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PropAngio: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma A proof of principle study

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PropAngio: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma

A proof of principle study

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Keywords: oncology; adult oncology; sarcoma; vascular medicine

ABSTRACT

Introduction: Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential and recurrence rate. Despite optimal treatment with surgery, with or without radiation, the prognosis remains poor and, therefore, new treatment strategies are warranted. Recently, propranolol has effectively been repurposed for the treatment of hemangioma. Propranolol is a nonselective antagonist of the β-adrenergic receptor, a receptor that is highly expressed in hemangioma. Angiosarcoma has several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth. As a result, propranolol has been administered small-scale in individual angiosarcoma cases with promising results. The precise effect of propranolol, however, is not yet established.

Methods and analysis: The goal of this neoadjuvant window of opportunity study is to prospectively evaluate the activity of propranolol monotherapy in patients with cutaneous angiosarcoma. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical response and histological response, without a significant delay in standard anti-cancer treatment. Fourteen patients with primary, recurrent or metastatic cutaneous angiosarcoma will be included. Propranolol will be administered orally in an escalating dose during three to six weeks, before the initiation of standard treatment. The primary endpoint is clinical response according to RECIST, as measured on consecutive coloured photographs or CT/MRI. The histological response will be determined as secondary endpoint, comparing the difference in proliferation index before and after propranolol. The study will be considered positive when at least 3 patients have a response to propranolol.

Ethics and dissemination: Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

Trial registration number: Registry through the Netherlands Trial Register (Trial no. NL8118).

Strengths and limitations of this study

- Drug repurposing is a process of developing approved drugs for new medical indications.
- This proof-of-principle study will help to elucidate the effect of a well-known drug (propranolol) for a new indication (cutaneous angiosarcoma).
- Propranolol is a generic and therefore relatively cheap product with a favourable safety profile.
- The neoadjuvant setting provides the opportunity to evaluate the antitumor response without delaying the standard treatment.

INTRODUCTION

Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential. The estimated incidence of angiosarcoma is 0.4 per million patients per year, making it a very rare disease.[1] The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.[2] Physicians and researchers are, therefore, in urgent need to find better treatment options for these patients.

Various additional drugs for systemic treatment have been investigated before.[2–4] Although the role of (neo)adjuvant chemotherapy remains controversial for localised disease, neoadjuvant chemotherapy is often administered for locally advanced angiosarcoma.[3,5–8] Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.[2–4] However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.[3,5–10] Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Propranolol hydrochloride, a synthetic nonselective β-adrenergic receptor antagonist, was registered by the Food and Drug Agency (FDA) decades ago for the treatment of hypertension. Drug repurposing is a drug development strategy focused on the reuse of existing drugs for new medical indications. Recently, propranolol has been repurposed and is now used in the treatment of hemangioma. Hemangioma is a benign vascular tumour and propranolol dosed 3 mg/kg led to a complete to near complete resolution in approximately 88% of the treated infants with hemangioma.[11,12] The pharmacological effects of propranolol in hemangioma are presumed to cause vasoconstriction, a decreased expression of vascular growth endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), inhibition of migration and proliferation of tumour cells and induction of apoptosis of endothelial cells.[12–16] Angiosarcoma have several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.[11,17]

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. In these case reports, patients with locally advanced or

metastatic angiosarcoma have been treated with propranolol, in combination with various chemotherapy regimens, including combination therapy with cyclophosphamide, etoposide, paclitaxel and vinblastine-based chemotherapy. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.[18–23] In one case there was a response after 1 week of propranolol monotherapy 40 mg twice a day (BID).[20] These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.[24] Furthermore, there was a reduction in the proliferative index of 34%, stabilization of tumour growth and less necrosis.[20] Additionally, one case described a patient with metastatic cardiac angiosarcoma who showed a long term response (>12 months) to propranolol monotherapy, while the mean survival time is only four months.[25]

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

METHODS AND ANALYSIS

Aim and objectives

The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.

Study design and study treatment

This is a prospective proof of principle study with neoadjuvant propranolol monotherapy in cutaneous angiosarcoma patients. We will use the neoadjuvant window as an opportunity to explore the activity of propranolol monotherapy, without delaying the standard treatment. The

duration of treatment will be three to six weeks. In this single arm trial, angiosarcoma patients will be treated with propranolol monotherapy in an intrapatient escalating dose, which will be adjusted to the tolerability of propranolol. The treatment plan of propranolol is provided in *Table 1*. The treatment plan was designed based on doses used in previous literature [15,19,22,23] and not exceeding the maximum maintenance dose of 320 mg/day for the registered indication hypertension (maximum daily dose in our study 240 mg/day).[24] In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level.

Table 1. Propranolol treatment plan.

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

*All patients start on day 1 with dose level 1.

The tolerability will be assessed during weekly visits in the outpatient clinic (*figure 1*). Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Propranolol will be tapered off after the biopsy, to prevent withdrawal symptoms (*table 1*).

Patient selection

The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast. Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.

Sample size calculation

An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.

Study endpoints

Primary endpoint

The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).(26) A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial.

Secondary endpoint

The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response.

Exploratory endpoints

To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (ADRB1, ADRB2, and ADRB3) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.[13,20] With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens *in vitro*. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.

Study logistics

Patient recruitment and study duration

Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit. As this is a monocenter study, all patients will be included in the Netherlands Cancer Institute (NKI). Approximately 20 new angiosarcoma patients are seen in the NKI yearly. As a result, the expected duration of the study is two years. Enrolment started on 27 December 2019.

Safety assessments

All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done

according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.

Data management

The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.

Study monitoring

Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication.

The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.

Termination of the study

An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.

Patient and public involvement statement

The trial protocol and other trial documents were developed in collaboration with the Dutch sarcoma patient advocacy group. They evaluated the specific patient need for this trial. They fully support this trial and the concept of exploring drug-repurposing strategies to improve outcome in sarcoma. The patient advocacy group will be informed about the progress of the study and the study time lines.

The study is funded by a Belgian non-profit organisation: the Anticancer Fund. Their mission is to complement current cancer care with patient-first thinking and a focus on evidence-based potential for new treatments. Financially, the Anticancer Fund is completely dependent on donations and private funding. The Anticancer Fund supports diverse clinical trials, mainly in under-prioritised treatment groups (such as in rare tumours), with non-conventional therapies and repurposed drugs. The trial was registered in the Netherlands Trial Register (NL71090.031.19).

Ethics and dissemination

Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

All essential documents (including patient files, the Investigator Study File, CRF's and electronic study data), data management and statistical files will be kept for 15 years.

CONCLUSION

Angiosarcoma is an extremely rare and aggressive malignancy with a high metastatic potential and a dismal prognosis. The current standard treatment cannot sufficiently manage the disease. Therefore, new strategies are warranted. Drug repurposing is a process of developing approved drugs for new medical indications. A strong rationale for repurposing of propranolol for the treatment of angiosarcoma patients exists. The precise effect of propranolol monotherapy is not yet established. In this study, we will therefore address the question about the efficacy of propranolol as neoadjuvant monotherapy in patients with cutaneous angiosarcoma. If this study shows positive results, further clinical trials are needed to establish the role of propranolol in the treatment of angiosarcoma, possibly even in combination with other agents such as chemotherapy, targeted therapy or immunotherapy.

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Authors' contributions

KH, NIJ, AMK, WG, RH, JB, AH, WH and NS each made substantial contributions to the conception or design of the study protocol. NIJ and KH wrote the first draft of the protocol and this paper. AMK, WG, RH, WH, JB, AH and NS provided critical input regarding the design of the study or study logistics. KH, NIJ, AMK, WG, RH, JB, AH, WH and NS revised the protocol critically and approved the final version to be published.

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Competing interest statements

The authors declare that they have no conflicts of interest related to this study.

REFERENCES

1. Rouhani P, Fletcher CD, Devesa SS, et al. Cutaneous soft tissue sarcoma incidence patterns in the U.S. : an analysis of 12,114 cases. *Cancer* 2008;113(3):616–27.
2. Young RJ, Brown NJ, Reed MW, et al. Angiosarcoma. *Lancet Oncol* 2010;11(10):983–91.
3. Oxenberg J, Khushalani NI, Salerno KE, et al. Neoadjuvant chemotherapy for primary cutaneous/soft tissue angiosarcoma: Determining tumor behavior prior to surgical resection. *J Surg Oncol* 2015;111(7):829–33.
4. Stacchiotti S, Palassini E, Sanfilippo R, et al. Gemcitabine in advanced angiosarcoma: a retrospective case series analysis from the Italian Rare Cancer Network. *Ann Oncol* 2012;23(2):501–8.
5. DeMartelaere SL, Roberts D, Burgess MA, et al. Neoadjuvant chemotherapy–specific and overall treatment outcomes in patients with cutaneous angiosarcoma of the face with periorbital involvement. *Head Neck* 2008;30(5):639–46.
6. Fayette J, Martin E, Piperno-Neumann S, et al. Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. *Ann Oncol* 2007;18(12):2030–6.
7. Guadagnolo BA, Zagars GK, Araujo D, et al. Outcomes after definitive treatment for cutaneous angiosarcoma of the face and scalp. *Head Neck 2*011;33(5):661–7.
8. Sinnamon AJ, Neuwirth MG, McMillan MT, et al. A prognostic model for resectable soft tissue and cutaneous angiosarcoma. *J Surg Oncol* 2016;114(5):557–63.
9. Li H, Yang S, Chen H, et al. Survival after heart transplantation for non-metastatic primary cardiac sarcoma. *J Cardiothorac Surg* 2016;11(1):145.
10. Abu Saleh WK, Ramlawi B, Shapira OM, et al. Improved Outcomes With the Evolution of a Neoadjuvant Chemotherapy Approach to Right Heart Sarcoma. *Ann Thorac Surg* 2017;104(1):90–6.
11. Léauté-Labrèze C, Hoeger P, Mazereeuw-Hautier J, et al. A Randomized, Controlled Trial of Oral Propranolol in Infantile Hemangioma. *N Engl J Med* 2015;372(8):735–46.
12. Léauté-Labrèze C, de la Roque ED, Hubiche T, et al. Propranolol for Severe Hemangiomas of Infancy. *N Engl J Med* 2008;358(24):2649–51.
13. Chisholm KM, Chang KW, Truong MT, et al. β-Adrenergic receptor expression in vascular tumors. *Mod Pathol* 2012;25(11):1446–51.

14. Stiles JM, Amaya C, Rains S, et al. Targeting of beta adrenergic receptors results in therapeutic efficacy against models of hemangioendothelioma and angiosarcoma. *PLoS One* 2013;8(3):e60021.
15. Amaya CN, Perkins M, Belmont A, et al. Non-selective beta blockers inhibit angiosarcoma cell viability and increase progression free- and overall-survival in patients diagnosed with metastatic angiosarcoma. *Oncoscience* 2018;5(3–4):109–19.
16. Pasquier E, Ciccolini J, Carre M, et al. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget* 2011;2(10):797–809.
17. Weidema ME, Flucke UE, van der Graaf WTA et al. Prognostic Factors in a Large Nationwide Cohort of Histologically Confirmed Primary and Secondary Angiosarcomas. *Cancers (Basel)* 2019;11(11):epub 12 Nov 2019.
18. Daguzé J, Saint-Jean M, Peuvrel L, et al. Visceral metastatic angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *JAAD Case Reports* 2016;2(6):497–9.
19. Pasquier E, André N, Street J, et al. Effective Management of Advanced Angiosarcoma by the Synergistic Combination of Propranolol and Vinblastine-based Metronomic Chemotherapy: A Bench to Bedside Study. *EBioMedicine* 2016;6:87–95.
20. Chow W, Amaya CN, Rains S, et al. Growth Attenuation of Cutaneous Angiosarcoma With Propranolol-Mediated β-Blockade. *JAMA dermatology* 2015;151(11):1226–9.
21. Pramanik R, Gogia A, Malik PS, et al. Metastatic Primary Angiosarcoma of the Breast: Can We Tame It the Metronomic Way. *Indian J Med Paediatr Oncol* 2017;38(2):228–31.
22. Banavali S, Pasquier E, Andre N. Targeted therapy with propranolol and metronomic chemotherapy combination: sustained complete response of a relapsing metastatic angiosarcoma. *Ecancermedicalscience* 2015;9:499.
23. Daguzé J, Saint-Jean M, Dréno B. Large nose angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *J Eur Acad Dermatology Venereol* 2018;32(2):e52–4.
24. Nederland Zorginstituut. Farmacotherapeutisch Kompas - Propranolol (cardiovasculair of neurologisch).[Internet]. Available from: https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/p/propranolol__ca

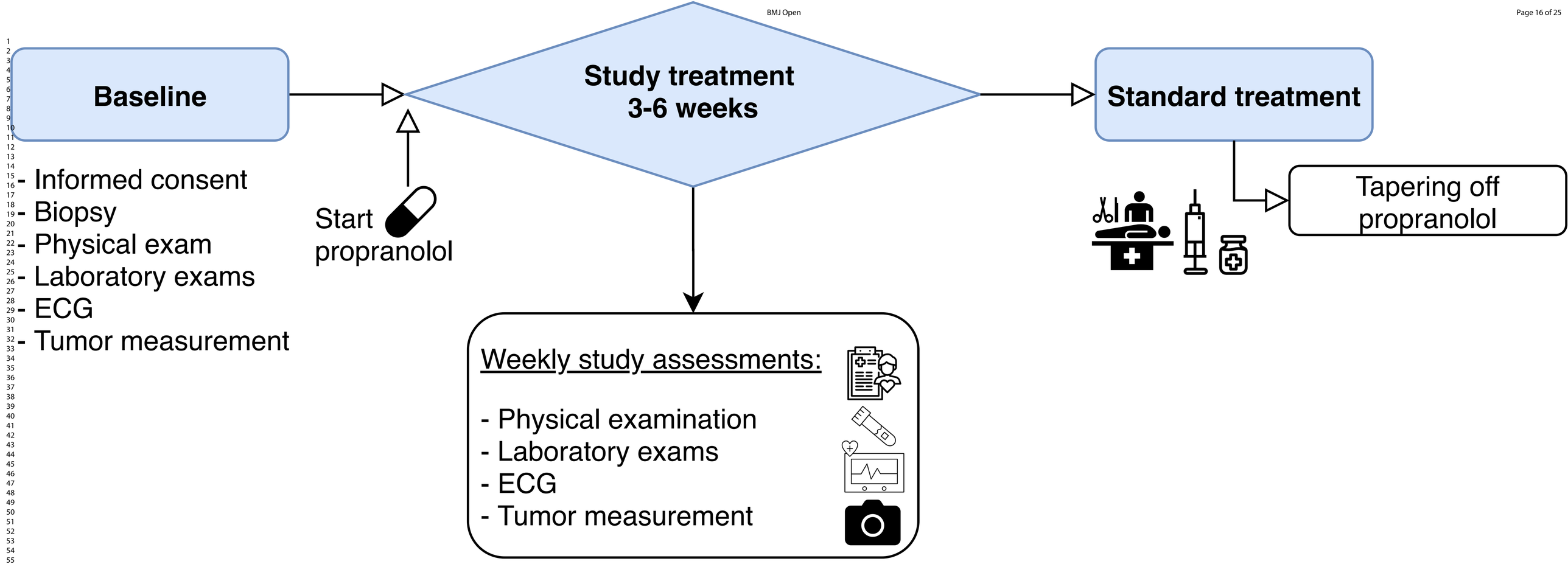
rdiovasculair_of_neurologisch_#eigenschappen. Accessed on 31 March 2020.

25. Galván DC, Ayyappan AP, Bryan BA. Regression of primary cardiac angiosarcoma and metastatic nodules following propranolol as a single agent treatment. *Oncoscience* 2018;5(9–10):262.

26. Schwartz LH, Seymour L, Litière S, et al. RECIST 1.1 - Standardisation and disease-specific adaptations: Perspectives from the RECIST Working Group. Eur J Cancer. 2016;62(Jul):138–45.

Legends

Figure 1. Study assessments. Figure 1 gives an overview of the study assessments, which are planned at baseline, during study treatment or when the standard treatment is initiated.


Baseline
- Informed consent
- Biopsy
- Physical exam
- Laboratory exams
- ECG
- Tumor measurement
Start propranolol
Study treatment 3-6 weeks
Weekly study assessments:
- Physical examination
- Laboratory exams
- ECG
- Tumor measurement
Standard treatment
Tapering off propranolol

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	PropAngio: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma A proof of principle study
Trial registration	2a	Registry through the Netherlands Trial Register (Trial no. NL8118).
	2b	https://www.trialregister.nl/trial/8118
Protocol version	3	Version 2.0, 7 October 2019
Funding	4	The study is funded by the Anticancer Fund from Belgium.

Roles and responsibilities	5a	Kimberley M. Heinhuis[1,5*], Nikki S. IJzerman[1,2,5*], Anne Miek Koenen[3], Winette T.A. van der Graaf[1], Rick L. Haas[4], Jos H. Beijnen[5,6], Alwin D.R. Huitema[5,7], Winan J. van Houdt[3], Neeltje Steeghs[1]. *These authors contributed equally. Affiliations 1. Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 2. Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands 3. Department of Surgical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 4. Department of Radiotherapy, the Netherlands Cancer Institute, Amsterdam, the Netherlands 5. Department of Pharmacy & Pharmacology, Netherlands Cancer Institute, Amsterdam, the Netherlands. 6. Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands 7. Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
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	5b	Anticancer Fund Belgium, Liese Vandeborne, Research Manager, +32 2 268 48 16, www.anticancerfund.org, Boechoutlaan 221, 1853 Strombeek-Bever, Belgium.
	5c	The study sponsor and funders will not have ultimate authority in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.
	5d	Not applicable.

Introduction

Background and rationale

6a Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential.(1) The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.(2)

Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.(2–4) However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.(3,5–10) Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Recently, propranolol has been repurposed and is now successfully used in the treatment of hemangioma.(11,12) Angiosarcoma have several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.(11,17)

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.(18–23) These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.(24)

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

[Reference numbers refer to references list in paper].

6b This is a proof of principle study with no comparator because there is no current standard neoadjuvant treatment for angiosarcoma.

Objectives	7	The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.
Trial design	8	This is a single armed, phase II, proof of principle study.

Methods: Participants, interventions, and outcomes

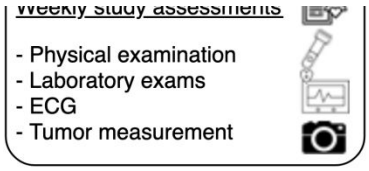
Study setting	9	This is a monocenter study and all patients will be treated in the Netherlands Cancer institute in Amsterdam, Antoni van Leeuwenhoek hospital.
Eligibility criteria	10	The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast. Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.
Interventions	11a	All patients will follow the same scheme (single armed study):

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

11b In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level. A patient can stop study treatment by withdrawing the informed consent at any time.

11c The tolerability will be assessed during weekly visits in the outpatient clinic. Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated

11d Treatment with other beta blockade therapy or anticancer therapies are prohibited (except hormonal therapy for breast cancer).

Outcomes	12	Primary endpoint The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).(26) A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial. Secondary endpoint The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response. Exploratory endpoints To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (ADRB1, ADRB2, and ADRB3) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.(13,20) With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens in vitro. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.
Participant timeline	13	

Sample size	14	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
Recruitment	15	The sarcoma unit of the NKI was trained for the study. Patientenplatform sarcomen, DSSG and Anticancer Fund Belgium are also informed and will provide information to possible patients.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Not applicable
Allocation concealment mechanism	16b	Not applicable
Implementation	16c	Not applicable
Blinding (masking)	17a	Not applicable
	17b	Not applicable

Methods: Data collection, management, and analysis

Data collection methods	18a	Data collection will be performed according to the figure as stated in section 13.
	18b	The follow up data will also be recorded in the eCRF by the investigators of the study.
Data management	19	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.
Statistical methods	20a	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
	20b	Not applicable
	20c	Not applicable

Methods: Monitoring

Data monitoring	21a	Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
	21b	An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Harms	22	All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.
Auditing	23	Frequency and procedures for auditing trial conduct will be done yearly by the MEC of the NKI and will be independent from investigators and the sponsor.

Ethics and dissemination

Research ethics approval	24	Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute.
Protocol amendments	25	Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.All essential documents (including patient files, the Investigator Study File, CRF's, amendements and electronic study data), data management and statistical files will be kept for 15 years.

Consent or assent	26a	Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit.
	26b	Not applicable
Confidentiality	27	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
Declaration of interests	28	No conflicts of interests to disclose related to this study
Access to data	29	Study investigators will have access to the final data. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Ancillary and post-trial care	30	The investigator has a liability insurance which is in accordance with article 7 of the WMO. The AVL as sponsor of this study also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.
Dissemination policy	31a	Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
	31b	Colleagues meeting the ICMJE criteria for authorship will become co-author. No professional writers will be involved.
	31c	The study was registered through the Netherlands Trial Register (Trial no. NL8118). https://www.trialregister.nl/trial/8118
Appendices		
Informed consent materials	32	Patient information folder in Dutch is attached
Biological specimens	33	All data regarding the clinical records and biological specimens will be stored anonymously for future research, if the patient gives consent in the patient information folder.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT

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PropAngio study protocol: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma
A proof of principle study

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PropAngio study protocol: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma

A proof of principle study

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ABSTRACT

Introduction: Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential and recurrence rate. Despite optimal treatment with surgery, with or without radiation, the prognosis remains poor and, therefore, new treatment strategies are warranted. Recently, propranolol has effectively been repurposed for the treatment of infantile hemangioma. Propranolol is a β3-sparing antagonist of the β-adrenergic receptor, a receptor that is highly expressed in infantile hemangioma. Angiosarcoma has several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth. As a result, propranolol has been administered small-scale in individual angiosarcoma cases with promising results. The precise effect of propranolol, however, is not yet established.

Methods and analysis: The goal of this neoadjuvant window of opportunity study is to prospectively evaluate the activity of propranolol monotherapy in patients with cutaneous angiosarcoma. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical response and histological response, without a significant delay in standard anti-cancer treatment. Fourteen patients with primary, recurrent or metastatic cutaneous angiosarcoma will be included. Propranolol will be administered orally in an escalating dose during three to six weeks, before the initiation of standard treatment. The primary endpoint is clinical response according to RECIST, as measured on consecutive coloured photographs or CT/MRI. The histological response will be determined as secondary endpoint, comparing the difference in proliferation index before and after propranolol by measuring the change in immunohistochemistry staining of Ki-67. The study will be considered positive when at least 3 patients have a response to propranolol.

Ethics and dissemination: Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

Trial registration number: Registry through the Netherlands Trial Register (Trial no. NL8118).

Strengths and limitations of this study

- The neoadjuvant setting provides the opportunity to evaluate the antitumor response of propranolol monotherapy without delaying the standard treatment.
- The propranolol dose will be escalated to optimize the safety profile of the treatment.
- As it is a window of opportunity study, the study duration will be relatively short.
- A limitation of the current design (proof of principle study), is the absence of randomisation.

INTRODUCTION

Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential. The estimated incidence of angiosarcoma is 0.4 per million patients per year, making it a very rare disease.[1] The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.[2] Physicians and researchers are, therefore, in urgent need to find better treatment options for these patients.

Various additional drugs for systemic treatment have been investigated before.[2–4] Although the role of (neo)adjuvant chemotherapy remains controversial for localised disease, neoadjuvant chemotherapy is often administered for locally advanced angiosarcoma.[3,5–8] Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.[2–4] However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.[3,5–10] Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Propranolol hydrochloride, a synthetic β3-sparing-adrenergic receptor antagonist, was registered by the Food and Drug Agency (FDA) decades ago for the treatment of hypertension. Drug repurposing is a drug development strategy focused on the reuse of existing drugs for new medical indications. Recently, propranolol has been repurposed and is now used in the treatment of infantile hemangioma. Infantile hemangioma is a benign vascular tumour and propranolol dosed 3 mg/kg led to a complete to near complete resolution in approximately 88% of the treated infants with infantile hemangioma.[11,12] The pharmacological effects of propranolol in infantile hemangioma are presumed to cause vasoconstriction, a decreased expression of vascular growth endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), inhibition of migration and proliferation of tumour cells and induction of apoptosis of endothelial cells.[12–16] Angiosarcoma have several similarities with infantile hemangioma, including its high β-adrenergic receptor expression and the suggested important role of VEGF in malignant growth.[14,17,18]

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. In these case reports, patients with locally advanced or metastatic angiosarcoma were treated with propranolol, in combination with various chemotherapy regimens, including combination therapy with cyclophosphamide, etoposide, paclitaxel and vinblastine-based chemotherapy. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.[19–24] In one case there was a response after 1 week of propranolol monotherapy 40 mg twice a day (BID).[22] These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.[25] Furthermore, there was a reduction in the proliferative index of 34%, stabilization of tumour growth and less necrosis.[22] Additionally, one case described a patient with metastatic cardiac angiosarcoma who showed a long term response (>12 months) to propranolol monotherapy, while the mean survival time is only four months.[26]

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

METHODS AND ANALYSIS

Aim and objectives

The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.

Study design and study treatment

This is a prospective proof of principle study with neoadjuvant propranolol monotherapy in cutaneous angiosarcoma patients. We will use the neoadjuvant window as an opportunity to explore the activity of propranolol monotherapy, without delaying the standard treatment. The duration of treatment will be three to six weeks. In this single arm trial, angiosarcoma patients will be treated with propranolol monotherapy in an intrapatient escalating dose, which will be adjusted to the tolerability of propranolol. The treatment plan of propranolol is provided in *Table 1*. The treatment plan was designed based on doses used in previous literature [15,21,24,26] and not exceeding the maximum maintenance dose of 320 mg/day for the registered indication hypertension (maximum daily dose in our study 240 mg/day).[25] In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level.

Table 1. Propranolol treatment plan.

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

*All patients start on day 1 with dose level 1.

The tolerability will be assessed during weekly visits in the outpatient clinic (*figure 1*).
Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Propranolol will be tapered off after the biopsy, to prevent withdrawal symptoms (*table 1*).

Patient selection

The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast (radiation induced). Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.

Sample size calculation

An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.

Study endpoints

Primary endpoint

The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).[27] A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial.

Secondary endpoint

The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response.

Exploratory endpoints

To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (β1-AR, β2-AR, β3-AR) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.[13,22] With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.

Study logistics

Patient recruitment and study duration

Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit. As this is a monocenter study, all patients will be included in the Netherlands Cancer Institute (NKI). Approximately 20 new angiosarcoma patients are seen in the NKI yearly. As a result, the expected duration of the study is two years. Enrolment started on 27 December 2019.

Safety assessments

All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done

according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.

Data management

The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.

Study monitoring

Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication.

The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.

Termination of the study

An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.

Patient and public involvement statement

The trial protocol and other trial documents were developed in collaboration with the Dutch sarcoma patient advocacy group. They evaluated the specific patient need for this trial. They fully support this trial and the concept of exploring drug-repurposing strategies to improve outcome in sarcoma. The patient advocacy group will be informed about the progress of the study and the study time lines.

The study is funded by a Belgian non-profit organisation: the Anticancer Fund. Their mission is to complement current cancer care with patient-first thinking and a focus on evidence-based potential for new treatments. Financially, the Anticancer Fund is completely dependent on donations and private funding. The Anticancer Fund supports diverse clinical trials, mainly in under-prioritised treatment groups (such as in rare tumours), with non-conventional therapies and repurposed drugs. The trial was registered in the Netherlands Trial Register (NL71090.031.19).

Ethics and dissemination

Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

All essential documents (including patient files, the Investigator Study File, CRF's and electronic study data), data management and statistical files will be kept for 15 years.

Summary

Angiosarcoma is an extremely rare and aggressive malignancy with a high metastatic potential and a dismal prognosis. The current standard treatment cannot sufficiently manage the disease. Therefore, new strategies are warranted. Drug repurposing is a process of developing approved drugs for new medical indications. A strong rationale for repurposing of propranolol for the treatment of angiosarcoma patients exists. The precise effect of propranolol monotherapy is not yet established. In this study, we will therefore address the question about the efficacy of propranolol as neoadjuvant monotherapy in patients with cutaneous angiosarcoma. If this study shows positive results, further clinical trials are needed to establish the role of propranolol in the treatment of angiosarcoma, possibly even in combination with other agents such as chemotherapy, targeted therapy or immunotherapy.

Acknowledgments

The authors would like to thank the Dutch sarcoma patient advocacy group (Patiëntenplatform Sarcomen) and the Anticancer Fund for their contributions to the study.

Authors' contributions

KH, NIJ, AMK, WG, RH, JB, AH, WH and NS each made substantial contributions to the conception or design of the study protocol. NIJ and KH wrote the first draft of the protocol and this paper. AMK, WG, RH, WH, JB, AH and NS provided critical input regarding the design of the study or study logistics. KH, NIJ, AMK, WG, RH, JB, AH, WH and NS revised the protocol critically and approved the final version to be published.

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Competing interest statements

The authors declare that they have no conflicts of interest related to this study.

REFERENCES

1. Rouhani P, Fletcher CD, Devesa SS TJ, et al. Cutaneous soft tissue sarcoma incidence patterns in the U.S. : an analysis of 12,114 cases. *Cancer* 2008;113(3):616–27.
2. Young RJ, Brown NJ, Reed MW, et al. Angiosarcoma. *Lancet Oncol* 2010;11(10):983–91.
3. Oxenberg J, Khushalani NI, Salerno KE, et al. Neoadjuvant chemotherapy for primary cutaneous/soft tissue angiosarcoma: Determining tumor behavior prior to surgical resection. *J Surg Oncol* 2015;111(7):829–33.
4. Stacchiotti S, Palassini E, Sanfilippo R, et al. Gemcitabine in advanced angiosarcoma: a retrospective case series analysis from the Italian Rare Cancer Network. *Ann Oncol* 2012;23(2):501–8.
5. DeMartelaere SL, Roberts D, Burgess MA, et al. Neoadjuvant chemotherapy–specific and overall treatment outcomes in patients with cutaneous angiosarcoma of the face with periorbital involvement. *Head Neck* 2008 May;30(5):639–46.
6. Fayette J, Martin E, Piperno-Neumann S, et al. Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. *Ann Oncol* 2007;18(12):2030–6.
7. Guadagnolo BA, Zagars GK, Araujo D, et al. Outcomes after definitive treatment for cutaneous angiosarcoma of the face and scalp. *Head Neck* 2011;33(5):661–7.
8. Sinnamon AJ, Neuwirth MG, McMillan MT, et al. A prognostic model for resectable soft tissue and cutaneous angiosarcoma. *J Surg Oncol* 2016;114(5):557–63.
9. Li H, Yang S, Chen H, et al. Survival after heart transplantation for non-metastatic primary cardiac sarcoma. *J Cardiothorac Surg* 2016;11(1):145.
10. Abu Saleh WK, Ramlawi B, Shapira OM, et al. Improved Outcomes With the Evolution of a Neoadjuvant Chemotherapy Approach to Right Heart Sarcoma. *Ann Thorac Surg* 2017 Jul;104(1):90–6.
11. Léauté-Labrèze C, Hoeger P, Mazereeuw-Hautier J, et al. A Randomized, Controlled Trial of Oral Propranolol in Infantile Hemangioma. *N Engl J Med* 2015 Feb 19;372(8):735–46.
12. Léauté-Labrèze C, de la Roque ED, Hubiche T, et al. A. Propranolol for Severe Hemangiomas of Infancy. *N Engl J Med* 2008;358(24):2649–51.
13. Chisholm KM, Chang KW, Truong MT, et al. β-Adrenergic receptor expression in vascular tumors. *Mod Pathol* 2012;25(11):1446–51.

14. Stiles JM, Amaya C, Rains S, et al. Targeting of beta adrenergic receptors results in therapeutic efficacy against models of hemangioendothelioma and angiosarcoma. *PLoS One* 2013;8(3):e60021.
15. Amaya CN, Perkins M, Belmont A, et al. Non-selective beta blockers inhibit angiosarcoma cell viability and increase progression free- and overall-survival in patients diagnosed with metastatic angiosarcoma. *Oncoscience* 2018;5(3–4):109–19.
16. Pasquier E, Ciccolini J, Carre M, et al. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget* 2011;2(10):797–809.
17. Greenberger S, Bischoff J. Pathogenesis of infantile haemangioma. *Br J Dermatol* 2013;169(1):12–19.
18. 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.
19. Daguzé J, Saint-Jean M, Peuvrel L, et al. Visceral metastatic angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *JAAD Case Reports* 2016;2(6):497–9.
20. Pasquier E, André N, Street J, et al. Effective Management of Advanced Angiosarcoma by the Synergistic Combination of Propranolol and Vinblastine-based Metronomic Chemotherapy: A Bench to Bedside Study. *EBioMedicine* 2016;6:87–95.
21. Chow W, Amaya CN, Rains S, et al. Growth Attenuation of Cutaneous Angiosarcoma With Propranolol-Mediated β-Blockade. *JAMA dermatology* 2015;151(11):1226–9.
22. Pramanik R, Gogia A, Malik PS, et al. Metastatic Primary Angiosarcoma of the Breast: Can We Tame It the Metronomic Way. I*ndian J Med Paediatr Oncol* 2017;38(2):228–31.
23. Banavali S, Pasquier E, Andre N. Targeted therapy with propranolol and metronomic chemotherapy combination: sustained complete response of a relapsing metastatic angiosarcoma. *Ecancermedicalscience* 2015;9:499.
24. Daguzé J, Saint-Jean M, Dréno B. Large nose angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *J Eur Acad Dermatology Venereol* 2018;32(2):e52–4.
25. European Medicines Agency. Propranolol 40 mg film-coated tablets - Summary of Product

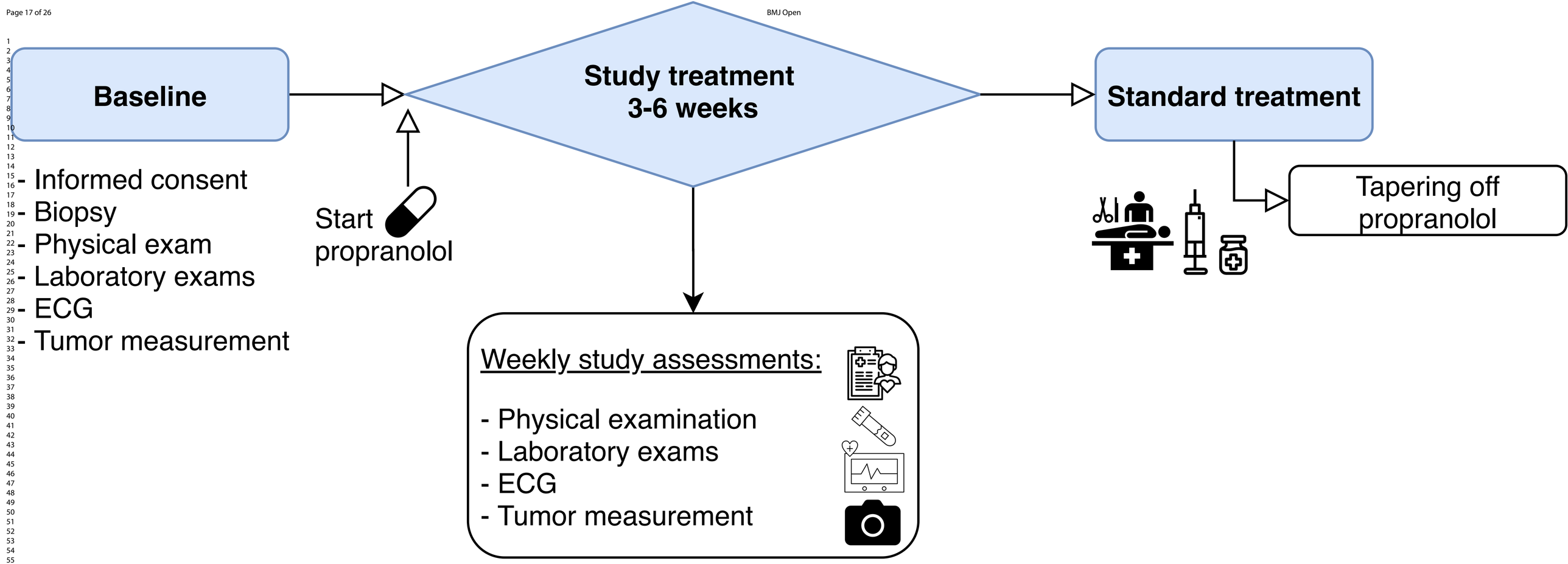
Characteristics (SmPC) - (eMC) [Internet]. Available from: https://www.medicines.org.uk/emc/product/2904/smpc

26. Galván DC, Ayyappan AP, Bryan BA. Regression of primary cardiac angiosarcoma and metastatic nodules following propranolol as a single agent treatment. *Oncoscience* 2018;5(9–10):262.

27. Schwartz LH, Seymour L, Litière S, et al. RECIST 1.1 - Standardisation and disease-specific adaptations: Perspectives from the RECIST Working Group. *Eur J Cancer.* 2016;62(Jul):138–45.

Legends

Figure 1. Study assessments. Figure 1 gives an overview of the study assessments, which are planned at baseline, during study treatment or when the standard treatment is initiated.


Baseline
- Informed consent
- Biopsy
- Physical exam
- Laboratory exams
- ECG
- Tumor measurement
Start propranolol
Study treatment 3-6 weeks
Weekly study assessments:
- Physical examination
- Laboratory exams
- ECG
- Tumor measurement
Standard treatment
Tapering off propranolol

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	PropAngio: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma A proof of principle study
Trial registration	2a	Registry through the Netherlands Trial Register (Trial no. NL8118).
	2b	https://www.trialregister.nl/trial/8118
Protocol version	3	Version 2.0, 7 October 2019
Funding	4	The study is funded by the Anticancer Fund from Belgium.

Roles and responsibilities	5a	Kimberley M. Heinhuis[1,5*], Nikki S. IJzerman[1,2,5*], Anne Miek Koenen[3], Winette T.A. van der Graaf[1], Rick L. Haas[4], Jos H. Beijnen[5,6], Alwin D.R. Huitema[5,7], Winan J. van Houdt[3], Neeltje Steeghs[1]. *These authors contributed equally. Affiliations 1. Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 2. Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands 3. Department of Surgical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 4. Department of Radiotherapy, the Netherlands Cancer Institute, Amsterdam, the Netherlands 5. Department of Pharmacy & Pharmacology, Netherlands Cancer Institute, Amsterdam, the Netherlands. 6. Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands 7. Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
		Authors' contributions KH, NIJ, AMK, WG, RH, JB, AH, WH and NS each made substantial contributions to the conception or design of the study protocol. NIJ and KH wrote the first draft of the protocol and this paper. AMK, WG, RH, WH, JB, AH and NS provided critical input regarding the design of the study or study logistics. KH, NIJ, AMK, WG, RH, JB, AH, WH and NS revised the protocol critically and approved the final version to be published.
	5b	Anticancer Fund Belgium, Liese Vandeborne, Research Manager, +32 2 268 48 16, www.anticancerfund.org, Boechoutlaan 221, 1853 Strombeek-Bever, Belgium.
	5c	The study sponsor and funders will not have ultimate authority in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.
	5d	Not applicable.

Introduction

Background and rationale

6a Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential.(1) The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.(2)

Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.(2–4) However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.(3,5–10) Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Recently, propranolol has been repurposed and is now successfully used in the treatment of hemangioma.(11,12) Angiosarcoma have several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.(11,17)

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.(18–23) These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.(24)

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

[Reference numbers refer to references list in paper].

6b This is a proof of principle study with no comparator because there is no current standard neoadjuvant treatment for angiosarcoma.

Objectives	7	The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.
Trial design	8	This is a single armed, phase II, proof of principle study.

Methods: Participants, interventions, and outcomes

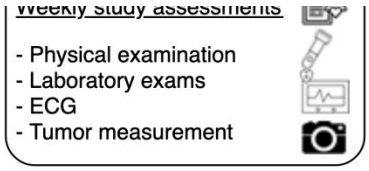
Study setting	9	This is a monocenter study and all patients will be treated in the Netherlands Cancer institute in Amsterdam, Antoni van Leeuwenhoek hospital.
Eligibility criteria	10	The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast. Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.
Interventions	11a	All patients will follow the same scheme (single armed study):

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

11b In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level. A patient can stop study treatment by withdrawing the informed consent at any time.

11c The tolerability will be assessed during weekly visits in the outpatient clinic. Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated

11d Treatment with other beta blockade therapy or anticancer therapies are prohibited (except hormonal therapy for breast cancer).

Outcomes	12	Primary endpoint The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).(26) A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial. Secondary endpoint The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response. Exploratory endpoints To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (ADRB1, ADRB2, and ADRB3) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.(13,20) With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens in vitro. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.
Participant timeline	13	

Sample size	14	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
Recruitment	15	The sarcoma unit of the NKI was trained for the study. Patientenplatform sarcomen, DSSG and Anticancer Fund Belgium are also informed and will provide information to possible patients.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Not applicable
Allocation concealment mechanism	16b	Not applicable
Implementation	16c	Not applicable
Blinding (masking)	17a	Not applicable
	17b	Not applicable

Methods: Data collection, management, and analysis

Data collection methods	18a	Data collection will be performed according to the figure as stated in section 13.
	18b	The follow up data will also be recorded in the eCRF by the investigators of the study.
Data management	19	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.
Statistical methods	20a	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
	20b	Not applicable
	20c	Not applicable

Methods: Monitoring

Data monitoring	21a	Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
	21b	An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Harms	22	All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.
Auditing	23	Frequency and procedures for auditing trial conduct will be done yearly by the MEC of the NKI and will be independent from investigators and the sponsor.

Ethics and dissemination

Research ethics approval	24	Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute.
Protocol amendments	25	Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.All essential documents (including patient files, the Investigator Study File, CRF's, amendements and electronic study data), data management and statistical files will be kept for 15 years.

Consent or assent	26a	Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit.
	26b	Not applicable
Confidentiality	27	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
Declaration of interests	28	No conflicts of interests to disclose related to this study
Access to data	29	Study investigators will have access to the final data. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Ancillary and post-trial care	30	The investigator has a liability insurance which is in accordance with article 7 of the WMO. The AVL as sponsor of this study also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.
Dissemination policy	31a	Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
	31b	Colleagues meeting the ICMJE criteria for authorship will become co-author. No professional writers will be involved.
	31c	The study was registered through the Netherlands Trial Register (Trial no. NL8118). https://www.trialregister.nl/trial/8118
Appendices		
Informed consent materials	32	Patient information folder in Dutch is attached
Biological specimens	33	All data regarding the clinical records and biological specimens will be stored anonymously for future research, if the patient gives consent in the patient information folder.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT

BMJ Open

PropAngio study protocol: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma
A proof of principle study

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Evidence based practice
Keywords:	ONCOLOGY, Adult oncology < ONCOLOGY, Sarcoma < ONCOLOGY, VASCULAR MEDICINE

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PropAngio study protocol: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma

A proof of principle study

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Keywords: oncology; adult oncology; sarcoma; vascular medicine

ABSTRACT

Introduction: Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential and recurrence rate. Despite optimal treatment with surgery, with or without radiation, the prognosis remains poor and, therefore, new treatment strategies are warranted. Recently, propranolol has effectively been repurposed for the treatment of infantile hemangioma. Propranolol is a β3-sparing antagonist of the β-adrenergic receptor. In infantile hemangioma, the β1, β2 and β3-receptors are highly expressed. Angiosarcoma has several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth. As a result, propranolol has been administered small-scale in individual angiosarcoma cases with promising results. The precise effect of propranolol, however, is not yet established.

Methods and analysis: The goal of this neoadjuvant window of opportunity study is to prospectively evaluate the activity of propranolol monotherapy in patients with cutaneous angiosarcoma. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical response and histological response, without a significant delay in standard anti-cancer treatment. Fourteen patients with primary, recurrent or metastatic cutaneous angiosarcoma will be included. Propranolol will be administered orally in an escalating dose during three to six weeks, before the initiation of standard treatment. The primary endpoint is clinical response according to RECIST, as measured on consecutive coloured photographs or CT/MRI. The histological response will be determined as secondary endpoint, comparing the difference in proliferation index before and after propranolol by measuring the change in immunohistochemistry staining of Ki-67. The study will be considered positive when at least 3 patients have a response to propranolol.

Ethics and dissemination: Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

Trial registration number: Registry through the Netherlands Trial Register (Trial no. NL8118).

Strengths and limitations of this study

- The neoadjuvant setting provides the opportunity to evaluate the antitumor response of propranolol monotherapy without delaying the standard treatment.
- The propranolol dose will be escalated to optimize the safety profile of the treatment.
- As it is a window of opportunity study, the study duration will be relatively short.
- A limitation of the current design (proof of principle study), is the absence of randomisation.

INTRODUCTION

Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential. The estimated incidence of angiosarcoma is 0.4 per million patients per year, making it a very rare disease.[1] The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.[2] Physicians and researchers are, therefore, in urgent need to find better treatment options for these patients.

Various additional drugs for systemic treatment have been investigated before.[2–4] Although the role of (neo)adjuvant chemotherapy remains controversial for localised disease, neoadjuvant chemotherapy is often administered for locally advanced angiosarcoma.[3,5–8] Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.[2–4] However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.[3,5–10] Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Propranolol hydrochloride, a synthetic β3-sparing-adrenergic receptor antagonist, was registered by the Food and Drug Agency (FDA) decades ago for the treatment of hypertension. Drug repurposing is a drug development strategy focused on the reuse of existing drugs for new medical indications. Recently, propranolol has been repurposed and is now used in the treatment of infantile hemangioma. Infantile hemangioma is a benign vascular tumour and propranolol dosed 3 mg/kg led to a complete to near complete resolution in approximately 88% of the treated infants with infantile hemangioma.[11,12] The pharmacological effects of propranolol in infantile hemangioma are presumed to cause vasoconstriction, a decreased expression of vascular growth endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), inhibition of migration and proliferation of tumour cells and induction of apoptosis of endothelial cells.[12–16] Angiosarcoma have several similarities with infantile hemangioma, including its high β-adrenergic receptor expression and the suggested important role of VEGF in malignant growth.[14,17,18]

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. In these case reports, patients with locally advanced or metastatic angiosarcoma were treated with propranolol, in combination with various chemotherapy regimens, including combination therapy with cyclophosphamide, etoposide, paclitaxel and vinblastine-based chemotherapy. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.[19–24] In one case there was a response after 1 week of propranolol monotherapy 40 mg twice a day (BID).[22] These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.[25] Furthermore, there was a reduction in the proliferative index of 34%, stabilization of tumour growth and less necrosis.[22] Additionally, one case described a patient with metastatic cardiac angiosarcoma who showed a long term response (>12 months) to propranolol monotherapy, while the mean survival time is only four months.[26]

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

METHODS AND ANALYSIS

Aim and objectives

The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.

Study design and study treatment

This is a prospective proof of principle study with neoadjuvant propranolol monotherapy in cutaneous angiosarcoma patients. We will use the neoadjuvant window as an opportunity to explore the activity of propranolol monotherapy, without delaying the standard treatment. The duration of treatment will be three to six weeks. In this single arm trial, angiosarcoma patients will be treated with propranolol monotherapy in an intrapatient escalating dose, which will be adjusted to the tolerability of propranolol. The treatment plan of propranolol is provided in *Table 1*. The treatment plan was designed based on doses used in previous literature [15,21,24,26] and not exceeding the maximum maintenance dose of 320 mg/day for the registered indication hypertension (maximum daily dose in our study 240 mg/day).[25] In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level.

Table 1. Propranolol treatment plan.

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

*All patients start on day 1 with dose level 1.

The tolerability will be assessed during weekly visits in the outpatient clinic (*figure 1*).
Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Propranolol will be tapered off after the biopsy, to prevent withdrawal symptoms (*table 1*).

Patient selection

The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast (radiation induced). Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.

Sample size calculation

An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.

Study endpoints

Primary endpoint

The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).[27] A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial.

Secondary endpoint

The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response.

Exploratory endpoints

To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (β1-AR, β2-AR, β3-AR) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.[13,22] With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.

Study logistics

Patient recruitment and study duration

Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit. As this is a monocenter study, all patients will be included in the Netherlands Cancer Institute (NKI). Approximately 20 new angiosarcoma patients are seen in the NKI yearly. As a result, the expected duration of the study is two years. Enrolment started on 27 December 2019.

Safety assessments

All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done

according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.

Data management

The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.

Study monitoring

Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication.

The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.

Termination of the study

An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.

Patient and public involvement statement

The trial protocol and other trial documents were developed in collaboration with the Dutch sarcoma patient advocacy group. They evaluated the specific patient need for this trial. They fully support this trial and the concept of exploring drug-repurposing strategies to improve outcome in sarcoma. The patient advocacy group will be informed about the progress of the study and the study time lines.

The study is funded by a Belgian non-profit organisation: the Anticancer Fund. Their mission is to complement current cancer care with patient-first thinking and a focus on evidence-based potential for new treatments. Financially, the Anticancer Fund is completely dependent on donations and private funding. The Anticancer Fund supports diverse clinical trials, mainly in under-prioritised treatment groups (such as in rare tumours), with non-conventional therapies and repurposed drugs. The trial was registered in the Netherlands Trial Register (NL71090.031.19).

Ethics and dissemination

Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute. Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.

All essential documents (including patient files, the Investigator Study File, CRF's and electronic study data), data management and statistical files will be kept for 15 years.

Summary

Angiosarcoma is an extremely rare and aggressive malignancy with a high metastatic potential and a dismal prognosis. The current standard treatment cannot sufficiently manage the disease. Therefore, new strategies are warranted. Drug repurposing is a process of developing approved drugs for new medical indications. A strong rationale for repurposing of propranolol for the treatment of angiosarcoma patients exists. The precise effect of propranolol monotherapy is not yet established. In this study, we will therefore address the question about the efficacy of propranolol as neoadjuvant monotherapy in patients with cutaneous angiosarcoma. If this study shows positive results, further clinical trials are needed to establish the role of propranolol in the treatment of angiosarcoma, possibly even in combination with other agents such as chemotherapy, targeted therapy or immunotherapy.

Acknowledgments

The authors would like to thank the Dutch sarcoma patient advocacy group (Patiëntenplatform Sarcomen) and the Anticancer Fund for their contributions to the study.

Authors' contributions

KH, NIJ, AMK, WG, RH, JB, AH, WH and NS each made substantial contributions to the conception or design of the study protocol. NIJ and KH wrote the first draft of the protocol and this paper. AMK, WG, RH, WH, JB, AH and NS provided critical input regarding the design of the study or study logistics. KH, NIJ, AMK, WG, RH, JB, AH, WH and NS revised the protocol critically and approved the final version to be published.

Funding statement

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Competing interest statements

The authors declare that they have no conflicts of interest related to this study.

REFERENCES

1. Rouhani P, Fletcher CD, Devesa SS TJ, et al. Cutaneous soft tissue sarcoma incidence patterns in the U.S. : an analysis of 12,114 cases. *Cancer* 2008;113(3):616–27.
2. Young RJ, Brown NJ, Reed MW, et al. Angiosarcoma. *Lancet Oncol* 2010;11(10):983–91.
3. Oxenberg J, Khushalani NI, Salerno KE, et al. Neoadjuvant chemotherapy for primary cutaneous/soft tissue angiosarcoma: Determining tumor behavior prior to surgical resection. *J Surg Oncol* 2015;111(7):829–33.
4. Stacchiotti S, Palassini E, Sanfilippo R, et al. Gemcitabine in advanced angiosarcoma: a retrospective case series analysis from the Italian Rare Cancer Network. *Ann Oncol* 2012;23(2):501–8.
5. DeMartelaere SL, Roberts D, Burgess MA, et al. Neoadjuvant chemotherapy–specific and overall treatment outcomes in patients with cutaneous angiosarcoma of the face with periorbital involvement. *Head Neck* 2008 May;30(5):639–46.
6. Fayette J, Martin E, Piperno-Neumann S, et al. Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. *Ann Oncol* 2007;18(12):2030–6.
7. Guadagnolo BA, Zagars GK, Araujo D, et al. Outcomes after definitive treatment for cutaneous angiosarcoma of the face and scalp. *Head Neck* 2011;33(5):661–7.
8. Sinnamon AJ, Neuwirth MG, McMillan MT, et al. A prognostic model for resectable soft tissue and cutaneous angiosarcoma. *J Surg Oncol* 2016;114(5):557–63.
9. Li H, Yang S, Chen H, et al. Survival after heart transplantation for non-metastatic primary cardiac sarcoma. *J Cardiothorac Surg* 2016;11(1):145.
10. Abu Saleh WK, Ramlawi B, Shapira OM, et al. Improved Outcomes With the Evolution of a Neoadjuvant Chemotherapy Approach to Right Heart Sarcoma. *Ann Thorac Surg* 2017 Jul;104(1):90–6.
11. Léauté-Labrèze C, Hoeger P, Mazereeuw-Hautier J, et al. A Randomized, Controlled Trial of Oral Propranolol in Infantile Hemangioma. *N Engl J Med* 2015 Feb 19;372(8):735–46.
12. Léauté-Labrèze C, de la Roque ED, Hubiche T, et al. A. Propranolol for Severe Hemangiomas of Infancy. *N Engl J Med* 2008;358(24):2649–51.
13. Chisholm KM, Chang KW, Truong MT, et al. β-Adrenergic receptor expression in vascular tumors. *Mod Pathol* 2012;25(11):1446–51.

14. Stiles JM, Amaya C, Rains S, et al. Targeting of beta adrenergic receptors results in therapeutic efficacy against models of hemangioendothelioma and angiosarcoma. *PLoS One* 2013;8(3):e60021.
15. Amaya CN, Perkins M, Belmont A, et al. Non-selective beta blockers inhibit angiosarcoma cell viability and increase progression free- and overall-survival in patients diagnosed with metastatic angiosarcoma. *Oncoscience* 2018;5(3–4):109–19.
16. Pasquier E, Ciccolini J, Carre M, et al. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget* 2011;2(10):797–809.
17. Greenberger S, Bischoff J. Pathogenesis of infantile haemangioma. *Br J Dermatol* 2013;169(1):12–19.
18. 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.
19. Daguzé J, Saint-Jean M, Peuvrel L, et al. Visceral metastatic angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *JAAD Case Reports* 2016;2(6):497–9.
20. Pasquier E, André N, Street J, et al. Effective Management of Advanced Angiosarcoma by the Synergistic Combination of Propranolol and Vinblastine-based Metronomic Chemotherapy: A Bench to Bedside Study. *EBioMedicine* 2016;6:87–95.
21. Chow W, Amaya CN, Rains S, et al. Growth Attenuation of Cutaneous Angiosarcoma With Propranolol-Mediated β-Blockade. *JAMA dermatology* 2015;151(11):1226–9.
22. Pramanik R, Gogia A, Malik PS, et al. Metastatic Primary Angiosarcoma of the Breast: Can We Tame It the Metronomic Way. I*ndian J Med Paediatr Oncol* 2017;38(2):228–31.
23. Banavali S, Pasquier E, Andre N. Targeted therapy with propranolol and metronomic chemotherapy combination: sustained complete response of a relapsing metastatic angiosarcoma. *Ecancermedicalscience* 2015;9:499.
24. Daguzé J, Saint-Jean M, Dréno B. Large nose angiosarcoma treated effectively with oral cyclophosphamide combined with propranolol. *J Eur Acad Dermatology Venereol* 2018;32(2):e52–4.
25. European Medicines Agency. Propranolol 40 mg film-coated tablets - Summary of Product

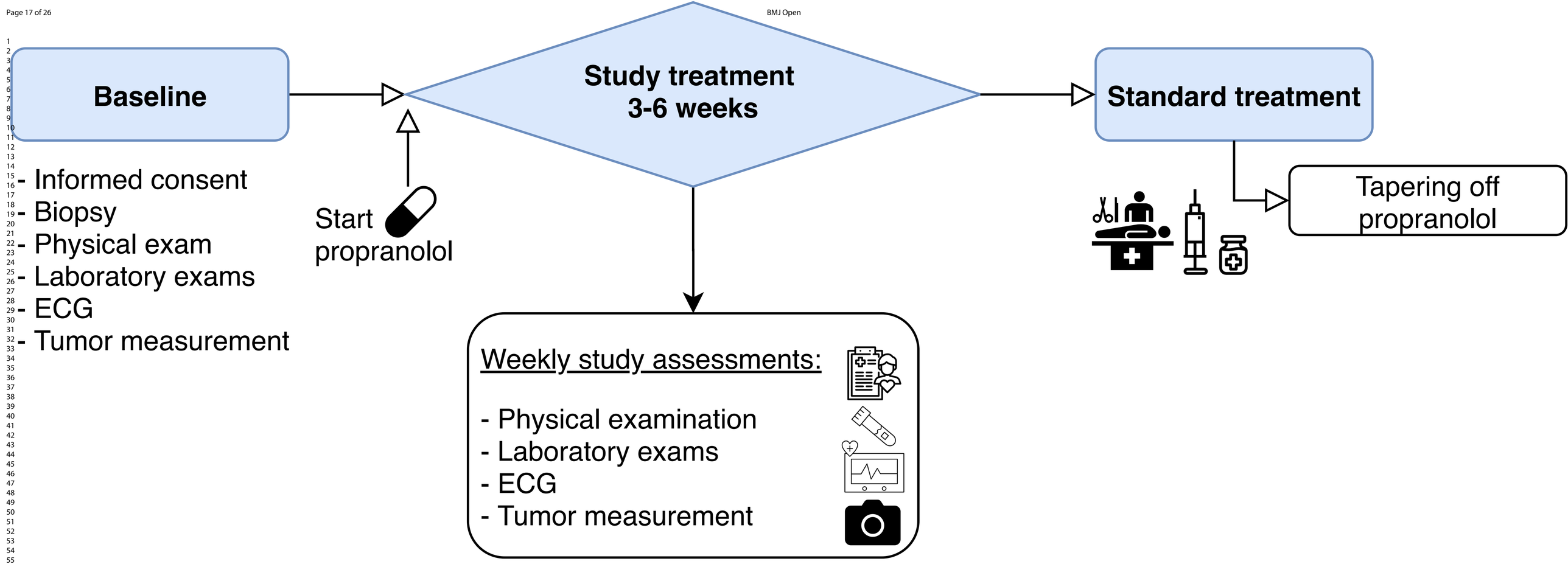
Characteristics (SmPC) - (eMC) [Internet]. Available from: https://www.medicines.org.uk/emc/product/2904/smpc

26. Galván DC, Ayyappan AP, Bryan BA. Regression of primary cardiac angiosarcoma and metastatic nodules following propranolol as a single agent treatment. *Oncoscience* 2018;5(9–10):262.

27. Schwartz LH, Seymour L, Litière S, et al. RECIST 1.1 - Standardisation and disease-specific adaptations: Perspectives from the RECIST Working Group. *Eur J Cancer.* 2016;62(Jul):138–45.

Legends

Figure 1. Study assessments. Figure 1 gives an overview of the study assessments, which are planned at baseline, during study treatment or when the standard treatment is initiated.


Baseline
- Informed consent
- Biopsy
- Physical exam
- Laboratory exams
- ECG
- Tumor measurement
Start propranolol
Study treatment 3-6 weeks
Weekly study assessments:
- Physical examination
- Laboratory exams
- ECG
- Tumor measurement
Standard treatment
Tapering off propranolol

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	PropAngio: a neoadjuvant trial on the efficacy of propranolol monotherapy in cutaneous angiosarcoma A proof of principle study
Trial registration	2a	Registry through the Netherlands Trial Register (Trial no. NL8118).
	2b	https://www.trialregister.nl/trial/8118
Protocol version	3	Version 2.0, 7 October 2019
Funding	4	The study is funded by the Anticancer Fund from Belgium.

Roles and responsibilities	5a	Kimberley M. Heinhuis[1,5*], Nikki S. IJzerman[1,2,5*], Anne Miek Koenen[3], Winette T.A. van der Graaf[1], Rick L. Haas[4], Jos H. Beijnen[5,6], Alwin D.R. Huitema[5,7], Winan J. van Houdt[3], Neeltje Steeghs[1]. *These authors contributed equally. Affiliations 1. Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 2. Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands 3. Department of Surgical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 4. Department of Radiotherapy, the Netherlands Cancer Institute, Amsterdam, the Netherlands 5. Department of Pharmacy & Pharmacology, Netherlands Cancer Institute, Amsterdam, the Netherlands. 6. Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands 7. Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
		Authors' contributions KH, NIJ, AMK, WG, RH, JB, AH, WH and NS each made substantial contributions to the conception or design of the study protocol. NIJ and KH wrote the first draft of the protocol and this paper. AMK, WG, RH, WH, JB, AH and NS provided critical input regarding the design of the study or study logistics. KH, NIJ, AMK, WG, RH, JB, AH, WH and NS revised the protocol critically and approved the final version to be published.
	5b	Anticancer Fund Belgium, Liese Vandeborne, Research Manager, +32 2 268 48 16, www.anticancerfund.org, Boechoutlaan 221, 1853 Strombeek-Bever, Belgium.
	5c	The study sponsor and funders will not have ultimate authority in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.
	5d	Not applicable.

Introduction

Background and rationale

6a Angiosarcoma is a rare and aggressive malignancy with a high metastatic potential.(1) The standard of care for localised angiosarcoma is currently complete surgical resection with or without radiation. Unfortunately, despite the current standard of care, only 60% of patients with localized disease survive for more than five years.(2)

Several cytotoxic drugs, including anthracyclines, taxanes and gemcitabine, have shown activity in angiosarcoma in the locally advanced and metastatic setting, with overall response rates varying from 17 to 89%.(2–4) However, for the treatment of resectable angiosarcoma, none of the previous studies show a prolonged disease-free survival or overall survival.(3,5–10) Improved treatment in the neoadjuvant setting might reduce the local and distant recurrence rates by treating micrometastases at an early stage and by improving the resection margins, potentially leading to higher survival rates. As a result, new drugs are urgently needed to prolong the survival.

Recently, propranolol has been repurposed and is now successfully used in the treatment of hemangioma.(11,12) Angiosarcoma have several similarities with hemangioma, including its high β-adrenergic receptor expression and the supposedly important role of VEGF in malignant growth.(11,17)

Several small case reports and case series have confirmed the idea that propranolol could be repurposed to treat angiosarcoma. The dose of propranolol in combination therapy varied between 80 to 120 mg per day.(18–23) These doses of propranolol are much lower than the standard maintenance dose of 160-320 mg daily for patients with hypertension.(24)

Since literature regarding the activity and mode of action of propranolol as a single agent for angiosarcoma is scarce, our aim is to evaluate the activity of propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma before they proceed to their standard anti-cancer treatment. The neoadjuvant setting provides a good opportunity to rapidly evaluate both the clinical and histological response, without delaying the standard anti-cancer treatment. Additional advantages of propranolol therapy would be the ease of use and the relatively mild toxicity profile. If this study turns out to be positive, further (randomized) clinical trials are thereby substantiated and highly recommended.

[Reference numbers refer to references list in paper].

6b This is a proof of principle study with no comparator because there is no current standard neoadjuvant treatment for angiosarcoma.

Objectives	7	The aim of this study is to investigate the effect of neoadjuvant propranolol monotherapy in patients with primary, recurrent or metastatic cutaneous angiosarcoma, before they proceed to their standard anti-cancer treatment (e.g. isolated limb perfusion, chemotherapy, targeted therapy, surgical resection or radiotherapy). The primary objective is to determine the clinical response of propranolol monotherapy and the histologic response will be evaluated as secondary objective.
Trial design	8	This is a single armed, phase II, proof of principle study.

Methods: Participants, interventions, and outcomes

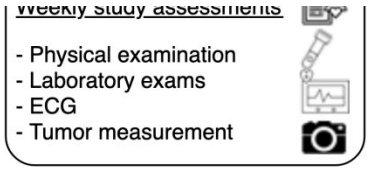
Study setting	9	This is a monocenter study and all patients will be treated in the Netherlands Cancer institute in Amsterdam, Antoni van Leeuwenhoek hospital.
Eligibility criteria	10	The study population consists of patients with primary, recurrent or metastatic cutaneous angiosarcoma, including angiosarcoma of the breast. Only patients with cutaneous angiosarcoma can participate, since these tumours are easily measurable on coloured photographs for clinical response evaluation. Patients are eligible if they are at least 18 years old; have a good performance status (world health organization (WHO-PS) of 0-2); have an adequate blood count, kidney and renal function; have a window of at least three weeks between their diagnosis and the start of the standard anti-cancer treatment and have evaluable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients with primary visceral angiosarcoma, contraindications for β-blockade therapy or current treatment with β-blockade therapy (both selective and non-selective β-blockade therapy) or other anti-cancer treatment are excluded.
Interventions	11a	All patients will follow the same scheme (single armed study):

Dose escalation scheme		
Period	**Dose level**	**Dose**
Day 1 – Day 7	1*	2x/day 40 mg
Day 8 – Day 14	2*	2x/day 80 mg
Day 15 – Day of surgery or biopsy	3*	3x/day 80 mg
Tapering off scheme after surgery/biopsy		
Period	**Dose**	
Day 1 - Day 7	2x/day 80 mg	
Day 8 - Day 14	2x/day 40 mg	

11b In case of hypotension (blood pressure <90/60 mmHg) or bradycardia (heart rate <55 bpm), or symptoms of bradycardia or hypotension (dizziness, syncope) the dose will be reduced to the previous dose level. In case of serious bradycardia (heart rate <50 bpm), the treatment will be stopped until an acceptable heart rate (>55 bpm) is reached and propranolol will be restarted in the dose of the previous level. A patient can stop study treatment by withdrawing the informed consent at any time.

11c The tolerability will be assessed during weekly visits in the outpatient clinic. Each visit consists of a physical examination, blood draw for safety assessment (hematology, hepatic- and renal function), vital signs, electrocardiogram (ECG), toxicity assessment, concomitant medication registration and tumour response assessment. After the study treatment, a biopsy will be obtained to evaluate the histologic response to propranolol monotherapy. Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated

11d Treatment with other beta blockade therapy or anticancer therapies are prohibited (except hormonal therapy for breast cancer).

Outcomes	12	Primary endpoint The clinical response will be determined according to RECIST 1.1 criteria (PD is >20% increase in size, PR is >30% decrease in size, SD is in between while CR is no measurable disease).(26) A response is defined as CR, PR, or SD with an improvement in clinical characteristics, like thickness, erythema, necrosis or edema of the inflicted area. Documentation will be performed with colour photography, including a ruler to measure the size of the lesion. Radiologic assessment will only be done if the patient has radiologic evaluable disease at the beginning of the study treatment. If the study turns out to be positive, this treatment modality is highly interesting and should be tested further in a randomized trial. Secondary endpoint The histologic response on propranolol treatment is defined as difference in proliferation index. This will be assessed by measuring the change in immunohistochemistry staining of Ki-67 and the tumour activity between the post-treatment biopsy (obtained during surgery if applicable) and the diagnostic biopsy before the study treatment. A decrease of >30% of the Ki-67 staining will be considered as a positive histologic response. Exploratory endpoints To obtain additional data regarding the primary objectives, the percentage of adrenergic receptors (ADRB1, ADRB2, and ADRB3) in the pre-treatment biopsies will be measured with immunohistochemically staining and the correlation with the anti-tumour response of the angiosarcoma patients will be investigated.(13,20) With this correlation analysis, the predictive value of adrenergic receptor expression in tumour tissue on the anti-tumour response will be assessed. Furthermore, we plan to assess the drug sensitivity with and without different chemotherapy regimens in vitro. Finally, we will compare the PET response before start of treatment and at the end of propranolol treatment.
Participant timeline	13	

Sample size	14	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
Recruitment	15	The sarcoma unit of the NKI was trained for the study. Patientenplatform sarcomen, DSSG and Anticancer Fund Belgium are also informed and will provide information to possible patients.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Not applicable
Allocation concealment mechanism	16b	Not applicable
Implementation	16c	Not applicable
Blinding (masking)	17a	Not applicable
	17b	Not applicable

Methods: Data collection, management, and analysis

Data collection methods	18a	Data collection will be performed according to the figure as stated in section 13.
	18b	The follow up data will also be recorded in the eCRF by the investigators of the study.
Data management	19	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor.
Statistical methods	20a	An exact single-stage phase II design will be used with a one-sided significance level α of 0.05 and a power of 80%. The maximum response rate that would be of no interest was assumed to be 5% and the minimum required response rate 30%. A total of 14 patients will be included in the study. If 3 or more out of these 14 patients have a response as defined in the study endpoints, the study is considered positive.
	20b	Not applicable
	20c	Not applicable

Methods: Monitoring

Data monitoring	21a	Monitoring of the study will be performed according to ICH GCP by the Clinical Research Monitor of the NKI or the person to whom the monitoring tasks have been delegated. Amongst others the following will be reviewed: compliance with the protocol, ICH GCP and all applicable regulatory requirements; consent procedures, including date of consent and signatures; study progress; (Serious) Adverse Events; completion of the (e)CRFs and verification of data against the source data; and storage, dispensing and accountability of study medication. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
	21b	An interim analysis is planned after the treatment of seven patients. If there are already three or more responses at this time point, the study will be stopped and stated positive. Otherwise an additional seven patients will be included. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Harms	22	All adverse events will be recorded in the electronic case report forms (eCRF) during the weekly outpatient clinic visits. We will perform extra blood draws, ECGs and measurements of the vital signs during these visits for safety assessments. The recording of the adverse events will be done according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0.
Auditing	23	Frequency and procedures for auditing trial conduct will be done yearly by the MEC of the NKI and will be independent from investigators and the sponsor.

Ethics and dissemination

Research ethics approval	24	Ethical approval was obtained from the Medical Ethical Committee of the Netherlands Cancer Institute.
Protocol amendments	25	Independent of the outcome, results of this study will be shared and submitted for publication in an international peer-reviewed journal.All essential documents (including patient files, the Investigator Study File, CRF's, amendements and electronic study data), data management and statistical files will be kept for 15 years.

Consent or assent	26a	Treating physicians will identify patients as possible candidates and inform patients about the study. If patients agree to participate and fulfil the selection criteria, patients will be included during an outpatient clinic visit.
	26b	Not applicable
Confidentiality	27	The original results will also be recorded in the eCRF by the investigators of the study. The data entry will be supervised by the Clinical Research Monitor. The Medical Ethical Committee of the NKI will review the study every year throughout the complete study duration. During this review, the committee will focus on monitoring of the safety of patients and evaluate the balance between the efficacy and the harmfulness of propranolol.
Declaration of interests	28	No conflicts of interests to disclose related to this study
Access to data	29	Study investigators will have access to the final data. Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
Ancillary and post-trial care	30	The investigator has a liability insurance which is in accordance with article 7 of the WMO. The AVL as sponsor of this study also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.
Dissemination policy	31a	Results of the study will be shared and be submitted for publication in an international peer-reviewed journal.
	31b	Colleagues meeting the ICMJE criteria for authorship will become co-author. No professional writers will be involved.
	31c	The study was registered through the Netherlands Trial Register (Trial no. NL8118). https://www.trialregister.nl/trial/8118
Appendices		
Informed consent materials	32	Patient information folder in Dutch is attached
Biological specimens	33	All data regarding the clinical records and biological specimens will be stored anonymously for future research, if the patient gives consent in the patient information folder.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT