Supplemental Online Content

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Supplement 1. Trial protocol and statistical analysis plan

This supplemental material has been provided by the authors to give readers additional information about their work.

Royal Hospital for Women

STUDY PROTOCOL

VERSION 7.0

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A DOUBLE BLINDED, PLACEBO CONTROLLED RANDOMISED TRIAL ON THE EFFICACY OF THE MONALISA TOUCH*TM* PROCEDURE FOR THE TREATMENT OF VAGINAL ATROPHY SYMPTOMS

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STUDY SUMMARY

This protocol outlines a randomised, double blind trial with a 12-month follow-up period to evaluate the efficacy of intra-vaginal fractional carbon dioxide treatment (marketed as the MonaLisa Touch™ (MLT) procedure) for the treatment of vaginal atrophy symptoms in post-menopausal women.

The MLT procedure is a newly described vaginal laser treatment for a range of symptoms, most commonly associated with the menopause. To date, there are a small number of publications on the efficacy of the MLT procedure, prospective cohort studies, mainly focusing on vaginal atrophy symptoms.[1,2] The data from these studies are promising, but randomised trial data to confirm the efficacy of the MLT procedure are yet to be obtained.

Despite this deficiency in the scientific validation of this procedure, it has been rapidly disseminated and performed throughout Australia and many other countries. As the MLT laser used in this study is FDA and TGA approved, this will be a phase II clinical study evaluating efficacy in a limited number of women. Safety will not be a primary focus of this study where a substantially greater number of women would be required for inclusion, given the data currently available.

A total of 78 women (minimum) will be enrolled into the study. Subjects enrolled into the study will be post-menopausal women with vaginal atrophy symptoms, who:

1) Previously trialled topical oestrogen preparations without alleviation of symptoms; OR
2) May be contra-indicated to the use of topical oestrogens (e.g. those with oestrogen receptor positive tumours who have been directed to avoid this treatment); OR
3) Are not willing to accept topical hormonal treatment.

At the time of enrolment, women will receive an examination to assess for possible vaginal prolapse (exclusion criteria). At the time of this examination, a 3mm Tischler Biopsy Forceps will be used to biopsy a sample of the right mid-vaginal wall and sent to histopathology. Bleeding from the site will initially be treated with pressure, then silver nitrate application if required. Following enrolment, women will undergo three treatments, each 4 weeks apart with half the group having the active MLT treatment and the other half of the group having a sham treatment. Prior to each treatment, the woman will be assessed

for her Vaginal Health Index. One month after the third treatment, a 3mm Tischler biopsy forceps will be used to biopsy a sample of the left mid-vaginal wall and sent to histopathology. Bleeding from the site will initially be treated with pressure, then silver nitrate application if required. Clinical follow-up will for a minimum of 12 months after the initial treatment with assessors blinded to the initial treatment type. Treatment outcomes will include:

1. Symptom intensity
2. Vaginal Health Index scores
3. Quality of life scores
4. Treatment discomfort
5. Treatment acceptability
6. Treatment complications.
7. Histological changes in vaginal mucosa

The treatment protocol will follow the following timeline shown below:

	Baseline	**Treat 1**	**Treat 2**	**Treat 3**	**F/U 1**	**F/U 2**
Time (w/m)	-	0 w	4 w	8 w	6 m	12 m
History	X					
Inclusion/ Exclusion criteria	X	X				
Demographic data	X					
Symptom assessment VAS	X	X	X	X	X	X
VSQ score	X	X	X	X	X	X
POPQ assessment	X					
VHI Score		X	X	X	X	X
Vaginal Biopsy	X				X	
QoL assessment	X				X	X
FSSQ	X	X	X	X	X	X
Treatment complication evaluation		X	X	X	X	X
Treatment discomfort evaluation		X	X	X		
Treatment acceptability evaluation					X	X

INTRODUCTION

According the product description brochure, the MonaLisa Touch*TM* (MLT) procedure is "a new non-hormonal, non-surgical treatment for patients of all ages suffering from the symptoms of atrophic vaginitis".[3] The actual procedure is a fractional carbon dioxide laser treatment to the vaginal epithelium. This 5-minute intra-vaginal laser treatment is suggested to provide long lasting improvements for women suffering from conditions such as:

1. Dyspareunia
2. Urinary incontinence
3. Vaginal itchiness and burning
4. Dryness and loss of lubrication
5. Recurrent thrush and UTIs
6. Prolapse
7. Vaginal laxity
8. Vaginal and vulval pain.

These are common conditions that may adversely affect many post-menopausal women. Consequently, there is substantial interest in new treatments that are minimally invasive such as the MLT procedure and alternatives to hormonal options and as a result there has already been rapid uptake in Australia and around the world for the MLT procedure.

There remains a paucity of high-grade scientific evidence to support the procedure's efficacy, despite this uptake and utilisation. Two prospective cohort studies of the MLT procedure provide supportive data for the treatment's efficacy in the treatment of vaginal atrophy symptoms in post-menopausal women. Short-term data (up to 1 month after the third of 3 laser treatments, each one month apart) are available for this procedure relating to symptoms that include vaginal burning, vaginal itching, vaginal dryness, dyspareunia and dysuria. It has been estimated that up to 50% of post-menopausal women experience at least one of these symptoms.[4,5] As such, the need for robust randomised controlled data to support the use of treatments for vaginal atrophy symptoms is imperative, both to assess treatment efficacy, longevity and safety.

Vaginal atrophy results from decreased levels of oestrogen following menopause. The symptoms of vaginal atrophy usually arise 4-5 years after menopause and, unlike other

menopausal symptoms, may worsen with the passage of time.[6] Decreased oestrogen levels result in thinning of the vaginal mucosa and the loss of vaginal rugae. Blood flow to the vaginal mucosa is decreased with associated decrease in vaginal secretions. Changes in the vaginal micro biota with resultant changes in vaginal pH make the vagina susceptible to opportunistic infection.[7] Treatments of vaginal atrophy symptoms aim to restore or mimic premenopausal physiological conditions in the vagina.

The mainstay treatment for vaginal atrophy symptoms is oestrogen replacement therapy, topical or systemic; topical oestrogen therapy is usually prescribed where vaginal atrophy symptoms predominate. Topical oestrogen reverses vaginal mucosal atrophy and alleviates symptoms; furthermore, this treatment appears to be safe. However, longer-term compliance with topical oestrogen treatment is sub-optimal and the safety of this treatment after 12 months is unknown.[1] Additionally, data on the use of topical oestrogen in high-risk patients (e.g., history of VTE, breast cancer, etc.) is also lacking.

The MLT procedure provides a superficial application of CO_2 laser to the vaginal skin by a specifically designed vaginal probe. Treatments last about 5 minutes are reported to be almost painless and otherwise safe. It is reported that the most uncomfortable part of the procedure is the insertion of the laser probe into the vagina. The laser treatment is being increasingly used to manage vaginal atrophy symptoms.[3] Histological changes in the vaginal mucosa after the MLT procedure include: restoration of thick glycogen containing vaginal squamous stratified epithelium; synthesis of new components of the extracellular matrix including collagen and ground substance molecules by activated fibroblasts in the lamina propria; and newly-formed papillae of connective tissue indenting the epithelium with penetrating capillaries penetrating the papillae.[9] These histological features are appear similar to those seen in pre-menopausal vaginal skin, and may to be secondary to fibroblast activation from heating of the submucosa by the laser pulses.[9] These findings, coupled with clinical relief of vaginal atrophy symptoms in studies from the same researchers, are suggestive of a mechanistic link due to the laser treatment.[1,2] These changes in the vaginal mucosa are hoped to be long-lasting.[1] However, there are no data relating to long-term outcomes with publications reporting follow-up to only 1 month after the final treatment (i.e., 3 months after the first of 3 treatments).[1,2,8,9] Furthermore, there are no robust randomised controlled data to conclusively prove a link between the MLT procedure and decreased vaginal atrophy

symptoms.

This study will be a double blinded, placebo-controlled randomised controlled trial with a follow up until 12 months after the initial laser treatment. The sham treatment will involve the exact same steps of the MLT procedure but on settings which will deliver no energy to the tissue. The procedure is reported to be painless with women unaware of the actual laser application. However, women will have topical anaesthetic administered to the introitus to minimise discomfort. Only the treating doctor and the patient will be present in the room at time of treatment and a curtain will be used to separate the subject from seeing the machine settings to maintain blinding. Lights, sounds, and treatment movements will be identical between the laser and sham treatments. A plume evacuator will be activated in both treatments to remove any olfactory differences.

The treatment group will be compared to the sham group for the response to treatment of vaginal atrophy symptoms, Vaginal Health Index, sexual function, Vaginal mucosal histology, quality of life scores, treatment discomfort, treatment acceptability and treatment complications. All components used in this study are FDA & TGA approved. The results of this trial have the potential to provide the necessary robust evidence to guide the treatment of post-menopausal vaginal atrophy symptoms.

OBJECTIVES

The primary objective of this Phase II clinical investigation is to evaluate the efficacy of the MLT procedure for the treatment of vaginal atrophy symptoms before, during and after treatment via the following methods:

a) Visual analogue scale (VAS) scores of symptoms (vaginal burning, vaginal itching, vaginal dryness, dyspareunia and dysuria) intensity
b) Vulvovaginal Symptom Questionnaire (VSQ) scores (symptom, emotion, life and sexual impact domains)

The secondary objectives are to evaluate the:

c) Assessment of Quality of Life - 6D (AQoL-6D) scores (6 dimensions)
d) Overall satisfaction with the procedure
e) Level of procedure discomfort/acceptability
f) Vaginal Health Index (VHI) scores (based on vaginal elasticity, fluid volume, pH,

epithelial integrity and moisture

g) Complication rate
h) Changes in Vaginal Mucosal Histology

Study numbers

A total of at least 78 women will be enrolled in this study. Women will be randomly allocated to each treatment arm by a computer-generated randomisation program in balanced blocks of 8, with third party randomisation occurring centrally after inclusion and exclusion criteria have been met to ensure concealment.

Duration of study

The enrolment period is expected to be up to 6 months or until the required sample size is reached, and all study subjects will be followed up for 12 months (10 months beyond the final laser treatment). It is therefore anticipated that the entire study will take 18-24 months to complete.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

a) Post-menopausal women who have not had a menstrual period for at least 12 months (with an intact uterus)

   OR

   Women who have previously had a hysterectomy or an endometrial ablation and have an elevation of FSH (>30 IU/mL) consistent with a post-menopausal state.

b) Women with vaginal atrophy symptoms (at least one of vaginal dryness, vaginal burning, vaginal itching, dyspareunia, or dysuria) significant enough for them to present to their healthcare provider.
c) Women who do not wish to use vaginal moisturisers or personal lubricants

   OR

   Women who have previously used vaginal moisturisers or personal lubricants without alleviation of atrophy symptoms

d) Women who do not wish to use topical intra-vaginal oestrogen preparations

   OR

   Women who have previously trialled topical intra-vaginal oestrogen preparations without alleviation of atrophy symptoms or with side effects to the treatment

OR

Women who have contraindications to topical intra-vaginal oestrogen preparations.

e) Women who understand the basis of the study and are willing to participate for the length of the prescribed term of follow-up.
f) Women who are willing and able to give informed consent.

Exclusion Criteria:

a) Use of local hormone replacement therapy in the six months prior to study intervention
b) Use of vaginal moisturisers or personal lubricants in the 30 days prior to study intervention.
c) Current or history of recurrent urinary tract infections.
d) Active genital infections.
e) Pelvic organ prolapse (most substantial prolapse) POPQ system stage 2 or greater.
f) Previous vaginal prolapse surgery or where there is mesh repair in the pelvis.
g) Chronic disease states that may interfere with compliance (including intercurrent malignancy).

STUDY DESIGN

This is a double-blind, placebo controlled randomised trial. A minimum of 78 subjects will be enrolled over an anticipated 6-month recruitment period. All subjects must satisfy the inclusion/exclusion criteria and will be followed for a minimum of 12 months post-initial treatment. Women will be randomised in a 1:1 ratio with at least 38 subjects in each group.

Subjects will be considered for enrolment according to the clinical findings and following informed consent. At the time of consent, each subject will understand their right to know which group they have been randomised to is waived, unless their doctor deems it medically necessary to inform them.

A randomisation schedule will be kept and controlled by the investigator. When the next eligible subject signs the consent form, and meets the inclusion/ exclusion criteria she will be randomised by a third party telephone randomisation service to maintain concealment

to either the MLT procedure or sham groups. Any deviation from the assigned treatment will be reported as a deviation from Protocol.

The MonaLisa Touch™ (MLT) laser system is a commercially available, TGA approved device. All components of the system have been approved for sale and use throughout Australia. The MLT laser system for this study was obtained through the manufacturer's local distributor.

STUDY TREATMENT PROCEDURES

The study treatment procedures will be performed identically for the MLT procedure group and the sham group, except the laser will not be activated during the sham treatment.

Treatment group:

At the time of admission for the procedure, subjects will be brought to the outpatient department at the Royal Hospital for Women where the investigating team will oversee all aspects of treatment and recovery.

1. All demographic and pre-treatment data will be reviewed and entered into the CRF (see attachment).
2. The vaginal probe of the laser is slowly inserted to the vagina until it reaches the cervix or the vaginal vault (dependent on whether the woman has a uterus or not).
3. The laser central unit will be separated from the subject by a curtain divider to ensure the device settings are not visible. A full explanation will be given that the woman may halt the procedure at any time by raising her hand and all treatment will be suspended and clarification of any problems, questions or concerns sought. Following a satisfactory response, the procedure will continue as it did before.
4. The laser will be activated as it is withdrawn from the vagina in order to provide a complete treatment of the vaginal walls.
5. The laser beam reflects off a 45° oriented mirror placed at the tip of the probe so that the uterine cervix is not treated)
6. Energy is applied to the vaginal walls using the probe matrix (this is predetermined by the perforations in the cover that allow the laser energy to be transmitted) that covers the laser probe. This ensures even spacing of the laser energy and prevents confluent areas of vaginal skin being treated. The probe is rotated in a known and identical fashion as it withdrawn from the vagina at regular increments

and the laser refired.

7. The laser settings will be dot power 40 watt, dwell time 1000 µs, dot spacing 1000 µm and the smart stack parameter from 2 on DekaPulse emission mode (fluence 5.26J/cm^2).
8. The total treatment time is anticipated to be approximately 5 min from probe insertion to probe removal
9. There may be discomfort associated with the insertion of the probe and there is minimal pain associated with laser activation.
10. Once the laser procedure is completed, the subject will be accompanied to a recovery area where she will be monitored until she has met the discharge criteria.
11. The woman must void and be comfortable prior to discharge.
12. She will be counselled regarding analgesia, provided with contact numbers in case of emergency and to avoid sexual intercourse for at least 3 days after her treatment since there may be a mild inflammatory reaction that may last up to 48 hours.
13. The treatment requires three laser applications 4 weeks apart.
14. The woman will be rebooked for her subsequent procedure and the above protocol will again occur with her next treatment cycle.

Sham group:

At the time of admission for the procedure, subjects will be brought to the outpatient department at the Royal Hospital for Women where the investigating team will oversee all aspects of treatment and recovery.

1. All demographic and pre-treatment data will be checked.
2. The vaginal probe of the laser is slowly inserted to the vagina until it reaches the cervix or the vaginal vault (dependent on whether the woman has a uterus or not).
3. The laser central unit will be separated from the subject by a curtain divider to to ensure the device settings are not visible. A full explanation will be given that the woman may halt the procedure at any time by raising her hand and all treatment will be suspended and clarification of any problems, questions or concerns sought. Following a satisfactory response, the procedure will continue as it did before.
4. The laser will be activated as it is withdrawn from the vagina in order to provide a complete treatment of the vaginal walls.
5. The sham treatment settings will be dot power 0.5 watt, dwell time 100 µs, dot

spacing 2000 µm and the smart stack parameter from 1 on SmartPulse emission mode, delivering no energy (fluence 0J/cm^2).

6. The total treatment time is anticipated to be approximately 5 min from probe insertion to probe removal
7. There may be discomfort associated with the insertion of the probe
8. Once the laser procedure is completed, the subject will be accompanied to a recovery area where she will be monitored until she has met the discharge criteria.
9. The woman must void and be comfortable prior to discharge
10. She will be counselled regarding analgesia, provided with contact numbers in case of emergency and to avoid sexual intercourse for at least 3 days after her treatment since there may be a mild inflammatory reaction that may last up to 48 h.
11. The treatment requires three complete treatments spaced 4 weeks apart.
12. The woman will be rebooked for her subsequent procedure and the above protocol will again occur with her next treatment cycle.

SUBEQUENT TREATMENTS

The above procedure will be repeated at four-weekly intervals for a total of three complete cycles. Women will be invited to present for their treatment and a research team member who is not performing their treatments will be completing their CRF including symptom questionnaires and quality of life data. The research team completing the CRF pertaining to clinical incidents, side effects or any associated complications will be blinded as to the subject's group allocation at all times.

The MLT or sham treatment will be undertaken by a clinician who is not collecting any data and will be blinded as to the symptom outcomes and data given by the subject. The above protocol relating to the insertion and delivery of laser or sham will be undertaken and the women will receive the same information and follow-up instructions on all three visits.

All follow-up data collection will be taken by a research team member who is not involved in the delivery of the MLT or sham procedure to ensure that there is double blinding.

INFORMED CONSENT

The Investigating team will detail the purpose of the study, proposed duration of the study, follow-up schedule, method of application and randomisation of study groups to the subject prior to her inclusion in the study. The team will discuss foreseeable risks involved in participation, as well as potential benefits that result from the use of the device. The Investigator will also inform all patients that, should an unanticipated adverse device-related event occur during the study that presents unreasonable risks to subjects as determined by the study co-ordinators steering the trial, all study subjects will be notified and study enrolment will be terminated. Subject information will be used during the analysis of the results of the clinical study but the confidentiality of the subject will be maintained at all times. Subjects will be informed by the Investigator that they are free to refuse participation in this study; and if they should participate, that they may withdraw from the study at any time without compromising further medical care. The Investigator or his/her designee will obtain a signed and dated Informed Consent Form from the subject prior to enrolment into this study. The original signed and dated information sheet and subject consent will be kept by the Investigator. A copy will be provided to the patient. A revocation of consent will also be provided to the subject at the time of recruitment and returned at any time during the study. This revocation will be kept on file should the subject withdraw from the study.

DATA CAPTURE AND STORAGE

All data will be recorded on Case Report Forms. The Investigator will complete and sign forms at the time of each protocol-specified visit. The following evaluations will be carried out at the times indicated in the timeline detailed above:

- Inclusion and exclusion criteria
- Demographic details
- Medical history
- Symptom intensity assessment (VAS)
- Vaginal health index
- Quality of life by validated questionnaire (see appendix A)
- Adverse events during the treatment cycle
- Treatment acceptability
- Treatment discomfort

All information on general medical, procedure and device related complications will be documented and tabulated. All information on complications (date of occurrence, description, severity, related to study device, treatment and resolution) will be recorded at the time of occurrence. Any SAE's will be immediately reported to the HREC for consideration and action with study continuation pending review of the SAE.

ENROLLMENT OF SUBJECT

At the time of subject identification, the Investigator will complete a review of the inclusion and exclusion criteria for each individual subject. A pre-operative physical examination will be conducted within 1 month prior to the initial treatment. A subject will be identified as a participant in this clinical trial upon signing a study Informed Subject Consent Form and upon being randomised into the study. The randomisation date will be used as the enrolment date for each patient. Subjects will be blinded as to which group she is assigned to, unless it is deemed medically necessary for her to be told. Should a subject be randomised and withdraw from the study before the initiation of treatment, the reason for withdrawal will be documented and no further follow-up will be obtained.

Contemporaneous collection, transfer and monitoring of clinical trial data will be undertaken. At each intervention, data review and the completion of questionnaires and CRFs follow-up will be assessed. The Principal Investigator will routinely review case Report Forms for completeness and accuracy, as well as any indication of patient risk. Data discrepancies will be resolved with the Investigator. When the data are incomplete, attempts will be made to obtain the data whenever possible.

Monitoring procedures will ensure that each subject returns for her follow-up visits according to the Evaluation Schedule noted in this protocol. Documentation of subjects who voluntarily withdraw from the study or who are lost to follow-up will be obtained on a Study Completion Form.

The Investigation is scheduled for approximately 24 months, with 4-6 months for participant accrual and 12 months for participant treatment and follow-up (10 months follow up after the last of 3 laser treatments).

Subjects will be advised that they may voluntarily withdraw from the study at anytime, for any reason (that they are not obligated to Investigator) and it will not affect their medical care. However, in such cases, appropriate effort will be made to determine the reason for voluntary withdrawal form the study. The Investigator may request the withdrawal from study form from the subject that is attached to the informed consent, noting her desire to withdraw from the study. The last known status of these subjects will be reported with the study results and all attempts to locate subjects lost to follow up will also be documented. Subjects will be informed that should they withdraw from the study they should remain under the care of an appropriately experienced doctor.

The following are circumstances for which a subject would be identified as not continuing her participation in the study:

- Study Completed / Terminated
- Death
- Voluntary Withdrawal
- Unable to Return
- Unwilling to Return
- Intercurrent Illness
- Lost to follow-up

Additionally, the subject may withdraw or be withdrawn from the clinical study for the following reasons:

- The subject may withdraw if she relocates to another geographic area, which requires a change of doctor.
- The subject may withdraw, or be withdrawn by the Investigator, if she is unable to continue participation in the study due to some condition unrelated to this study.

A Study Completion Form will be completed for all subjects who withdraw from the study.

POSTPROCEDURAL COMPLICATIONS/CONCURRENT MEDICAL EVENTS

Post-procedure complications/concurrent medical events will be treated with appropriate medical care and are to be reported on the Case Report Form. Should any unanticipated adverse device effects occur, the Investigator will ensure that these are documented and reported to the reviewing HREC as soon as possible. The Investigators will conduct an

evaluation of such effects and following this evaluation, if it is determined that an unanticipated adverse device effect presents an unreasonable risk to subjects, the study will be terminated and reported to the HREC. Termination shall occur no later than five working days after the Investigator makes this determination and no later than fifteen working days after the Investigator first receives notice of the unanticipated adverse effect. HREC approval will be obtained prior to resuming a terminated Investigation.

STATISTICAL METHODS

Sample Size Calculation

For this study, intensity of vaginal atrophic symptoms such as dryness and itch has been chosen as the primary endpoint. Previous work using this device in this population have reported a decrease in the intensity of these symptoms by at least 50% during the study period of 12 weeks.[2,3] The power calculation made using the ABS calculator, with a treatment effect of 50% reduction in vaginal atrophic symptoms, a SD of 1, a CI of 0.05%, and a dropout rate of 20%, indicates that 48 subjects in total would need to be recruited with 24 in each arm. Dropout rate is approximate only and it is recognised that additional subjects may need to be recruited should the drop-out rate be greater than this number.

Enrolment and Randomisation

At least 78 subjects will be enrolled to this study. Enrolment is defined as completion of informed consent and randomisation. The randomisation procedure to each allocated treatment type (MLT procedure or sham) will be undertaken by a third party to ensure concealment using a computer-generated randomisation block in balanced groups of 8. The randomisation scheme will insure that during the enrolment period the ratio of the number of cases allocated to the two groups remains approximately equal.

Data Analysis

Data from each CRF will be reviewed and entered into a secure electronic database. The data will be summarised and comparisons presented according to treatment type (MLT procedure or sham) for:

1. Visual analogue scale (VAS) scores of symptom intensity
2. Vulvovaginal symptom questionnaire (VSQ) score
3. Vaginal Health Index (VHI) scores
4. Assessment of Quality of Life – AQoL-6D score

5. Monash Female Sexual Satisfaction Questionnaire (FSSQ) score
6. Treatment discomfort (VAS taken at the time of the procedure for pain during insertion of probe and pain during active treatment phase – this will be during probe withdrawal in both groups).
7. Treatment satisfaction as determined on a VAS
8. Treatment complication rate

Frequency and percent distributions will be presented in tabular form for categorical variables and the chi-square test for 2-way contingency tables will be used for comparison of the study groups. Mixed model analyses will be used for outcomes assessing repeated measures of symptom progression throughout the study, including within and intra-group comparison. The mean, median, standard deviation, minimum and maximum will be presented by study group for quantitative variables and the appropriate parametric or non-parametric statistical test will be used, depending on the distribution of the data. Analyses will be undertaken using an intention to treat analysis will be performed using all patients enrolled.

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FINAL STATISTICAL ANALYSIS PLAN

Trial: "A double-blind, randomised, placebo-controlled trial on the efficacy of fractionated laser treatment for vaginal symptoms in postmenopausal women with or without breast cancer."

Principal Investigator	Professor Jason Abbott B Med (Hons) FRANZCOG FRCOG PhD School of Women's and Children's Health UNSW Sydney
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ANZCTR Trial registration number	ACTRN12616001403426, registered 10/10/2016
SAP Version	2.4 Dated 31 August 2020
Protocol Version	7.0 Dated 16 February 2017

1. INTRODUCTION

1.1 Background and rationale

Up to 50% of postmenopausal women are affected by vulvovaginal symptoms associated with menopause (1, 2). Vulvovaginal symptoms impact a woman's physical comfort and can be detrimental to a woman's self-esteem, emotional resilience and sexual function, which can consequently place great stress on relationships, social support and self-confidence (3).

Fractionated CO2 laser is a novel vaginal interventional treatment for a range of clinical conditions, including vaginal symptoms associated with menopause. This procedure is marketed as a 5-minute intra-vaginal laser treatment that provides long-lasting improvements for women suffering from vulvovaginal symptoms of menopause, including vaginal itchiness, burning and dryness, dyspareunia, recurrent thrush and UTI's and vaginal laxity (4). Data to date has reported marked improvement in symptoms, with small prospective studies suggesting at least 50% efficacy of this treatment on vaginal atrophy symptoms (5-10).

However, there is no rigorous scientific evidence to support the use of these technologies for this purpose. High quality data from a randomised, double-blinded placebo-controlled trial is necessary to scientifically evaluate fractionated CO2 laser vaginal treatment against placebo to determine its true efficacy, demonstrate safety, acceptability and longevity of the intervention. This will be beneficial to guide clinical practice.

1.2 Aim

The primary aim of the study will be to investigate the effect of the fractionated CO2 laser treatment on women suffering from postmenopausal vaginal symptoms.

1.3 Research hypothesis

The null hypothesis is that there is no difference between fractionated CO2 laser and placebo in the treatment of vaginal symptoms in postmenopausal women.

1.4 Study objectives

The primary objective of this trial is to determine the effectiveness of the fractionated CO2 laser for postmenopausal vaginal symptoms.
Primary objectives will best be evaluated using quantitative standardised measures before, during and after treatment, for symptomatic comparison:

- Visual Analog Scale (VAS) for symptom severity

- Vulvovaginal Symptom Questionnaire (VSQ) for presence of vulvovaginal symptoms and their impact

Secondary objectives will include evaluation of:

- Other participant-reported outcomes including:
    - Monash Women's Health Program Female Sexual Satisfaction Questionnaire (WHP FSSQ)) for sexual satisfaction and experience
    - Assessment of Quality of Life for general impact on quality of life
    - Participant satisfaction with the treatment
    - Lubricant use
- Physician-reported outcomes
    - Vaginal Health Index (VHI) for features on physical examination, including subjective scoring of moisture, fluid volume, epithelial integrity and elasticity, and objective scoring of pH
- Laboratory-based outcomes
    - Pre- and post-treatment histology for menopausal features of the tissue on histologic examination – classified as 'pre-menopausal', 'early postmenopausal' and 'postmenopausal' by specialist gynaecological pathologist
    - Pre- and post-treatment cytology for Vaginal Maturation Index (VMI) to determine the Vaginal Maturation Value (VMV) by specialist gynaecological pathologist
- Safety outcomes

2. TRIAL METHODS

2.1 Trial design

This study is a double blinded, placebo controlled randomised controlled trial with a 12-month study duration.

2.2 Randomisation

Participants will be randomly allocated to either intervention or placebo arm in a 1:1 ratio to each treatment arm by a computer-generated randomisation program in balanced blocks of 8, with third party telephone randomisation occurring centrally after inclusion and exclusion criteria have been met to ensure concealment.

A randomisation schedule will be kept and controlled by the investigator. Any deviation from the assigned treatment will be reported as a deviation from Protocol.

2.3 Sample size

For this study, intensity of vaginal symptoms associated with menopause, such as dryness and dyspareunia, has been chosen as the primary endpoint. Previous work using this device in this population have reported a decrease in the intensity of these symptoms by at least 50% during the study period of 12 weeks (4, 5). The power calculation made using the ABS calculator, with a treatment effect of 50% reduction in vaginal atrophic symptoms, a SD of 1, a CI of 95%, and a dropout rate of 20%, indicates that 48 subjects in total would need to be recruited with 24 in each arm.

Following review by the ethics committee and independent statistical evaluation, the recommendation for this study was to increase the recruitment sample size to a minimum of 78 subjects, to reduce risk of Type II error.

2.4 Framework

This is a randomised, placebo controlled clinical trial of fractionated CO2 laser compared with placebo treatment for vaginal symptoms of menopause.

2.5 Statistical interim analyses and stopping guidance

We are not intending Interim analyses in this trial, given the anticipated length of this study.

Post-procedure complications/concurrent medical events will be treated with appropriate medical care and are to be reported on the Case Report Form. Should any unanticipated

adverse device effects occur, the Investigator will ensure that these are documented and reported to the reviewing HREC as outlined in the ethics submission. The Investigators will conduct an evaluation of such effects and following this evaluation, if it is determined that an unanticipated adverse device effect presents an unreasonable risk to subjects, the study will be terminated and reported to the HREC. Termination shall occur no later than five working days after the Investigator makes this determination and no later than fifteen working days after the Investigator first receives notice of the unanticipated adverse effect. HREC approval will be obtained prior to resuming a terminated Investigation.

2.6 Timing of final analysis

Final analysis of the outcomes of intervention compared to placebo treatment will occur following completion of data collection. The main report of the trial will be prepared in summary of all data to 12-month follow-up collectively.

2.7 Timing of outcome assessments

	Baseline	Treat 1	Treat 2	Treat 3	F/U 1	F/U 2
Event	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Time (w/m)	**-**	**0w**	**4w**	**8w**	**6m**	**12m**
History	X					
Inclusion/exclusion criteria	X	X				
Demographic data	X					
Symptom assessment VAS	X	X	X	X	X	X
Symptom assessment VSQ	X	X	X	X	X	X
POPQ assessment	X					
VHI Score		X	X	X	X	X
Vaginal biopsy	X				X	
QoL assessment	X				X	X
FSSQ	X	X	X	X	X	X
Lubricant use	X	X	X	X	X	X
Treatment complication evaluation		X	X	X	X	X
Treatment discomfort evaluation		X	X	X		
Treatment acceptability evaluation					X	X

3. STATISTICAL PRINCIPLES

3.1 Confidence intervals and p-values

All applicable statistical tests will be two-sided and will be performed using an alpha significance level of 5%, and beta error of 20%. 95% confidence intervals will be reported. Primary outcomes (VAS, VSQ) will be analysed using repeated measures mixed model method analyses. Secondary outcomes with repeated measures (FSSQ, AqoL-6D, VHI) will be analysed in the same way.

For any other continuous outcomes assessed with repeated analyses, the Holm-Bonferroni method will be used to adjust for multiplicity.

3.2 Adherence and protocol deviation

Compliance is assessed based on the percent of subjects who have taken the scheduled number of treatments. It is defined as: % compliance = (number of treatments taken / number of treatments supposed to have been administered)*100%.

Descriptive statistics on the percent compliance (N, mean, SD, median, minimum, maximum) will be summarised by randomisation group.

The following are pre-defined major protocol violations with a direct bearing on the primary outcome:
1) Use of concurrent vaginal oestrogen therapies during course of the study;
2) Incomplete treatment course or delivery of treatment other than that as per randomisation allocation;
3) Incomplete follow-up at 6-months;
4) Unblinding of participant or assessment doctor, not in accordance with protocol.

Protocol deviations are classified prior to unblinding of treatment. The number (and percentage) of patients with major and minor protocol deviations will be summarised by treatment group with details of type of deviation provided. The patients that are included in the ITT analysis data set will be used as the denominator to calculate the percentages. No formal statistical testing will be undertaken.

3.3 Analysis population

The intention-to-treat population will include all randomised patients, regardless of their eligibility, according to the treatment they were randomised to receive.

4. TRIAL POPULATION

4.1 Reporting of screening data

Potential participants for this trial will be screened based on postmenopausal status, experience of vaginal symptoms due to menopause, and acceptance of conditions of trial (study design; trial length). No screening data will be collected for this trial.

Number of patients screened will be reported in the CONSORT flow diagram.

4.2 Summary of eligibility criteria

Inclusion criteria

Postmenopausal women who have not had a menstrual period for at least 12 months (with an intact uterus) and/or have vulvovaginal symptoms (vaginal dryness; vaginal burning; vaginal itching; dyspareunia or dysuria) associated with menopause, substantial enough for them to present to their health care provider due to symptoms, AND any of 1-4:

1. Women who have previously trialled topical intra-vaginal oestrogen preparations without alleviation of symptoms or with side effects to the treatment
2. Women who have contraindications to topical intra-vaginal oestrogen preparations.
3. Women who have previously had a hysterectomy and have vaginal atrophy symptoms and have an elevation of FSH (>30) consistent with a postmenopausal state.
4. Women who have symptoms of vaginal atrophy and do not wish to use topical oestrogen.

Key exclusion criteria

1. Use of systemic or local hormone replacement therapy in the six months prior to study intervention;
2. Use of vaginal moisturisers or other preparations in the 30 days prior to study intervention;
3. Current urinary tract infection;
4. Active genital infections;
5. Current genital herpes infection;
6. Pelvic organ prolapse (most substantial prolapse) POPQ system stage 2;
7. Previous vaginal prolapse surgery where there is mesh repair in the pelvis; or
8. Chronic disease states which will interfere with compliance to study.

4.3 Information to be included in CONSORT flow diagram

A CONSORT flow diagram will be used to summarise the number of patients who were:

- Assessed for eligibility at screening
- Eligible at screening

- Ineligible at screening
- Eligible and randomised
- Eligible but not randomised
- Received the randomised allocation
- Did not receive the randomised allocation
- Lost to follow-up
- Discontinued the intervention
- Randomised and included in the primary analysis
- Randomised and excluded from the primary analysis
- Reasons will be provided

4.4 Withdrawal/follow-up

Level, timing and reason of withdrawal will be reported in the CONSORT flow diagram, using the categories as follows:

- Withdraw from intervention (no continuation with follow-up)
- Withdraw from follow-up but allow data collected to date to be used
- Withdraw from follow-up and withdraw consent for data collected to date to be used
- Lost to contact/follow-up

The following are circumstances for which a subject would be identified as not continuing her participation in the study:

- Study Completed / Terminated
- Death
- Voluntary Withdrawal
- Unable to Return
- Unwilling to Return
- Intercurrent Illness
- Lost to follow-up

Additionally, the subject may withdraw or be withdrawn from the clinical study for the following reasons:

- The subject may withdraw if she relocates to another geographic area, which requires a change of doctor.
- The subject may withdraw, or be withdrawn by the Investigator, if she is unable to continue participation in the study due to some condition unrelated to this study.

4.5 Baseline patient characteristics

Baseline characteristics to be summarised, separately for two randomised groups:

- Age (years)
- Ethnicity, Caucasian
- Married/de-facto
- Tertiary education
- Age at menarche
- Age at menopause
- Years since menopause
- Induced menopause (chemical or surgical)
- Nulliparous
- Parity
- Sexually active
- Regular alcohol consumption
- Non-smoker, current
- Regular exercise
- Other medical co-morbidities
- Previous hysterectomy

Categorical data will be summarised by numbers and percentages. Continuous data will be summarised by mean, SD and range if data are normal and median, IQR and range if data are skewed. Minimum and maximum values will also be presented for continuous data. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

5. ANALYSIS

5.1 Outcomes

5.1.1 Primary outcome

The primary objective of this Phase II clinical investigation is to evaluate the efficacy of the fractionated CO2 laser procedure for the treatment of vaginal symptoms before, during and after treatment as outlined in Table 1.

Table 1

Outcome	Timing	Measurements/units	Derivation of outcome
VAS	All visits (screening visit; treatment visits 1-3; follow-up visits at 6 and 12 months)	Score out of 100	-
VSQ	All visits (screening visit; treatment visits 1-3; follow-up visits at 6 and 12 months)	Score out of 20	Standard scoring (11)

5.1.2 Secondary outcomes

The secondary objectives include other participant-reported outcomes to determine the impact of vaginal symptoms on menopausal women; physician-reported outcomes determined on physical examination; and laboratory-based outcomes to determine any cytological or histological changes secondary to treatment, as outlined in Table 2.

Table 2

Type	Outcome	Timing	Measurements/ units	Derivation of outcome
Physician-reported outcomes	VHI	Treatment visits 1-3; follow-up visits at 6 and 12 months	Score out of 25 Pre-defined domains out of 5	Standard scoring (12)
Other Participant-reported outcomes	AqoL-6D	Screening visit; follow-up visits at 6 and 12 months	Score out of 100	Standardised scoring (13)
	Monash WHP FSSQ	All visits (screening visit; treatment visits 1-3; follow-up visits at 6 and 12 months)	Score out of 54	Standard scoring (14)
	Treatment discomfort	Treatment visits 1-3	5-point Likert scale	-
	Treatment acceptability	Follow-up visits at 6 and 12 months	5-point Likert scale	-
	Lubricant use	All visits (screening visit; treatment visits 1-3; follow-up visits at 6 and 12 months)	Yes/no	
	Satisfaction	Follow-up visits at 6 and 12 months	5-point Likert scale	-
Laboratory-based outcomes	Pathology	Screening visit; follow-up visit at 6 months	Categorised as 'pre-menopausal; early postmenopausal' or 'postmenopausal'	-
	VMI	Screening visit; follow-up visit at 6 months	Vaginal Maturation index ratio of parabasal : intermediate : superficial cells	Calculation of VMV = (0 x Parabasal % + 0.5 x

				Intermediate % + 1 x Superficial %)

5.2 Analysis methods

The analysis will be performed using an intention-to-treat method.

Frequency and percent distributions will be presented in tabular form for categorical variables and the chi-square test for 2-way contingency tables will be used for comparison of the study groups. All outcomes will be presented using descriptive statistics; normally distributed numerical data by the mean, standard deviation (SD) and range; and skewed distributions by the median and interquartile range (IQR). Binary and categorical variables will be presented using counts and percentages. Shapiro-Wilk test and normality plots will be used to assess the data distribution.

The primary outcomes are represented by continuous data with repeated measures (VAS, VSQ) and will be assessed using mixed model method of analysis to assess outcomes over time. Study subjects will be considered as random effects, treatment group and visit number as fixed effects. The estimated difference in mean change from baseline to 12 months and the corresponding 95 % confidence interval (CI) will be presented.

Secondary outcomes also represented by continuous data with repeated measures (VHI, AQoL-6D, FSSQ, VMV) will be assessed in the same method as the primary outcome. Secondary outcomes represented by categorical data (satisfaction, lubricant use, histological category) will be assessed using Pearson Chi-Square test (or Fisher's exact test if less than 5 in any category).

For related paired samples, continuous variables will be assessed using Wilcoxon signed-rank test for non-parametric data, and t-test for parametric data. Continuous outcomes of independent samples will be analysed using Mann-Whitney-U test for non-parametric data or student's t-test for parametric data. If these analyses are performed on multiple timepoints, they will be adjusted using the Holm-Bonferroni Method to reduce risk of Type 1 error; un-adjusted and adjusted p-values will be reported.

All tests were based on a significance level of 5% (p-value<0.05). SPSS statistical software was used for the analyses.

Absolute risk reduction will be calculated and reported.

5.3 Adjustment for covariates

As this is a randomised study with 1:1 treatment allocation, adjustment for covariate will only be performed if there is a clinically significant difference in the baseline population demographics of each group.

5.4 Sensitivity analyses

A sensitivity analysis will be performed to compare earliest and latest administered treatments, to identify any difference between timing of treatments in relation to operator learning curve.

5.5 Subgroup analyses

Subgroup analyses will be performed to identify any difference between natural and iatrogenic (chemical/surgical) menopause groups. Further subgroup analyses may be considered, in context of outcomes.

5.6 Missing data

Given the primary outcomes were assessed with repeated measures over several timepoints, linear mixed model analysis will be used for analysis using the full data set, through which missing data will be managed by pairwise deletion, prior to maximum likelihood estimation.

5.7 Harms

Adverse events will be collected at visits V2-V6 and reported quantitatively. Moderate and severe adverse events will be escalated to the clinical supervisor. Adverse events will be described quantitatively, and rate of events will be compared between groups using Pearson Chi-square analysis to determine any difference in frequency and severity between groups.

5.8 Statistical software

All analysis will be performed using SPSS statistical analysis software version 26.0 (IBM Corp., Armonk, NY, USA).

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