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APOLLO: neo-adjuvant pembrolizumab for primary vulvar squamous cell carcinoma—a multicenter, single-arm, phase II, clinical proof-of-concept study

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ABSTRACT

Background Vulvar squamous cell carcinoma (VSCC) is a rare cancer for which the cornerstone of treatment is surgery with high complication rates. The unmet need is a less radical and more effective treatment for VSCC.

Primary Objectives To investigate the impact of mono-immunotherapy pembrolizumab as neoadjuvant treatment for primary resectable VSCC patients.

Study Hypothesis Some primary VSCC patients display a specific immune profile which is associated with better survival. In other tumors, this profile is associated with a better response to programmed cell death protein 1 (PD-1) checkpoint blockade which may reinvigorate tumor-specific T cells. This potentially results in a reduced tumor load and less radical surgery and/or adjuvant treatment in patients with this immune profile.

Trial Design This is an investigator-initiated, prospective, single arm, multicenter, phase II clinical trial.

Inclusion Criteria Patients with VSCC clinical stage International Federation of Gynecology and Obstetrics (FIGO) I-III (2021) eligible for primary surgery, with at least one measurable lesion of at least one dimension ≥10 mm in the largest diameter, are included in this study.

Main Exclusion Criteria Patients not suitable for surgery and/or previously treated with immunomodulatory agents, and/or who suffer from comorbidities that may interfere with PD-1 blockade, are excluded from the study.

Endpoints The clinical efficacy of neoadjuvant pembrolizumab in VSCC is measured by an objective change in tumor size according to the Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST 1.1) and documented by calipers using standardized digital photography with a reference ruler. In addition, the activation, proliferation, and migration of T cells in the tumor will be studied. The secondary endpoints are pathological complete responses at the time of surgery, feasibility, and safety.

Sample Size 40 patients with FIGO I-III (2021) primary VSCC will be enrolled.

Estimated Dates for Completing Accrual and Presenting Results The intervention phase started in July 2023 and will continue until July 2025. The expected completion of the entire study is July 2026.

Trial Registration Number NCT05761132

INTRODUCTION

Vulvar squamous cell carcinoma (VSCC) is a relatively rare gynecological cancer type that accounts for 10% of all gynecological cancers, with an annual incidence of 2–3 per 100 000 women. The majority of these patients (90%) have a T1 (restricted to the vulva, not involving the urethra, vagina, or anus), N0-1, M0 tumor. Patients with International Federation of Gynecology and Obstetrics (FIGO) stage (2009) I/II VSCC have approximately a 70% 5-year survival, which decreases drastically to 40% in stage III disease when lymph nodes are involved.[1] Surgical resection of the primary vulvar tumor and excision of sentinel inguinal lymph nodes or full inguinal lymph node dissection depending on clinicopathological features remains the standard-of-care for VSCC, and approximately 20% of the patients receive adjuvant radiotherapy.[2] The prognosis of VSCC has not improved over the past decades and post-operative morbidity remains a lingering burden, consisting of wound complications, infections, positive margins, vital tissue damage to clitoris, urethra, and/or anus, and associated psychosocial issues and sexual dysfunction.[3]

In the end, up to 40% of VSCC patients will develop recurrent disease and eventually die of their disease and/or accompanied complications.[4]

To improve outcome, as well as reduce short-term and late morbidity after cancer treatment, there is a need to study potential therapeutic agents that may result in less (surgical or treatment-related) morbidity as well as decreased recurrences.

We recently showed that patients treated by surgery and if needed adjuvant radiotherapy displayed better overall survival and recurrence-free survival when their VSCC was strongly infiltrated by T cells and showed signs of active immune signaling.[5–7] In other solid tumors, immunotherapy in the form of checkpoint blockade works best in patients with such an inflamed/hot tumor,[8] especially when it is applied in a neoadjuvant setting where it is associated with partial and complete responses in substantial

numbers of patients.[9–13] Based on these data, we hypothesized that programmed cell death protein 1 (PD-1) blockade may activate and boost T cell immunity when patients have an inflamed/hot VSCC, and consequently show a reduction in tumor size prior to surgery. This may lead to less radical surgery, thereby reducing the deleterious post-surgical morbidity and meanwhile limiting the recurrence rate of VSCC. Therefore, we initiated APOLLO, a prospective, multicenter, phase II, non-controlled clinical trial with pembrolizumab, to evaluate the efficacy and anti-tumor response in VSCC in a neoadjuvant setting.

METHODS

Trial Design

Patients with primary VSCC who present at the outpatient clinic of participating centers will be informed of the study by their treating physician. For those patients who are potentially eligible and interested in the study, further information and counseling will be done by the gynecological oncologist and medical oncologist. Written informed consent will be obtained after a reflection period (week 2). At baseline, the tumor size will be recorded by calipers using digital photography. Pre-treatment biopsies will be obtained for confirmation of VSCC and for translational studies.

The planned dose of pembrolizumab for this study is 200 mg intravenously (IV) 3-weekly, based on safety and feasibility data generated from the Keytruda development program. Patients will receive standard-of-care surgery after two cycles of neoadjuvant pembrolizumab approximately 3–4 weeks after the second dose. The duration of pre-operative treatment of approximately 6 weeks has been chosen as this is a short time period unlikely to result in significant disease progression which might preclude complete surgical resection.

Clinical responses are categorized according to the Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST 1.1)[14] and documented by calipers using standardized digital photography with a reference ruler as either a complete response with disappearance of all target lesions, in which any pathological lymph nodes (whether target or non-target) must have a reduction in short axis to <10 mm, or a partial response with at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. Progressive disease is defined as at least a 20% increase in the sum of diameters of target vulvar lesions, taking as reference the smallest-sum-on-study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered as progression. Finally, stable disease in which neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum diameters while on study, is observed. Responders have the option to fall into an extension cohort and, after consultation with the multidisciplinary team, will be treated with adjuvant pembrolizumab from week 16 until week 58 (400 mg IV, 6-weekly, seven times). These patients will be monitored for long-term effects, including blood collections for safety values and digital photography of the vulva. Non-responders and responders who will not have extended pembrolizumab will visit the hospital at week 13 and 16 to monitor potential long-term effects of pembrolizumab, including blood collections for safety values and for oncological follow-up in accordance with standard-of-care guidelines.

Baseline immunological parameters are measured from two fresh pre-treatment 5 mm wide biopsies (one diagnostic and one translational biopsy) that will be taken at week 3 and paraffin embedded using the standard workflow for diagnostic pathology including a p16-, p53-, and programmed death-ligand 1 (PD-L1) immunohistochemistry. At surgical resection, diagnostic and translational material of the tumor will be paraffin embedded. Part of the tumor at surgical resection (if the tumor size allows for this) will be used to make a fresh tumor digest. Peripheral blood mononuclear cells are isolated, washed, and cryopreserved according to standard operating procedures. A graphical representation of the study is provided in (Figure 1).

Participants

Patients who are clinically diagnosed with FIGO I-III (2021) will be accrued from the Leiden University Medical Center, the University Medical Center Groningen, and the Erasmus University Medical Center. Subjects will be recruited by the gynecologist-oncologist. Included are women with a histologically confirmed primary diagnosis of VSCC with at least one lesion that can be measured in at least one dimension with ≥10 mm in largest diameter and clinical stage FIGO I-III (2021) who are eligible for primary surgery. Women should not be pregnant or breastfeeding and will be excluded when they have received prior therapy with an anti-PDL-1 or anti-PD-L2 agent, or other systemic anti-cancer therapies within 4 weeks prior to allocation. Also prior radiotherapy or major surgery within 2 weeks of the start of the study treatment, and a live vaccine within 30 days before the first dose of pembrolizumab, are exclusion criteria. In addition, an immunodeficiency or chronic systemic steroid therapy (≥10 mg daily of prednisone equivalent) are excluded (see full list of inclusion/exclusion criteria in (Table 1).

Outcomes

Primary Endpoints

The primary endpoint of the study is: (1) the clinical efficacy of neoadjuvant pembrolizumab in VSCC, measured by an objective change in tumor size (according to RECIST 1.1) and documented by calipers using standardized digital photography with reference ruler at the time of surgery; and (2) the activation, proliferation, and migration of tumor-specific T cells on PD-1 blockade.

Secondary Endpoints

In addition to the clinical response, the pathological response in tumor tissue derived from surgery (week 10) will be assessed by a gynecological pathologist, blinded to other study data. The pathological tumor response (pTR) is defined as the presence of tumor cell necrosis and keratinous debris with giant cell/histiocytic reaction, quantified as a percentage of the overall tumor bed (area pathologic response/area pathologic response plus viable tumor): pTR-0 (<10%), pTR-1 (10–49%), pTR-2 (≥50%), pTR-3 (100%, complete response). Moreover, the feasibility (defined as delay in planned surgery and surgical outcome) and safety according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTAE) version 5.0 of pembrolizumab in a neoadjuvant setting will be assessed. Moreover, we will study the activation,

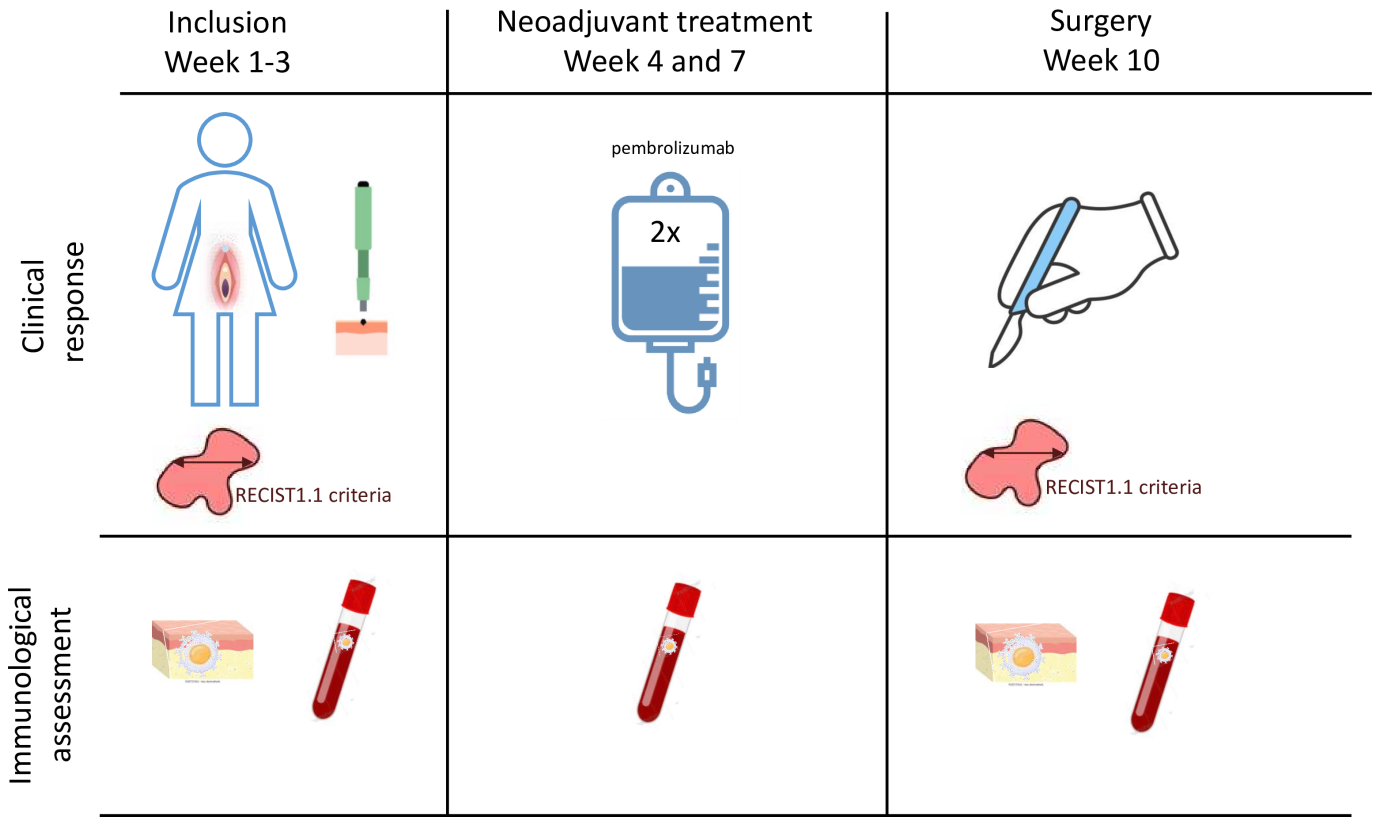

Figure 1 Overview of study design. After the enrollment of patients in the study, the tumor is measured according to RECIST 1.1 criteria and documented by calipers using standardized digital photography with a reference ruler. Both vulvar biopsies and blood samples are taken. In short, pembrolizumab is given in week 4 and week 7 following the collection of the blood samples. In week 10 surgery is planned with a second measurement of the tumor, together with the obtainment of pre-surgery blood samples and resection material (see trial design). RECIST 1.1, Response Evaluation Criteria In Solid Tumors, version 1.1.

proliferation, and migration of other cell populations on PD-1 blockade.

Exploratory Endpoints

To study the on-treatment effect of neoadjuvant PD-1 blockade on the tumor microenvironment in fresh resection material and peripheral blood. To discover potential future predictors for minimal residual disease and/or relapse we will study the utility of circulating tumor DNA. The quality of life (European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC QLQ) C-30 and QLQ-VU34) of patients enrolled in this study will be assessed.

Sample Size

The clinical hypothesis is that the group of VSCC patients with an inflamed tumor (that is strongly T cell infiltrated tumor with 'hot' immune transcriptional signature) is more likely to respond to PD-1/PD-L1 blockade in a neoadjuvant setting.[5–7] At this stage a conservative estimation is that 25% of primary FIGO I-III (2009) VSCC displays such an inflamed tumor.[5–7] Assuming that 50% of these patients with an inflamed tumor (12.5%) should respond, a <1% spontaneous (base rate) response and an α=0.05, then n=29 (power 90%) patients are required to establish clinical outcome. For biomarker studies the power calculations (α=0.05, β=0.2, power 80%) show that with a responder to non-responder ratio of 1:3 (10 vs 30 out of 40), a positive observation in 80% of the responders and in maximally 30% of the non-responders (background) would require n=36 patients (n=9 responders and n=27 non-responders) to adequately identify potential biomarkers. Based on these calculations a total of 40 primary VSCC patients will be included in this clinical proof-of-concept study.

Randomization and Blinding

Not applicable

Statistical Methods

In order for patients to be eligible for evaluation of the primary clinical endpoint they should have received at least one cycle of pembrolizumab. All data collected until week 16 will be used for the analyses. Continuous variables will be summarized using descriptive statistics for all assessments (mean, SD, quartiles, minimum, and maximum). Categorical variables will be summarized using counts and percentages. Non-parametric (Wilcoxon signed-rank or Mann-Whitney test for two samples and Friedman or Kruskal-Wallis with Dunn's multiple comparison test for multiple samples) and parametric (paired or unpaired t test for two samples or repeated measures one-way analysis of variance (ANOVA) or ordinary one-way ANOVA with Tukey's multiple comparison test for multiple samples) tests are performed as appropriate. P values <0.05 are marked as significant. Objective response rates (ORRs) will be calculated using standard statistics for ORR (point estimate and confidence interval). The confidence level will be set at 95%.

Table 1 Inclusion and exclusion criteria of the APOLLO trial

Inclusion criteria		Exclusion criteria
Signed written informed consent prior to performance of study-specific procedures or		Locally advanced tumor not amenable to surgical therapy
Age ≥18 years at the day of signing informed consent		A woman of childbearing potential who has a positive urine pregnancy test within 72 hours prior to allocation. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required
Histologically confirmed primary vulvar squamous cell carcinoma, with all of the following characteristics:	At least one lesion that can be measured in at least one dimension with ≥10 mm in largest diameter	Prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T cell receptor (eg, CTLA-4, OX 40, CD37)
	Clinical stage FIGO I-III	Prior systemic anti-cancer therapy including investigational agents within 4 weeks prior to allocation
	Documentation confirming the absence of distant metastasis (M0) as determined by institutional practice. Routine exams to discard metastases will be performed according to Investigator judgment but are mandatory in case of suspicion of metastatic disease	Prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis
	Vulvar cancer eligible for primary surgery	Major surgery within 2 weeks of starting study treatment and patients must have recovered from any effects of any major surgery
	In the case of a multifocal tumor (defined as the presence of two or more foci of cancer on the vulva), the largest lesion must be ≥10 mm and all lesions ≥10 mm are designated as 'target' lesion(s) for all subsequent tumor evaluations and biopsies	A live vaccine within 30 days before the first dose of study intervention. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, bacillus Calmette-Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist) are live attenuated vaccines and are not allowed
ECOG performance 0–1		Currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment. Note: participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent

Continued

Table 1 Continued

Inclusion criteria			Exclusion criteria	
Have adequate organ function as measured within 28 days prior to administration of study treatment	System	Laboratory value	Diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug	
	Hematological		A known additional malignancy that is progressing or has required active treatment within the past 3 years. Note: participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin on a location other than the vulva, or carcinoma *in situ* (eg, of the breast, cervix or bladder) that have undergone potentially curative therapy are not excluded.	
	Hemoglobin	≥10.0 g/dL or >6.2 mmol/L, with no blood transfusions the past 28 days	Severe hypersensitivity (≥grade 3) to pembrolizumab and/or any of its excipients	
	Absolute neutrophil count	≥1500/µL	Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease (eg, colitis or Crohn's disease), diverticulitis (with the exception of diverticulosis), systemic lupus erythematosus, sarcoidosis syndrome, or Wegener syndrome (granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc)). The following are exceptions to this criterion:	Patients with vitiligo or alopecia
	Platelets	≥100 000/µL, with no platelet transfusion in the past 28 days		Patients with hypothyroidism (eg, following Hashimoto syndrome) stable on hormone replacement
	Hepatic			Any chronic skin condition that does not require systemic therapy
	Total bilirubin	≤1.5 x ULN (not applicable to Gilbert's syndrome with an upper limit of 2.5 ULN)		Patients without active disease in the last 5 years may be included but only after consultation with the study physician
	AST (SGOT) and ALT (SGPT)	≤2.5 x ULN		Patients with celiac disease controlled by diet alone
	Renal		History of (non-infectious) pneumonitis that required steroids or has current pneumonitis	
	Creatinine clearance (using the Cockcroft-Gault equation or 24 hour urine clearance)	≥51 mL/min	An active infection requiring systemic therapy	
			Known history of HIV. Note: no HIV testing is required unless mandated by local health authority	

Continued

Table 1 Continued

Inclusion criteria		Exclusion criteria
		Known history of hepatitis B (defined as hepatitis B surface antigen (HBsAg) reactive) or known active hepatitis C virus (defined as HCV RNA (qualitative) is detected) infection. Note: no testing for hepatitis B and hepatitis C is required unless mandated by local health authority
A female participant is eligible to participate if she is not pregnant or breastfeeding, and at least one of the following conditions applies:	Is not a woman of childbearing potential, or	A history or current evidence of any condition, therapy, or laboratory abnormality or other circumstance that might confound the results of the study, interfere with the subject's participation for the full duration of the study in such it is not in the best interest of the subject to participate, in the opinion of the treating investigator
	Is a woman of childbearing potential and using a contraceptive method that is highly effective (with a failure rate of <1% per year), with low user dependency, or be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long-term and persistent basis), as described in appendix 11 during the intervention period and for at least the time needed to eliminate each study intervention after the last dose of study intervention and agrees not to donate eggs (ova, oocytes) to others or freeze/store for her own use for the purpose of reproduction during this period. The length of time required to continue contraception for each study intervention is 120 days for pembrolizumab	Is pregnant or breastfeeding or expecting to conceive within the projected duration of the study, starting with the screening visit through 120 days after the last dose of trial treatment
		Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial
		Has had an allogenic tissue/solid organ transplant

ALT (SGPT), alanine aminotransferase (serum glutamic-pyruvic transaminase); AST (SGOT), aspartate transaminase (serum glutamic-oxaloacetic transaminase); CTLA-4, cytotoxic T-lymphocyte associated protein 4; ECOG, Eastern Cooperative Oncology Group; FIGO, International Federation of Gynecology and Obstetrics; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; ULN, institutional upper limit of normal.

DISCUSSION

This a clinical proof-of-concept study, and the first trial that studies checkpoint blockade in primary VSCC in a neoadjuvant setting. While only sporadic responses have been reported for adjuvant checkpoint therapy in metastatic VSCC, we expect that neoadjuvant treatment will be much more successful. The rationale behind this hypothesis lies in our observations that patients with an inflamed tumor (around 45% of all VSCC[5]) exhibit more favorable responses to standard-of-care treatments. As such, this patient population is less likely to have metastatic or recurrent VSCC[5 7] and are therefore not incorporated in previous adjuvant checkpoint studies. Importantly, the literature on successful (neoadjuvant) checkpoint therapy in other cancer types indicates that patients with inflamed tumors are more likely to respond.[9 10 12 13]

The application of only two 3-weekly doses of checkpoint therapy in a neoadjuvant setting will likely not compromise surgical outcome in case of non-responding VSCC and may already lead to clinical responses. Importantly, recent studies, in which 1–2 doses

of checkpoint therapy have been given in a neoadjuvant setting for other types of tumors, suggest that this may be enough to obtain complete and partial responses in a substantial portion of patients.[9 10 12 13] By treating VSCC patients with checkpoint therapy in a neoadjuvant approach, also the patients with inflamed tumors will receive immunotherapy. If successful, neoadjuvant checkpoint therapy of VSCC is high-gain because then we would have—for the first time in decades—a new treatment option for VSCC and be able to facilitate more sparing and less morbid surgery. In addition, the study may lead to the discovery of biomarkers that could predict clinical responsiveness in this patient group.

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Correction notice This article has been corrected since it was first published. Author name Anna K L Reyners has been corrected.

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Contributors MvP: conceptualization, data curation, writing. KK: investigation, data curation, writing. HvD: data curation, writing. HN: data curation, writing. IB: data curation, writing. AR: data curation, writing. PEG: data curation, writing. BJ: data curation, writing. TB: data curation, writing. MW: data curation, writing. JK: conceptualization, data curation, writing. SvdB, conceptualization, writing. SvdB accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Ethical Committee, Leiden University Medical Center NL82378.058.22. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Commissioned; internally peer reviewed.

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