CONFIDENTIAL

# ANVU

## (Australian National Vulvar Cancer Trial)

## A Phase II Randomised Clinical Trial of Ultrasound Groin Monitoring versus Groin Lymph Node Dissection to De-Escalate the Extent of Surgery in Vulvar Cancer

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| **Study Title** | **A Phase II randomised clinical trial of ultrasound groin monitoring versus groin lymph node dissection to de-escalate the extent of surgery in vulvar cancer** |
| **Abbreviated Title** | ANVU |
| **Clinical Trials Registration** | TBD |
| **Protocol Version/Date** | Version 0.9. 21 June 2024 |
| **Study Sponsor** | The University of Queensland |
| **Coordination** | |
| Coordinating Centre | Queensland Centre for Gynaecological Cancer<br>UQCCR, Building 71/918<br>Royal Brisbane and Women's Hospital<br>Herston QLD Australia 4029<br>Ph: +61 7 3346 5590 |
| **Study Team** | |
| Study Chair | Professor Andreas Obermair[1,2,3,4]<br>Email: a.obermair@uq.edu.au |
| Deputy Chair | Professor Neville F Hacker[5] |
| Study Statisticians | Professor Val Gebski MStat[4,6,7]<br>Professor Andrew Martin [2] |
| Coordinating Study Investigators | Professor Alexandra McCarthy [2,8]<br>Professor Sandra Hayes[4,10]<br>Professor Monika Janda[2]<br>Professor Haitham Tuffaha[11]<br>Dr Shohreh Rezai[3]<br>Dr Sarah Lord[6]<br>Dr Eva Baxter[2]<br>Dr Zoe West[2]<br>Professor Paul A. Cohen[12, 13]<br>Associate Professor Orla McNally [13, 14] |
| Other Trial Contacts | ANVU Trial Manager: Brianna Armstrong<br>Phone: 07 3346 5063<br>Email: ANVUtrial@uq.edu.au<br><br>ANVU Trial Monitor: TBD<br>Phone: TBD |

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| | Email: TBD |
| Trial Management Committee | Professor Andreas Obermair[1]<br>Professor Neville F Hacker[5]<br>Professor Val Gebski [4,6,7]<br>Professor Alexandra McCarthy [2,8]<br>Professor Sandra Hayes[4,10]<br>Professor Monika Janda[2]<br>Professor Haitham Tuffaha[11]<br>Dr Shohreh Rezai[3]<br>Dr Sarah Lord[6]<br>Dr Eva Baxter[2]<br>Dr Zoe West[2]<br>Professor Paul A. Cohen[4,12,]<br>Associate Professor Orla McNally[4,13]<br>Dr Alison Evans [4]<br>John Andrews [4]<br>Fran Butt[14]<br>Merran Williams[15] |
| Site Principal Investigators | Prof Andreas Obermair[16]<br>Dr Andrea Garrett [3, 17]<br>Dr Michelle Harris[18]<br>Dr Nicole Krzys[19]<br>Dr Stuart Salfinger[12]<br>Associate Professor Orla McNally[13]<br>Dr Martin Oehler[20]<br>Dr Rachel O'Sullivan[9]<br>Dr Greg Gard[21]<br>Dr Adam Pendlebury[22] |
| Affiliations | 1. Queensland Centre for Gynaecological Cancer, RBWH, Herston, QLD Australia 4029<br>2. The University of Queensland, St Lucia, QLD Australia 4067<br>3. Royal Brisbane and Women's Hospital, Herston, QLD Australia 4029<br>4. Australia New Zealand Gynaecological Oncology Group (ANZGOG), Camperdown, NSW Australia 2050<br>5. The University of New South Wales, Kensington, NSW Australia 2052<br>6. NHMRC Clinical Trials Centre, Camperdown, NSW Australia 2006<br>7. The University of Sydney, Camperdown, NSW Australia 2006<br>8. Mater Hospital Brisbane, South Brisbane, QLD Australia 4101 |

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|---|---|
| | 9. John Hunter Hospital, New Lambton Heights, NSW Australia 2305<br>10. Cancer Council Queensland, Brisbane, QLD<br>11. Centre for the Business and Economics of Health, The University of Queensland, St Lucia, QLD Australia 4067<br>12. St John of God Hospital, Subiaco, WA Australia 6008<br>13. The Royal Women's Hospital, Parkville, VIC Australia 3052<br>14. Vulvar/Vaginal Cancer Consumer Group, Queensland Centre for Gynaecological Cancer, RBWH, Herston, QLD Australia 4029<br>15. Consumer-Led Research Group, Queensland Centre for Gynaecological Cancer, RBWH, Herston, QLD Australia 4029<br>16. St Andrew's War Memorial Hospital, Spring Hill, QLD Australia 4000<br>17. The Wesley Hospital, Auchenflower, QLD Australia 4066<br>18. Royal Darwin Hospital, Tiwi, NT Australia 0810<br>19. Royal Hobart Hospital, Hobart, TAS Australia 7000<br>20. Royal Adelaide Hospital, Adelaide, SA 5000<br>21. Royal North Shore Hospital, St. Leonards, NSW 2065<br>22. Mercy Hospital for Women, Heidelberg, VIC 3084 |

| Protocol Version History | | | |
|---|---|---|---|
| **Version Number** | **Version Date** | **Amendment Number** | **Summary of Changes** |
| | | | |

# Contents

## Protocol Authorisation

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| **Project Title** | A Phase II randomised clinical trial of ultrasound groin monitoring versus groin lymph node dissection to de-escalate the extent of surgery in vulvar cancer |
| **Short Title** | Australian National Vulvar Cancer Trial |
| **Protocol ID** | ANVU |
| **Protocol Version and Date** | [Insert the HREC-approved version and date.] |

The design of this study as outlined by this protocol has been reviewed and approved by the responsible personnel as indicated in the signature table below.

**Coordinating Principal Investigator**

| **Name (PRINT)** | **Signature** | **Date** |
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**Statisticians**

| **Name (PRINT)** | **Signature** | **Date** |
|---|---|---|

| **Name (PRINT)** | **Signature** | **Date** |
|---|---|---|

## Investigator Signature Page & Confidentiality

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By signing this Protocol, the Investigator(s) acknowledges and agrees:

The Protocol contains all necessary details for conducting the study. The Investigator will conduct this study as detailed herein, in compliance with Good Clinical Practice (ICH-GCP) and the applicable regulatory requirements and will make every reasonable effort to complete the study within the time designated.

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QCGC Research will have access to any source documents from which Case Report Form information may have been generated. The Case Report Forms and other data pertinent to this study are the sole property of QCGC Research, which may utilise the data in various ways, such as for submission to government regulatory authorities or in publication of the results of the study.

The conduct and results of this study will be kept confidential. The results of this study may be published. Upon completion of the Study, it is the intention of the parties to prepare a joint publication regarding or describing the Study and all the results there from and both parties shall cooperate in this regard.

**Site Name**

**Principal Investigator**

| **Name (PRINT)** | **Signature** | **Date** |
|---|---|---|

# 1. Protocol Summary

## 1.1 Synopsis

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| Short Study Title | Australian National Vulvar Cancer Trial |
| Full Study Title | A phase II randomised clinical trial of ultrasound groin monitoring versus groin lymph node dissection to de-escalate the extent of surgery in vulvar cancer |
| Protocol Identification | ANVU |
| Development Phase | Phase II |
| Indication | Vulvar Cancer – Clinical Stage 1 or 2 |
| General aim | This clinical trial will determine whether groin ultrasound monitoring (USM) is (1) effective and safe to replace invasive groin lymph node dissection (LND) to manage vulvar cancer; and (2) decreases the morbidity and its cost associated with vulvar cancer surgery, without compromising survival. |
| Study Objectives | **Primary objective:**<br>• To determine the incidence of palpable, fixed to skin and histologically positive groin nodes at 12 months in women with vulvar cancer randomised to serial high-resolution groin USM versus standard surgical groin LND<br>**Secondary objectives:**<br>• Compare Patient Reported Outcomes (PROMS) and Health-Related Quality of Life (HRQL) between the groups<br>• Compare pain and adverse events (morbidity) between the groups<br>• Compare lower limb lymphoedema between the groups<br>• Compare cost and cost effectiveness between the groups<br>• Determine the diagnostic accuracy of pre-operative, standardised high-resolution groin ultrasound to identify groin node metastasis.<br>**Translational research objective:**<br>• To assess the role of blood and tissue molecular biomarkers to accurately determine the burden of disease (positive lymph nodes) in vulvar cancer, assist with risk stratification and aid disease surveillance |
| Study Endpoints | **Primary endpoint:**<br>• Incidence of clinically palpable, fixed to skin and histologically positive groin lymph nodes (node metastasis) at 12 months after surgical excision of the primary vulvar tumour |

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| | **Secondary endpoints:**<br>• Health-Related Quality of Life (HRQL) and Patient Reported Outcomes (PROMS) as measured by the EQ-5D and FACT-V at baseline, 8 weeks, 6 months, and 12 months<br>• Morbidity at 12 months after surgery<br>• Lower limb lymphoedema incidence up to 12 months after surgery<br>• Cost and cost effectiveness at 12 months post-surgery<br>• Clinical accuracy of high-resolution serial ultrasound to predict groin lymph node involvement<br>• Disease free and overall survival 12 months post-surgery<br>**Translational research endpoints:**<br>• Utility of biomarkers to reliably reflect the presence or absence of positive groin lymph nodes.<br>• Explore novel biomarkers for vulvar cancer |
| Translational research | All participants (pending consent) will be asked to donate blood samples and Formalin-Fixed Paraffin Embedded (FFPE) tissue (vulvar tissue with/without lymph nodes) to be banked for the purpose of future molecular and biomarker research.<br><br>Tumour with/without groin lymph nodes will be collected at the diagnostic biopsy procedure and/or during surgery. Throughout the study, clinical trial coordinators at each site will arrange for blocks for each episode of biopsy or excision to be extracted (vulvar tissue, lymph nodes) and couriered to a central laboratory in QLD in batches as required and will undergo centralised histopathology review.<br><br>For participants who receive standard upfront groin LND, blood will be collected at two time points: prior to surgery (baseline) and 8 weeks post-surgery. For participants who have serial high-resolution groin USM, blood will be collected prior to surgery (baseline) and at each ultrasound scan (7-time points total). Blood samples will be processed and stored at local labs, and then couriered to a central laboratory in QLD in batches as required. Specimens will be stored for an indefinite period following the last participant completing the trial.<br><br>All translational research using these samples will be subject to a separate ethics application. |
| Study Design | A phase II, open label, domestic and international, three-group, randomised clinical trial. |
| Randomisation | Participants with a normal/negative baseline groin node ultrasound will be randomised 2:1 to receive radical wide local excision or radical vulvectomy with either: |

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| | • bilateral groin USM every 2 months (groin lymph nodes are not removed) in conjunction with clinical examinations every 3 months for a period of 12 months<br>OR<br>• groin LND (based on clinical standards: either a sentinel node biopsy (SNB) or an inguinofemoral lymph node dissection (IFL)),<br><br>Randomisation will be stratified by clinical stage, HPV status, Charlson Comorbidity Index, and histological cell type. |
| Eligibility Criteria (Inclusion and Exclusion) | **Inclusion Criteria:**<br>• Females, over 18 years, with histologically confirmed squamous cell carcinoma (SCC), adenocarcinoma, or melanoma of the vulvar<br>• Clinically stage 1b or 2 on medical imaging (CT or MRI scan of pelvis, abdomen, and chest), without evidence of regional or distant metastatic disease<br>• Willing to undergo IFL/SNB according to local clinical practice management guidelines<br>• Willing and able to comply with all study requirements, timing and/or nature of required assessments<br>• Signed written informed consent<br>• Negative (serum or urine) pregnancy (BHCG) test ≤ 30 days of surgery ONLY in pre-menopausal women and women < 2 years after the onset of menopause<br>**Exclusion Criteria:**<br>• Women with non-invasive vulvar conditions (e.g. non-invasive non-mammary Paget's disease)<br>• Squamous cell carcinoma (SCC) of the vulvar with depth of invasion ≤1 mm<br>• Clinical or medical imaging evidence of regional and/or distant metastatic disease<br>• Serious concomitant systemic disorders incompatible with the study (at the discretion of the investigator)<br>• Other prior malignancies <5 years before inclusion, except for successfully treated keratinocyte skin cancers, or ductal carcinoma in situ<br>• Estimated life expectancy ≤ 6 months |
| Study intervention | Eligible women (n=240) will receive a bilateral groin node ultrasound up to 30 days prior to planned surgery.<br>• If the groin node ultrasound is suspicious/indeterminate, the participant will receive an upfront full groin LND or SNB, which is current standard treatment (according to local clinical practice management guidelines; estimated n=60). |

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| | • If the groin ultrasound is negative/normal (n=180), participants will be randomised 2:1 to receive either a groin LND (n=60) or bilateral groin USM every 2 months along with clinical examination every 3 months for a period of 12 months (n=120; no upfront groin LND).<br><br>All participants will receive appropriate surgical excision of the primary tumour (radical wide local excision or radical vulvectomy). All lymph nodes will undergo central pathology review [1]. Postoperative treatment will follow local institutional treatment guidelines. |
| Stage details and number of participants | 240 participants in total:<br>• Ultrasound negative/normal groin nodes (120 undergoing groin USM every 2 months along with clinical examination every 3 months and 60 upfront groin IFL/SNB)<br>• Ultrasound suspicious/indeterminate groin nodes (60 having upfront groin IFL/SNB) |
| Study Duration | Enrolment for this study is projected to conclude within a 4-year timeframe. The study will be deemed complete once 180 participants have been enrolled, randomised, and successfully completed a 12-month follow-up period. |

## 1.2 Abbreviations

| Abbreviation | Definition of Term |
|---|---|
| **AE** | Adverse event |
| **ANZGOG** | Australia New Zealand Gynaecological Oncology Group |
| **ASAR** | Australian Sonographers Accreditation Registry |
| **BIS** | Bioimpedance Spectroscopy |
| **BMI** | Body Mass Index |
| **CDMU** | Central Data Management Unit |
| **CI** | Chief Investigator |
| **CRF** | Case Report Form |
| **CT** | Computed Tomography |
| **CTCAE** | Common Terminology Criteria for Adverse Events |
| **ECOG** | Eastern Cooperative Oncology Group |
| **eCRF** | Electronic Case Report Form |
| **EDTA** | Ethylenediamine Tetraacetic Acid |
| **ESMO** | European Society for Medical Oncology |
| **EV** | Extracellular Vesicles |
| **FACT** | Functional Assessment of Cancer Therapy |
| **FBC** | Full Blood Count |
| **FoR** | Fear of Recurrence |
| **HREC** | Human Research Ethics Committee |
| **HRQL** | Health Related Quality of Life |
| **ICH-GCP** | International Council for Harmonisation - Good Clinical Practice |
| **IDSMC** | Independent Data Safety Monitoring Committee |
| **IFL** | Inguino-Femoral Lymphadenectomy |
| **IGCS** | International Gynaecological Cancer Society |
| **LFT** | Liver Function Tests |
| **LND** | Lymph Node Dissection |
| **MRI** | Magnetic Resonance Imaging |
| **NPV** | Negative Predictive Value |
| **PCR** | Polymerase Chain Reaction |
| **PET** | Positron Emission Tomography |
| **PI** | Principal Investigator |
| **PROMS** | Patient Reported Outcomes |
| **PM** | Project Manager |
| **PPV** | Positive Predictive Values |
| **QCGC** | Queensland Centre for Gynaecological Cancer |
| **QALY** | Quality Adjusted Life Year |
| **QLD** | Queensland |
| **QoL** | Quality of Life |
| **SAE** | Serious Adverse Event |
| **SCC** | Squamous Cell Carcinoma |
| **SNB** | Sentinel Node Biopsy |
| **SGO** | Society of Gynaecologic Oncology |
| **TMC** | Trial Management Committee |
| **UQ** | University of Queensland |
| **US** | Ultrasound |
| **USM** | Ultrasound Monitoring |
| **UQRDM** | The University of Queensland Research Data Manager |

## 1.3 Trial Schema

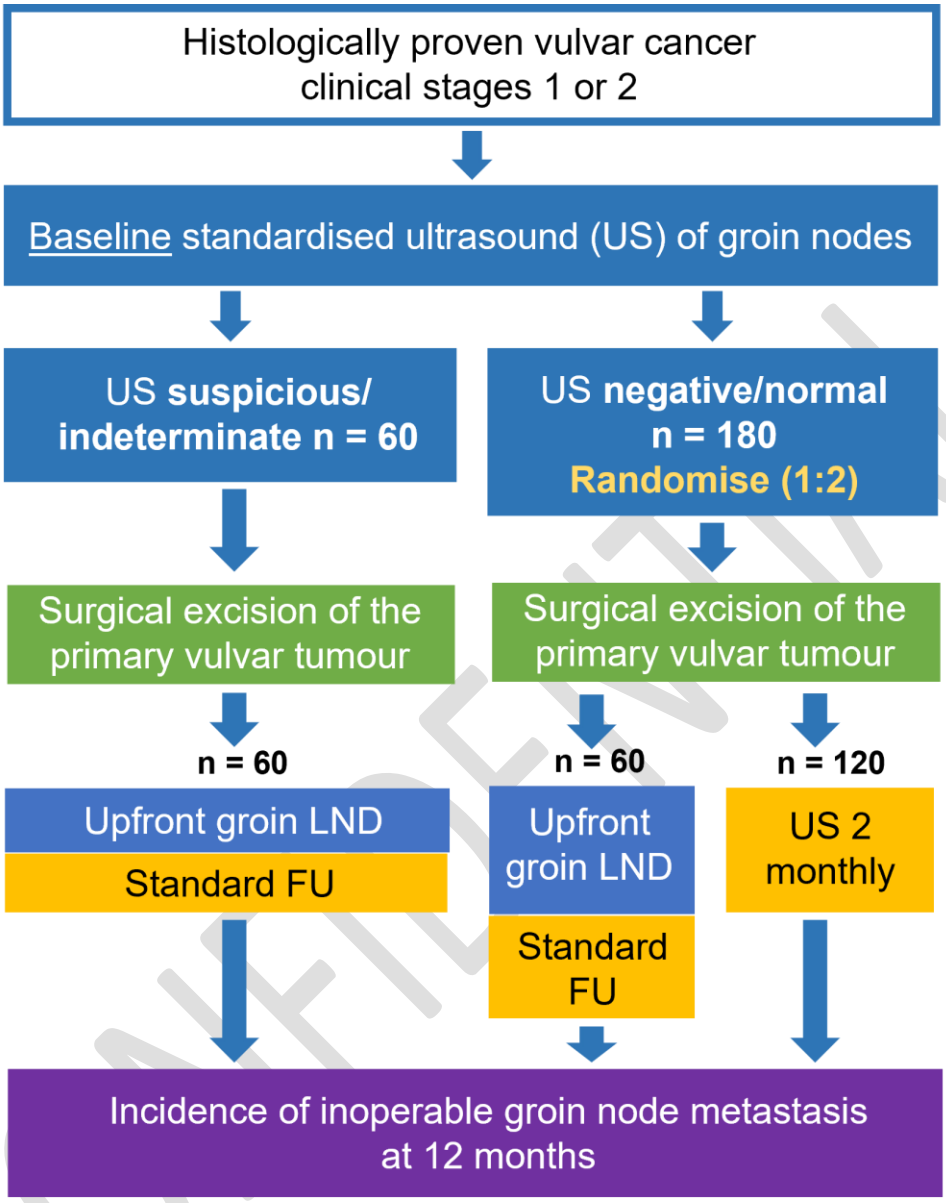


Figure 1. Trial Schema

# 2. Justification of the ANVU Trial

Treatment of vulvar cancer causes significant morbidity. Despite being a rare cancer at least 2500 survivors of vulvar cancer live in Australia [2]. Most survivors must live with the detrimental, life-long impacts resulting from their cancer treatment because there are currently no alternatives to mitigate these impacts. The personal and societal burden this entails is significant. To control such cancer-associated burden is a national priority [3].

Clinical palpation of the groin region and medical imaging scans do not reliably detect groin node involvement [4, 5]. Hence, current clinical guidelines recommend all women diagnosed at apparent Stage 1 or 2 have a surgical groin LND [6]. This can be performed as a full IFL to remove all groin nodes,

or as SNB to remove selected (one or two) nodes [7-9]. SNB is less invasive, however, is appropriate only for vulvar cancers <4cm in diameter and unifocal tumours (~50% of all Stage 1 and 2 vulvar cancers) [4, 8, 9]. SNB is also associated with a false negative rate (10%) that increases the risk of undetected nodes [4, 7]. While groin LND is often curative, most survivors will report debilitating and long-term side effects including persistently painful and disfiguring limb swelling, impaired mobility and increased susceptibility to infections [10-13] [7, 14-16]. Strategies to prevent these side effects without compromising survival are lacking and are the focus of this clinical trial.

In 20% of cases (~80 Australian women per year), groin LND will reveal positive nodes (i.e., metastases) [8, 9], which triggers a referral for radiation treatment. If positive groin nodes are missed, and over time become enlarged, clinically palpable and attached to the overlying skin, >90% of women will die within 12 months despite subsequent treatment [4, 5]. Conversely, if groin node involvement is detected early (e.g., by ultrasound) while still small, survival outcomes are excellent [10]. Ultrasound technology is potentially as accurate as LND due to recent advances in resolution and technologies such as 2D/3D volumetric assessments and tissue flow. Furthermore, ultrasound is superior to magnetic resonance imaging (MRI), and to standard computed tomography (CT) and positron emission tomography (PET) scans in capturing groin node involvement because it has a higher resolution, avoids harmful radiation, and the technology is readily accessible outside of high-volume metropolitan areas [11].

We propose to reduce surgical morbidity by replacing upfront groin LND for vulvar cancer patients with serial high-resolution ultrasound to detect groin metastases. Groin LND will only be required for the few women with ultrasound-detected metastasis when they are still small. The ANVU trial will determine the value of serial groin ultrasound examinations in stage 1 and 2 vulvar cancer patients and whether it is feasible and safe to de-escalate the extent of vulvar cancer surgery to achieve improved outcomes for affected patients.

# 3. Introduction

## 3.1 Background

Vulvar cancer is rare disease that, by its intimate nature, is also largely hidden from public discourse. With an incidence of 2.5/100,000, approximately 400 Australian women are diagnosed every year [2, 4, 5]. Standard treatment of invasive vulvar cancer comprises of surgical removal of the primary tumour plus surgical groin LND [4, 5, 12]. Groin LND can be delivered through a full Inguino Femoral Lymph Node Dissection (IFL) (removing as many nodes as possible) or through a Sentinel Node Biopsy (SNB) (removing only one or two targeted groin lymph nodes). While the gold standard is often curative, groin LND is associated with debilitating short and long-term morbidity for virtually all women requiring this procedure (see 3.3 below) [13-19].

Adverse outcomes range from painful and disfiguring limb swelling, compromised function, impaired mobility, and greater susceptibility to infection. Sparing women a groin LND could greatly reduce the incidence and burden of these problems, improve recovery time, and facilitate their return to normal daily activities [2, 7]. It is not possible at present to offer treatment that lessens morbidity without exposing affected patients to the risks of impaired survival.

Australian women with this rare cancer bear an unacceptably high (and potentially avoidable) treatment-related burden. This clinical trial (ANVU) is the first step to address this problem in a novel yet pragmatic way. The overall outcome of ANVU is a novel, less invasive alternative to vulvar cancer LND associated with significantly less morbidity, without compromising survival.

## 3.2 Importance of Groin Nodes

Vulvar cancer develops from vulvar skin. It can spread locally to adjacent structures (vagina, urethra, anus) or via lymphatic channels to regional lymph nodes in the groin [2, 5]. If groin nodes are involved, the cancer is upgraded to Stage 3. This indicates a worse prognosis compared to tumours confined to local involvement [4]. Patients with groin node metastasis require further treatment. Unfortunately pre-treatment detection via groin clinical examination (palpation) and medical imaging scans do not reliably detect microscopic groin node involvement [2, 5]. Hence current clinical guidelines mandate that all patients (except those with Stage 1A disease) require surgical groin LND to mitigate the risk of missing groin node metastases [12]. In 30% of cases (~120 Australian women per year) a groin LND reveals positive (involved) nodes [8, 9], and a concomitant need for postoperative radiation treatment. Should positive groin nodes be missed and become enlarged and clinically palpable, more than 90% of patients will die within 12 months despite subsequent treatment [2, 5]. Conversely, if groin node involvement is detected early while still small, survival outcomes are expected to be excellent. For those reasons, the detection of clinically palpable, enlarged groin nodes is an appropriate marker for oncological safety in vulvar cancer (primary outcome of this clinical trial).

## 3.3 Outcomes of Groin Lymph Node Dissection

While effective, groin LND inevitably leads to substantial short- and long-term morbidity [2, 5]. Groin LND can be performed as an IFL; removing all groin nodes or SNB to remove selected (one or two) nodes [7-9]. For SNB and IFL, respectively, adverse outcomes include rates of wound breakdown in 11.7% vs 34%; cellulitis 4.5% vs 21.3%; and life-long swelling of the legs (lymphoedema) in 60% of patients [7-9]. The incidence of surgery-related morbidity is higher in patients who undergo IFL [7]. Less invasive SNB, however, is appropriate in only 50% of cases. SNB is also associated with a false negative rate (up to 10%) that increases the risk of undetected nodes (1% vs. 5%) [4, 7]. For the ~70% to 80% of patients who have negative groin nodes (i.e., no cancer within the nodes), groin LND has no clinical benefit and patients still suffer from the potential adverse outcomes of undergoing this procedure.

## 3.4 Preliminary Data

Our comprehensive literature review focussed on the five critical areas of the proposed ANVU study:

**Surgical morbidity:** Our comprehensive review of 35 studies found >50% of women developed one or more complications from groin LND [18]. A prospective, longitudinal gynaecological cancer cohort study (n=546, funded by Cancer Australia APP519711) [15] undertaken by team members supported this, finding that 91% of women with vulvar cancer either self-reported lower limb swelling (45%) without a formal diagnosis; 25% received a formal clinical diagnosis of lymphoedema; and a further 41% reported both. Other common adverse outcomes included long length of hospital stay (8-13 days), wound breakdown (12%-34%), and cellulitis (5%-21%) [7, 9, 13].

**Quality of life (QoL):** Andersen and Hacker's (1983) earliest work flagged that women who had experienced vulvar surgery registered emotional distress in the 88th percentile [20] as a result of these morbidities. We subsequently assessed the more detailed QoL impact of vulvar cancer in a mixed-method program that developed a reliable and valid vulvar-cancer specific QoL subscale to accompany the Functional Assessment of Cancer Therapy (FACT) [15-17, 19, 21]. In this study, women reported substantial reductions in emotional, physical and social functioning, activities of daily living, as well as sexual wellbeing and perceptions of body image associated with standard vulvar cancer treatment [14]. Return to work was often impaired [17]. In a complementary qualitative study of QoL after treatment, we found that women with lymphoedema or iatrogenic leg pain reported significantly worse (5-30% worse) QoL in the social, physical and sexual domains than women who did not report these problems [22]. This study identified four main themes: fear, body image concerns, altered sexuality and lymphoedema, with the most detrimental impact reported by women who experienced radical vulvar excision, multiple vulvar procedures and/or who developed lymphoedema [14].

**Ultrasound technology** is likely superior to standard CT,PET, and MRI for capturing groin node involvement because it is cheaper, has higher resolution and avoids harmful radiation [11]. Nodal assessment via ultrasound is already routine in head and neck, breast, and other cancers, where it consistently yields high sensitivity rates (>90%) [12, 23, 24]. Our comprehensive review of the accuracy of ultrasound in the preoperative assessment of groin nodes in vulvar cancer uncovered eight original studies [23, 25-31].While the data in these studies are promising, only two studies [25, 27] were prospective and their small samples (n=20 and 44) and age (published 2000 and 2003) preclude generalisation and potential translation into clinical practice.

**Women with vulvar cancer fear the outcomes of LND** [33]. In previous work, we interviewed 10 women who recently completed vulvar cancer treatment, capturing their views on their experience. Four concerns predominated: (1) Women genuinely fear LND, expressing their "terror" of its consequences; (2) Participants described life after LND as traumatic: "I thought I'd be back to my normal life within a very short time. And I'm still struggling past the 12-month mark"; (3) Many women were confident in the advice received from their surgeon; however, they wanted more treatment options: "I'm grateful that the surgeon was willing to entertain an alternative or a slightly different course from the usual ... I'm pleased I put my foot down to the [LND] surgery"; and (4) While they feared recurrence, women's treatment decision-making was not necessarily driven by thoughts of mortality. Equally cogent was their likelihood of avoiding lymphoedema, and thereby their capacity to maintain a good quality of life through an active lifestyle, a functional career, and being there for significant others. "I said no to the [LND] surgery. I'm not going to have that happen for the fact that I'm only 41. I've got a three-year-old, I'm not going to take the risk to end up with lymphoedema."

**Ultrasound technology is potentially as accurate as LND** due to recent advances in resolution and technologies such as 2D/3D volumetric assessments and tissue flow. Furthermore, ultrasound is superior to MRI, CT, and PET scans in capturing groin node involvement because it has a higher resolution, avoids harmful radiation and the technology is readily accessible outside of high-volume metropolitan areas.[18]

**Our pilot data confirm that groin ultrasound is a clinically feasible alternative to LND** [17]. CIs Obermair and Hacker undertook a pilot study to determine whether 3-monthly groin USM could be performed in lieu of upfront groin LND after a negative bilateral groin ultrasound in 32 women with apparent Stage 1 or 2 vulvar cancer. Three positive nodes (9.4%) were detected during a median

follow-up of 37 months (none lost to follow-up) and 29 patients could be spared a groin LND. All ultrasound-detected positive nodes developed within 12 months. One participant died of her groin recurrence (3.1%). The sensitivity of serial groin USM was 100% (95% CI: 44-100%), with specificity of 97% (95% CI: 83-99%) and negative predictive value of 100% (95% CI: 88-100%). These compelling pilot data justify a larger study on serial groin ultrasound in lieu of upfront groin LND in selected patients with vulvar cancer. In this study sensitivity of the monitoring via 2-monthly ultrasonography was calculated as the proportion of true-positive ultrasonic scans (confirmed histologically) of the total number of true-positive and false-negative scans. Similarly, the specificity was calculated as the proportion of true-negative scans of the total number of true-negative and false-positive scans. A false-positive scan was determined after fine needle cytology was negative; two nodes were surgically removed and found to be negative histologically.

**Standardisation of groin ultrasound is achievable**. Encouraged by our pilot study, CIA Obermair recently completed a prospective feasibility study of synoptic reporting of groin ultrasound findings in women with vulvar cancer (pre-ANVU study, ACTRN12622000886785; publication in preparation). It applied 10 rigorous criteria for groin node ultrasound. Eligible women underwent groin ultrasound prior to planned groin LND. All 20 women had all 10 criteria reported. This successful feasibility and quality assurance study warrants using those 10 criteria in our ANVU trial. Furthermore, the pre-ANVU study attracted the award of the Australian Society of Gynaecological Oncologists Tony McCartney Surgical Innovation prize in 2023, citing the work represents the “perfect synthesis of reducing significant morbidity associated with vulvar cancer surgery … that enables de-escalation of vulvar cancer surgery [that is] safe for patients”.

# 4. Expected Outcomes and Health Impact

This randomised clinical trial will evaluate bilateral groin ultrasound to predict groin lymph node involvement in vulvar cancer patients. The knowledge embodied in the project outcome is a first step towards facilitating a change in current clinical practice towards equally safe but less invasive management of vulvar cancer with superior outcomes for women.

Consistent with the objectives of our study, outcomes include:

1. Information on post-intervention quality of life and function that is crucial for women’s and clinicians’ decision-making.
2. Affirmation of the safety of serial high-resolution groin USM of patients randomised to serial groin USM versus groin LND.
3. Affirmation of the diagnostic accuracy of high-resolution ultrasound to predict groin lymph node involvement in vulvar cancer patients.
4. Optimising patient outcomes and minimise the incidence of morbidity and costs associated with groin LND, by providing a thorough evaluation of how current surgical practice can be improved.
5. Early data on the utility of novel blood and tissue biomarkers for vulvar cancer, similar to those available for ovarian, bowel and other cancers.

The critical knowledge embodied in project outcomes is the first step towards translation into clinical practice. The findings will enable the stakeholders to weigh the benefits more accurately versus risks

of each procedure in terms of prognostic sensitivity, safety, surgical recovery, pain, length of hospital stay, quality of life and function.

If groin USM is demonstrated to be feasible and safe, the ANVU trial will also have an impact on improving the quality of care for Australian and international vulval cancer patients by providing high quality evidence to support shared decision-making which is a central component of patient-centred care. Shared decision-making involves describing alternative options for care and providing patients with the best available evidence about the benefits, harms, and uncertainty for each option and supporting patients to consider their values and preferences to achieve an informed decision.

The ANVU trial will also provide Australian and international vulvar cancer patients with the opportunity to benefit from innovative medical research—this is the only clinical intervention trial to focus on the minimisation of iatrogenic harm in this under-researched group.

**Post-trial options:** If the trial demonstrates feasibility and safety of groin USM, there are several options of continuing towards clinical practice management change:

- Continue enrolment into the ANVU trial as a phase 3 randomised clinical trial to reach 790 patients with a 4-year survival primary endpoint.
- Continue enrolment into the ANVU trial with an unspecified number of patients and observe survival outcomes.
- Monitor survival outcomes of the 240 enrolled patients for 4 years.
- Perform a phase 4 non-randomised, observational study on serial groin ultrasound.
- Develop a subsequent phase 2/3 randomised controlled trial with molecular biomarkers for stratification.
- Co-develop a decision-making tool with consumer representatives and other stakeholders to provide patients with information on the probabilities of important outcomes associated with groin USM and LND, and related uncertainty, based on trial primary and key secondary endpoints, and uncertainty, in a format to elicit patients' values and preferences to support shared decision-making.

# 5. Trial Design

## 5.1 Primary Research Question

Is incidence of groin metastases (defined as enlarged, clinically palpable, fixed to skin and histologically positive nodes) comparable between women randomised to serial groin USM or upfront groin LND?

## 5.2 Aim

To determine whether groin USM (1) is effective and safe to replace invasive groin LND to manage vulvar cancer; (2) decreases the morbidity associated with vulvar cancer surgery; and (3) is cost effective.

## 5.3 Primary Objective

Determine the incidence of palpable, fixed to skin and histologically positive groin nodes in women with vulvar cancer randomised to serial high-resolution groin USM compared to standard upfront surgical groin LND at 12 months.

## 5.4 Secondary Objective

1. Compare Patient Reported Outcomes (PROMs) and Health Related Quality of Life (HRQL) between the groups
2. Compare pain and adverse events (AEs) (morbidity) between the groups
3. Compare incidence of lower limb lymphoedema at 12 months post-surgery between the groups
4. Compare cost and cost effectiveness between all groups
5. To assess the role of molecular biomarkers to accurately determine the burden of disease (positive lymph nodes) in vulvar cancer, assist with risk stratification and aid disease surveillance
6. Determine the diagnostic accuracy of pre-operative, standardised high-resolution groin ultrasound to identify groin node metastasis.

## 5.5 Primary Endpoint

Incidence of enlarged clinically palpable groin nodes (groin metastasis) in women randomised to serial high-resolution groin USM compared to surgical groin LND 12 months after surgery.

## 5.6 Secondary Endpoint

1. HRQL and PROMS as measured by the EQ-5D and FACT-V at baseline, 8 weeks, 6 months, and 12 months
2. Morbidity at 12 months after surgery
3. Lower limb lymphoedema: incidence up to 12 months after surgery
4. Cost and cost effectiveness at 12 months post-surgery
5. Clinical accuracy of high-resolution ultrasound to predict groin lymph node involvement
6. Disease-free survival (DFS) and overall survival (OS)
7. Utility of biomarkers to reliably reflect the presence or absence of positive groin lymph nodes
8. Explore novel biomarkers for vulvar cancer

# 6. Study Design

A phase II, open label, multicentre, three-group, randomised clinical trial (ANVU).

## 6.1 Study Population

The following study population will be identified through gynaecological oncology clinics in Australian (QLD, NT, NSW, VIC, TAS, WA, and SA), and international sites.

## 6.2 Inclusion Criteria

- Females, over 18 years, with histologically confirmed SCC, adenocarcinoma, or melanoma of the vulvar
- Clinically stage 1 or 2 on medical imaging (CT or MRI scan of pelvis, abdomen, and chest), without evidence of regional or distant metastatic disease
- Undergo IFL/SNB according to local clinical practice management guidelines
- Willing and able to comply with all study requirements, timing and/or nature of required assessments.
- Signed written informed consent
- Negative (serum or urine) pregnancy (BHCG) test ≤ 30 days of surgery ONLY in pre-menopausal women and women < 2 years after the onset of menopause.

## 6.3 Exclusion Criteria

- Women with non-invasive vulvar conditions (e.g. non-invasive non-mammary Paget's disease)
- SCC of the vulvar with depth of invasion ≤1 mm
- Clinical or medical imaging evidence of regional and/or distant metastatic disease
- Serious concomitant systemic disorders incompatible with the study (at the discretion of the investigator)
- Other prior malignancies <5 years before inclusion, except for successfully treated keratinocyte skin cancers, or ductal carcinoma in situ
- Estimated life expectancy of ≤6 months

## 6.4 Randomisation

Eligible participants will be required to provide signed informed consent before being enrolled. Prior to enrolment, each patient will be screened for eligibility according to the inclusion and exclusion criteria. Participants will then receive a bilateral groin node ultrasound, participants who are found to have negative groin nodes will be randomised using a secure online randomisation system 2:1 to groin USM vs standard groin LND:

- ~120 participants will receive radical wide local excision or radical vulvectomy with serial high-resolution bilateral groin USM every 2 months in conjunction with clinical examinations every 3 months (groin lymph nodes are NOT removed).
- ~60 participants will receive radical wide local excision or radical vulvectomy with groin LND (Based on local clinical practice management guidelines.: either a SNB or an IFL).

Randomisation will be stratified by:

- Clinical stage
  - Depth of invasion (1-2 mm)
  - Depth of invasion (2.1-3 mm)
  - Depth of invasion 3.1 + mm
- HPV status
  - HPV positive
  - HPV negative
- Charlson Comorbidity Index (<5, ≥5)

- Histological cell type
  - SCC
  - All other cell types (i.e. adenocarcinoma, melanoma).

Participants with a groin node ultrasound that is suspicious/indeterminate will not be randomised but receive standard groin LND (SNB or IFL as appropriate, as per institutional guidelines).

## 6.5 Intervention

### 6.5.1 Protocol of Inguino-Femoral Lymphadenectomy/Sentinel Node Biopsy

All participants will receive appropriate surgical excision of the primary vulvar tumour (radical wide local excision or radical vulvectomy).

For participants who receive standard groin LND, patients can have either a full IFL or a SNB (tracer agent not specified) as per institutional guidelines, unilaterally or bilaterally as appropriate. Patients for SNB need to have tumours less than 4 cm in largest diameter and must be unifocal. Sentinel nodes will undergo ultra-staging [1].

### 6.5.2 Postoperative Adjuvant Treatment:

Patients who have had a full IFL and have one positive node without extracapsular spread require no adjuvant radiation [32]. Patients with 2 or more positive nodes or with any extracapsular spread should have bilateral groin and pelvic radiation [33]. Patients with a positive sentinel node 2 mm or less in diameter may be treated with bilateral groin and pelvic radiation. Patients with a positive sentinel node greater than 2 mm diameter should have a full IFL and adjuvant radiation if they have any further positive nodes identified [34].

### 6.5.3 Protocol for Groin Ultrasound Examinations:

All groin ultrasound examinations will be performed by registered sonographers with a postgraduate degree in ultrasound recognised by the Australian Sonographers Accreditation Registry. Minimum requirements for ultrasonic machines include a) Linear transducer/s, 7 and 9 MHz frequencies; b) Curved 5 MHz transducer; c) Colour and Power Doppler; d) Standard linear and volumetric measurements; and e) Looped video.

Ten ultrasound criteria will be captured for each identified groin node on a trial specific case report form: (1) shape (globular vs non globular); (2) homogeneity of echo structure; (3) hyper- or hypoechoic areas within the lymph nodes; (4) hilum abnormalities; (5) cortical thickening; (6) nodal grouping; and for the most suspicious or largest lymph node (7) long (L) and short (S) axis (L/S ratio, values of <2 are considered abnormal); (8) perinodal hyperechoic ring (inflammation); (9) cortical interruption (possibility of extra-nodal spread); and (10) nodal vascularity.

An overall assessment will be made by the radiologist into normal or suspicious/positive. Radiologists will send their report to the local PI within 5 working days of ultrasound completion. All sites are required to enter the completed ultrasound eCRF on REDCap within 5 working days of ultrasound completion. Additionally, they must upload a copy of a signed and de-identified ultrasound report to REDCap, where QCGC Research will verify ultrasound completion.

If the participant is randomised to serial groin USM, patients will have surgical excision of the primary vulvar tumour followed by bilateral groin ultrasound every two months and clinical examinations every three months for the 12 months.

If suspicious, indeterminate, or positive nodes or any other concerning indicators are detected, the participant will be referred to either the local PI or the specialist gynaecological oncologist to discuss their options. Depending on the clinical judgment of the clinician, the participant may be recommended for LND (standard treatment) or may opt to continue with bilateral groin ultrasound every two months and clinical examinations every three months (remaining on trial). Participants undergoing LND will no longer need bilateral groin ultrasound and blood collections every two months and clinical examinations every three months. However, their data will still be collected up to the original 12-month point. Data collected will include documentation of the status of disease, AEs, pain level, health status and quality of life and lymphoedema. Please refer to section 10.6.5.1, titled 'Evaluating and Reporting Positive Nodes', for further information.

Patients that fail to comply with the serial ultrasound and clinical examination schedule will be removed from the trial and referred back to their gynaecological oncologist for alternative treatment for safety reasons.

# 7. Project Methodology

## 7.1 Study Procedures and Assessments

Prospective participants will be screened at the gynaecological oncology clinic based on the above inclusion and exclusion criteria. Prospective participants are identified based on this criterion by Investigators and/or research staff. Once a prospective participant has been identified as being potentially eligible, a member of the clinical care team will approach the participant, enquire if they consent to being contacted by a research team member regarding the study, and provide them with an information pack containing the Participant Information and Consent Form (PICF). Subsequently, the prospective participant will either be approached in person by a research team member or contacted later via phone or email to discuss the study. Each prospective participant will be given a full explanation of the study and will be given adequate time to thoroughly read the PICF and discuss with family and friends (if applicable) prior to agreeing to participate. Time for questions throughout the discussion will be given and all questions will be adequately addressed before proceeding. The participant will not be coerced to participate in the study. Participants may choose to consent on the same day as being given the PICF, provided the member of staff taking consent is satisfied that the participant understands the study. Alternatively, prospective participants will be provided with the option to take the PICF home for further consideration and return the signed consent form via express post (envelopes to be provided by the site).

Prospective patients will be informed that they are free to refuse participation in this study and, if they choose to participate, that they may withdraw from the study at any time without compromising their medical care. Participants who agree to the study will be provided with a copy of the information sheet and their signed consent form. Participant data will be collected over the period of the study. This may be obtained from the participants themselves, other health institutions, medical practices,

laboratory results etc. No third party will be provided with access to any data on participants. Consent to obtain this information will have been provided by participants when they sign the consent form. No data is collected/retained on participants prior to this.

## 7.2 Pre-Operative (Visit 1)

### 7.2.1 Screening/Baseline

The following procedures will be performed within 75 days prior to surgery:

- Screen eligibility of patients (see section 6.1 Study Population)
- Histologically confirmed SCC, adenocarcinoma, or melanoma of the vulvar without evidence of regional or distant metastatic disease (clinical Stage 1b or 2)
- CT or MRI of Chest, Abdomen and Pelvis
- Sign HREC approved informed consent form

The following procedures will be performed within 30 days prior to surgery:

- Physical examination
- Negative (serum or urine) pregnancy (BHCG) test ONLY in pre-menopausal women and women < 2 years after the onset of menopause
- Standard preoperative laboratory workup as determined by each institution (e.g. full blood count (FBC), liver function tests (LFT's))
- Record all current medications
- Record surgical, medical, and gynaecological history
- Review Eastern Cooperative Oncology Group (ECOG) performance score
- Assess participants for their individual pain level using Numeric Pain Rating Scale
- Height and Weight to calculate Body Mass Index (BMI) = kg/m2
- Lymphoedema assessments via bioimpedance spectroscopy (BIS), leg circumference measurements (participating sites only) and/or Self-Report Questionnaire
- Provide participant with Baseline Questionnaire booklet (demographics, PROMs, HRQL, lymphoedema).

## 7.3 Ultrasound (Visit 2)

- Participants will undergo a groin ultrasound examination up to 30 days prior to planned surgery.
- Following review of the ultrasound images, patients with negative/normal groin ultrasound will be randomised.
- Notify the participants' primary care physicians if they are assigned to the Interventional Group (with bi-monthly groin USM in conjunction with clinical examination every three months).
- Optional blood sample for translational research (baseline) for all consented participants

## 7.4 Surgery (Visit 3)

- Perform surgical excision of the primary vulvar tumour (radical wide local excision or radical vulvectomy)
- If participant is receiving groin LND, perform IFL or SNB according to local clinical practice management guidelines
- Record all intraoperative information (including operation details, length of surgery, intraoperative complications, transfusion details and any other special conditions around the operation)
- Record histopathology findings

## 7.5 Follow-Up (Visits 4-9)

All enrolled participants, irrespective of treatment group will be followed-up for 12 months. Participants without groin LND will be provided with medical imaging request forms to receive groin ultrasound examinations every 2 months. **Changes in ultrasound patterns from "normal" to "suspicious" will alert the gynae-oncologist and trigger further clinical action, such as groin LND and histological assessment of the groin nodes**. **Groin USM will not be offered to women who had a groin LND (IFL or SNB)** as it is **not standard practice for these patients**. If ultrasound scan surveillance following sentinel node biopsy is included in the standard care protocol at any of the study sites, participants randomised to lymph node dissection (LND) can undergo ultrasound scans as part of their standard care, though it is not mandatory for this study.

Participants will be assessed for local, regional, or distant recurrence, quality of life status, and treatment morbidity for 12 months. Data collected will include documentation of the status of disease, AEs, pain level, health status, quality of life and lymphoedema.

Participants randomised to serial groin USM will be seen every 3 months by a gynaecological oncologist or gynaecologist for clinical examination as per routine standard. Participants receiving standard groin LND (either SNB or LND) will be seen at 8 weeks, 3, 6, 9 and 12 months as per standard clinical practice. Follow up will be handed over to the treating gynaecological oncologist after 12 months.

### 7.5.1 Postoperative Week 8 ± 3 days (Visit 4) - All Participants

Can be obtained via medical visit, telephone contact or postal correspondence:

- Provide participant with booklet containing PROMS, HRQL and lymphoedema questionnaires
- Lymphoedema assessments via BIS and leg circumference measurements (participating sites only) and/or Self-Report Questionnaire
- Review ECOG performance score
- Assess participants for their individual pain level using Numeric Pain Rating Scale
- Record disease status
- Collect all AEs (assess using Common Terminology Criteria for Adverse Events (CTCAE) V5.0)
- Record hospitalisations
- Record FBC, Urea and Electrolytes and LFT's if available
- Collect all serious adverse events (SAEs)
- Update new medications since surgery

- Optional post-operative translational research blood collection for all consented participants
- Serial high-resolution groin ultrasound (interventional treatment)
- Evaluate symptoms and conduct a physical examination in alignment with European Society for Medical Oncology (ESMO) guidelines.

### 7.5.2. Postoperative Month 3 ± 7 days (Visit 5) - Interventional Arm

Evaluate symptoms and conduct a physical examination in alignment with ESMO guidelines (interventional treatment):

To mitigate the likelihood of participants experiencing unfavourable outcomes, we have implemented precautionary measures. Participants in the interventional arm are required to have clinical examinations every three months following surgery to remove the primary vulvar tumour. This requirement persists for a duration of 12 months. Ideally, participants will be scheduled to undergo their physical examination on-site. However, for those who reside far away and cannot return every three months, they can alternatively arrange an appointment with their local gynaecologist. A signed and de-identified visit report must be uploaded to REDCap within 5 working days, where QCGC Research will verify the completion of the clinical examination.

If any concerning indicators are detected, the participant will be referred to either the local PI or the specialist gynaecological oncologist to discuss their options. Depending on the clinical judgment of the clinician, the participant may be recommended for LND (standard treatment) or may opt to continue with bilateral groin ultrasound every two months and clinical examinations every three months (remaining on trial). Participants undergoing LND will no longer need bilateral groin ultrasound and blood collections every two months and clinical examinations every three months. However, their data will still be collected up to the original 12-month point. Data collected will include documentation of the status of disease, AEs, pain level, health status, quality of life, and lymphoedema.

### 7.5.3 Postoperative Month 4 ± 7 days (Visit 6) - All Participants

Can be obtained via medical visit, telephone contact or postal correspondence:

- Review ECOG performance score
- Assess participants for their individual pain level Numeric Pain Rating Scale
- Record disease status
- Collect all AEs (assess using CTCAE V5.0)
- Record hospitalisations
- Collect all SAEs
- Update new medications since their last visit
- Blood collection for interventional treatment participants (translational studies)
- Serial high-resolution groin ultrasound (interventional treatment)

### 7.5.4 Postoperative Month 6 ± 7 days (Visit 7) - All Participants

Can be obtained via medical visit, telephone contact or postal correspondence:

- Review ECOG performance score
- Provide participant with Questionnaire booklet to collect PROMS, HRQL and lymphoedema

- Lymphoedema assessments via BIS, leg circumference measurements (participating sites only) and/or Self-Report Questionnaire
- Assess participants for their individual pain level Numeric Pain Rating Scale
- Record disease status
- Collect all AEs (assess using CTCAE V5.0)
- Record hospitalisations
- Collect all SAEs
- Update new medications since their last visit
- Blood collection for interventional treatment participants (translational studies)
- Weight if available
- Record FBC, Urea and Electrolytes and LFT's if available
- Evaluate symptoms and conduct a physical examination in alignment with ESMO guidelines (interventional treatment)
- Serial high-resolution groin ultrasound (interventional treatment)

### 7.5.5 Postoperative Month 8 ± 7 days (Visit 8) - All Participants

Can be obtained via **medical visit**, **telephone contact** or **postal correspondence**:

- Review ECOG performance score
- Assess participants for their individual pain level Numeric Pain Rating Scale
- Record disease status
- Collect all AEs (assess using CTCAE V5.0)
- Record hospitalisations
- Update new medications since their last visit
- Blood collection for interventional treatment participants (translational studies)
- Serial high-resolution groin ultrasound (interventional treatment)

### 7.5.6. Postoperative Month 9 ± 7 days (Visit 9) - Interventional Arm

Evaluate symptoms and conduct a physical examination in alignment with ESMO guidelines (interventional treatment).

### 7.5.7 Postoperative Month 10 ± 7 days (Visit 10) - All Participants

Can be obtained via medical visit, telephone contact or postal correspondence:

- Review ECOG performance score
- Assess participants for their individual pain level Numeric Pain Rating Scale
- Record disease status
- Collect all AEs (assess using CTCAE V5.0)
- Record hospitalisations
- Update new medications since their last visit
- Blood collection for interventional treatment participants (translational studies)
  Serial high-resolution groin ultrasound (interventional treatment)

### 7.5.8 Postoperative Month 12 ± 7 days (Visit 11) - All Participants

Can be obtained via medical visit, telephone contact or postal correspondence:

- Provide participant with Questionnaire booklet to collect PROMS, HRQL and lymphoedema

- Lymphoedema assessments via BIS, leg circumference measurements (participating sites only) and/or Self-Report Questionnaire
- Review ECOG performance score
- Collect Fear of Recurrence (FoR)
- Weight if available
- Assess participants for their individual pain level Numeric Pain Rating Scale
- Record FBC, Urea and Electrolytes and LFT's if available
- Evaluate symptoms and conduct a physical examination in alignment with ESMO guidelines (interventional treatment)
- Record disease status
- Collect all AEs (assess using CTCAE V5.0)
- Record hospitalisations
- Update new medications since their last visit
- Blood collection for interventional treatment participants (translational studies)
- Serial high-resolution groin ultrasound (interventional treatment)

# 8. Patient Reported Outcomes (PROMS) and Health Related Quality of Life (HRQL)

PROMs are questionnaires that all participants complete at baseline, 8 weeks, 6 months, and 12 months post-surgery. In Australia, PROMs are an emerging method of assessing the quality of health care. They ask for the patient's assessment of how health services and interventions have, over time, affected their quality of life, daily functioning, symptom severity, and other dimensions of health which only patients can know. PROMs promise to fill a vital gap in our knowledge about outcomes and about whether healthcare interventions can a difference to people's lives [35, 36].

## 8.1 EuroQoL-5D (EQ-5D)

The EQ-5D is one of the most frequently used generic, preference-based instruments for measuring the health utilities of patients in economic evaluations [37]. EQ-5D is a standardised instrument for use as a measure of health outcomes. It provides a descriptive profile and a single index value for health status. EQ-5D will measure changes in health status and quality of life to calculate the quality-adjusted life years (QALYs) gained with the intervention. The EQ-5D will be administered at baseline, 8 weeks, 6 months, and 12 months. This valid HRQL tool is also a utility measure in cost-effectiveness studies. For our short-term primary outcome, we will specifically focus on return to usual daily activities specified in the EQ-5D-5L with 5 levels (unable to do, severe-, moderate-, slight-, or no-restrictions), as consumers in our focus groups rated it as most relevant.

## 8.2 Functional Assessment of Cancer Therapy-Vulvar (FACT-V)

HRQL will be measured by Functional Assessment of Cancer Therapy-Vulvar (FACT-V) [31], a validated and reliable FACT-V includes 15 vulvar cancer-specific items [38] captures dimensions of physical, social, emotional, and functional wellbeing, as well as pain/discomfort, discharge, odour, swelling, and urination. Coding of the 4-point Likert scale measure follows the FACT manual [39]. The FACT-V will be administered at baseline, 8 weeks, 6 months, and 12 months.

## 8.3 Lymphoedema Assessment

Lymphoedema will be self-assessed using validated questionnaires successfully used in our previous studies [16]. Where trained staff and appropriate equipment are available at sites, a subset of participants will have leg circumference measured as per the standard measurement protocol provided by the Australasian Lymphology Association, measuring every 10cm from the heel working proximally. The sum of leg circumferences will be calculated and compared to pre-treatment measurements. In clinical practice, an increase by 5% or more in those measurements is considered indicative of lymphoedema development, leading to initiation of treatment. If the increase is less than 5%, the participant is observed before initiating treatment. Also, when possible, the ImpediMed SOZO BIS device will be used to assess electrical impedance to extracellular fluid in the upper and lower limbs. As per the SOZO standard protocol, lymphoedema is indicated when the right or left ratio of impedance of the upper and lower limb exceeds one standard deviation of mean ratio from normative data. When this threshold is exceeded, referral to treatment will occur. Lymphoedema will be assessed at baseline, 8 weeks, 6 months, and 12 months after surgery.

### 8.3.1 Measurement Protocol for Lymphoedema Assessment

BIS refers to the process of sending non-detectable electrical currents (n=256), at a range of frequencies (3 kHz to 1000 kHz) through the body, allowing precise measurement and analysis of impedance to currents by extracellular fluid.

BIS measurements will be taken with the patient standing or, when patient is unstable or exceeds weight limits, sitting.

- Standing measurements are taken with the patient standing with legs apart, body weight distributed evenly on both feet, arms relaxed with elbows at their side and hands making contact with the electrodes.
- Sitting measures are taken with patient sitting fully upright, balanced in a non-metal chair. Body weight is to be distributed evenly on both hips, knees bent at a right angle, plus or minus 10 degrees.

For both sitting and standing measures, feet (no stockings/pantyhose or socks) are placed onto SOZO step, ensuring each foot is flat and in full contact with each electrode plate. Both hands are placed onto the SOZO touch with thumbs securely wrapped around the corners of each recess. Each hand must be flat and in full contact with each electrode plate.

All measurements take approximately 20 seconds and once complete and cole plots are deemed acceptable data (R0, Ri, Rinf, fat mass (FM), fat-free mass (FFM), %FM, %FFM) will be stored for analysis. Lymphoedema status will be determined by using R0 in the following equations:

Dominant side of the body:
predictor = -18.41 + 5.58 (arm R0/leg R0) - 0.45 (male=1|female=0) + 0.03 (age) + 0.24 (BMI)
probability = exp (predictor)/(1+exp(predictor))
probability >0.045= lymphedema
Non-dominant side of the body:
predictor = -18.59 + 6.11(arm R0/leg R0) - 0.44 (male=1|female=0) + 0.02 (age) + 0.24 (BMI)
probability = exp (predictor)/(1+exp(predictor))
probability >0.042= lymphedema

BIS will be administered (if available and participant is on site) at baseline, 8 weeks, 6 months, and 12 months after surgery. These measures must be done at baseline, 6 months, and 12 months at least.

Where trained staff and appropriate equipment are available at sites, a subset of participants will have leg circumference measured as per the standard measurement protocol provided by the Australasian Lymphology Association, measuring every 10cm from the heel working proximally. The sum of leg circumferences will be calculated and compared to pre-treatment measurements. In clinical practice, an increase by 5% or more in those measurements is considered indicative of lymphoedema development, leading to initiation of treatment. If the increase is less than 5%, the participant is observed before initiating treatment. Lymphoedema will be assessed at baseline, 8 weeks, 6 months, and 12 months after surgery.

Participating Sites Only: Leg Circumference Measurements will be administered at baseline, 8 weeks, month 6 and month 12. These measures must be done Baseline, 6 months, and 12 months at a minimum.

## 8.4 Fear of Recurrence (FoR)

This four-item short-form (FCR-4) will be given to patients at 12 months post-surgery. It has been tested for its reliability and validity using classical psychometric and item response theory methods, and due to its brevity, poses low patient burden.

## 8.5 Decision-Making Tool

We will use the trial findings to develop a decision-making tool to support patient shared decision-making for groin USM or LND. This tool will be developed to align with the International Patient Decision Aid Standards (IPDAS) recommendations. It will include information about the probability of all important outcomes for each option including palpable groin node metastases and secondary endpoints such as lymphoedema and morbidity, and uncertainty, and support patients to consider their values and preferences for each outcome in order to achieve an informed decision.

In preliminary work, we have used a decision analysis approach to explicitly frame the treatment choice between groin USM and LND as a trade-off between the potential increased risk of nodal recurrence for USM versus the increased risk of lymphoedema and morbidity for LND using a decision tree. We used the decision tree to explore the impact of existing uncertainty about the probabilities of important outcomes and impact on health-related quality of life for each option on the expected value of USM versus LND. We will use the trial findings to build on the decision tree including explicit incorporation of patient preferences. We plan to use the decision tree approach to facilitate the co-development of an ANVU patient decision tool with consumer representatives, gynaeoncologists, surgeons, nurse consultants, and other stakeholders. Trial participants from Australian sites will be invited to opt in to receive an invitation to participate in a focus group for the development of the decision aid following the completion of their initial 12-month follow-up period.

Three focus groups will be formed, and each group will attend 4 x 2-hour sessions. Group 1 will comprise of 20 consumers previously treated for vulvar cancer. Group 2 will comprise of 20 certified gynaecological oncologists, nurse consultants, sonographers, and cancer service managers. Group 3

will comprise of 20 women randomised to the Intervention Group of ANVU. For equity, the sample will include rural/remote, younger, and older Australians to contribute important perspectives.

In Session 1 of the focus groups, we will present participants with practical information about US monitoring and LND options and a protype decision tool based on our preliminary decision model data for the probabilities of groin node recurrence, lymphoedema, and QoL measures, summarised in an 'option grid' with graphics to communicate benefits, harms, and level of uncertainty. After trial completion, these data will be updated with actual trial results. Participants will assess the clinical, behavioural, cultural, and environmental fit of the proposed tool's components and processes with the lived experience of consumer or clinical decision making; refining these in Session 2, until consensus. In Session 3, guided by the Consolidated Framework for Implementation Research (CFIR) and IPDAS33, the groups will investigate five factors that might influence the implementation of the tool in practice; namely: (1) The evidence of the tool's quality and validity as perceived by stakeholders; (2) Consumer and clinical need; (3) Existing networks, cultures, desire for change and resources; (4) Key individuals and their capacity to advocate for or against the tool; and (5) Planning and engagement necessary to implement the tool. A draft implementation strategy associated patient information and decision-making resources will be developed from Session 3 data, which will be presented at Session 4 for revision and ratification.

The decision-making tool and its implementation strategy will be approved by all focus group participants (n=60). They will then be asked to complete the Acceptability, Appropriateness, and Feasibility of Intervention Measures (AAFIMs), with 12 items to assess whether respondents consider the tool and implementation strategy acceptable and appropriate. The AAFIMs and focus group data will be used to finalise the decision-making tool to support shared decision making and finalise an implementation strategy to enhance the uptake of the tool in the wider Australian and International setting.

# 9. Translational Research

In addition to the above, participants have the option to provide samples for use in future translational research. We will collect blood samples from participants who consent to be banked for the purpose of future molecular and biomarker research into vulvar cancer. Participants who receive standard groin LND will have blood collected at two time points: baseline (pre-operatively) and 8 weeks after surgery. For participants who have serial high-resolution groin USM, blood will be collected prior to surgery (baseline) and at each ultrasound scan (7-time points total). Participants can still participate in this study if they choose not to provide blood samples. Participants who consent to provide blood samples will be sent a blood collection request form for their preferred commercial pathology laboratory. Each participant will be asked to donate blood (up to 50 ml) at their convenience. Participants' blood samples will be collected for germline DNA (baseline only), circulating tumour DNA, plasma, and serum. For participants who have consented for the future use of blood samples, analyses will include examining circulating factors, such as extracellular vesicles and their contents.

Blood samples will be forwarded to a local pathology laboratory for processing and storage. Throughout the study, samples will be retrieved and stored at one or more of our central laboratories in Queensland, Australia, these include Mater Pathology and The University of Queensland Centre for Clinical Research. The blood samples will be assigned a unique code, which will be used in all analyses,

thus removing identifying information about the participant, and thereby protecting their privacy. Recruited participants will also be asked to provide FFPE tissue samples for use in future translational research. Tissue (vulvar tumour, lymph nodes) will be collected from all participants at the diagnostic biopsy procedure or during surgery (if applicable) as per standard clinical care.

These patients will be asked to participate in this project because, as part of their planned medical procedure, they will have their tumour biopsied or are undergoing surgery to have their tumour removed. Their scheduled biopsy or surgery is part of a medical treatment that they agreed upon with their doctor. During the procedure, cancer tissue will be removed, and their tissue blocks samples will be requested from pathology laboratories throughout the study. Analyses will include genotyping for HPV, and analysing biomarkers by immunohistochemistry, DNA sequencing, and other technologies.

# 10. Safety Reporting

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of AE or a SAE as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

The investigator/researcher must report all SAEs to QCGC Research within 24 hours of the site becoming aware of the SAE, in accordance with the study protocol and GCP guidelines.

## 10.1 Definition of an Adverse Event (AE)

An AE is any untoward medical occurrence in a patient, which does not necessarily have a causal relationship with this treatment. AE will be categorised using the CTCAE V5.0 and be collected for 12 months after surgery. An AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. For marketed medicinal products, this also includes failure to produce benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of participants' previous therapeutic regimen).

Examples of an AE do not include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.

- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to hospital).

## 10.2 Definition of a Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that:

- results in death,
- is life threatening,

Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

- requires hospitalisation or prolongation of an existing hospitalisation,

Note: In general, hospitalisation signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalisation are AEs. If a complication results in prolonged hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious. Hospitalisation for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

- results in disability/incapacity,

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.

- is a congenital abnormality/birth defect, or

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependence or abuse.

- Any event deemed by the investigator as being a significant medical event.

SAEs will be categorised using the CTCAEs V5.0 and be collected for 6 months after surgery.

## 10.3 Reporting Requirements

Any AE considered Serious by the local Investigator, or which meets the previous SAE criteria must be reported to Central Data Management Unit (CDMU) /QCGC Research and reviewing HREC according to local HREC policy.

The following must occur within 24 hours from the time that the site personnel first become aware of the serious adverse event:

The site personnel must complete the SAE case report form and submit this to the Lead Site/ CDMU below:

| **ANVU Trial Manager** |
| --- |
| Queensland Centre for Gynaecological Cancer (QCGC) Research<br>UQCCR, Building 71/918<br>Royal Brisbane and Women's Hospital<br>Herston QLD Australia 4029<br>Email: ANVUtrial@uq.edu.au |

As well as providing the SAE form to CDMU the site is also required to email a brief notification of SAE data transmission. All SAE's will be reported by the CDMU to a central HREC according to central HREC policy and the Independent Data Safety Monitoring Committee (IDSMC).

SAEs will be categorised using the CTCAE V5.0 and collected for 6 months after surgery.

## 10.4 Time Period, Frequency, Method of Detecting AEs, and SAEs

All AEs will be recorded between the time of surgery and the 12-month follow-up visit and SAEs will be recorded up to 6 months after surgery.

Each participant will be monitored regularly by the investigator and study personnel for AEs occurring throughout the study. The investigator or designee will enquire about AEs and SAEs by asking a non-leading question, for example:

"How are you feeling?"

At subsequent scheduled intervals participants will be asked:

"Since you were last asked, have you felt unwell or different from usual?"

## 10.5 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator or designee to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator or designee will then record all relevant information regarding an AE/SAE into the CRF/eCRF.

It is not acceptable for the investigator to send photocopies of the participant's medical records to QCGC Research in lieu of completion of the appropriate AE/SAE CRF/eCRF pages. However, there may be instances when copies of medical records for certain cases are requested by QCGC Research. In this instance, all participant identifiers will be blinded on the copies of the medical records prior to submission to QCGC Research.

For each AE, start and stop dates, action taken, outcome, severity, and relationship to study treatment/intervention must be documented. If an AE changes in frequency or intensity during a study, a new entry of the event must be made in the CRF/eCRF.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms should be documented.

All details of any treatments initiated due to the adverse event should be recorded in the participant's notes and the CRF/eCRF.

## 10.6 Evaluating AEs and SAEs

### 10.6.1 Assessment of Severity

The investigator or designee will assess intensity for each AE and SAE reported during the study. The assessment will be based on the investigators or designee's clinical judgement.

Intensity of adverse events will be graded using the National Cancer Institute CTCAE V5.0 and reported in detail as indicated on the CRF/eCRF.

If an adverse event occurs which is not contained in the CTCAE V5.0, the five-point scale below will be used:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort, and not interfering with everyday activities
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event which is incapacitating and prevents normal everyday activities
- Life Threatening: represents an immediate threat to life
- Death

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilised for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as "serious" when it meets one of the predefined outcomes as described in section 10.1 "Definition of an SAE".

### 10.6.2 Assessment of Causality

The investigator or designee is obligated to assess the relationship between treatment and the occurrence of each AE/SAE. They will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the treatment will be considered and investigated.

The causal relationship to the treatment assessed by the Investigator or designee should be assessed using the following classifications:

- Not Related: In the Investigator's opinion, there is not a causal relationship between the study treatment and the adverse event
- Unlikely: The temporal association between the AE and study treatment is such that the study treatment is not likely to have any reasonable association with the AE
- Possibly: The adverse event could have been caused by the study participant's clinical state or the study treatment
- Probably: The AE follows a reasonable temporal sequence from the time of study treatment administration, abates upon discontinuation of the study treatment and cannot be reasonably explained by the known characteristics of the study participant's clinical state
- Definitely: The AE follows a reasonable temporal sequence from the time of study treatment

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report to QCGC Research. However, it is very important that the investigator always assesses causality for every event prior to transmission of the SAE form to QCGC Research. The investigator may change her/his opinion of causality in light of follow-up information, amending the SAE form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

### 10.6.3 Exemptions from AE/SAE Reporting

Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs:

Laboratory test value abnormalities will not be reported as AEs, unless there is an associated clinical condition for which the participant is given treatment, concomitant treatment is altered, or the event is considered a serious adverse event.

The investigator or designee will exercise their medical and scientific judgement in deciding whether abnormal laboratory findings, or other abnormal assessment is clinically significant.

### 10.6.4 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator or designee is required to proactively follow each participant and provide further information to QCGC Research on the participant's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilises, until the event is otherwise explained, until the participant is lost to follow-up, or 30 days post 12 month follow up visit. Once resolved, the appropriate AE/SAE CRF/eCRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

New or updated information for SAEs will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator or designee. The updated SAE form should be resent to QCGC Research.

### 10.6.5 Safety Monitoring

QCGC Research will provide safety information to the Trial Management Committee (TMC) and the Independent Data Safety Monitoring Committee (IDSMC) on a periodic basis for review.

Trial safety data will be monitored to identify:

- new adverse reactions to the trial treatment regimen or individual trial treatments
- SAEs
- trial related events that are not considered related to the trial treatment regimen.

Should QCGC Research identify or suspect any issues concerning participant safety at any point throughout the trial, the Chief Investigator (CI) or TMC will be consulted for their opinion.

#### 10.6.5.1 Evaluating and Reporting Positive Nodes

The senior medical imaging specialists will review the serial high-resolution bilateral ultrasound scans, evaluating for the presence of positive lymph nodes or any suspicious findings, relying on their clinical expertise. Radiologists will send their report to the local PI within 5 working days of ultrasound completion. All sites are required to enter the completed ultrasound eCRF on REDCap within 5 working days of ultrasound completion. Additionally, they must upload a copy of a signed and de-identified ultrasound report to REDCap, where QCGC Research will verify ultrasound completion. All sites should also provide a copy of de-identified US images to QCGC Research through The University of Queensland Research Data Manager platform (UQRDM) for quality assurance purposes. UQRDM is a secure platform designed for storing, managing, and sharing research data. It ensures compliance with data protection regulations and facilitates efficient data access and collaboration among researchers.

As explicitly stated in section 6.5.3. of the protocol, if suspicious, indeterminate, or positive nodes or any other concerning indicators are detected, the participant will be referred to either the local PI or the specialist gynaecological oncologist to discuss their options. Depending on the clinical judgment of the clinician, the participant may be recommended for LND (standard treatment) or may opt to continue with bilateral groin USM every two months and clinical examinations every three months (remaining on trial).

Participants receiving interventional treatment (serial groin USM in conjunction with clinical examination) who develop suspicious, indeterminate, or positive nodes are not removed from the trial as we still collect their data. The requirement for bi-monthly bilateral ultrasound, physical examination and blood tests will be discontinued.

# 11. Statistical Considerations

## 11.1 Sample Size & Stopping Rules

Incurable groin recurrence has a >90% mortality within 12 months [21]. We estimate the probability of incurable groin recurrence within 12-month of an upfront LND, i.e., $P(\text{recur}|\text{LND})$, to plausibly be up to 3% in usual practice. This is based on a 32% prevalence of positive nodes in clinical stage I/II vulva cancer [41] and that the false negative rate for routinely used SNB is up to 8.8% [7,42]. A

recurrence probability given groin USM, i.e., $P(\text{recur}|\text{USM})$, of 6% would be acceptable to avoid the side-effects of LND based on our decision analysis using best available evidence, expert judgement, and consumer input. $P(\text{recur}|\text{USM})$ will be characterised using a Bayesian Beta-Binomial model with priors informed by our pilot study (1/32 recurrences) [10]. We will declare groin USM acceptably safe if the probability that $P(\text{recur}|\text{USM}) < 0.06$ is 80%. A Bayesian Monitoring approach will ensure participant safety (MD Anderson Biostatistics BTOX routine www.trialdesign.org/#software). Stopping early due to unacceptably safety will be triggered if there is more than a 70% probability that $P(\text{recur}|\text{USM})$ exceeds 0.06 (i.e., recurrence rate of at least 5/40, 6/60, 7/80, 9/100, or 10/120). We anticipate that $P(\text{recur}|\text{USM})$ is truly 3% and thus calculate that ANVU has less than a 2% probability of incorrectly stopping early for unacceptable safety and a 0.85 probability of correctly declaring acceptable safety at N=120. The statistical design accommodates expansion beyond N=120 USM participants (e.g., as a protocol amendment) if neither safety criterion is reached and the predicted probability of success given expansion is at least 80%.

## 11.2 Statistical Analysis

Analyses will adhere to the intention-to-treat principle and a Statistical Analysis Plan will be prepared using the estimand framework (ICH E9-R1 addendum).

### 11.2.1 Incidence of Enlarged, Palpable Groin Nodes

Groin USM will be deemed acceptably safe if the probability that $P(\text{recur}|\text{USM}) < 0.06$ is 80% using the approach described in section 11.1. This will be supplemented by conventional frequentist methods to estimate the proportion of women with clinically enlarged palpable groin node metastases at 12 months from randomisation for each group together with 95% confidence intervals. The difference in proportions between groups will also be calculated and presented with confidence intervals.

### 11.2.2 Impact of Ultrasound Surveillance on Quality of Life and Function, Pain, Lymphoedema and Morbidity

Demographics, behavioural characteristics and comorbidities will be captured at enrolment (baseline), 8 weeks, 6 months and 12 months, PROMs including HRQL and FoR (only at 12 months) will be assessed by a study-specific survey incorporating validated instruments:

PROMs will be scored according to their manuals to construct scales. Comparisons between randomised groups on PROMs scales will be performed using a linear mixed model for repeated measures accounting for stratification factors. Analysis will also be performed using a time-integrated approach to HRQL outcomes assessment pioneered by Professor Gebski [44]. Logistic regression will be used to compare groups on categorical outcomes (or an exact test if there are few events). Categorical outcomes include: return to usual activities at 8 weeks post-surgery; and the proportion of women experience a clinical relevant change in PROMs from baseline [45] at 8-weeks post-surgery, 6 months post-surgery, and 12 months post-surgery.

### 11.2.3 Morbidity of Intervention

Post-surgery, patients will be assessed daily until discharge from hospital. Perioperative outcomes including duration of surgery (time from skin incision to wound closure), length of hospital stay,

estimated intraoperative blood loss, requirements for blood transfusions, intraoperative complications, and crossovers (including the clinical necessity for a comprehensive LND) will be recorded. Intraoperative injuries, postoperative AEs and SAEs will be recorded according to the CTCAE V5.0, at predefined time points for up to 12 months. We will compare the incidence of (a) intraoperative; (b) postoperative; and (c) serious AEs at 8 weeks and 6 months and 12 months post-surgery.

### 11.2.4 Lower Limb Lymphoedema

Lymphoedema will be self-assessed using validated questionnaires successfully used in our previous studies [16]. We will use our previously developed self-reported lymphoedema tool (questions on swelling in legs, pelvis or vulvar; 14 lower-limb symptoms including pain, tingling, weakness) to analyse lymphoedema at 8 weeks, and 6 months, and 12 months.

We will compare the prevalence of subjective symptoms of lymphoedema over time using a general linear mixed modelling approach. Similar analyses will be conducted on the subgroup of patients for whom we have objective assessment of lymphoedema (using leg measurements).

### 11.2.5 Determine the Clinical Accuracy of Preoperative, Standardised, High-Resolution Ultrasound to Identify Vulvar Cancer Patients with Positive (Involved) Groin Nodes

In women who receive a groin LND, overall ultrasound assessment scores will be compared to histopathology of the groin nodes. Sensitivity, specificity, positive and negative predictive values of the ultrasound outcomes compared to histopathology, will be evaluated together with receiver-operating characteristic.

### 11.2.6 Cost and Cost Effectiveness

Health resources utilised between study groups will be identified, measured, and valued to obtain the total costs of each intervention. Length of stay and subsequent hospital admissions up to 12 months post-surgery will be valued (national hospital pricing model) and prices adjusted based on 3% annual inflation. The use of all health services (including adjuvant treatments) within 12 months of surgery will be collected and valued at each site in $AUD. Cost of AEs will be allocated depending on their severity as determined using the CTCAE V5.0. To assess the value of health outcomes for each surgical option, the EQ-5D-5L will be used to estimate utility scores and calculate gains in quality adjusted life years (QALYs) associated with the intervention.

A Markov model will simulate how patients progress through health states and incur costs and QALYs. Model input parameters (e.g., transition probabilities, costs, and utility scores) will be obtained from this trial and other relevant published literature. The incremental costs and QALYs will be compared to calculate the incremental cost effectiveness ratio (ICER). A willingness to pay threshold of $50,000/QALY, a commonly used threshold in the literature, will be used as a decision rule [46]. Probabilistic sensitivity analysis will be conducted to characterise the uncertainty in the results of the economic evaluation. Value of implementation analysis will be conducted to estimate the expected net health and monetary benefits gained with the adoption of groin node ultrasound surveillance.

### 11.2.9 Disease-Free and Overall Survival (DFS, OS)

Participants will be followed for 12 months and assessed for local, regional, or distant recurrence. Participants will be seen every 3 to 6 months. Clinical assessments will be performed at each visit. Clinical assessment, radiological work-up ± histological confirmation of recurrence will prove the presence of recurrent disease.

DFS is defined as time until the first recurrence or death from any cause. In patients without recurrence, or lost to follow-up, DFS will be censored on the date of the last follow-up visit. DFS will be compared between treatment arms using methods for the analysis of censored data. Variables to be evaluated for effect on DFS will include clinical management group (groin LND, ultrasound groin surveillance), age, medical comorbidities.

# 12 Trial Monitoring and Oversight

## 12.1 Patient Safety

The number of enlarged, palpable groin node metastases/recurrences in all three groups will be monitored continuously and trial stopping rules are in place if these numbers exceed the upper limit of normal. Ultrasound is non-invasive and does not use radiation. In Australia, all ultrasound scans will be performed by registered sonographers with a postgraduate degree recognised by the Australian Sonographers Accreditation Registry (ASAR) and monitored by the TMC. At International sites, all ultrasound scans will be performed by sonographers with equivalent credentials. Blood tests will be performed up to 30 days prior to surgery (as part of routine blood tests), 8 weeks post-operation and then every 2 months for ultrasound patients. The risk of participants in having the intervention is to develop a clinically apparent groin node recurrence that would grow to a large size undetected and that would lead to poor survival outcomes (potentially leading to death). To mitigate this risk, we have implemented precautionary measures. Following surgery to remove the primary vulvar tumour, participants in the intervention group will have a groin ultrasound scan every 2 months and a clinical examination every 3 months, for a period of 12 months. Eligibility for participation in this study requires a commitment from patients to adhere to these frequent follow-up intervals and only participants with a normal/negative baseline groin node ultrasound are eligible to be randomised.

The prospective study's design, stringent inclusion and exclusion criteria, a well-defined follow-up schedule involving bi-monthly ultrasounds and clinical examinations every three months, use of a synoptic reporting format for groin ultrasound findings and the appropriate allocation of resources, including dedicated study staff, along with the comprehensive training of sonographers will effectively reduce the likelihood of participants experiencing unfavourable outcomes. However, it's important to mention that even when patients undergo groin node dissection (LND) as part of their standard treatment, the risk of subsequent groin node metastasis and the associated risk of death fall within the range of 3% to 10%, as indicated in published literature [6].

## 12.2 Quality Assurance

A central histopathology review will categorise involved nodes into positive, micro-metastasis, or isolated tumour cells.

## 12.3 Trial Management Committee (TMC)

The role of the TMC is to provide overall supervision of the trial. The TMC will include the CI, clinicians, and experts from relevant specialties and ANVU trial staff from QCGC Research. The TMC will be responsible for overseeing the trial and ensuring that the trial is conducted to the required standards. The group will meet approximately two to three times a year (more if required) and will provide updates to PIs via newsletters and/or at Investigator meetings.

Following each meeting, the TMC will recommend that the study continues according to the protocol or may suggest changes to the protocol based on the outcome of the data review. The TMC will review substantial amendments to the protocol prior to submission to the HREC. All PIs will be kept informed of substantial amendments through their nominated responsible individuals.

The TMC will also review the recommendations of the Independent Data Safety Monitoring Committee (IDSMC) and, on consideration of this information, recommend any appropriate amendments/actions for the trial as necessary.

## 12.4 Independent Data Safety Monitoring Committee (IDSMC)

The trial executive will monitor the incurable groin recurrence within 12-months according to the scheduled presented in Section 11.1.

The IDSMC will provide independent advice on enrolment, patient safety, data completeness and efficacy, scientific validity, and data integrity, as well as critical efficacy endpoints. The members of the IDSMC are not nor have ever been participants in the ANVU Trial.

The first safety analysis will be reviewed at 6 months or after 20 participants have completed surgical treatment (whichever comes first). The IDSMC will then meet approximately once or twice a year, or more often if required.

Following each meeting, the committee will provide a written report to the TMC and recommend continuation of the trial or protocol changes based on the outcome of the data review. The IDSMC is advisory to the TMC and in exceptional cases can recommend premature closure of the trial to the TMC due to safety reasons.

The IDSMC membership includes the following:

- Two independent gynaecological oncologists who are otherwise not involved with the trial.
- At least one independent statistician who is otherwise not involved with the trial.
- At least two specialists from a profession related to gynaecological oncology (pathology, medical imaging, medical oncology, radiation oncology, etc.) who is otherwise not involved with the trial.
- Additional members may be co-opted as necessary.

All IDSMC members will be required to agree to an IDSMC Terms of Reference.

## 12.5 Recurrence Adjudication Committee (RAC)

The objective of adjudication is that a well-recognised group of experts in the field of Gynaecological Oncology provide their input based on their best judgement and expertise in vulvar cancer, the location of the recurrence, and the date of this recurrence in participants accrued to the ANVU trial. The members of the RAC are not nor have ever been participants in the ANVU Trial. Their review will provide an independent, unbiased, clinical review of documented recurrences and this will be blinded to randomised treatment. The RAC review will be scheduled every 6 months from year 2 onwards for the duration of the study. A summary of each recurrence, including all correlating source documents (and translation forms if applicable), will be provided for the clinical review.

Adjudication ensures accuracy due to the high level of expertise of members, independency to avoid any bias, and consistency by using a centralised committee.

The RAC is responsible for reviewing each reported recurrence. Their work is independent and impartial. This type of review is to ensure accuracy and consistency of events reported in this study.

All RAC members will be required to read and agree to the RAC Terms of Reference.

## 12.7 Missing Ultrasound or Physical Examination

If a participant allocated to the interventional group misses their ultrasound appointment or physical examination by ±7 days, both the trial PI and the site will be notified. The PI will investigate, with trial management assisting in promptly rescheduling the ultrasound appointment or physical examination within 7 days. Actions will be implemented to mitigate the risk, which may include the possibility of removing the patient from the trial.

We will allocate dedicated staff to ensure participants adhere to and comply with all study monitoring schedules, ensuring ultrasound scan appointments or physical examinations are conducted within a ±7-day window, and that all eCRFs and reports are promptly received. Should any issues arise, the site PI will be immediately informed.

# 13. Dissemination of Findings

We expect the results of this study will be disseminated by publication in a peer reviewed journal, presentation at national or international conferences, and circulation throughout patient and professional organisations relevant to gynaecological oncology care. The investigators agree that publications or presentations of any of the results from the study will take into account the co-operative nature of the conduct of the study and the overall objective of increasing public knowledge and shall be in accordance with accepted scientific practice, academic standards and customs and in accordance with the protocol and with any more specific publication/presentation guidelines developed by the trial management committee during the course of the study, including but not limited to the following:

As the study is a multi-centre study, any results from a single centre must not be published before the publication of results from all centres and before the trial PI does not give explicit written permission to the site/investigator to do so.

Individuals making a substantial contribution to the study will be recognised with co-authorship in publications from the study unless they elect not to be recognised.

# 14. Ethical Consideration

All sampling and recruitment procedures will comply with ethical requirements, including respect for privacy and consent, and to reduce any risk for harm. The wellbeing and safety of participants will be paramount considerations at all times. Participants will be informed of the potential risk prior to participating and will be advised they can withdraw from the study at any time and their decision to participate/not participate will not affect their relationship with The University of Queensland or their treating doctors. Potential participants will be given an opportunity to ask questions about the study and what their participation would involve, and have their questions answered to their satisfaction.

There is the possibility of finding an unsuspected abnormality during the scanning process. In the event of such an abnormality being discovered as a result of the ultrasound examination, we will advise a participant and a relevant medical practitioner, or GP may be contacted, and a participant may be referred, if necessary, to an appropriate clinician.

The research team will have access to the data but will de-identify it prior to analysis. No identifiable information will be published. All comments and responses are anonymous and will be treated confidentially unless required by law.

Individual results from this study will be confidential, and no material which could personally identify participants will be used in any reports on this study. Study records will be confidential and stored securely.

Participation in the study will be voluntary. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2023), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2018), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2013.

To this end, no patient will be recruited to the study until all the necessary approvals have been obtained and the patient has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a participant. In this circumstance the PI and HREC must be advised immediately.

# 15. Recording of Data and Retention of Records

It is the responsibility of all PIs to ensure data and all essential documents relating to the ANVU trial held at their site are retained securely for a minimum of 15 years after the end of the trial, and in accordance with local legislation.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.

QCGC Research will notify sites when trial documentation held at sites may be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

# 16. Study Schedule

| | Screening/ Eligibility | T0 Baseline | T1 week8 | T2 FOLLOW UP (MONTH 3) | T3 FOLLOW UP (MONTH 4) | T4 FOLLOW UP (MONTH 6) | T5 FOLLOW UP (MONTH 8) | T6 FOLLOW UP (MONTH 9) | T7 FOLLOW UP (MONTH 10) | T8 FOLLOW UP (MONTH 12) |
|---|---|---|---|---|---|---|---|---|---|---|
| Medical History | × | | | | | | | | | |
| Cancer Diagnosis | × | | | | | | | | | |
| Eligibility Criteria | × | | | | | | | | | |
| Informed Study Consent | × | | | | | | | | | |
| Biopsy Within 75 Days Prior To Ultrasound | × | | | | | | | | | |
| Medical Imaging (CT or MRI) of Chest/Abdo/Pelvis Within 75 Days Prior To Ultrasound | × | | | | | | | | | |
| Negative (Serum Or Urine) Pregnancy (BHCG) Test ≤ 30 Days Of Surgery ONLY in Pre-Menopausal Women and Women < 2 Years After The Onset Of Menopause | × | | | | | | | | | |
| Demographic Questionnaire | | × | | | | | | | | |
| FBC, Urea and Electrolytes, and LFT's | | × | × | | | × | | | | × |
| ECOG Performance Score | | × | × | | × | × | × | | × | × |
| Height | | × | | | | | | | | |
| Weight | | × | × | | | × | | | | × |
| Pain Scale | | × | × | | × | × | × | | × | × |
| Blood Samples for Translational Research (See Section 9) (LND/SNB) | | × | × | | | | | | | |
| Blood Samples for Translation Research (See Section 9) (US) | | × | × | | × | × | × | | × | × |
| Bilateral Groin Node Ultrasound Up To 30 Days Prior To Planned Surgery | | × | | | | | | | | |

| | | Screening/ Eligibility | T0 Baseline | T1 week8 | T2 FOLLOW up (MONTH 3) | T3 FOLLOW up (MONTH 4) | T4 FOLLOW up (MONTH 6) | T5 FOLLOW up (MONTH 8) | T6 FOLLOW up (MONTH 9) | T7 FOLLOW up (MONTH 10) | T8 FOLLOW up (MONTH 12) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| a) US Suspicious/ Indeterminate: SNB/IFL | | | × | | | | | | | | |
| b) Negative Groin Ultrasound: Randomisation To Upfront Groin LND OR 2 Monthly Groin USM + 3 Monthly Physical Examination | | | × | | | | | | | | |
| Surgical Excision of The Primary Tumour - Planned Surgery | | | × | | | | | | | | |
| Central Histopathology Review | | | × | | | | | | | | |
| Groin USM (Interventional Treatment) | | | | × | | × | × | × | | × | × |
| Current Medications | | | × | × | | × | × | × | | × | × |
| Adverse Events | | | | × | | × | x | × | | x | × |
| Hospitalisations | | | | × | | × | × | × | | × | × |
| Serious Adverse Events | | | | × | | × | × | | | | |
| Physical Examination (Interventional Treatment) | | | | × | × | | × | | × | | × |
| Disease Status | | | | × | | × | × | × | | × | × |
| PROMS & HRQL | FACT-V | | × | × | | | × | | | | × |
| | EuroQol-5D (EQ-5D) | | × | × | | | × | | | | × |
| | Lymphoedema Assessment | | × | × | | | × | | | | × |
| | Fear of Recurrence (FCR-4) | | | | | | | | | | × |

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