i-NITRITE THERAPY FOR TREATMENT OF PERIPHERAL ARTERIAL DISEASE

by

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*We also certify that written approval has been obtained for any proprietary material contained therein.
Dedicated to my family and teachers for their love and support!
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i-Nitrite Therapy for Treatment of Peripheral Arterial Disease

Abstract

by

Neeti Maan

Peripheral Arterial Disease (PAD) is a chronic disorder associated with reduced blood flow to the extremities which results in serious complications such as critical limb ischemia, limb amputation and in some cases may lead to death. Approximately 18 million patients in US were affected by PAD in 2010 and with an increasing aging population this number is estimated to increase to 24 million by 2030. There is an urgent need for the development of safe and effective novel drug therapies targeting the stimulation of new vessel growth and help in revascularization of ischemic found in PAD.

The disease is a result of atherosclerosis and endothelial dysfunction leading to decreased nitric oxide bioavailability, an important modulator of vascular tone. TheraVasc has come up with a novel pharmaceutical composition TV1001 that is a nitric oxide prodrug and enhances nitric oxide bioavailability in ischemic region of limbs associated with PAD. TV1001 induces angiogenesis and enhances tissue perfusion specifically in ischemic tissue.
1. INTRODUCTION

a. About TheraVasc Inc

TheraVasc Inc. is a privately held biotechnology C-Corp located in Cleveland Ohio working towards translation of innovative research to products that will improve the quality of life of unserved people. TheraVasc vision is to promote today’s innovations that will be tomorrow’s valuable products for better health of people. TheraVasc is dedicated to reducing the risk, cost and time associated with drug development by focusing on developing repurposed drugs for unserved markets. By focusing its efforts on drugs with established safety profiles, targeting diseases that result in a terrible quality of life or rapidly progress to death, and for which no current therapies exist.

TheraVasc management believes that both patients and clinicians will readily embrace its products, which in turn will facilitate development by increasing enrollment into clinical trials. In addition, the regulatory path with the FDA will be simplified due to an established safety profile with the agency. TheraVasc initial product, TV1001 is an oral pharmaceutical composition of sodium nitrite and targets patient populations with poor blood flow to the extremities, such as those with vascular complications of diabetes or PAD. The Company is currently pursuing clinical trials for the treatment of PAD and other problems associated with poor blood flow to the limbs, including diabetes.
\textit{b. Internship at TheraVasc}

I joined TheraVasc in 2010 as a part of my internship. At TheraVasc I have contributed to variety of projects from grant writing to intellectual property matters.

I carried out extensive scientific literature searches, clinical trial searches and helped in writing the pharmacology section of company’s recently filed IND. In addition to this, I wrote federal and ADA grants which are currently being reviewed. I also performed patent searches and provided assistance in intellectual property matters. Besides that, I am working closely with the clinical trial management team for the Company’s upcoming clinical trial.

\textit{c. Scope of Thesis}

This thesis will provide an overview of severity of PAD and its social economic impact. The document will address the problem with current available treatment as well as therapeutic potential of emerging inorganic nitrite. Further it will describe the innovation behind the TheraVasc product. Furthermore the need, market potential and value proposition of TheraVasc pharmaceutical compositions are discussed. In this section competition from current treatments and new entrants are also analyzed. Lastly, TheraVasc’s project plan and companies IP strategy is described.
2. PERIPHERAL ARTERIAL DISEASE (PAD)

PAD is a chronic disorder associated with reduced blood flow to the extremities which results in serious complications such as critical limb ischemia, limb amputation and in some cases may even lead to death (1). The predominant cause of PAD is systemic atherosclerosis that results in arterial stenoses in the arteries supplying muscles of lower extremities (2). Atherosclerosis is caused by the oxidation of low-density lipoproteins in the vessel walls. This oxidation initiates cascades of inflammatory and proatherogenic events. These events increase the uptake of low density lipoproteins by macrophages which result in the foam cell formation, smooth cell proliferation and thus plaque formation. This can further cause atheromatous plaque ruptures and thrombus formation in the arteries. Gradually atherosclerosis causes the critical narrowing of arteries, limiting their ability to supply blood and oxygen to tissues resulting in tissue ischemia (3).

*Figure-1 Difference between normal artery and artery with atherosclerosis (4)*
Further, a significant contributor to the development of the disease is endothelial cell dysfunction involving defects in vascular reactivity due to decreased nitric oxide bioavailability which further enhances atherosclerosis (5).

Patients with PAD often feel pain because of intermittent claudication, in this case lower extremities arteries cannot deliver blood and oxygen to meet the tissue demands. Moreover complete obstruction of an artery by thrombus can further lead to tissue necrosis. This can further lead to open sores, ulcers and gangrene (Figure-2).

![Figure-2 PAD patient foot showing gangrene (6)](image)

Critical limb ischaemia (CLI) is a severe manifestation of PAD. The patients with CLI experience chronic ischemic pain even at rest and often have ischemic skin lesions such as ulcers and gangrenes (7). These patients have higher mortality as the artherosclerosis is systemically spread and is not localized to a single vessel. Most of the patients with CLI need surgical revascularization to prevent limb loss. Studies have reported that 25% of patients die within one year of onset of CLI and 25% require a major amputation (8).
Moreover, one third of the amputated PAD patients die within one year of the surgical procedure (7).

The common risk factors associated with PAD include age, smoking, diabetes mellitus, hypertension, hypercholesterolemia and chronic renal insufficiency (9). The best predictor of the disease is aging, as the population continues to age; the incidence of PAD will also continue to increase. However, PAD is also reported in patients less than 40 years of age. Increasing prevalence of obesity and diabetes may help to explain the recent increase in incidence among younger people (10).

Patients with PAD are at greater risk of cardiovascular diseases including myocardial infarction and stroke (11). Half of the PAD patients have coronary artery disease (12).

Various imaging tests such as Doppler ultrasound, Duplex ultrasound, angiography and MRI are used in the diagnosis of PAD (13). PAD is most commonly diagnosed by ankle brachial index (ABI) (14). It is a simple non invasive test in which blood pressure measurements are taken at arms and ankle (Figure-3). ABI is the ratio of the systolic blood pressure in the ankle to systolic blood pressure in the brachial artery of arms. ABI less than 0.9 indicate that patient is having PAD and ABI below 0.5 suggests severe occlusion in the arteries of legs.
Figure-3 Illustration of Ankle Brachial Index Test (14)
3. THERAVASC INNOVATION

a. Role of nitric oxide in vascular health

Nitric oxide (NO) is an important regulator of vascular health in humans and defects in its bioavailability are responsible for many vascular diseases (15). NO is synthesized in mammals by nitric oxide synthases (16). Nitric oxide synthases are of three types, endothelial isoforms (eNOS), neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS). Nitric oxide is synthesized in vascular endothelial cells by endothelial nitric oxide synthase (Figure-4) and is responsible for maintaining vascular tone in humans.

Figure -4 is copy written please see reference number-17 for further information

_Figure: 4 Nitric oxide synthesis in endothelial layer of arteries (17)_
Nitric oxide synthase converts L-Arginine to L-Citrulline resulting in nitric oxide formation. This reaction is mediated by co-factors such as BH₄, NADPH, Ca²⁺ and oxygen (18).

NO is mediator of various important functions in vascular system. NO oxide diffuses to smooth muscle cells and carries out vasodilation by increasing the amount of cyclic GMP (Figure-5).

Figure -5 is copy written please see reference number-19 for further information

*Figure: 5 NO oxide signaling pathway (19)*

Nitric oxide (NO) is an important player in regulating physiological and therapeutic angiogenesis and works through various signal transduction pathways, including VEGF dependent angiogenesis (20).

Nitric oxide itself has not proven to be a viable strategy for ischemic disorders for a number of reasons, including expense and the difficulties in site specific delivery to
achieve desired effects (21). Moreover, NO is not stable and is associated with systemic side effects such as hypotension and possible cytotoxicity at high doses. The use of current NO donors are also significantly limited as these agents cannot achieve site specificity to tissue compartments thus requiring the use of larger doses with a narrow therapeutic range. Moreover, long term use of organic nitrates such as nitroglycerin is entirely different from inorganic nitrite as nitroglycerin is metabolized in a completely different manner and its use is associated with tolerance (22).
b. i- Nitrite Therapy

Relation between NO and Nitrite
Nitrite is endogenously generated either by oxidation of nitric oxide or by reduction of salivary nitrate (Figure-6). The nitric oxide derived from L-Arginine-NO pathway is oxidized to nitrite in the presence of oxygen and ceruplasmin. Besides that, commensal bacteria in the salivary glands reduce nitrate into nitrite with the help of nitrate reductase enzymes present in them. Recently, inorganic nitrite has also been demonstrated to be a selective nitric oxide donor during hypoxia and acidosis (23, 24,25).

Figure -6 is copy written please see reference number-25 for further information

*Figure: - 6 Mammalian nitrogen oxide cycle (25)*
Normally, nitrite is derived from nitrate rich diet. Foods such as green leafy vegetables, beets, celery, broccoli and cauliflower are good source of nitrates and nitrites (26). Dietary nitrate is absorbed from the upper part of the intestine, and transported via plasma into the salivary glands where it is ultimately reduced to nitrite by oral symbiotic bacteria (27). This nitrite is further absorbed by the GI tract and accumulates in the circulation (Figure-7).

Figure -7 is copy written please see reference number-27 for further information

*Figure:*-7 Enterosalivary circulation showing the locations and reactions that occur in the bioactivation of nitrate to nitrite and thence NO. After ingestion of oral inorganic nitrate (NO$_3^-$; 1), the NO$_3^-$ is swallowed and enters the gastrointestinal (GI) tract (2), where it is then absorbed and accumulates in the circulation (3). Approximately 75% of the ingested NO$_3^-$ is excreted via the kidneys (4) and the remaining 25% concentrated in salivary glands (5). This NO$_3^-$ enters the saliva where it is reduced to NO$_2^-$ by oral bacteria (6). The NO$_2^-$ is then swallowed thereby entering the GI tract (7) and subsequently resulting in the accumulation of NO$_2^-$ in the circulation (8). Once within the
circulation the NO$^{2-}$ is reduced to nitric oxide (NO) in the vasculature where it activates soluble guanylate cyclase (sGC) resulting in bioactivity (27).

Besides the synthesis of NO through L-Arginine/NOS pathway; recent studies strongly suggest that nitrite generates nitric oxide under acidic and low oxygen concentrations (28). Nitrite is reduced to nitric oxide by number of mechanisms such as acidic disproportion (29), heme dependent nitrite reduction (30) or enzymatic reduction by enzymes like xanthine oxido reductase (31). Various studies have concluded that nitric oxide generated by nitrite exhibit cytoprotective and vasodilatory effects under ischemic conditions.

Figure -8 is copy written please see reference number-29 for further information

*Figure-8 Nitrite is reduced to NO under ischemic condition and potentially nitrosylates and nitrates proteins and lipids along the physiological oxygen and pH gradient, ultimately modulating important signal transduction pathways, physiological functions and disease (29).*
**Therapeutic potential of inorganic nitrite**

Nitrite is a circulating and tissue storage form of the gaseous nitric oxide molecule, a key regulator of cardiovascular health and therapeutic angiogenesis (32). Thus, nitrite is an endogenous modulator with the potential to treat cardiovascular diseases (33).

The beneficial effects of sodium nitrite therapy have been linked to the stimulation of angiogenesis, nitric oxide-induced increased blood flow at the site of injury, and robust and selective blood reperfusion in ischemic tissues. This is due to the selective conversion of sodium nitrite back to nitric oxide only in the ischemic region without any effect on non-ischemic tissues (34).

**Plasma Nitrite levels lower in vascular disease patients**

Moreover, when circulating levels of nitrite were compared in normal volunteers, diabetic patients, patients with PAD and diabetic patients with PAD, it was found that circulating nitrite levels were lower in all three groups of patients than in normal subjects. The lowest levels were observed in patients with PAD and diabetes. This article also reported that while nitrite levels increased in normal subjects following exercise, plasma nitrite levels did not increase in the PAD and diabetic PAD patient populations (35).

It is also known that risk factors associated with cardiovascular disease are inversely correlated to circulating plasma levels of nitrite (36). These studies suggest that circulating nitrite levels are important modulators of endothelial health.
**Nitrite as Vasodilator**

Inorganic nitrite therapy has emerged as a prime candidate to act as a selective nitric oxide donor during hypoxia and acidosis. Numerous studies have reported nitrite mediated ischemic vasodilation in animal models (37, 38) and humans (39, 40). Studies have also confirmed that nitrite acts as a vasodilator particularly in hypoxic conditions (41). Moreover, Rifkind et al. have shown that nitrite infusions led to increases in cerebral blood flow in rats (42). Recently Presley et al. demonstrated the ability of oral nitrate to increase plasma nitrite as well as to increase cerebral blood flow within the white matter of elderly humans (43).

**Nitrite’s cytoprotective role**

Various animal studies suggest an important cytoprotective role for nitrite in ischemia-reperfusion injury of the heart (44), liver (45) and kidney (46). A study done by Jung et al. clearly demonstrated a neuroprotective effect of sodium nitrite on the ischemic brain in rats by decreasing infract size and enhancing cerebral blood flow (47).

Another study done by Dezfulian et al. suggests a beneficial role of sodium nitrite as a neuroprotectant in brains of mice using the I/R model (48). Even a small rise in plasma nitrite of ~350 nM increased forearm blood flow in humans and non human primates (49).

Figure -9 is copy written please see reference number-33 for further information

*Figure-9 Therapeutic potential of the inorganic anion nitrite in various disease states (33)*
Role of nitrite in other disease states

A recent study done by Mack et al. suggested the potential therapeutic role of sodium nitrite in treating sickle cell disease which is also characterized by decreased NO bioavailability as well as increased oxidative stress. This study showed that nitrite is able to augment forearm blood flow and is also potent to increase systemic nitrite level changes (50).

Another study done by Ohtake et al. describes the role of inflammatory disease such as experimental colitis. This study demonstrated that administration of a high dose of nitrite
in a mouse model of colitis was able to induce protection against tissue damage and restored tissue nitrite levels (51).

**Current clinical trials with Sodium nitrite**

At present there are 12 clinical studies underway to investigate the therapeutic potential of inorganic nitrite therapy for diseases such as MI, cerebral vasospasm, pulmonary hypertension and sickle cell disease (52). While the data from these studies may provide evidence for clinical efficacy, no information is available on the possible use of inorganic nitrite for treating chronic ischemic vascular disorders such as cerebral vascular disorders in diabetic patients.
Sodium Nitrite safety profile

Sodium nitrite is an FDA approved drug as an active ingredient in cyanide antidote kits for treating cyanide poisoning (53). The dose of nitrite administered to humans to treat cyanide poisoning is significantly higher than doses administered to stimulate hypoxic blood flow, protect tissues from ischemic injury and promote angiogenesis (0.5-10μM). At these low doses no toxicity has been observed in rodents, primates or humans. There are three major safety concerns surrounding the use of therapeutic sodium nitrite; methemoglobinemia, cancer and lowering blood pressure.

Methemoglobinemia

The conversion of ferrous hemoglobin to methemoglobin can occur when erythrocytes are exposed to high levels of nitrite. Although, there is a reasonable safety window for levels of methemoglobin before serious adverse events occur, the generation of methemoglobin does represent the single largest concern with nitrite therapies. Normally, methemoglobin represents about 1% of globin levels found in the blood. When methemoglobin levels reach about 10-15% of the total globin levels, headaches and dizziness can occur. At methemoglobin levels of 50%, a patient can become comatose and possibly die.

Again, the doses that lead to these adverse events are measured in grams while the doses to be used in TheraVasc trial are substantially less. Kohn et al. has predicted that sodium nitrite doses necessary for methemoglobin-related symptoms to occur would be 15.9 and 11.0 mg/kg in male and female rats respectively (54). This is five to ten times higher than the maximal dose (80 mg or approximately 1-2 mg/kg) contemplated for this study.
Nitrosamine formation

The other risk associated with nitrite is the formation of carcinogenic nitrosamines. This concern was mitigated by the results of a two-year chronic oral nitrite dosing study of rodents, performed by the National Toxicology Program, which saw no evidence of cancers at doses 10-100-fold higher than those contemplated in the present study (55).

Lowering of Blood Pressure

Lastly, the use of sodium nitrite may result in a decrease in blood pressure, since the compound is a vasodilator. However, the concentrations and doses required to support hypoxic blood flow, inhibit tissue injury and accelerate angiogenesis are a log order lower than those known to lower blood pressure. Nevertheless, blood pressure will be closely monitored during the trial.
d. TheraVasc Science

Studies done by several laboratories have established the cytoprotective and vasodilatory therapeutic potential of sodium nitrite. TheraVasc’s scientific founder, Dr. Chris Kevil’s lab has also reported the cytoprotective effects of sodium nitrite in heart and liver of mice (56,57). Dr. Kevil’s lab study results strongly suggest that sodium nitrite mediates cytoprotection in a nitric oxide dependent manner. Knowing that nitric oxide is an important mediator of angiogenesis further intrigued Dr. Kevil lab members and they hypothesized that sodium nitrite therapy may augment angiogenesis in a disease such as PAD, characterized by defective vascular perfusion leading to chronic tissue ischemia.

Remarkably the results of this hypothesis were exciting, Dr. Kevil has discovered that inorganic sodium nitrite therapy serves as a potent and selective NO prodrug which robustly augments blood flow and induces angiogenesis specifically in ischemic tissues (57). Importantly, sodium nitrite therapy had no effect on angiogenic activity in non-ischemic tissues, while angiogenic stimulation in the ischemic tissue occurs rapidly, within few days. These findings highlight sodium nitrite as novel therapeutic approach to stimulate ischemic tissue specific angiogenesis.

The primary etiology of PAD is reduced blood flow to tissues resulting in tissue ischemia. Sodium nitrite represents an effective therapy for the treatment of PAD as nitrite can improve blood flow by regeneration of blood vessels and ischemic vasodilation.

Figure -10 is copy written please see reference number-56 for further information
**Figure-10** *Figure of a physiologically healthy vessel compared with a diseased vessel and the effect nitrite therapy has on diseased vessels (56).*

**Preclinical studies behind TheraVasc Science**

To evaluate the effect of sodium nitrite on tissue angiogenesis, sodium nitrite was tested in murine models of hind-limb ischemia. Femoral artery ligation was performed to reduce limb blood flow and decreased tissue perfusion which resulted in hind limb ischemia and necrosis. Ascending doses of sodium nitrite (8.25 µg/kg, 16.5 µg/kg, 165 µg/kg, and 3.3 mg/kg) were administered intraperitonially. It was observed that twice daily intraperitoneal sodium nitrite injections over a range of doses significantly increased perfusion of murine ischemic hind-limbs (Figure-11).
Figure- 11A) Changes in ischemic hind-limb blood flow after chronic ischemia PBS or sodium nitrite injections at various doses BID. Therapy regimens were begun within 2 hours of permanent femoral artery ligation. B) Scavenging of NO using carboxy-PTIO completely abrogated the ability of sodium nitrite to restore chronically ischemic hind limb blood flow demonstrating nitrite therapy works through in a NO dependent manner. *p<0.01 nitrite versus PBS control at each respective time point (n=10). #p<0.01 nitrite versus nitrite + c-PTIO at each respective time point (n=10)(57)

Importantly, this study further suggested that sodium nitrite significantly increase arteriogenesis by day 7 in murine ischemic gastrocnemius tissues compared with PBS control. Further plasma nitrite levels were found to be significantly increased in ischemic tissues as compared to non ischemic.
To study the effects of sodium nitrite on chronic arteriogenesis permanent artery ligation was performed and it was observed that sodium nitrite therapy increased the formation collateral branches as compared to PBS treatment (Figure 12). The arrow indicates the medial circumflex femoral artery (MCFA) with the red circle highlighting distal branches. Figure 12 A demonstrates that PBS therapy minimally alters MCFA size and perfusion. Figure 12B shows enhanced MCFA size and perfusion with nitrite therapy along with increased collateralization of the distal arterial branches. Figure 12 C further reports the number of arterial branches off of the MCFA between both treatments and demonstrates that continuous nitrite therapy significantly enhances collateral artery formation in the chronically ischemic hind limb.

Figure 12 is copy written please see reference number 57 for further information

*Figure: 12  A) Collateral artery formation and perfusion is shown using blue Microfil vascular casting resin for PBS treated ischemic hind limb at day 7. B) Collateral artery*
formation and perfusion is illustrated using Microfil vascular casting resin for 165 µg/kg sodium nitrite treated ischemic hind limb at day 7. C) Arterial branch points were counted originating from medial circumflex femoral artery (red circles). *p<0.05 sodium nitrite versus PBS treatments (n=10)(57)

TheraVasc believes that nitrite’s selective angiogenic activity is a true breakthrough in the field of vascular disease and offers an opportunity to effectively treat a large number of patients with PAD and DVD. The company has a major advantage over other drugs in which angiogenesis is not selectively induced, since abnormal blood vessel growth and associated side effects will be limited.
4. COMMERCIALIZATION PLAN

TheraVasc commercialization strategy is focused on reducing the risks and costs associated with the process of drug development. The company is working on the repurposed drug to meet the unmet needs of the vascular market. There is an urgent need for the development of safe and effective novel drug therapies aimed at the stimulation of new vessel growth and help in revascularization of ischemic region as is the case in PAD. The first product of Company is an oral pharmaceutical composition of sodium nitrite- “Vasodilator and Ischemic Tissue Angiogenesis Stimulator Drug- TV1001”. TV1001 is already being tested in a Phase I clinical trial in diabetic patients with peripheral vascular abnormalities.

a. Need

Socioeconomic impact of Peripheral Arterial Disease (PAD)

According to a recent analysis by The Sage Group, approximately 18 million U.S. citizens suffered from PAD in 2010 and this number is projected to increase to 24 million by 2030. Further, the number of Critical limb ischemia (CLI) currently is 2.8 - 3.5 million and is forecasted to increase to 4.5 and 5.6 million in US by 2030. Besides its severe outcomes, CLI poses a critical economic burden on the society. The CLI hospital treatment costs exceed $10 billion as reported at New Cardiovascular Horizons (NCVH) meeting in 2010. Furthermore, average inpatient charges for CLI are $55,000 and are higher than that of stroke and heart attack (58).
Patients with diabetes are also at a greater risk of developing PAD (59). Nearly 23.6 million people were reported to suffer from diabetes in US in 2010 and the prevalence of the disease is predicted to rapidly increase in the near future (60).

**Problem with Current Therapies**

Current PAD therapy focuses on initiating causes of disease such as high cholesterol, diabetes, smoking and high blood pressure (61). Pharmacological agents are used for symptomatic improvements (e.g. anti-platelets, statins, PDE inhibitors and β-blockers) with no drug currently approved to promote healing (62). Initial therapy consists of exercise and cilostazol therapy to improve blood flow with secondary anti-platelet therapy (63, 64). Since PAD is characterized by severe pain in the affected extremity, compliance is poor and the condition typically worsens over time. However, when symptoms deteriorate, surgical revascularization of the affected extremities by angioplasty or bypass graft is usually necessary.

In cases of critical limb ischemia (CLI), the standard of care initially involves treatment with heparin often leading to surgical intervention and in severe cases, amputation.

PAD is a fatal disease that gets worse with time. It affects a patient’s ability to work or have a satisfying social life. Unfortunately, there are no effective treatments available for this disease. Since the cause of the disease is reduced blood flow that results in a loss of vascularization and subsequent ischemic conditions in the extremities, improving blood flow through the regeneration of blood vessels represents a logical strategy for treating PAD and other ischemic
conditions. There is an urgent need for the development of safe and effective novel drug therapies aimed at treating these diseases.

b. Market

The global PAD therapeutics market was estimated to be approximately $606 million in 2009 and is forecasted to reach $1 billion by 2017. The market has grown at an approximate CAGR of 5.2% from $405 million in 2001, to 606 million in 2009 (65). PAD is an under diagnosed and undertreated disorder of arteries of lower extremities. Recently i-Data market research company reported that 80% of PAD patients remain undiagnosed suggesting that the true market size of PAD is underrepresented (66). A number of companies are working on new diagnostic tests that will allow for the identification and treatment of patients that do not yet have severe symptoms which will further increase the number of PAD patients.

PAD patients with diabetes develop ulcers in the extremities, due to the development of blockages in the arteries, also known as diabetic vascular disease (DVD). In US, 3.9-4.6 million people have diabetic foot ulcers and this number is estimated to increase further to 7.0 million by 2030. These patients are further at risks of adverse outcomes, such as non-healing ulcers, amputation and mortality. Ischemic and neuroischemic ulcers are diabetic foot ulcers accompanied by PAD. Further, 2.0-3.7 million U.S. citizens with PAD have diabetic foot ulcers. Moreover ~1 million diabetic foot ulcer patients suffer from critical limb ischemia with a greater risk of limb amputation and higher mortality (67). Further, PAD is a chronic condition, and given the likely safety profile of TV1001 it is anticipated
that patients will continue to take the drug unless or until the contributing risk factors such as hyperlipidemia, hypertension and diabetes are resolved. Considering the above facts TheraVasc believes that there is great opportunity in PAD market for which there are no effective therapeutics available.

c. Competition

Drugs for Peripheral Arterial Disease (PAD) treatment

The currently available therapies for PAD are focused on multidisciplinary approaches to control the pain and to manage the risk factors of the disease such as high cholesterol, diabetes, smoking and high blood pressure. Two drugs approved by the FDA for the treatment of intermittent claudication are pentoxifylline and cilostazol, which are often prescribe along with exercise or surgery to manage the disease. Currently there is no approved drug to promote healing. Cilostazol helps to dilate blood vessels and prevents platelet aggregation, again leading to decreased blood viscosity. Antiplatelet agents such as aspirin and clopidogrel, anticoagulants such as low molecular weight heparin are used in the most severe cases to reduce the clot/plaque build-up.

A number of companies such as CardioVascular Biotherapeutics (68) have advanced recombinant angiogenic growth factors, such as VEGF and FGF-2, in clinical trials. Companies such as Cardium Therapeutics (69) are attempting to promote angiogenesis with gene therapy products. There is limited success reported to date in both recombinant angiogenic factors as well as gene therapy products because of dose limiting toxicity associated with them (70,71). Recently,
the emphasis has been on cellular therapies using various stem cell therapies for treating PAD. It is still too early to predict whether stem cell therapy will have clinical benefits.

Also, few companies like Aries and Hope pharmaceutical are trying sodium nitrite inhaled formulations or injection for indications such as pulmonary hypertension and sickle cell disease. While these companies are working with sodium nitrite they are not a threat to the TheraVasc product as none of their drugs have an oral formulation and they are working on different indications.

*Surgical options for Peripheral Arterial Disease (PAD) treatment*

Surgical revascularization of the affected extremities by angioplasty or bypass surgery is usually necessary in cases of severe critical limb ischemia. In cases of acute limb ischemia, the standard of care initially involves treatment with heparin and could lead to surgical intervention and, in the most extreme cases, amputation.

In addition to competition from other therapeutics, devices are also likely to compete with TheraVasc’s products for the vascular disease market. These devices include peripheral drug-eluting stents, angioplasty, catheter-directed thrombolytics, endovascular embolization, mechanical atherectomy, and mechanical thromboectomy. Some of the leading companies such as Boston Scientific Abott, ev3 and Medtronic are working on devices for Peripheral Arterial Diseases (PAD).

In most cases a therapeutic option is preferred over a surgical option so there is little threat from competitors from PAD market. Moreover, in cases where
surgery and device implantation is warranted due to the severity of a patient’s condition, therapeutics are often administered after the surgery, and thus TV1001 may be used in combination with many of the above procedures such that the device is implanted to open up the vessel and allow immediate blood flow while TV1001 stimulates longer-term revascularization of the affected extremity.

d. Value Proposition
TheraVasc believes that its drug TV1001 will be long awaited solution for PAD. The TheraVasc product is an inorganic nitrite therapy based drug, which selectively promotes angiogenesis in ischemic tissue such as those found in patients with PAD. The TheraVasc drug will provide increased blood flow to the ischemic region resulting in rapid clinical improvement and a sustained effect through the delivery of naturally occurring growth factors and immune response in the newly vascularized tissue. The novel formulation of the drug will be relief for patients with PAD. A recent opinion article from leading Journal Nature has also appreciated TheraVasc finding stating that if this drug will become available it can be a lifeline to suffocating tissues (72). The following are the benefits of this novel pharmaceutical composition:
• **Strong Preclinical Studies Results**
  
  A number of preclinical studies done in various animal models suggest therapeutic potential of i-Nitrite therapy. These studies strongly support that sodium nitrite will be a clinical success.

• **First orally active drug to stimulate angiogenesis in ischemic region**
  
  TheraVasc TV1001 would be the first orally active PAD drug to stimulate new blood vessel growth.

• **Established Safety of Drug**
  
  Nitrite has been used for many years as part of the cyanide antidote kit in humans with a large amount of clinical data demonstrating the safety of high dose therapy in critically ill patients. Also, in a two-year chronic oral nitrite dosing study of rodents, the National Toxicology Program saw no evidence of cancer at dosages 10-100-fold higher than those contemplated in the present study. Lastly, neither methemoglobin nor significant blood pressure changes were observed when sodium nitrite was given to normal human subjects in the drinking water at levels 2 to 4 fold higher than those in this study. The facts above support that sodium nitrite will be a safe therapy.

• **Inexpensive Therapy**
  
  The cost of the active pharmaceutical ingredient of TV1001 is exceptionally low thus TV1001 drug will be an inexpensive therapy to treat PAD. Besides that costs associated with side effects resulting from current drugs will be much lower. TV1001 has ability to promote blood vessel growth and inhibit tissue necrosis that
will further reduce the number of surgical interventions and their significant high costs.

e. Project plan

TheraVasc has secured ~ 3 million till date including a grant from Global Cardiovascular Innovation Center (GCIC), GLIDE and Jumpstart. These funds are currently used to carry out the clinical trials of TV1001 drug in diabetic patients with PAD. TheraVasc is also working on the sustained release formulation of the drug.

Encouraging Phase I Results

TheraVasc is currently conducting a Phase Ib trial evaluating single dose of two different oral formulations of 80 mg sodium nitrite on pharmacokinetics and safety in Type 2 diabetic patients. In the Phase Ib trial following a single dose of either formulation, separated by a two-week wash out period, no adverse events have been observed, including no increase in methemoglobin levels or drop in blood pressure in the seven patients tested with both formulations to date. While five more patients will be enrolled in this study, the results are encouraging and suggest the maximum dose contemplated in the proposed study will be well-tolerated. Although pharmacokinetic data is not yet available from the diabetic patients treated in the Phase Ib trial, the half-life of circulating nitrite in non-diabetic patients is very short, ~1 hour, suggesting that no accumulation of nitrite in the blood will occur following chronic, twice daily dosing.
**Phase 11a trial**

TheraVasc’s TV1001 is initiating Phase IIa dose ranging clinical trial in diabetic patients with peripheral vascular abnormalities. The successful demonstration of safety and tolerability in this trial will help the company to raise more money and support the further development of TV1001.
5. INTELLECTUAL PROPERTY

TheraVasc has obtained an exclusive license for all fields in all territories for the TV1001 patent application covering the use of sodium nitrite for treating chronic disorders. The company has filed a series of additional PCT applications on new combinations, sustained release formulations to enhance its IP portfolio. TheraVasc has retained patent counsel from the law firm of Clark and Elbing.
6. SUMMARY

TheraVasc drug TV1001 seems to be a potent and novel treatment for PAD. Given the fact that there is enormous preclinical data in various animal models suggesting the therapeutic potential of i-Nitrite therapy; TheraVasc drug TV1001 will be highly promising. There are currently minimal therapeutic options available for PAD and with the significant discovery by company’s CSO Dr. Kevil that i-Nitrite therapy selectively induces angiogenesis and enhance tissue perfusion only in ischemic region has generated hope of great clinical utility of the drug in the PAD. Besides that TV1001 has an established safety profile and side effect associated with it are minimal. Moreover, TheraVasc drug is an oral formulation and will be more effective for clinical use. TheraVasc current clinical trial will further establish safety and clinical efficacy of the drug in PAD patients.
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