Protocol: Vasculera® in patients with Lipedema: an exploratory controlled case study

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Vasculera® in patients with Lipedema: an exploratory controlled case study

Protocol #: PLP-01

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Date: March 19, 2019

Version: 2

Protocol Signature and Financial Disclosure Page

This protocol has been written by Primus Pharmaceuticals, Inc.,
Director of Clinical Development, Robert M. Levy, M.D.

Robert M. Levy, M.D.

Director of Clinical Development signature Date

I have read and I understand all the provisions and requirements of this protocol,

Vasculera® in patients with Lipedema: an exploratory controlled case study, PLP-01 I agree to perform the investigator's responsibilities exactly as set forth in the protocol according to the principles of Good Clinical Practice. I further understand that all information pertaining to this protocol is confidential and may not be disclosed in personal communications or professional presentations without the prior written consent of Primus Pharmaceuticals, Inc.

Investigator signature Date

I certify that, other than the investigator stipend agreed upon in the contract and budget for this study, I have no other financial interest in Primus Pharmaceuticals, Inc. or any of its present or future products.

Investigator signature Date

Vasculera® in Patients with Lipedema: an exploratory controlled case study

Synopsis

Protocol #: PLP-01

Background

Lipedema is a condition characterized by abnormal accumulation of subcutaneous fat, predominantly in the legs, often associated with interstitial edema. The condition causes disfigurement, pain, functional impairment, emotional disturbance and marked reduction in overall quality of life. The cause is unknown but is believed to involve both genetic and acquired factors and may involve defective vascular function. Treatment modalities consist of surgical removal of large fatty deposits and decompression therapy such as compression garments, manual lymphatic drainage, and home sequential pneumatic compression devices; dietary control is rarely effective (Buck 2016).

Vasculera® is a prescription medical food product marketed for management of chronic venous insufficiency (CVI) and all its complications, including venous ulcers, and for edema of many etiologies (Lyseng-Williamson 2003). Diosmin, the primary ingredient in Vasculera, has been used in Europe for 40 years as a drug (Daflon®) for treatment of CVI. Its hemodynamic and molecular activities on veins, lymphatics, microvasculature and endothelial cells have been studied for many years (Colleridge-Smith 2000, Ramelot 2005). Anecdotal reports from physicians currently prescribing Vasculera and the use of diosmin in the Treatment, Research and Education of Adipose Tissue (TREAT) program at the University of Arizona, College of Medicine suggest that diosmin may have benefit in reducing the signs and symptoms of lipedema at doses of 500 and 600 mg once or twice daily. (Medications and Supplements 2019).

The glycocalyx is an ultrathin proteoglycan membrane covering all endothelial surfaces (Alphonsus 2014, Pillinger 2017). Although described more than 60 years ago, it is only within the past ~15 years that rigorous investigation of glycocalyx structure and function has been accomplished. Although endothelial cells have been generally thought to be a site of action for diosmin (Shoab 1999), several of the known features of the mechanism of action of diosmin and of glycocalyx activity suggest that the primary target of diosmin may be the glycocalyx rather than the endothelial cell itself. There are several commonly measured entities, of which we have chosen to measure two, syndecan-1 and heparan sulfate as exploratory outcomes (Mensah 2017).

Study design

This will be a three (3) month open-label case study. Patients with lipedema (see Appendix III for diagnostic criteria) present for at least one (1) year who have not taken any diosmin containing medications within the 1 year prior to the screening visit and meet all other inclusion and exclusion criteria will be eligible for participation. At the screening visit patients will have the study explained to them and those wishing to participate will sign an Informed Consent Document (ICD). Demographic information will be recorded, measurements of vital signs, weight, and Body Mass Index (BMI) and leg circumference will be recorded. Subjects will complete a Short WOMAC and complete a series of assessments on numerical rating scales of 0 to 10, as listed below under efficacy measurements. Women of child bearing potential will have a urine pregnancy test performed, results of which must be negative to continue in the study. A blood sample for markers of glycocalyx function will be drawn. Subjects will then take one (1) Vasculera tablet BID for the duration of the study. Subjects will return to the clinic monthly at which times they will again complete the rating scales. At the final visit a blood sample for glycocalyx function markers will again be drawn and investigators will complete a 5-point Response to Therapy Likert scale. Adverse events will be recorded at all follow-up visits.

If subjects are receiving any form of manual leg decompression therapy, the type and frequency should remain unchanged for one (1) week prior to the baseline visit and for the duration of the study. In addition, subjects should not have any decompression for one (1) week prior to any study related clinic visit.

Study objectives

This case study is designed to gain preliminary information via a uniform protocol regarding the clinical effects of Vasculera in patients with lipedema and the possible role of the glycocalyx as a physiological target for Vasculera activity. It is anticipated that the results of this case study will inform the development of a formal randomized, double-blind, placebo controlled trial.

Efficacy measurements

1- Subject weight
2- Leg circumference 2 and 12 inches above lateral malleoli of both legs
3- BMI
4- Short WOMAC
5- Subject-assessments, on a 0-10 rating scale, for:

- leg discomfort
- feeling of leg swelling/tightness
- leg tenderness
- leg bruising
- leg skin redness
- fatigue
- ability to perform activities of daily living (ADL)
- overall sense of well-being

6- Investigator assessment of response to therapy by 5-point Likert scale

Exploratory Measures
Plasma glycocalyx markers

Safety measurements
Adverse event recording

Study product dosing and rationale
Vasculera 630 mg BID
Vasculera is marketed for management of venous insufficiency at a dose of 630 mg (one tablet, 95% purity) daily. Most of the literature, almost all of which is from historical use in Europe uses doses of 500 mg (90% purity) BID. The literature suggests that 600 mg of 95% product is the equivalent of 500 mg of 90% product for treatment of the early stages of CVI but anecdotal evidence suggest that BID dosing of Vasculera performs better than QD dosing in more severe stages of CVI and other disorders.

Inclusion criteria
1- established diagnosis of lipedema (see Appendix III) for at least one (1) year
2- women, ages 20 to 70 years
3- score of at least 4 out of 10 on a rating scale of 0-10 (10=worst) for overall sense of well being
4- able to read and understand Informed Consent Document (ICD) and all questionnaires in English or with family/friend translator
5- not pregnant or lactating; women of child bearing potential must use an approved method of birth control (Appendix II).
6- be willing to stop compression therapy for one week prior to each visit

Exclusion criteria
1- other forms of leg enlargement, including lymphedema without coexisting lipedema
2- any primary systemic vasculopathy
3- history of exposure to Vasculera or other diosmin containing medication within one (1) year of the screening visit
4- concomitant use of warfarin, platelet inhibitors, factor Xa inhibitors or any medication intended to reduce blood coagulability
5- concomitant use of diclofenac, metronidazole or chlorzoxazone
6- uncontrolled hypertension (BP>170/110), unstable cardiac disease, active skin ulceration
7- any other disease or condition that, in the opinion of the investigator, might put the subject at risk by participation in this study OR confound evaluation of response to Vasculera
7- history of substance abuse within one (1) year of the screening visit or of current alcohol consumption more than one (1) unit daily. For purposes of this study, a unit of alcohol will be considered to be 12 oz of beer, 6 oz of wine or 1 ^ oz of hard spirits.

Number of subjects
It is estimated that 30 subjects will be sufficient to obtain a meaningful efficacy signal.

Length of study
It is estimated that it will take 2 months to recruit the required number of subjects. The total length of the

study is anticipated to be five (5) months.

Statistical considerations

Descriptive statistics will be presented for all relevant variables including demographic variables such as age, sex, weight and BMI for each group. Descriptive statistics for baseline and endpoint values for all study variables, including weight, BMI leg circumference, short WOMAC, investigator Likert and all subject reported outcomes per section 4.2 will also be presented. Data from baseline and final visit will be compared by Student's t-test (paired). P values </= 0.05 will be considered of significance.

Vasculera® in Patients with Lipedema: an exploratory case controlled study

Protocol #: PLP-01

1.0 Background

Lipedema is a condition characterized by abnormal accumulation of subcutaneous fat, predominantly in the legs, often associated with interstitial edema. The condition causes disfigurement, pain, functional impairment, emotional disturbance and marked reduction in overall quality of life. The cause is unknown but is believed to involve both genetic and acquired factors and may involve defective vascular function. Treatment modalities consist of surgical removal of large fatty deposits and decompression therapy such as compression garments, manual lymphatic drainage, and home sequential pneumatic compression devices; dietary control is rarely effective (Buck 2016).

Vasculera® is a prescription medical food product marketed for management of chronic venous insufficiency (CVI) and all its complications, including venous ulcers, and for edema of many etiologies (Lyseng-Williamson 2003). Diosmin, the primary ingredient in Vasculera, has been used in Europe for 40 years as a drug (Daflon®) for treatment of CVI. Its hemodynamic and molecular activities on veins, lymphatics, microvasculature and endothelial cells have been studied for many years (Colleridge-Smith 2000, Ramelot 2005).
Anecdotal reports from physicians currently prescribing Vasculera and the use of diosmin in the Treatment, Research and Education of Adipose Tissue (TREAT) program at the University of Arizona, College of Medicine suggest that diosmin may have benefit in reducing the signs and symptoms of lipedema at doses of 500-600 mg once or twice daily. (Medications and Supplements 2019).

The glycocalyx is an ultrathin proteoglycan membrane covering all endothelial surfaces (Alphonsus 2014, Pillinger 2017). Although described more than 60 years ago, it is only within the past ~15 years that rigorous investigation of glycocalyx structure and function has been accomplished. Although endothelial cells have been generally thought to be a site of action for diosmin (Shoab 1999), several of the known features of the mechanism of action of diosmin and of glycocalyx activity suggest that the primary target of diosmin may be the glycocalyx rather than the endothelial cell itself. Several components of GCX structure which are liberated into blood are commonly measured to assess GCX status. In this study, blood levels of syndecan-1 and heparin sulfate will be measured as exploratory outcomes (Mensah 2017).

2.0 Study objectives

This exploratory case study is designed to gain preliminary information via a uniform protocol regarding the clinical effects of Vasculera in patients with lipedema and the possible role of the glycocalyx as a physiological target for Vasculera activity. It is anticipated that the results of this case study will inform the development of a formal randomized, double-blind, placebo controlled trial.

3.0 Study product

All subjects will receive Vasculera 630 mg BID. No dose modifications are anticipated.

3.1 Dose rationale

Vasculera is marketed for management of venous insufficiency at a dose of 630 mg (one tablet, 95% purity) daily. Most of the literature, almost all of which is from historical use in Europe, uses doses of 500 mg (90% purity) BID. The literature also suggests that 600 mg of 95% product is the equivalent of 500 mg of 90% product for treatment of the early stages of CVI but anecdotal evidence suggest that BID dosing of Vasculera performs better than QD dosing in more severe stages of CVI and other disorders. Anecdotal reports from physicians currently prescribing Vasculera and the use of diosmin in the Treatment, Research and Education of Adipose Tissue (TREAT) program at the University of Arizona, College of Medicine suggest that diosmin may have benefit in reducing the signs and symptoms of lipedema at doses of 500-600 mg once or twice daily. (Medications and Supplements 2019).

4.0 Study design

This will be a three (3) month open-label case study. Patients with lipedema (see appendix III for diagnostic criteria) present for at least one (1) year who have not taken any diosmin containing medications within the 1 year prior to the screening visit and meet all other inclusion and exclusion criteria will be eligible for participation. At the baseline visit and all follow-up visits subjects will complete a Short WOMAC and self-assessments on a 0-10 rating scale for:

- leg discomfort
- feeling of leg swelling/tightness
- leg tenderness
- leg bruising
- leg skin redness
- fatigue
- ability to perform activities of daily living (ADL)
- overall sense of well-being

If subjects are receiving any form of decompression therapy such as compression garments, manual lymphatic drainage, or home sequential pneumatic compression devices; the type and frequency should remain unchanged for at least one (1) week prior to the baseline visit and for the duration of the study. In addition, subjects should not have any decompression for one (1) week prior to any study related clinic visit.

A blood sample (10 cc) will be drawn for glycocalyx markers (heparin sulfate and syndecan-1) at the baseline and final (3 month) visits. Investigators will complete a 5-point Likert scale for response to therapy at the 3 month visit.

4.1 Concomitant medications and procedures

All subjects may continue their usual medication regimen, including diuretics, but the dose of these should

remain constant throughout the duration of the study. Similarly, subjects receiving decompressive therapy should continue their usual regimen for the duration of the study. Any changes in medication or procedural regimen should be noted on the appropriate case report form together with the reason for the change.

4.2 Efficacy measurements

1- Subject weight
2- Leg circumference 2 and 12 inches above malleoli
3- BMI
4- Short WOMAC
5- Self-assessment on a 0-10 rating scale for:

- leg discomfort
- feeling of leg swelling/tightness
- leg tenderness
- leg bruising
- leg skin redness
- fatigue
- ability to perform activities of daily living (ADL)
- overall sense of well-being

6- Investigator assessment of response to therapy by 5-point Likert scale

Exploratory Measure
Plasma glycocalyx markers

4.3 Safety measurements

Adverse event recording

4.4 Number of subjects

It is estimated that 30 subjects will be sufficient to obtain a meaningful efficacy signal.

5.0 Study selection criteria

5.1 Inclusion criteria

1- established diagnosis of lipedema (see appendix III) for at least one (1) year
2- women, ages 20 to 70 years
3- score of at least 4 out of 10 on self-assessment on a 0-10 scale (10=worst) for overall sense of well being
4- able to read and understand all questionnaires in English or with family/friend translator
5- not pregnant or lactating; women of child bearing potential should use an approved method of birth control for the duration of the study

6- be willing to stop compression therapy for one week prior to each visit

5.2 exclusion criteria

1- other forms of leg enlargement, including lymphedema
2- any primary systemic vasculopathy
3- history of exposure to Vasculera or other diosmin containing medication within one (1) year of the screening visit
4- concomitant use of warfarin, platelet inhibitors, factor Xa inhibitors or any medication intended to reduce blood coagulability
5- concomitant use of diclofenac, metronidazole or chlorzoxazone
6- uncontrolled hypertension (BP>170/110), unstable cardiac disease, active skin ulceration
7- any other disease or condition that, in the opinion of the investigator, might put the subject at risk by participation in this study OR confound evaluation of response to Vasculera
8- history of substance abuse within one (1) year of the screening visit or of current alcohol consumption more than one (1) unit daily. For purposes of this study, a unit of alcohol will be considered to be 12 oz of beer, 6 oz of wine or 1 'A oz of hard spirits.

6.0 Study procedures

6.1 Screening visit

At the screening visit patients will have the study explained to them and those wishing to participate will sign an Informed Consent Document (ICD). Prospective subjects must be allowed to take the ICD home prior to signing to consider and/or discuss with family, if they wish to do so. Subject eligibility will be determined according to the established inclusion and exclusion criteria. Women of child bearing potential (WOCBP) will have a urine pregnancy test performed, results of which must be negative to continue in the study.

6.2 Baseline visit (visit 1)

The baseline visit may occur at the same time as the screening visit if the subject wishes to participate, signs the ICD and meets all study entry requirements. The following procedures will then be performed:

1- Brief physical examination
2- urine pregnancy test for WOCBP
3- Medical history and current medications
4- Vital signs (blood pressure, pulse)
5- Subject weight and height
6- Leg circumference 2 and 12 inches above the lateral malleoli on both legs
7- BMI (see appendix V)
9- Blood draw (10 cc) for glycocalyx markers
8- Short WOMAC
10- Self-assessment on a 0-10 rating scale for:

- leg discomfort
- feeling of leg swelling/tightness

- leg tenderness
- leg bruising
- leg skin redness
- fatigue
- ability to perform activities of daily living (ADL)
- overall sense of well-being

11- dispense study medication

6.3 Visit 2 (1 month)

At the one (1) month visit, the following procedures will be performed.

1- Vital signs
2- Subject weight
3- Leg circumference 2 and 12 inches above the lateral malleoli on both legs
4- All self-assessments as above
5- Record adverse events
6- Record changes/new medications
7- Collect and count used study medication
8- Dispense new bottle of study medication

6.4 Visit 3 (2 month)

At the two (2) month visit, the following procedures will be performed.

1- Vital signs
2- Subject weight
3- Leg circumference 2 and 12 inches above the lateral malleoli on both legs
4- All self-assessments as above
5- Record adverse events
6- Record changes/new medications
7- Collect and count used study medication
8- Dispense new bottle of study medication

6.5 Visit 4 (3 month, final)

1- Brief physical examination
2- vital signs (blood pressure, pulse)
3- Subject weight
4- Leg circumference 2 and 12 inches above the lateral malleoli on both legs
5- BMI (see appendix XXX for table)
6- Blood draw (10 cc) for glycocalyx markers
7- Investigator Likert for response to therapy

8- All self-assessments, as above
9- Record adverse events
10- Record changes/new medications
11- Collect and count used study medication

7.0 Study Withdrawal

Subjects may be withdrawn from the study for one or more of the following reasons:

- Noncompliance with the protocol
- Unacceptable adverse event. In this case the decision to remove the subject from the study may be made by the subject, the investigator or Primus.
- Determination by the investigator or Primus that termination of the subject's participation in the study is in the subject's best medical interest or for an administrative reason other than an adverse event.
- The use of any excluded medication. If the subject is discontinued prior to receiving the first dose of study drug AEs and SAEs will not be collected. If the subject has received study medication, AEs and SAEs will be collected until the termination visit or until 30 days after the last dose of study drug, whichever is longer. All AEs and SAEs must be followed until resolution or stability.
- Request for withdrawal by the subject for any reason whether or not related to an AE.
- Lost to follow-up.

According to FDA administrative law and the Declaration of Helsinki a subject in a clinical trial has the right to withdraw at any time and for any reason without prejudicing his/her future medical care at the investigative site. Investigators and sponsors may withdraw subjects because of intercurrent illness, AEs, treatment failure, protocol violation or other administrative reasons. In the case of early withdrawal of a subject, the investigator should make all reasonable efforts to have the subject attend a termination visit and document all details of the early termination on the appropriate CRF.

8.0 Early Termination Visit Procedures

If a subject wishes to end his/her participation in the study prematurely, whether because of an AE or any other reason, he/she should be asked to complete a termination visit at the next scheduled appointment or within 14 days, whichever is more convenient. The investigator should make every reasonable attempt to perform the final assessment. Whenever possible, all final visit procedures should be performed at the time of an early termination visit. All outstanding AEs should be followed to resolution or stability. The reason for early termination should be clearly stated on the Early Termination CRF.

9.0 Withdrawals Due to Adverse Events

All adverse events that result in a subject's withdrawal from the study must be reported to the Primus Director of Clinical Development, Robert Levy, MD, (480-415-6779) within 24-hours for SAEs and ten working days for non-serious AEs.

10.0 Definition of Adverse Event

An AE is any untoward or unexpected medical occurrence in a subject participating in a clinical study whether or not there is a possible relationship between the investigative agent and the event. For purposes of this study, AE collection will begin after the first dose of study product.

10.1 Serious Adverse Events

SAEs require special attention, management and documentation. All SAEs must be reported to Primus within 24 hours **whether or not** they are considered by the investigator to be related to the study agent (s).

An SAE is any medical event that:

- results in death
- is life threatening—the investigator believes the subject's life is in immediate danger (This does not include events that, had they been more severe, might have been life threatening)
- requires in-patient hospitalization or prolongation of an existing hospitalization
- results in permanent disability
- is a congenital anomaly or birth defect
- requires medical or surgical intervention to prevent one of the outcomes above

Although cancer is no longer considered an SAE by the FDA, Primus requires notification of such diagnoses within 48 hours of the time the investigator first learns of the event.

10.2 Severity of Adverse Events

The investigator must rate every AE for severity according to the following guidelines:

1 = Mild	The event does not interfere with daily activities.
2 = Moderate	The event discomfort and/or dysfunction is enough to interfere with, but not prevent, daily activities.
3 = Severe	The event causes significant impairment of ability to perform daily activities.
4 = Life-threatening	The event, if, in the opinion of the investigator or sponsor places the subject at immediate risk of death.

10.3 Assessment of Causality

Investigators will be responsible for determining the relationship of the study agent(s) to the AE and will record their conclusions on the CRF adverse event page. It is recognized that causality often cannot be established with certainty and relies on clinical judgement.

1 = Definitely Related	After careful medical evaluation there is little or no doubt that the study agent is the direct etiologic cause of the AE. This situation is most commonly seen when an AE recurs after re-challenge with study agent.
2 = Probably Related	After careful scrutiny of the available medical facts, there is a reasonable, but inconclusive, certainty that the event is related to the study agent.
3 = Possibly Related	Those events for which other etiologies cannot be clearly demonstrated and for which a relationship to the study agent cannot be positively excluded.
4 = Unlikely	Those AEs for which other causes are more likely.
5 = Not Related	Those AEs for which other etiologies can clearly and conclusively be defined. Investigators must use their best clinical judgment keeping in mind the following questions:

- Was study drug administered
- What was the temporal relationship between the administration of study drug and the AE? Was this consistent with the nature of the AE?
- Was there another obvious or possible cause for the AE?
- Was the AE consistent with the known effects of the study drug?
- Were there any features of the subject's clinical state, concomitant medications or diseases or environmental factors that could have caused or modified the AE?
- Did the AE resolve, improve, remain stable or worsen after the study drug was stopped or the dose reduced?
- If the subject was re-exposed to the study agent (re-challenge), did the AE recur, worsen or remain unchanged? Re-challenge should be attempted only after careful consideration and only after discussion with Primus.

10.4 Reporting Serious Adverse Events

SAEs must be reported to Primus by telephone within 24 hours from the time the investigator first learns of them. This should be followed by a faxed report outlining the details of the event and the investigator's assessment of the severity and relationship of the event to the study agent. All SAEs will be reported on both the AE and SAE pages of the case report forms. In addition:

- A new SAE form must be completed and faxed to Primus within 24 hours
- Follow-up SAE forms must be completed and faxed to Primus whenever new information becomes available about the event
- A final SAE report must be completed and faxed to Primus when the event has resolved or its consequences have stabilized and all relevant information (including hospital records, laboratory and pathology reports, autopsy reports, etc.) have been received. It is the responsibility of the investigator to obtain copies of all relevant information in a timely manner.
- It is the responsibility of the investigator to determine the severity and causality of the SAE.
- The initial SAE and all subsequent reports must be retained in the subject's study file together with a telephone log of all contacts with Primus and the subject. These should be recorded at the time of the conversation, dated and signed by the investigator or his/her representative and should include the name of the person with whom the conversation was held.

Telephone contact should be made with:
Robert Levy, MD, Director Clinical Development Tel:
480-483-1410 Mobile: 480-415-6779 Fax: 480-483-
2604

Primus will review all SAEs with the investigator and a decision will be made regarding the need for further action. Primus' primary concern will be subject safety, both for the subject having the event as well as for the other subjects in the study. If, after detailed review, it is determined that a subject safety issue exists, Primus will notify all investigators participating in any trial with the same study agent and may require one or more of the following:

- Discontinuation or suspension of the study
- Modification of the protocol
- Modification of the informed consent document to reflect additional risk(s) to be signed by current and future subjects
- Addition of newly discovered adverse events to the investigator's brochure

10.5 Follow-Up of Adverse Events

All adverse events, whether serious or non-serious, must be followed until resolution or stability. This follow-up may extend beyond the end of the study. Primus considers AE follow-up to be part of a study. Therefore, a study cannot be closed at an investigative site if an AE is outstanding.

Any serious AE occurring after the conclusion of a study must be reported to Primus if the investigator thinks the event ***may*** be causally related to the study agent.

10.6 Deaths

All deaths, regardless of cause, that occur between the time a subject signs an informed consent document and the final study visit or 30 days after the last dose of study product, whichever is longer, must be reported to Primus by telephone with a follow-up fax within 24 hours of the investigator becoming aware of the death. This applies whether or not the subject has received study product and whether or not the study product is thought to be causally related to the death.

It is the investigator's responsibility to obtain all relevant medical records and autopsy reports and forward them to Primus as soon as possible.

10.7 Reporting Safety Information to IRB

It is the responsibility of the investigator to notify his/her IRB of any unexpected or previously unknown risk to study subjects. This includes death from any cause and all SAEs. The investigator should make this report in accordance with the requirements of the IRB. The report should include any action taken after discussion of the SAE with Primus.

10.8 Managing Adverse Events

All AEs and SAEs should be medically managed according to the best medical standards available in the investigator's community. Except in the case of emergent treatment and routine care the management of the event should be discussed with Primus. Decisions regarding re-challenge with study product must be made in consultation with Primus.

10.9 Pregnancy

Although pregnancy is not considered to be an adverse event, Primus requires that investigators provide notice of any pregnancy in an active study subject or within 30 days of the last dose of study drug. Primus also requires investigators to provide follow-up information on the pregnancy and the health of the infant.

The informed consent document, signed by the patient, must contain information about the potential risks to pregnant women and/or to a fetus. Subjects must be advised to use contraception during and for 30 days after their participation in the study.

11. Ethical Considerations

This study will be conducted in accordance with the principles of the Declaration of Helsinki, ICH guidelines and Good Clinical Practices (GCPs) and US FDA regulations.

11.1 Informed Consent

All patients must read and sign the Research Subject Information and Consent Document (ICD) before they can be subjects in this research study. It is the investigator's responsibility to fully inform the patient about the procedures, risks and benefits of the study and to answer, in a manor clearly understandable to the patient, all the patient's questions. The ICD must be signed and dated by the patient, the investigator and a witness. A copy of the signed and dated ICD must be given to the subject. The IRB approved ICD must not be modified in any way from its original, IRB

approved form. The original copy of the ICD must be retained with the subject's study file and is subject to inspection by representatives of Primus and the regulatory agencies.

ICDs and all other written material distributed to subject during the study must be written in a language the subject understands.

A model informed consent form can be found on the IRB website.

11.1.1 Institutional Review Board (IRB)

Primus will assume responsibility for submitting the protocol and ICD to a central IRB. Before this can be done each investigator must submit the following to Primus:

- Signed and dated protocol signature page
- Completed, signed and dated FDA Form 1572
- Completed, signed and dated W-9 form
- Current curriculum vitae for investigator and all sub-investigators, signed and dated

If an investigator wishes to use an IRB other than that selected by Primus, he/she may do so after obtaining approval from Primus. In this case, the investigator will be responsible for all required submissions. Any changes to the model ICD must be first approved by Primus, in writing. The investigator must agree to all protocol amendments and, if necessary, make appropriate changes in the ICD. The IRB should be notified of any such changes and IRB approval must be sought for any changes involving subject safety. Serious or unexpected adverse events must be reported to the IRB within one week of their occurrence.

11.1.2 Subject Confidentiality

Subject identities should be protected at all times. This is the legal responsibility of the investigator. Subjects should be identified in study documents only by initials and study number on any document sent to Primus or a regulatory agency. Documents that are only for office use must be kept in strict confidence

Investigators must allow representatives of Primus or government regulatory agencies to inspect study records but the identity of the subjects should remain confidential.

12. Administrative and legal obligations

12.1 Pre-study Documentation

Before an investigator can begin screening subjects and before shipment of study agent can commence the investigator must complete and return the following to Primus:

- Signed and dated protocol signature page

- Completed, signed and dated FDA Form 1572
- Copy of IRB approved ICD
- Copy of IRB approval letter for the study and ICD
- Membership list of IRB (usually supplied with approval letter)
- Current CVs of the investigator and all sub-investigators, signed and dated
- Signed and dated contract to conduct the study under the conditions and financial arrangements specified
- Name and contact information of person(s) responsible for the day to day management of the study

12.2 Protocol Amendments

Protocol amendments may be made only by Primus. The investigator's IRB must be informed of any amendments that may affect subject safety or the conduct of the study. The IRB must issue a letter of approval of such amendments. The investigator must keep the original letter in the study's regulatory binder and forward a copy of the letter to Primus.

12.3 Study Termination

Both the investigator and Primus have the right to terminate their participation in the study. If this is done by the investigator he/she must provide a detailed explanation of the action to Primus and the IRB and a copy of this account must be retained with the regulatory file.

12.4 Case Report Forms (CRFs)

Primus will provide investigators with sets of NCR paper CRFs bearing information identifying the study site and the principle investigator. All data obtained during the course of the study will be recorded on the CRFs. The CRFs should be reviewed on site for completeness by the investigator or his/her representative. The original will be forwarded to Primus and a copy will remain with the subject's study data file.

CRFs contain the critical study data and are the basis from which results are tabulated, analyses performed and final reports made. Therefore, it is essential that data recorded on the CRFs be legible, complete and accurate. Forms should be typed or completed in black ball point ink. Corrections should be made by placing a single line through the error, adding the correct data then initialing and dating the correction. Under no circumstances should the original entry be erased, overwritten or obscured by white-out or some other method. CRFs should contain only subject initials and study number. Names or other identifying information should not appear. All data points must be answered. If the information is unknown or unavailable the terms "NA" (not available), "ND" (not done), "SU" (subject unable) or "DP" (data pending) may be used.

12.5 Final Study Documentation and Storage

Investigators must retain all study records in a secure location, safe from environmental damage, for a minimum of two (2) years or as required by Primus. If, for any reason, the investigator becomes unable to store study documents Primus will assume responsibility for storage. Study documents should never be destroyed without the written consent of Primus.

13. Summary of Investigator responsibilities

Investigator responsibilities include, but are not necessarily limited to the following. He/she will:

- conduct the study according to the protocol and will make no changes without the consent of the sponsor. This requirement is not intended to limit the investigator's ability to deviate from the protocol to protect the health and welfare of a subject.
- comply with all applicable regulatory requirements.
- personally supervise the conduct of the study.
- be responsive to input from study subjects as their observations are often helpful
- inform subjects that the study product is investigational.
- report any adverse events that occur in the course of the study. Non-serious AE's may be reported on the CRF's. Serious AE's must be reported to Primus within 24 hours and to the applicable IRB in accordance with the requirements of the IRB.
- promptly treat or arrange for treatment of any AE or SAE
- follow any AE until its resolution even if that endpoint occurs after the close of the study.
- make a statement on the AE reporting form as to whether, in the investigator's judgment, the AE was or was not related to the study agent
- use a properly constituted IRB for study approval and oversight.
- supply Primus with a current curriculum vitae.
- review all CRF's for accuracy and completeness.
- maintain complete and accurate records of all study related documents for two (2) years

14. Primus contacts

Questions related to SAE's, medical observations or interventions should be directed to:
Robert Levy, MD
Director of Clinical Development
Primus Pharmaceuticals, Inc.
7373 N. Scottsdale Rd.
Ste. B-200
Scottsdale, AZ 85253 Tel: 480-483-1410 Cell: 480-415-6779 Fax: 480-483-2604

Questions related to study operational issues may be addressed to Dr. Levy or to:
Mary Sanstead, RN, BSN, Project manager Tel: 480-843-1410 Cell: 480-250-6689 Fax: 480-483-2604

15. Publication/presentation of data

Primus regards all information obtained from the conduct of this study as confidential. If an investigator wishes to publish or present in oral or written form all or part of the data obtained during the study he/she must first submit a complete copy of such presentation to the director of Clinical Investigation for review and approval. This submission must occur no later than sixty (60) days before the anticipated date of presentation. The investigator will be required to incorporate any suggestions made by Primus consistent with its right to

protect the confidentiality of the information.

16. Statistical considerations

This is a pilot, exploratory case study designed to inform development of a formal RBCDB clinical trial. As such, only those variables clearly shown to be affected by the study treatment will be incorporated into the formal trial.

Descriptive statistics will be presented for all relevant variables including demographic variables such as age, sex, weight and BMI for each group. Descriptive statistics for baseline and endpoint values for all study variables, including weight, BMI leg circumference, short WOMAC, investigator Likert and all subject reported outcomes per section 4.2 will also be presented. Data from baseline and final visit will be compared by Student's t-test (paired). P values </= 0.05 will be considered of significance.

17. References

Alphonsus CS, Rodseth RN. The endothelial glycocalyx: a review of the vascular barrier.Anaesthesia.2014.69:777-84

Buck DW, Herbst KL. Lipedema: a relatively common disease with extremely common misconceptions. PlasReconstr Surg Glob Open. 2016. Doi: 10.1097/GOX.1043.

Colleridge-Smith PD. Micronized purified flavonoid fraction and the treatment of chronic venous insufficiency: microcirculatory mechanisms. Microcirc. 2000.7:S35-40.

Herbst KL. Medications and Supplements. (2019, March 18). Retrieved from https://treat.medicine.arizona.edu/treatments-therapies/

Herbst KL. Rare adipose disorders (RADs) masquerading as obesity. Acta Pharmacologica Sinica. 2012.33:155-72.

its use in chronic venous insufficiency, venous ulcers and haemorrhoids, Drugs. 2003.63:71100.

Lyseng-Williamson KA, Perry CM. Micronized purified flavonoid fraction: a review of

Mensah SA, Cheng MJ, Homayoni H, Plouffe BD, Coury AJ, Ebong EE. Regeneration of glycocalyx by heparan sulfate and sphingosine 1-phosphate restores inter-endothelial communication. ***PLoS One.*** 2017;12(10):e0186116. Published 2017 Oct 12. doi:10.1371/journal.pone.0186116

Pillinger NL, Kam PCA. Endothelial glycocalyx: basic science and clinical implications. Anaesth Intens Care. 2017.45:1-13.

Ramelot AA. Daflon 500 mg: symptoms and edema clinical update. Angiology.2005.56 (suppl 1):S25-32.y

Shoab SS, Porter J, Scurr JH, Coleridge-Smith PD. Endothelial activation response to oral micronized flavonoid therapy in patients with chronic venous disease—a prospective study. Eur J Vasc Endovsac Surg. 1999.17:313-8.

Wold LE, Hines EA, Jr., Allen EV. Lipedema of the legs: a syndrome characterized by fat legs and edema. Ann Intern Med. 1951.34:1243-50.

Appendix I

The World Medical Association Declaration of Helsinki

World Medical Association Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and as last revised by the World Medical Assembly in Seoul, Korea 2008

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The Purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.
    Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.
12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
3. In any medical study, every patient--including those of a control group, if any--should be assured of the best proven diagnostic and therapeutic method.
4. The refusal of the patient to participate in a study must never interfere with the physician- patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers--either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

Appendix II

Acceptable Contraceptive Measures

1- Oral contraceptives

2- Depo Provera

3- norplant implants

4- Intra-uterine device

5- Tubal ligation

6- Vasectomy

7- Condom plus diaphragm

8- condom plus contraceptive sponge or spermicidal jelly

Appendix III

Diagnostic Features of Lipedema

From Wold 1951; modified by Herbst 2012

- Women
- Usual onset at puberty, pregnancy or menopause
- Symmetrical adipose hypertrophy of lower extremities, sparing trunk and feet, with sharp demarcation at ankles (cuffing). Upper extremities may or may not be involved.
- Negative Stemmer sign
- Easy Bruising
- Minimal pitting edema
- Pain, often spontaneous, tenderness on palpation
- No response to leg elevation or weight loss

APPENDIX IV
SCHEDULE OF EVENTS

VISIT	V1	V2	V3	V4
DAY/WEEK	Screen/ Baseline	1 Month	2 Month	3 Month
Window	+/- 2d	+/- 2d	+/- 2d	+/- 2d
Informed Consent	X			
Demographics	X			
Current Medication	X			
Medical History	X			
Inclusion/Exclusion	X			
Vital Signs (BP, P)	X	X	X	X
Weight/Height	X	X	X	X
Brief Physical Exam	X			
Urine Pregnancy Test	X			
Leg Circumference1	X	X	X	X
BMI2	X			X
Leg Discomfort	X	X	X	X
Feeling of Leg Swelling/Tightness	X	X	X	X
Leg Tenderness	X	X	X	X
Leg Bruising	X	X	X	X
Leg Skin Redness	X	X	X	X
Fatigue	X	X	X	X
Ability to Perform ADLs	X	X	X	X
Overall Sense of Well-Being	X	X	X	X
Short WOMAC	X	X	X	X
Draw Bld for Glycocalyx Markers	X			X
Dispense Product	X	X	X	
Adverse Events		X	X	X
Concomitant Medications	X	X	X	X
Collect and Count Product		X	X	X
Likert Scale Investigator Response to Therapy				X

[1] Measure 2 and 12 inches above the lateral malleoli on both legs [2] Use only the chart provided

APPENDIX V: *Body Mass Index Table*

To use the table, find the appropriate height in the left-hand column labeled Height. Move across to a given weight. The number at the top of the column is the BMI at that height and weight. Pounds have been rounded off.

BMI	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	3
Height (inches)	Body Weight (pounds)																
58	91	96	100	105	110	115	119	124	129	134	138	143	148	153	158	162	16
59	94	99	104	109	114	119	124	128	133	138	143	148	153	158	163	168	17
60	97	102	107	112	118	123	128	133	138	143	148	153	158	163	168	174	17
61	100	106	111	116	122	127	132	137	143	148	153	158	164	169	174	180	18
62	104	109	115	120	126	131	136	142	147	153	158	164	169	175	180	186	19
63	107	113	118	124	130	135	141	146	152	158	163	169	175	180	186	191	19
64	110	116	122	128	134	140	145	151	157	163	169	174	180	186	192	197	20
65	114	120	126	132	138	144	150	156	162	168	174	180	186	192	198	204	21
66	118	124	130	136	142	148	155	161	167	173	179	186	192	198	204	210	21
67	121	127	134	140	146	153	159	166	172	178	185	191	198	204	211	217	22
68	125	131	138	144	151	158	164	171	177	184	190	197	203	210	216	223	23
69	128	135	142	149	155	162	169	176	182	189	196	203	209	216	223	230	23
70	132	139	146	153	160	167	174	181	188	195	202	209	216	222	229	236	24
71	136	143	150	157	165	172	179	186	193	200	208	215	222	229	236	243	25
72	140	147	154	162	169	177	184	191	199	206	213	221	228	235	242	250	25
73	144	151	159	166	174	182	189	197	204	212	219	227	235	242	250	257	26
74	148	155	163	171	179	186	194	202	210	218	225	233	241	249	256	264	27
75	152	160	168	176	184	192	200	208	216	224	232	240	248	256	264	272	27
76	156	164	172	180	189	197	205	213	221	230	238	246	254	263	271	279	28

Appendix VI
Blood Sample Processing

I. FREEZE PLASMA WITHIN ONE (1) HOUR OF DRAWING
II. Shipping boxes and exterior labeling will be provided.
III. Samples will be shipped in 2 separate batches.
IV. Draw and process as directed below.

1. Complete one lab requisition for each draw; v1 and v4
2. Draw 10 ml blood into lavender top K2 EDTA tube
3. Centrifuge for 15 minutes at 5000 rpm
4. Decant or pipette plasma into two (2) 3 ml cryovials
5. Label each cryovial with cryo-pen:
    a. Subject initials Ex: ABC or A-C
    b. 3-digit study number Ex: *site 1 subject 4 = 104 (*Site # = 1-digit, Subj # = 2-digits)
    c. Date and time of draw Ex: mm/dd/yy 9:05 am or 1:20 pm
    d. Visit number = 1 or 4 Ex: v1 or v4
6. Store at -70^{o} F within 1 hour of drawing

At study completion:

1. Retain one sample for each subject at -70^{o} F
2. Ship one sample in dry ice to:
   Attn: Eno Ebong Laboratory
   Chemical Engineering Department
   Northeastern University
   360 Huntington Ave., 335 ISEC
   Boston MA 02115-5000
3. After arrival of first shipment is confirmed, ship second batch.