PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

INSTRUCTIONS

- This should include a referenced systematic evidenced-based review when possible.
- If this study involves qualitative research explain the major constructs of your study.
- Do not state in this section what you plan to do in this study. This information should be entered later under "What will be done in this protocol?"
- Do not include the bibliography in this section.
- For studies submitted under the Expedited review criteria, this section need not be more than a few paragraphs.
- For those studies where data will be analyzed collaboratively by multiple sites doing a similar study for which there is no common protocol (Collaborative Site Analysis Study) include a description of the common scientific goals/ procedures/data points.
- If this is an update to current templates from Protocol Builder make sure the information throughout the protocol includes the most current information.

Answer/Response:

Nitropaste is a topical agent that contains 2% nitroglycerin. It is an effective vascular smooth dilator, with more powerful effect on venous vasculature than arterial vasculature.[1] While its main indication is for angina pectoris, there have been many studies showing improved survival of axial and random pattern flaps.[2,3] Furthermore, recent clinical studies highlight significantly decreased mastectomy flap wound complication and need for sharp debridement.[4,5] Nitropaste has low rates of side effects and is very well tolerated in general.[5,6] To this date, there's no study that investigates its utility on patients who are undergoing chest masculinizing surgery. The purpose of this study is to investigate the potential utility of nitropaste in reducing rates of wound complications in patients undergoing chest masculinizing surgery.

1. Erba M, Jungreis CA, Horton JA. Nitropaste for prevention and relief of vascular spasm. AJNR American journal of neuroradiology 1989;10:155-6.
2. Rohrich RJ, Cherry GW, Spira M. Enhancement of skin-flap survival using nitroglycerin ointment. Plast Reconstr Surg 1984;73:943-8.
3. Khavanin N, Yesantharao P, Kraenzlin F, Darrach H, Sacks JM. Quantifying the Effect of Topical Nitroglycerin on Random Pattern Flap Perfusion in a Rodent Model: An Application of the ViOptix Intra.Ox for Dynamic Flap Perfusion Assessment and Salvage. Plast Reconstr Surg 2021;148:100-7.
4. Gdalevitch P, Van Laeken N, Bahng S, et al. Effects of nitroglycerin ointment on mastectomy flap necrosis in immediate breast reconstruction: a randomized controlled trial. Plast Reconstr Surg 2015;135:1530-9.
5. Wang P, Gu L, Qin Z, Wang Q, Ma J. Efficacy and safety of topical nitroglycerin in the prevention of mastectomy flap necrosis: a systematic review and meta-analysis. Sci Rep 2020;10:6753-.

6. Kutun S, Ay AA, Ulucanlar H, et al. Is transdermal nitroglycerin application effective in preventing and healing flap ischaemia after modified radical mastectomy? South African journal of surgery Suid-Afrikaanse tydskrif vir chirurgie 2010;48:119-21.

Objectives/Hypothesis

INSTRUCTIONS:
If this study involves biomedical research clearly state the objectives and hypotheses and clearly define the primary and any secondary outcome measures. If this study involves qualitative research clearly state your research hypothesis or question.

This section should not include information already included in other sections such as background information or information from the procedures section.

Answer/Response:

Hypothesis: application of nitropaste for those who are undergoing chest masculinizing surgery (CMS) will reduce the rates of wound complication.

Primary outcome: rates of partial nipple graft loss, rates of complete nipple graft loss, rates of wound complications

Secondary outcome: surgical complication including hematoma, seroma, infection, and hypertrophic scarring; rates of sharp debridement; rates of revision; 30 day ED visit rate; 30 day admission rate

Study Design: Biomedical

1. Will controls be used?
Answer/Response: Yes

►IF YES, explain the kind of controls to be used.
Answer/Response: standard of care – chest masculinization surgery without nitropaste

2. What is the study design?

Example: case series, case control study, cohort study, randomized control study, single-blind, double-blind, met-analysis, systematic reviews, other. You may also view the IRB-HSR Learning Shot on this topic to help you answer this question.
Writing a Clinical Research Protocol

Answer/Response:
Randomized control study, single-blinded

3. Does the study involve a placebo?
Answer/Response:
No
►IF YES, provide a justification for the use of a placebo
Answer/Response:

Human Participants

Ages: _18 or older___
Sex: **_Any___**
Race: **_Any___**

Subjects- see below

INSTRUCTIONS: For question 1-4 below insert an exact #. Ranges or OPEN is not allowed. This # should be the maximum # you expect to need to enroll (i.e. sign consent) If you are only collecting specimens the number of participants should equate to the # of specimens you need. If you are collecting only data from a chart review the number should designate the number of subjects whose medical records you plan to review. Age/ Sex/Race criteria should designate the demographics of participants from whom you will obtain the specimen/data.

1. **Provide target # of subjects (at all sites) needed to complete protocol.**

   INSTRUCTIONS: If this is NOT a database protocol, this number should be the same as the number of subjects needed to obtain statistically significant results.

   Answer/Response:
   256 participants

2. **Describe expected rate of screen failure/ dropouts/withdrawals from all sites.**

   Answer/Response:
   Minimal, <5%

3. **How many subjects will be enrolled at all sites?**

   INSTRUCTIONS: This number must be the same or higher than the # from question # 1 in order to account for the # of screen failures, dropouts, withdrawals described in question # 2.

   Answer/Response:
   UVA, 270

4. **How many subjects will sign a consent form under this UVA protocol?**

   INSTRUCTIONS: If the protocol does not have a consent form- the number listed here should reflect such things as the number of subjects from whom specimens will be obtained, the number of charts to be reviewed etc.

   Answer/Response:
   All participants, 270

Inclusion/Exclusion Criteria

INSTRUCTIONS:

- The inclusion and exclusion criteria should be written in bullet format.
- *This item applicable if the study will require consent (verbal or written).* Unless there is a scientific reason for not recruiting a certain type of vulnerable population(e.g. not

enrolling fetuses, neonates or children in a study regarding Alzheimer's) list the following vulnerable populations under either Inclusion or Exclusion criteria below: pregnant women, fetuses, neonates, children, prisoners, cognitively impaired, educational or economically disadvantage, non- English speaking subjects .

- If you will not enroll subjects who do not speak English because certain procedures cannot be carried out if the subject does not speak English (e.g. a survey is not validated in other languages) insert the following as an Inclusion Criteria: Willingness and ability to comply with scheduled visits and study procedures.
- If this is a collection of only retrospective* specimens or data, the inclusion criteria must include a start and stop date for when specimens/ data will be collected.
- The stop date must be prior to the version date of this protocol.
- *Retrospective: all specimens are in a lab at the time this protocol is approved by the IRB. All data exists in medical records or records from previous studies at the time this protocol is approved by the IRB.

1. List the criteria for inclusion

Answer/Response:

- Adult (18 or older)
- Patients of any gender identity who are undergoing chest masculinizing surgery with double incision and free nipple grafting technique.

2. List the criteria for exclusion

Answer/Response:

- patients younger than age 18,
- anyone who's not getting free nipple grafting,
- anyone who's not utilizing double incision pattern,
- prisoners,
- anyone who is allergic to nitropaste,
- anyone who is taking phosphodiesterase inhibitor (ex)Sildenafil, tadalafil, vardenafil),
- anyone who's taking soluble guanylate cyclase stimulator riociguatdz
- anyone who is pregnant

3. List any restrictions on use of other drugs or treatments.

INSTRUCTIONS: List only those drugs or treatments that are prohibited while on study, not those listed as an exclusion criteria.

Answer/Response:

None

Statistical Considerations

1. Is stratification/randomization involved?

Answer/Response:

Yes

►**IF YES, describe the stratification/ randomization scheme.**

INSTRUCTIONS:

The stratification factors and/or the randomization plan should be identified. If there is no randomization component or important patient characteristics that will be used in treatment allocation or data analysis, a statement to this effect should be included.

Stratification factors: These are pretreatment patient characteristics which could be balanced across treatment arms by design or may be used to determine starting dose or treatment allocation.

If randomization is going to be used, the details of the randomization plan should be described.

The description should include:
--the method and timing of randomization
--the type of randomization scheme that will be used in the study
--whether or not the randomization masked/blinded/if so, then to whom is it masked/blinded
--who has access to the randomization scheme

Answer/Response:
Randomization will occur at the time of surgery. Random number generator will be used and even number will indicate control group while odd number will indicate experimental group. Randomization will be blinded to the patient.

►**IF YES, who will generate the randomization scheme?**

_____ Sponsor
_____ UVA Statistician. Insert name Answer/Response:
_____ UVA Investigational Drug Service (IDS)
__x___ Other: Specify Answer/Response: random number generator

2. What are the statistical considerations for the protocol?

The objectives section and the statistical section should correspond, and any objective for which analysis is unfeasible should be deleted. Also, the estimates and non-statistical assumptions of the statistical section should be supported by discussion in the background section.

The answer to this question should include:
--Study Design/Endpoints
--Recap of study objectives and endpoint definitions. An assessment of how study objectives will be assessed by identifying & defining which endpoints will be used to assess each component of the study objectives.
--The study design should include contingencies for early stopping, interim analyses, stratification factors (If applicable), and any characteristics to be incorporated in analyses.
--The power/precision of the study to address the major study endpoint(s), the assumptions involved in the determination of power/precision.

--If statistical hypothesis testing is included then specify the null and alternative hypotheses, the test statistic, and the type I and II error rates
--If precision of an estimate, then provide a definition for precision
--If other, then specify

Answer/Response:

The study will continue until we reach the goal number of participants of 256 participants. We will do interim analysis semi-annually to track progress and see if there's any significant and obvious benefit to the experimental group. If there's a statistically significant benefit to the experimental group, we may stop the study early and implement nitropaste as part of standard of care. Answer for question 3 will answer questions regarding the goal sample size.

3. Provide a justification for the sample size used in this protocol.

Include sample size calculations or statistical power estimation. If not applicable, please provide explanation.
Also include the anticipated accrual rate, the accrual goal for the study, including accrual goals by strata if appropriate, adjustments for drop-outs etc. and study duration.

Answer/Response:

Sample size was calculated using a two-sample comparison of proportions power calculation with power of 0.8 and significance level of 0.05. Based on the literature, we assumed any wound complication rate would be about 15% and nitropaste would reduce it by 50% (8%). This resulted in goal sample size of 256.

4. What is your plan for primary variable analysis?

Include primary outcome(s)/predictor variable(s), statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.

Answer/Response:

Primary outcomes include any wound complication (partial nipple graft loss, complete nipple graft low, superficial wound complication, deep wound complication, and delayed wound healing). We will compare the wound complication rates between the control vs. experimental groups and look for any statistical significance.

5. What is your plan for secondary variable analysis?

Include the following:
--Secondary outcome(s)/predictor variables, statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.
--For phase III studies, the power/precision of the study to address the secondary objective(s).

Answer/Response:

Secondary outcomes include any complications (hematoma, seroma, infection, hypertrophic scarring), sharp debridement rate, revision rate, and 30 day ED visit/ admissions.). We will compare the rates between the control vs. experimental groups and look for any statistical significance

6. Have you been working with a statistician in designing this protocol?

Consultation with a professional statistician is highly recommended to ensure good science of the study and facilitate the review process.

Answer/Response:
Yes

IF YES, what is their name?
Answer/Response: Clay Ford

7. Will data from multiple sites be combined during analysis?

Answer/Response: No

INSTRUCTIONS: IF YES, answer the following questions

7(a). Does the study involve randomization?

Answer/Response:

IF YES, will randomization be done at each site or among sites?

Answer/Response:

7(b). Has the sample size calculation considered the variation among sites?

Answer/Response:

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?

Answer/Response:

7(d). Is there a common protocol used in all sites?

Answer/Response:

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?

Answer/Response:

Study Procedures-Biomedical Research

1. What will be done in this protocol?

INSTRUCTIONS:
This should include everything that will be done as part of this protocol. Do not repeat information that is included in other sections such as Background or Hypothesis sections.

This section should include an indication of which research interventions if any offer a prospect for direct benefit and which interventions (invasive measurements, collection of blood, tissue, data, surveys, etc.) are being done solely to answer a research question and generate generalizable knowledge. If the interventions done solely for research purposes are associated with greater than minimal risk they need to be justified. Describe and justify any control and experimental arm and include method, dose, and duration of drug administration. Reference any claim of clinical equipoise if applicable.

If you are obtaining specimens or data, provide information regarding the type of specimen/data, amount of specimen needed and how the specimen/data will be obtained and what analysis will be done with the specimen/data.

Special note for studies with waiver of consent/waiver of documentation of consent: Include a statement regarding how subjects will be recruited. For other studies this information is captured in Recruitment does not need to be duplicated in this section.

Answer/Response:

Once a participant enrolls and signs the consent, randomization will be done on the day of surgery. The Study team will review potential participant's medical history and also go over the inclusion/exclusion criteria and any contraindications to nitropaste use with the subject to determine their eligibility. Those who are in control group will get standard chest masculinization surgery with double incision and free nipple grafting. The standard surgical dressing will consists of Xeroform bolster over the nipple grafts, telfa strips over the incisions, and then Tegederm over the bolster/ chest to hold everything in place. Those who are in experimental group they will get one time application of nitropaste around the free nipple graft/ chest intraoperatively (1 packet on each side = 15 mg x 2 = 30mg total) and get the same dressing applied. Surgical dressing will be identical for both groups and patients will be instructed to keep it on for 5 days until they come to clinic. At this time all dressing will be removed and their surgical sites will be examined. All participants will follow up at 2 weeks and 6 week post and again their surgical sites will be examined. Any secondary outcomes will be recorded. All patients will be followed up to 3 months post-op and any complications will be recorded.

2. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.**

Example: If the subject will be taking an investigational drug, will they need to be put back on an approved drug when they have completed the study? If yes, explain how this will be accomplished and who will cover the cost. If the subject has a device implanted will it be removed? Again- who will cover the cost of the removal?
Instructions: Answer NA if this study does not involve a study treatment.

Answer/Response: N/A

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.**
   (e.g. study team will administer study drug/ study interventions, study drug inventory of dispensed and returned drug, diary etc.)
   Answer/Response:
   The application of nitropaste is the only study procedure. The study team monitor for compliance with standard care postoperative instructions per documentation in the medical record.

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**
   (e.g. subject returns more than 20% of the study drug, subject misses 20% of study visits)
   Answer/Response: The study team will be able to identify whether the dressing was taken off prematurely and surgical site washed or not when they present to the clinic, which would be the only "noncompliance" event that the patient can have. Subject who remove the dressing prematurely and wash the surgical site will be removed from the study

Bibliography

INSTRUCTIONS: Provide a current bibliography supporting the hypothesis, background and methodology including references to papers and abstracts that have resulted from previous work by the investigator and references to the work of others.

1. Erba M, Jungreis CA, Horton JA. Nitropaste for prevention and relief of vascular spasm. AJNR American journal of neuroradiology 1989;10:155-6.
2. Rohrich RJ, Cherry GW, Spira M. Enhancement of skin-flap survival using nitroglycerin ointment. Plast Reconstr Surg 1984;73:943-8.
3. Khavanin N, Yesantharao P, Kraenzlin F, Darrach H, Sacks JM. Quantifying the Effect of Topical Nitroglycerin on Random Pattern Flap Perfusion in a Rodent Model: An Application of the ViOptix Intra.Ox for Dynamic Flap Perfusion Assessment and Salvage. Plast Reconstr Surg 2021;148:100-7.
4. Gdalevitch P, Van Laeken N, Bahng S, et al. Effects of nitroglycerin ointment on mastectomy flap necrosis in immediate breast reconstruction: a randomized controlled trial. Plast Reconstr Surg 2015;135:1530-9.
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6. Kutun S, Ay AA, Ulucanlar H, et al. Is transdermal nitroglycerin application effective in preventing and healing flap ischaemia after modified radical mastectomy? South African journal of surgery Suid-Afrikaanse tydskrif vir chirurgie 2010;48:119-21.