bjd.12435.
This reference may also provide a source of references for β-adrenergic receptor expression in IH.
For β-adrenergic receptor expression in angiosarcoma, Stiles et 2013, which is already referenced in the paper, can be used.

It would also be helpful to replace “supposedly important role of VEGF” with “identified” or “potential” or “suggested” since “supposedly” seems to negate the effort of other investigators to identify a role for VEGF in IH as well and angiosarcoma and put into question the potential role of VEGF.

Reply: We have rephrased ‘supposedly’ into ‘suggested’ and adjusted the references to Stiles et al., Greenberger et al. and Itakura et al. as requested.

3. In the abstract, please add that staining for Ki-67 will be used as an indication of proliferation to provide sufficient information regarding the method for a reader.

Reply: This was added to the abstract section.

4. Under strengths and limitations of the study, the authors state:
“This proof-of-principle study will help to elucidate the effect of a well-known drug (propranolol) for a new indication (cutaneous angiosarcoma).”
Can this statement be rewritten so that it more clearly addresses that this clinical trial will test the use of propranolol as a single-agent treatment? Propranolol is already being used to treat angiosarcoma patients in combination with chemotherapy, so it is not a “new” indication. The authors acknowledge this on page 4, line 54.

Reply: We have rewritten this statement to clarify that it concerns propranolol monotherapy (strengths and limitations section).

5. In the Introduction (line 33) the authors refer to propranolol as nonselective β-adrenergic receptor antagonist. While “nonselective” is often used to describe propranolol, the antagonist is actually highly selective for the β1- and β2-adrenergic receptors and has a relatively low affinity for the β3-receptor. Please be more specific. One can also refer to propranolol as β3-AR sparing.

Reply: ‘Nonselective’ was changed into ‘β3-sparing AR’ in the introduction and the abstract.

6. On page 5 for the sentence “metastatic angiosarcoma have been treated with propranolol, in combination with various,” it should read “were treated” since the study has been completed and reported.

Reply: We have changed ‘have been treated’ to ‘were treated’ to avoid confusion.

7. For the following, “These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.” Can another reference be provided as the reference provided was not accessible? Other references listed the dose as being 120-240 mg daily, which is more in line with the dose given to angiosarcoma patients.

Reply: We have changed the reference into the summary of product information of propranolol.

8. Under Exploratory Endpoints, p.8, the authors use the gene designation (ADRB1, ADRB2, ADRB3) rather than the protein designation for the receptors (β1-AR, β2-AR, β3-AR). Since the levels of the receptors are being measured by immunohistochemistry, the protein designation should be used.

Reply: We have replaced the gene designation for the protein designation.

9. On page 8 (line 26) the authors state, “Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens in vitro.” It is not clear what studies will be done here. Will the authors be using cell lines and performing drug synergy studies? If so, more details should be provided. If this is a separate study, the statement should be removed from the paper.

Reply: We have removed this sentence. The drug sensitivity assays will be done in cell lines as a separate part of the study. These experiments will not influence the outcome of the study.

Reviewer: 2

Reviewer Name: Hadrian Schepler
Institution and Country: Medical Center of the Johannes Gutenberg University, Mainz Department of Dermatology

The application of the beta-blocker propranolol as a neoadjunctive therapy for angiosarcoma seems to be an interesting and realistic challenge to treat this extremely aggressive tumor. Its efficacy has been proven in the past especially in the treatment of infantile hemangiomas.
The clinical and histological proximity of the hemangioma to angiosarcoma underlines the potential of propranolol to have a similar effect and may host a new therapeutic strategy. First case reports are encouraging.
In the presented study, 14 patients with primary, recurrent and metastatic cutaneous angiosarcoma are to undergo a neoadjunctive therapy with propranolol for a maximum period of 6 weeks. However, it is not clear to what significance radiation - induced angiosarcoma is considered. Since this kind of angiosarcoma will become more and more important in the future due to the breast-conserving therapy with mostly cutaneous manifestations, the question arises to what extent this entity will be considered in the study or rather defined as an exclusion criterion. Here the authors should point out their position. Besides, it would be an important enrichment.
Otherwise I am very curious whether patients will benefit from the proposed therapy in future.

Reply: Thank you for reviewing our manuscript, for your enthusiastic words and for providing us with your valuable suggestion to specify how we will address the radiation induced angiosarcomas. Indeed, it is likely that the prevalence of radiation induced angiosarcomas will continue to rise in the future. Available current literature shows no clear biological difference, in terms of beta-adrenergic receptor expression or in VEGF expression, between primary and secondary angiosarcoma. Therefore, we have no indication to expect that propranolol will have a different effect on one of these, and we decided to include all types of cutaneous angiosarcoma in this study. To clarify, we have added a comment to the patient selection paragraph.

Patient selection paragraph: “The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast (radiation induced)”.

VERSION 2 – REVIEW

REVIEWER	Erin Dickerson University of Minnesota, Twin Cities USA
REVIEW RETURNED	29-Jun-2020

GENERAL COMMENTS	Thank you for making the requested changes to your paper. It seems that the revised wording in the abstract suggests the β3-receptor is the only beta adrenergic receptor expressed in infantile hemangiomas. This should be reworded to avoid confusion. Propranolol is a β3-sparing antagonist of the β-adrenergic receptor, a receptor that is highly expressed in infantile hemangioma.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1:

Reviewer Name: Erin Dickerson
Institution and Country: University of Minnesota, USA

Thank you for making the requested changes to your paper. It seems that the revised wording in the abstract suggests the β3-receptor is the only beta adrenergic receptor expressed in infantile hemangiomas. This should be reworded to avoid confusion.
Propranolol is a β3-sparing antagonist of the β-adrenergic receptor, a receptor that is highly expressed in infantile hemangioma.

Reply: Thank you for your comment. We have adjusted the requested sentence into: “Propranolol is a β3-sparing antagonist of the β-adrenergic receptor. In infantile hemangioma, the β1, β2 and β3-receptors are highly expressed”.