VITAMIN D LEVELS IN SUBJECTS WITH VITAMIN D DEFICIENCY FOLLOWING TOPICAL APPLICATION OF THREE DOSES OF VITAMIN D GEL – A PROOF OF CONCEPT STUDY

Protocol No: VITD-001

Version: 2.0.

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SPONSOR-INVESTIGATOR CONTACT

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1. SYNOPSIS

Study Title:	Vitamin D Levels in Subjects with Vitamin D Deficiency Following Topical Application of Vitamin D Gel – A Proof of Concept Study
Protocol Number:	VITD-001
Development Phase:	Phase 1
Indication:	Subjects with known vitamin D deficiency (<20 μg/L Total 25-OH Vitamin D)
Study Drugs: (Including test, comparator, dosage form, dosing regimen and route)	A novel vitamin D_3 (cholecalciferol) is provided as a topical gel using ingredients that are identified in the USP/NF and/or designated in the list of Inactive Ingredients in FDA Approved Drugs. The ingredients in the topical gel are well known in the industry and have references in the pharmaceutical literature and some have available FDA drug master file information. A single applicator contains 0.75g of gel, containing 150,000 international units of vitamin D3 (cholecalciferol) to be applied to the upper arm. One dose consists of two applicators, one applied to each upper arm, for a total of 300,000 international units per dose. Subjects with known vitamin D deficiency of ≤20 ng/ml total 25-OH Vitamin D (50 nmol/liter) will be consented and enrolled. They will have a blood draw prior to dosing on Day 1 (to obtain a baseline vitamin D, calcium, albumin, and parathyroid hormone level) and will receive 300,000 units of topical vitamin D3. On Day 7, subjects will return to the clinic for a blood draw to assess Vitamin D level and to check for any signs of skin rash and/or reaction. If a subject has a skin rash and/or reaction prior to Day 7, he/she will be advised to return to the clinic for assessment as soon as possible. Any rash and/or skin reaction may be photographed and diagnosed. A second dose of 300,000 units of topical Vitamin D will be applied by the nurse at the day 7 visit. Subjects will return on Day 14 for a third dose and a third blood draw. A final blood draw to assess for Vitamin D level will be done on day 21. A study nurse or physician will apply each dose of topical Vitamin D. Prior to application of the gel, 2 strips of paper tape will be applied to the subject's upper arms. One strip will be applied approximately 2 inches below the subject's shoulder and the second strip will be applied approximately 2 inches above the subject's elbow. The entire contents of one syringe (0.25 mL of gel) will be applied onto the subjects arm in a straight line, from approximately 1 inch below the first strip of paper tape to approximately 1 inch above the second strip of paper tape. The subject will be instructed to gently rub the gel into their upper arm with their fingertips, while staying between the two pieces of paper tape, for approximately 2 minutes or until the gel has been absorbed. After completing the application process on both arms, the subject will wash his/her hands thoroughly.

No. Subjects:	20 subjects will participate. If the change from baseline in 25-OH vitamin D is consistently less than 10 μg/L in the first 10 subjects then the study will be discontinued.
No. Centers:	The study will be conducted at the University of Minnesota (single center)
Study Duration:	The maximum duration of treatment is 14 days (study Days 0, 7 and 14). Subjects will be followed for change in vitamin D blood level and for safety until Study Day 21. It is anticipated that the study will last 4 months to enroll and complete dosing and assessment of 20 patients.
Objectives of the Study:	The primary objective of the study is to determine the change in vitamin D blood levels following 3 doses of topical vitamin D3 treatment. The secondary objective is to assess the safety (based on skin rash and/or other reaction, as well as calcium and parathyroid hormone levels) following the application of 3 doses of vitamin D3 gel.
Study Endpoints: (Primary and Secondary)	The primary endpoint is to establish the average change in vitamin D blood levels following 3 doses of treatment with vitamin D gel. The secondary study endpoint is the number of subjects who have any skin rash and/or other skin reaction following treatment with the vitamin D gel.
Study Design:	The study is a pre vs. post open-label trial to assess the change in vitamin D blood levels following treatment with 3 doses of topical vitamin D gel. Subjects with known vitamin D deficiency will be consented and enrolled. They will have a blood draw prior to dosing on Day 0 (to obtain a baseline vitamin D level) and will receive topical vitamin D_3 on Days 0, 7 and 14. On Days 7, 14, and 21 subjects will return to the clinic for a post-dose blood draw and to be assessed for any signs of skin rash and/or reaction. If a subject has a skin rash and/or reaction at any time during the study, he/she should return to the clinic for assessment as soon as possible. Any rash and/or skin reaction will be photographed and diagnosed.
Eligibility Criteria (Inclusion and Exclusion):	Inclusion criteria: 1. Known vitamin D deficiency based on blood work obtained prior to study consent defined as serum 25-hydroxyvitamin D less than or equal to 20 ng/mL within 30 days prior to study entry. (Normal ≥ to 30 μg/L total 25-OH Vitamin D) 2. Adult, age 18 to 85 years, male or female 3. If on oral vitamin D therapy, have been on a stable dose for the previous 90 days.

Eligibility Criteria (continued):	Exclusion criteria: 1. History of chronic liver disease with elevated liver function tests, chronic kidney disease (stage 3 or greater, eGFR <60 mL/min), uncontrolled thyroid disease (elevated thyroid function tests), primary or secondary hyperparathyroidism, hypercalcemia, or multiple endocrine neoplasia. 2. Hypercalcemia defined as either elevated corrected serum calcium >10.2 mg/dL) measured within 3 months prior to study. 3. eGFR <60 mL/min within 3 months prior to study. 4. Active cancers 5. Women who are pregnant or breastfeeding. 6. Individuals who are unable to give informed consent 7. Individuals with psoriasis, active eczema or other skin disease, or who are currently receiving treatments or medications for skin disease. 8. Individuals who do not agree to refrain from using tanning beds for the duration of the study. 9. Individuals who do not agree to avoid submerging the gel site in water for 8 hours after gel application. 10. Family members of the study faculty or staff.

Study Procedures: (Including pharmacokinetic (PK) sampling times)	The following procedures will be performed during this study:
	• Chart review to assess for potential eligibility
	• Day 0
	  ○ Subject informed consent
	  ○ Measurement of height and weight and examination of skin for rash/appearance to confirm eligibility
	  ○ Record Fitzpatrick skin test score.
	  ○ Blood draw for baseline vitamin D levels (total vitamin D 25-OH and vitamin D3)
	  ○ Blood draw for serum creatinine (for GFR calculation), albumin (for corrected calcium) and parathyroid hormone
	  ○ Application of Vitamin D gel by study staff to inside of each arm
	  ○ Instruct subject on assessment of skin. If any rash or other skin reaction appears, contact the clinic as soon as possible for an assessment.
	• Day 7
	  ○ Assessment of skin for rash or other reaction
	  ○ Draw blood for Ca, vitamin D level.
	  ○ Application of Vitamin D gel by study staff to inside of each arm.
	• Day 14
	  ○ Assessment of skin for rash or other reaction
	  ○ Draw blood for Ca, vitamin D level.
	  ○ Application of Vitamin D gel by study staff to inside of each arm.
	• Day 21
	  ○ Assessment of skin for rash or other reaction
	  ○ Blood draw for post dose vitamin D, calcium, and parathyroid hormone level.

Safety	All subjects will be monitored for skin rash or other reactions. Any rash and/or reaction will be photographed by study staff and assessed/diagnosed by a physician. Patient will have serum calcium followed each week during the study. Although it is unlikely that calcium will change with an increase in topical Vitamin D, hypercalcemia presents a potential theoretic risk if absorption is high or unexpected variability in vitamin D absorption exists.
Laboratory Parameters/Analysis:	Laboratory analyses will consist of 25-hydroxyvitamin D blood levels (D2 and D3) and parathyroid hormone levels. For safety, an albumen adjusted calcium determination will be measured on day 0, 7, 14, and 21.
Total Blood Volume:	Approximately 5 mL will be required at day 0, 7, 14, and 21 to assess for vitamin D parameters and safety.

Sample Size Determination: (If applicable)	The study will have 20 subjects who are evaluable for changes in vitamin D blood levels.
Statistical Analyses: (Brief Description)	Two sample paired t test of pre and post total vitamin D and parathyroid hormone with an alpha of .05. We do not expect any significant change in adjusted calcium.

2. STUDY SCHEDULE

Table 1: Schedule of Events

		Testing Visits			
Clinical/Study Assessment	Prescreening	Day 0	Day 7	Day 14	Day 21
Chart review for potential eligibility	X				
Physical Exam of skin* ·Skin integrity assessment ·Fitzpatrick skin score		X**	X**	X**	X**
Blood Draw: · Serum 25-OH vitamin D · Serum calcium		X	X	X	X
· Serum creatinine (eGFR) · Serum albumin		X			
· Serum parathyroid hormone		X			X
Topical Treatment Application		X	X	X	

* Subjects will be instructed to contact the site as soon as possible if he/she notices a skin rash or any other reaction.
**A digital photograph may be taken to record any signs of irritation or reaction.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
µg	Microgram(s)
AE	Adverse event
CRF	Case report form
g	Gram(s)
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
kg	Kilogram(s)
L	Liter
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram(s)
mL	Milliliter
SAE	Serious adverse event

5. INTRODUCTION

5.1 Vitamin D Deficiency

Vitamin D insufficiency is an emerging and compelling public health issue. Recent studies suggest that 19% of the U.S. population is at risk for vitamin D inadequacy [1], although this may underestimate the number of people at risk from suboptimal vitamin D levels[2]. More than 40% of U.S. and European elderly men and women living in the community are deficient in Vitamin D[3], and more than 50% of postmenopausal women taking medication for osteoporosis[4]. Diverse population subgroups are deficient in Vitamin D including Arab women and lactose intolerant individuals who consume less vitamin D from fortified products[5]. In a Boston hospital, 25% of healthy students, physicians, and residents were found to be vitamin D–deficient, despite drinking a glass of milk and taking a multivitamin daily and eating salmon at least once a week[6]. By the end of the winter, it has been estimated that 42% of 15- to 49-year-old black females throughout the United States have 25-hydroxyvitamin D levels below 20 ng per milliliter. The implications of this are far-reaching. In addition to well-known impacts on bone health, vitamin D status is thought to potentially influence a number of common diseases, including cancers, autoimmune disorders, and cardiovascular disease. Vitamin D deficiency may increase the risk of injury from falls as people age[7], while early in life Vitamin D deficiency has been associated with increased risk of Type 1 diabetes[8,9]. Other patient populations at increased risk for vitamin D insufficiency include individuals with fat malabsorption, chronic kidney disease, or obesity[10,11,12] .

The Endocrine Society defines vitamin D deficiency as a 25(OH)D of 20 ng/ml (50 nmol/liter) or below, and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (525–725 nmol/liter)[13]. They recommend measuring vitamin D using the serum circulating 25-hydroxyvitamin D (25(OH)D). The Institute of Medicine reports that persons are potentially at risk for inadequacy at serum 25OHD levels between 12 and 20 ng/mL (30 and 50 nmol/L)[14]. In sun-rich environments, normal levels have been estimated to be 54-90 ng/ml[15]. Although it is not known what the safe upper value for 25(OH)D is for avoiding hypercalcemia, most studies in children and adults have suggested that the blood levels need to be above 150 ng/ml before any concern arises[16].

Of all of the forms of vitamin D, cholecalciferol (Vitamin D3) has been viewed as having the best overall combination of safety and efficacy, assuming normal kidney and liver function. In the US, cholecalciferol has primarily been available as an oral supplement at fairly low doses (most commonly 1000 – 2000 IU). In otherwise healthy patients, a common US standard of care for treatment of low serum vitamin D is to give 50,000 IU Vitamin D orally per week for four weeks (total dose 200,000 IU) until levels are brought up into the adequate

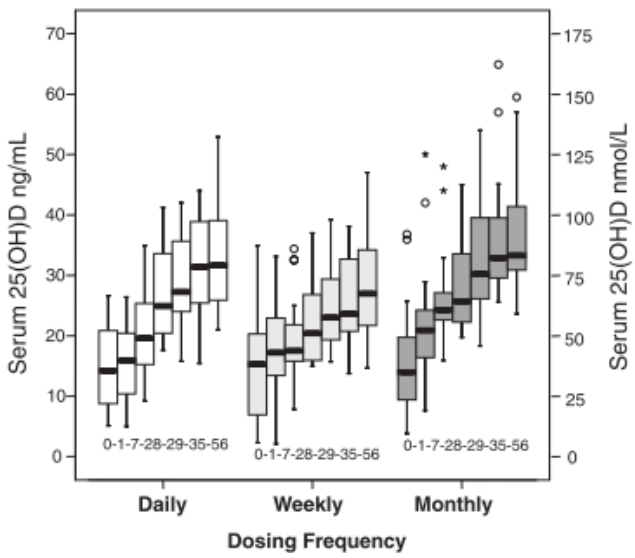

Figure 1. Absorption kinetics of oral Vitamin D3.

range, followed by daily supplements of 2,000 IU. This is often provided as commercially available Vitamin D2, however supplementation with Vitamin D3 appears to be more potent and may provide other benefits[17].

Despite the widespread availability of oral supplementation, the frequency of vitamin D deficiency remains high. Some individuals have difficulty taking pills, while others, especially the elderly, may be overwhelmed by their number of oral medications. A need exists for a more convenient delivery mechanism that enhance access to effective long term treatment of vitamin D deficiency. The proposed study aims to investigate both safety and efficacy of a topically applied vitamin D that is likely to be more effective, convenient, or acceptable for many individuals.

A wide variety of dosing schedules for Vitamin D have been documented[18]. Recently Vieth's team conducted a study with oral cholecalciferol in 48 elderly women (81 ± 8 years)[19]. The study tested oral cholecalciferol (D3) up to 45,000 IU once every 28 days. The study showed that a single oral dose of 45,000 IU dissolved in alcohol generally produced a rise in serum vitamin D of about 10 ng/ml. A second oral dose 28 days later further increased the serum vitamin D level by about another 10 ng/ml. Thus, the total increase after two oral doses (90,000 IU) was about 20 ng/ml. Although following oral dosing, rises in serum Vit D can be seen up to one month following administration, Vieth's study demonstrated that most of the rise (>60%) in Vitamin D occurs within 7 days. (Figure 1). No one receiving oral monthly dosing of 45,000 IU Vitamin D3 developed hypercalcemia.

Vitamin D Product Development

Innovative Pharmacy Services, Inc. has developed a vitamin D3 topical gel using ingredients that are identified in the USP/NF and/or designated in the list of Inactive Ingredients in FDA Approved Drugs. The ingredients in the topical gel are well known in the industry and have references in the pharmaceutical literature and some have available FDA drug master file information. A single applicator contains 0.75 g of gel, containing 150,000 international units of vitamin D3 (cholecalciferol). One dose will consist of two applicators. One applicator will be applied to each of the subject's upper arm for a total dose of 300,000 units.

5.2 Study Rationale and Selection of Dose

5.2.1 Rationale for Study

The purpose of this investigation is to demonstrate that a novel topical vitamin D3 (cholecalciferol) gel is bioavailable to the systemic circulation and in sufficient quantities to treat hypovitaminosis D. In Phase Ia, healthy volunteers will be dosed with a topical gel to determine increases of serum 25-OH vitamin D that can be expected from three doses. The application will take place in the winter months in Minnesota. Although ambient levels of light are low at that time, subjects will be asked to avoid attending tanning booths, and to wear a comfortable shirt that covers their upper arms at least to the elbow if they choose to go outside during daylight hours.

5.2.2 Rationale for Selected Dose

A wide variation in Vitamin D dosing has been used in clinical practice[12]. If we assume that the topical gel vitamin D dosing has no greater bioavailability than intramuscularly injected dosing, then the maximal effect of 100,000 IU of Vitamin D3 applied topically would be expected to reflect that of a single 100,000 IU injected dose[19]. The supplementation protocol most often used in France to correct vitamin D levels of $\geq$ 10 ng/ml and <20 ng/ml is three doses of 100,000 IU of Vitamin D3 given intramuscularly one week apart[20]. For values $\leq$ 10ng/ml four doses of 100,000 IU are administered. An observational study of this common French protocol by Rouillon evaluated 204 patients receiving 3-4 doses of 100,000 IU Vitamin D3 weekly for vitamin deficiency (Groups 1 and 2)[21]. Rouillon found the protocol to be safe with no episodes of hypercalcemia. Two patients from the total of 257 studied developed Vitamin D levels over 90 ng/ml (one 92 mg/ml and one 94 ng/ml), with both resolving within one month. .

The proposed study will use a topical application of Vitamin D which is unlikely to be as completely absorbed as intramuscular injection. Furthermore, topical application of Vitamin D provides important additional physiologic mechanisms that reduce the potential for possible hypervitaminosis. Upon exposure to UVB radiation, previtamin D3 is synthesized from 7-DHC in the stratum basale and stratum spinosum layers of the epidermis. Previtamin D3 thermoisomerizes to vitamin D3 with heat or sunlight exposure at the ultraviolet region of the solar spectrum around 316-330 nm [22]. Vitamin D3 is then available for binding with a vitamin D binding protein (DBP) and transported into the circulation[23]. In the skin, the DBP acts as a regulatory mechanism preventing over absorption of Vitamin D into the circulation by providing a threshold when the binding protein has been fully occupied. Upon exposure to sunlight, vitamin D3 itself is rapidly degraded to other photoproducts, including 5,6-trans-vitamin D3, suprasterol I, and suprasterol II [24]. Although winter sunlight is not strong enough to produce vitamin D3 in the skin, it is strong enough to promote the photodestruction of existing vitamin D [24]. Neither binding with DBP nor other serum components prevent photodestruction.).

In the process of normal physiologic production, previtamin D3 can isomerize in a reversible reaction to form the photoproducts, tachysterol-3 or lumisterol-3. These isomers do not enhance intestinal calcium absorption and do not significantly enter the circulation. The inactive metabolic products are sloughed off when skin cells are naturally shed. Removal of previtamin D3 provides a mechanism to prevent vitamin D toxicity from prolonged sun

exposure[25], and may further increase the potential safety of topical Vitamin D3 administration. The Mayo Clinic reports that Vitamin D is 'likely' safe when applied to the skin[12].

The effectiveness of topical absorption for the proposed product is unknown, however topical absorption is substantially less effective than the intramuscular injection of Vitamin D3. In the first three candidates, 300,000 units applied over three weeks resulted in an insignificant average change in Vitamin D level, as follows.

Baseline	Week 1	Week 2	Week 3	Av change
10.2	9.4	10.4	12.1	1.2

Oral administration of 50,000 IU should produce a 10 ng/ml increase in total serum Vitamin D [13]. The topical application os 300,000 IU, therefore, was substantially less effective than one 50,000 IU oral dose. . The study will use three doses of 300,000 IU applied one week apart with safety and outcome measures after each. This is hoped to provide a greater difference in vitamin D level than that seen in one oral 50,000IU supplement, but should produce less change than the typical US replacement dosing of three oral supplements, If the change in total serum Vitamin D level after the topical application of 900,000 IU in the first 10 subjects is consistently less than 10 mg/ml then the study will be discontinued.

6. STUDY OBJECTIVES

The primary purpose of the study is to assess the change in serum 25-OH vitamin D concentrations following 3 doses of 300,000 IU of a novel vitamin D3 gel. The secondary objective is to assess the safety (based on any skin rash or other skin reactions, as well as calcium and parathyroid hormone levels) following treatment.

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7. INVESTIGATIONAL PLAN

7.1 Overall Study Design

20 healthy adults with known vitamin D deficiency (defined as serum 25-OH vitamin D concentration < 20 ng/ml) will receive 3 doses of 300,000 IU of topical vitamin D once per week for three weeks. Each dose will be made up of two applications of 150,000 IU cholecalciferol in .75 ml of gel applied consecutively to the upper arms (one 150,000 IU application per arm).

Blood will be drawn at baseline prior to the first dose (Day 0) and on Days 7, 14, and 21. Total serum 25-OH vitamin D will be assayed at each draw. Parathyroid hormone level will be assayed at baseline and on day 21.

7.2 Study Duration

The maximum duration of treatment is 14 days (study Days 0, 7 and 14). Subjects will be followed for change in vitamin D blood level and for safety until Study Day 21. It is anticipated that the study will last 4 months to enroll and complete dosing and assessment of 20 patients.

8. STUDY POPULATION

8.1 Inclusion Criteria

A subject must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Known vitamin D deficiency based on blood work obtained prior to study consent defined as serum 25-hydroxyvitamin D less than or equal to 20 ng/mL within 30 days prior to study entry.
2. Adult, age 18 to 85 years, male or female
3. If on oral vitamin D therapy, have been on a stable dose for the previous 90 days.

8.2 Exclusion Criteria

Subjects are excluded from this study if any of the following conditions are met:

1. History of acute or chronic liver disease with elevated liver function tests, chronic kidney disease (stage 3 or greater, eGFR <60), uncontrolled thyroid disease (elevated thyroid function tests), primary or secondary hyperparathyroidism, hypercalcemia, or multiple endocrine neoplasia.
2. Hypercalcemia defined as elevated corrected serum calcium (>10.2 mg/dl) measured within 3 months prior to study.
3. eGFR <60 mL/min within 3 months prior to study.
4. Active cancers
5. Women who are pregnant or breastfeeding
6. Individuals who are unable to give informed consent
7. Individuals with psoriasis, active eczema or other active skin disease, or who are currently receiving treatments or medications to control skin disease
8. Individuals who do not agree to refrain from using tanning beds for the duration of the study.
9. Individuals who do not agree to avoid submerging the gel site in water for 8 hours after gel application.
10. 10. Family members of the study faculty or staff.

8.3 Criteria for Withdrawal

A subject has the right to withdraw fully or partially from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

Withdrawal of full consent for a study means that the subject does not wish to receive further investigational treatment and does not wish to or is unable to continue further in study participation. Subject data up to withdrawal of consent will be included in the analysis of the

study. The Investigator will discuss with the subject appropriate procedures for withdrawal from the study. Withdrawal of partial consent from a study means that the subject does not wish to take investigational product or other protocol-required therapy any longer but is still willing to collaborate in providing further data by continuing on study. Should a subject request or decide to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information should be reported on the applicable case report form (CRF). Reasons for removal from protocol-required treatment or observation might include:

- partial/full withdrawal of consent
- administrative decision by the Sponsor-Investigator
- pregnancy
- ineligibility
- significant protocol deviation
- subject noncompliance
- adverse event, including rash or other skin reaction
- Death

8.4 Replacement of Subjects

Subjects who withdraw or are removed from the study prior having their Day 7 blood draw for a reason other than a drug-related adverse event will be replaced.

9. TREATMENT OF SUBJECTS

9.1 Treatment Assignment

This is an open-label, nonrandomized study in which all subjects will receive topical vitamin D gel.

9.2 Treatment Administration

Subjects will be provided with written and verbal instructions about Vitamin D deficiency during their baseline/Day 1 visit. A study nurse or physician will supervise each treatment application to ensure that the gel is applied correctly. Prior to application of the gel, 2 strips of paper tape will be applied to the subject's upper arms. One strip will be applied approximately 2 inches below the subject's shoulder and the second strip will be applied approximately 2 inches above the subject's elbow. The entire contents of one syringe (0.75 mL of gel) will be applied onto their arm in a straight line, from approximately 1 inch below the first strip of paper tape to approximately 1 inch above the second strip of paper tape. The subject will be instructed to gently rub the gel into their upper arm with their fingertips, while staying between the two pieces of paper tape, for approximately 2 minutes or until the gel has been absorbed. After completing the application process on both arms, the subject will wash his/her hands thoroughly.

A complete description of the administration process can be found in Appendix 1.

10. STUDY DRUG MATERIALS AND MANAGEMENT

10.1 Description of Study Drug

A novel topical vitamin D3 (cholecalciferol) gel has been developed and is being provided by Innovative Pharmacy Solutions, Inc. The ingredients in the gel are well-known in the industry and have references in the pharmaceutical literature and some have available FDA drug master file information. The formulation for the topical vitamin D gel is:

Gel Formula	
Vitamin D3 1,000,000 IU/gm	20.0 gm
Phosol 53 MCT (lipoid)	20 gm
Cholesterol USP-NF	0.8 gm
Isopropyl myristate USP-NF	5 gm
Poloxamer 407 NF	7.5 gm
Purified Water USP	46.5 gm
Methylparaben USP-NF	0.18 gm
Propylparaben USP-NF	0.02 gm

The previous anhydrous formula was changed to a hydrous base to provide a less greasy product. The new formula is a simple wash off base that provides phospholipids that are able to self-associate into micelles and liposomes.

10.2 Packaging and Labeling

The topical vitamin D gel is provided in a syringe as a single application of 0.75g of gel, containing 150,000 IU of vitamin D3 (cholecalciferol) that will be applied to each of the subject's upper arms, totaling 300,000 IU per dose.

Labeling of study drug will comply with regulatory requirements as follows:

II.G. Labeling
II.G. 1. Anhydrous Base

Hydrous Base

Date Made 04-10-2014
Exp 07-10-14
Compounded Product
Lot # 20140410
IND# 110937
KEEP REFRIGERATED
Caution New Drug –
Limited by Federal Law to
Investigational Use

II.G. 2. Drug Product

1. The label for the syringe with the final product will include the product name, strength and quantity, mixing date, beyond use date, name of the pharmacy compounding the product (Innovative Pharmacy Solutions), IND number, RPh name that compounded and dispensed and auxiliary labels "Keep Refrigerated" and "CAUTION New Drug – Limited by Federal law to Investigational Use."

> Innovative Pharmacy Solutions
> IND# 110937
> Vitamin D3 in Hydrous Base 200,000 IU/mL
> Quantity: 0.25ml 50,000 IU VitaminD3
> Mix Date: 7.28.2015 Beyond Use Date: 7.28.2015
> RPh name: Toll Free Phone 1-800-844-9845
> KEEP REFRIGERATED
> **CAUTION New Drug - Limited by Federal Law to Investigational Use**

2. The ziplock bag label will contain the compounding pharmacy name (Innovative Pharmacy Solutions), address, phone number and after hours phone number, IND number, "Keep Refrigerated" and "CAUTION New Drug – Limited by Federal law to Investigational Use."

> Innovative Pharmacy Solutions
> IND# 110937
> 323 Jackson Avenue Elk River, MN 44330
> Minnesota Pharmacy License # 263570
> Pharmacy Phone 763-421-4788
> Steve Cell 612-270-5504 Toll Free Phone 1-800-805-9845
> KEEP REFRIGERATED
> **CAUTION New Drug - Limited by Federal Law to Investigational Use**

3. The 1 mL syringe labels will contain the product name and "For Left Arm administration" or "For Right Arm administration", IND number, and "CAUTION New Drug – Limited by Federal law to Investigational Use."

> For Left Arm Administration
> 50,000 IU Vitamin D3 in Anhydrous Base 0.25 ml
> IND# 110937
> **CAUTION New Drug - Limited by Federal Law to Investigational Use**

For Right Arm Administration
50,000 IU Vitamin D3 in Anhydrous Base 0.25 ml
IND# 110937
CAUTION New Drug - Limited by Federal Law to Investigational Use

10.3 Study Drug Administration

A study nurse or physician will supervise each treatment application to ensure that the gel is applied correctly. Prior to application of the gel, 2 strips of paper tape will be applied to the subject's upper arms. One strip will be applied approximately 2 inches below the subject's shoulder and the second strip will be applied approximately 2 inches above the subject's elbow. The entire contents of one syringe (0.75 mL of gel) will be applied onto each arm in a straight line, from approximately 1 inch below the first strip of paper tape to approximately 1 inch above the second strip of paper tape. The subject will be instructed to gently rub the gel into their upper arm with their fingertips, while staying between the two pieces of paper tape, for approximately 2 minutes or until the gel has been absorbed. After completing the application process on both arms, the subject will wash his/her hands thoroughly.

Handling and Storage of Study Drug

Specific instructions for preparing and administering the topical vitamin D gel are provided in Appendix 1.

11. STUDY ASSESSMENTS AND PROCEDURES

The schedule of study assessments and procedures is shown in Table 1.

11.1 Baseline Assessments

A chart review of subjects with known vitamin D deficiency will be performed prior to Day 0 in order to assess for potential study eligibility. Informed consent must be obtained before any study procedures are performed.

11.1.1 Study Day 0

The following procedures should be completed on Day 0:

- informed consent
- assessment of height, weight and skin to assess for study eligibility
    - Anthropometric Assessment
        - Patients are measured for height (cm) and weight (kg) following standardized procedures as outlined in the Anthropometric Standardization Reference Manual (Lohman et al., 1991). Body weight is measured with a stand-on digital scale and height is measured by a stadiometer. Height is measured to the nearest 0.1 cm and weight to the nearest 0.1 kg.
    - Recording of Fitzpatrick skin-type score.
- Blood draw for vitamin D level, parathyroid hormone level, calcium, and albumin.
- Application of the topical vitamin D gel, including instructions for dosing at home

11.1.2 Study Day 7 and Study Day 14

- Evaluation of skin site where gel applied. If the subject has any skin rash or other reaction, he/she should contact study personnel as soon as possible for photographs and an assessment/diagnosis
    - Photographs of application sites
        - A digital photograph may be taken of the gel application site following application to record any signs of irritation or reaction. Identifying marks on or near the application site (i.e. tattoos, birthmarks, scars etc.) should be avoided when a photograph is taken. Digital photographs will be uploaded to the REDcap database and stored on the secure server.
- Blood draw for vitamin D level and calcium
- Application of the topical vitamin D gel

11.1.3 Study Day 21

- Assessment of skin for rash or any other reaction
- Blood draw for vitamin D, parathyroid hormone level, and calcium.

12. EFFICACY ASSESSMENTS

Efficacy assessments should be performed according to the Schedule of Events (Table 1).

12.1 Vitamin D Levels

Assessments of serum 25-OH vitamin D levels (D2 and D3) will be done and on Day 0, 7, 14, and 21. Venous blood draws will be performed by a trained University of Minnesota Physicians (UMP) technician and delivered to HealthEast laboratory for analysis.

13. ASSESSMENT OF SAFETY

13.1 Safety Assessments

13.1.1 Skin Rash or Other Reaction

An assessment of each subject's skin will be performed on Days 7, 14 and 21 using the following scoring system for topical irritation developed per FDA guidance:

Basic Score:	**Description:**
0	No visible reaction
0.5	Doubtful or negligible erythema reaction
1.0	Mild or just perceptible macular erythema reaction in a speckled/follicular, patchy or confluent pattern (slight pinking)
2.0	Moderate erythema reaction in a confluent pattern (definite redness)
3.0	Strong or brisk erythema reaction that may spread beyond the test site

Supplemental Scores:	**Description:**	**Label:**
0.5	Edema	E
0.5	Papules	P
0.5	Vesicles	V
0.5	Bullae	B

Supplemental scores will be added to the basic scores if the associated symptoms are present. The final score will be the sum of the basic and supplemental scores.

As part of the general safety assessment, dermal irritation will be evaluated by the Investigator or designated research staff using the outlined scale. The likelihood of allergic reaction not manifested as a dermal irritation is extremely low. Given that all the ingredients used to make the topical vitamin D gel are considered GRAS, we do not anticipate non-local allergic reactions. In the event that a non-dermal allergic reaction occurs, standard medical procedures utilized for such situations will be followed.

If a subject develops a dermal response of 1.5 up to 2.5, he or she will be given the option to permanently discontinue the study treatment and withdraw from the study, or to allow sufficient time for the rash to resolve before he or she reinitiates the phase of the study he or she was in at the time that the rash developed. If a participant develops a dermal response of 2.5 or greater, he or she will be required to permanently discontinue the study treatment and he or she will be withdrawn from the study. In both cases, the study participant will be encouraged to seek medical care from his or her primary care physician. In addition, we will ask subjects who decide to withdraw from the study to return for their next scheduled blood draw, as per protocol. We will also follow up with any study participant who withdraws from the study due to AE occurrence through telephone contact at 1 week intervals until the AE resolves.

All other adverse events will be assessed by self-report from subjects.

We will initially administer 100,000 units topically to subjects, and check serum Vitamin D and calcium levels one week later. Dosing will be discontinued if a patient achieves a Vitamin D level of greater than normal (> 90 ng/ml) or if serum calcium rises above normal (> 10.2 mg/dl). Additional doses of 100,000 units will be administered on days 7 and 14. The outcome of interest will be the effect on total serum Vitamin D at 21 days. Although a change in calcium is unlikely, calcium will be monitored for safety.

Vitamin D may alter glucose values and improve glucose control in individuals with diabetes[26]. Although individuals with diabetes will not be excluded, we will instruct any subjects with diabetes to more closely monitor glucose values during the course of the study.

Parathyroid hormone will be measured at baseline and on Day 21 to determine if vitamin D correction changes the level of this metabolic calcium regulatory hormone.

13.2 Reporting of Safety Information

13.2.1 Definition of an Adverse Event (AE)

Any untoward medical occurrence in a clinical investigation subject, temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- A new condition detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE)

Examples of an AE do not include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to hospital, such as for PK sampling convenience)

In this study, AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e., invasive procedures, modification of a subject's previous therapeutic regimen).

13.2.2 Definition of a Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that, at any dose:

a. results in death

b. is life-threatening

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

c. requires hospitalization or prolongation of an existing hospitalization

Note: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-subject setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. results in disability/incapacity

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

e. is a congenital abnormality / birth defect

f. any event deemed by the Investigator as being a significant medical event.

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

13.2.3 Recording of AEs and SAEs

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

For each AE, start and stop dates, action taken, outcome, intensity (see Section 13.2.3.1) and relationship to study product (causality) (see Section 13.2.3.2) must be documented. If an AE changes in frequency or intensity during a study, a new entry of the event must be made in the CRF.

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

All details of any treatments initiated because of the AE should be recorded in the subject's notes and the CRF.

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

13.2.3.1 Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgment. Adverse events from the first dose of topical vitamin D gel will be assesed based on this general guideline:

- Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily
- Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living
- Life-threatening consequences; urgent intervention indicated
- Death related to AE

Severity should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as "serious" when it meets one of the pre-defined outcomes as described in Section 13.2.2 "Definition of an SAE."

13.2.3.2 Assessment of Causality

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

The causal relationship to the study product assessed by the Investigator (or medically qualified delegate) should be assessed using the following classifications:

Not Related In the Investigator's opinion, there is not a causal relationship between the study product and the AE.

Unlikely The temporal association between the AE and study product is such that the study product is not likely to have any reasonable association with the AE.

Possible The AE could have been caused by the study subject's clinical state or the study product.

Probable The AE follows a reasonable temporal sequence from the time of study product administration, abates upon discontinuation of the study product and cannot be reasonably explained by the known characteristics of the study subject's clinical state.

Definitely The AE follows a reasonable temporal sequence from the time of study product administration or reappears when study product is reintroduced.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

13.2.3.3 Assessment of Expectedness

Expected An adverse reaction, the nature or severity of which is consistent with the applicable product information (e.g., Investigators' Brochure for an unapproved medicinal product).

Unexpected An adverse reaction, the nature or severity of which is not consistent with information in the relevant source document (e.g., Investigators' Brochure for an unapproved medicinal product).

13.2.4 Reporting of SAEs

Once a Sponsor-Investigator becomes aware that an SAE has occurred in a study subject, he/she will assess the event and determine if it meets regulatory reporting requirements. The Investigator must also notify the reviewing Investigational Review Board (IRB) and site governance office (if required) of any SAEs according the guidelines of the IRB.

14. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Data for this study will be entered into a REDCap database, which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

The general analytical approach for all endpoints will be descriptive in nature. No formal statistical hypothesis testing will be conducted. No p-values will be presented due to the small sample size of this study. Data analyses will be provided for all study subjects combined wherever appropriate.

For continuous variables, summary statistics will include number of subjects, mean, median, standard deviation, minimum, and maximum. For categorical variables, descriptive statistics will include number of subjects and percentages.

In regards to handling of missing data, there will be no imputation of missing values.

14.1 Analysis Populations

Safety Population: all subjects who receive at least 1 dose of study drug and who have at least one post-dosing safety evaluation. All safety endpoints, baseline characteristics, and demographic data will be summarized using the Safety Population.

Efficacy Evaluable Population: all subjects who receive all 3 doses of study drug and have a follow up blood draw for vitamin D levels, or discontinue prior to completing dosing due to a drug-related adverse event. Vitamin D level data will be analyzed using the Efficacy Evaluable Population.

14.2 Study Endpoints

The primary endpoint is to establish the change in vitamin D blood levels following 3 doses of treatment with vitamin D gel.

Secondary study endpoints include assessment of safety, including change in parathyroid hormone level and the number of subjects who have any skin rash and/or other skin reaction following treatment with the vitamin D gel.

14.3 Data Analysis

14.3.1 Subject Disposition

The number and percentage of subjects entering and discontinuing the clinical study will be presented. The reasons for discontinuation will also be summarized.

14.3.2 Demography and Baseline Characteristics

Demographic and baseline characteristics will be descriptively summarized using the Safety Population. Quantitative and/or categorical summaries will be presented for demographics and other baseline characteristics.

14.3.3 Treatment Exposure

Treatment exposure will be summarized. Measures of extent of exposure include the total number of doses per subject, cumulative dose per subject, and compliance (percent of target dose received per cycle). The reason(s) for dose(s) missed will also be summarized.

14.3.4 Safety Analyses

Safety parameters will be listed and summarized using standard descriptive statistics.

Adverse event terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by dose. Incidence of AEs occurring during the study will be summarized by system organ class (SOC) and preferred term. Adverse events will also be summarized by causality and grade. Serious adverse events and AEs leading to discontinuation of study treatment will be listed separately.

Additional analyses will be performed if warranted upon review of the data.

14.3.5 Analysis of Efficacy

The primary outcome will be the change in total Vitamin D following the topical application of 100,000IU Vitamin D3 weekly for three weeks.

14.4 Determination of Sample Size

A change from baseline of 10 μg/L in total 25-OH Vitamin D levels in subjects with Vitamin D deficiency is thought to be a clinically relevant improvement. Assuming that the standard deviation for pre and post measures of total 25-OH Vitamin D is 4 μg/L, then the standard deviation of the difference is 5.6 μg/L so 10 subjects should be adequate to show an effect size of 6.4 μg/L with alpha of .05 and a power of 90%. However the standard deviation of the second measure is unknown since absorption may vary by individual. Assuming the standard deviation of the second measure increases to 12, then 20 subjects would be needed to detect an effect size of 10 μg/L. In order to be most conservative, 20 subjects will be treated in this proof-of-concept study.

ETHICS

14.5 Ethical Considerations

This study will be carried out according to the Declaration of Helsinki, the National Statement on Ethical Conduct in Human Research (2007) and the Notes for Guidance on Good Clinical Practice as adopted by the Australian Therapeutic Goods Administration (TGA; 2000) (CPMP/ICH/135/95) and the ICH GCP Guidelines[27].

14.6 Ethical Review Committee

The Protocol will be submitted for review to the University of Minnesota Investigational Review Board (IRB), and written approval will be obtained from both the IRB and Governance Office (as required), before subjects are recruited and enrolled. The Investigators will receive all the documentation needed for submitting the protocol to the IRB and/or Governance Office. A copy of the respective approval letters will be obtained before starting the study. If approval is suspended or terminated by the IRB or Governance Office, the Sponsor-Investigator will have responsibility for notifying the Food and Drug Administration.

It is the responsibility of the Investigator to report study progress to the IRB or Governance Office as required.

The Sponsor-Investigator, or his/her nominee, will be responsible for reporting any SAEs to the IRB as required.

14.7 Informed Consent

Before recruitment and enrollment into the study, each prospective subject will be given a full explanation of the nature and purposes of the study. Once the essential study information has been provided, and the Investigator is assured that the volunteer understands the implications of participating in the study, the subjects will be asked to give consent to participate in the study by signing the Informed Consent Form (ICF)/Patient information sheet. The consent form will be signed and dated by the appropriate parties. A notation that written informed consent has been obtained will be made on the subject's medical record and CRF. The completed consent form will be retained by the Investigator. A copy of the completed ICF will be provided by the Investigator to the subject.

15. STUDY CONDUCT

15.1 Regulatory Authorities

This study will be conducted under an Investigational New Drug (IND 110937).

15.2 Monitoring Procedures and Quality Assurance

The organization, monitoring, supply of study materials and quality assurance of the present clinical study is the responsibility of Innovative Pharmacy Solutions or its designee.

Monitoring may be conducted to ensure compliance with the protocol, ICH GCP and applicable regulatory requirements.

In order to ensure the accuracy of data, direct access to source documents by the representatives of Innovative Pharmacy Solutions, Inc. and regulatory authorities is mandatory.

The Investigator is required to submit to the Reviewing IRB, annual (or more frequent if requested) reports of the study.

15.3 Curriculum Vitae

A current, signed and dated Curriculum Vitae for all study staff will be provided and maintained in the files at Innovative Pharmacy Solutions, Inc. (or designee). A Staff Signature List will be maintained at each Investigator's site.

15.4 Investigator Responsibilities

Except where the Principal Investigator's signature is specifically required, it is understood that the term 'Investigator' as used in this Protocol and on the CRFs refers to the Principal Investigator or an appropriately qualified member of the staff that the Principal Investigator designates to perform specified duties of the Protocol. The Principal Investigator is ultimately responsible for the conduct of all aspects of the study.

Each Investigator will comply with the local regulations regarding clinical trials and the Investigator responsibilities outlined in the ICH GCP guidelines.

15.5 Emergency Contact with Investigators

All subjects will be provided with a Participant Emergency Contact Card with contact details of whom to contact in the case of an emergency.

15.6 Notification of Primary Care Physician

With the consent of the subject, it is the Investigator's responsibility to notify the primary care physician of the subjects' involvement in the study, provided that such a physician can be identified for the subject. A letter will be sent to the physician stating the nature of the study, treatments, expected benefits or AEs and concomitant drugs to be avoided. A copy shall be retained by the study site.

15.7 Protocol Compliance

The instructions and procedures specified in this protocol require diligent attention to their execution. Any subject treated in a manner that deviates from the protocol, or who is enrolled into the study but is not qualified according to the Protocol may be ineligible for analysis and thereby compromise the study.

Only when an emergency occurs that requires a departure from the Protocol for an individual will there be such a departure. The nature and reasons for the Protocol violation/deviation shall be recorded in the CRF.

The Investigator and designees will comply with all applicable federal, state and local laws.

15.8 Termination of the Study

The Sponsor-Investigator reserves the right to terminate the clinical study at any time. Reasons for termination may include, but are not limited to, the following:

- the incidence or severity of AEs in this study
- serious or persistent noncompliance by the Investigator with the protocol, clinical research agreement, or applicable regulatory guidelines in conducting the study
- IRB decision to terminate or suspend approval for the investigation or the Investigator
- Investigator request to withdraw from participation

In addition the Sponsor-Investigator reserves the right to discontinue the study prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must contact all participating subjects promptly, and written notification must be sent to the Reviewing IRB and relevant Governance Offices (as required).

16. ADMINISTRATIVE CONSIDERATIONS

16.1 Protocol Amendments

Protocol modifications that impact on subject safety or the validity of the study will be approved by the IRB. If a Protocol amendment requires changes to the Informed Consent Form, the revised Informed Consent Form, prepared by the Investigator, must be approved by the IRB and site governance offices (as required).

Once the final Protocol has been issued and approved by the Sponsor-Investigator and the authorized signatories, it shall not be informally altered. Protocol amendments are alterations to a legal document and have the same legal status. Therefore, they must pass through appropriate steps before being implemented. In general, any important change that theoretically increases risk to subjects constitutes an amendment.

It is the responsibility of the Investigator to submit the amendment to the IRB for their approval; written approval should be obtained. The original signed copy of amendments will be kept in the Study File with the original Protocol. It should be noted that where an amendment to the Protocol substantially alters the study design or the potential risks to the subjects, each subject's consent to continue participation should be obtained.

16.2 Archiving of Records

All source documents, CRFs and trial documentation will be kept by the Investigator for the appropriate retention period as stipulated by local regulations and ICH-GCP.

16.3 Study Report

The Sponsor-Investigator or designee will produce the clinical study report at the completion of the study. In addition, it is intended that the outcome of the study will be submitted for publication in appropriate peer review journals and scientific meetings, as accepted.

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