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RESEARCH ARTICLE

High dose intravenous vitamin C for lyme disease: a safety and tolerability study with an exploratory assessment of treatment efficacy.

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ABSTRACT

Lyme disease is the most common vector-borne disease in the United States with an estimated 476,000 new cases per year. Delays in diagnosis and treatment are common and there is a lack of consensus on the antibiotic therapy protocols to ensure successful outcomes. Much of this discord is due to the observation that more than 50 percent of people treated with conventional antibiotic protocols, which have changed little in the last 30 years, report persistent and/or recurring symptoms within 6 months of completing treatment. Labeled as chronic Lyme or post-treatment Lyme disease, it is a syndrome that afflicts more than 2 million people in the United States and can have a devastating impact on both quality of life and socioeconomic capacity. Despite these known treatment failures, the research community has lacked the funding necessary to advance our understanding of this syndrome or to explore innovative options to improve success. In this regard, complementary and alternative therapies, which are generally not patentable and have little potential for producing large economic gain, are generally disregarded in favor of new designer drugs or old reworked protocols. Intravenous vitamin C is one such example that has research validated effectiveness for a wide range of clinical conditions ranging from infection to cancer care. Achieving serum levels much higher than could be obtained orally, high dose intravenous vitamin C has the capacity to generate a concentration of intracellular peroxide that exerts both cytotoxic and immune stimulating effects. Unfortunately, these high doses of intravenous vitamin C have never been evaluated to establish safety and tolerability in Lyme positive patients. This study's primary aim is to examine this question with the unique additions of DMSO and calcium EDTA into the formula to enhance tissue penetration and address biofilm formation respectively, both of which are known barriers to antibiotic success. The secondary aim of the study is to determine whether subjects gain relief from Lyme-associated symptoms by tracking changes in both the PROMIS-29 and the Horowitz-Lyme-MSIDS questionnaires. The exploratory objective is to assess changes in Lyme specific labs including the standard immunoblot and the T-cell based Elispot as well as the CD57+ lymphocyte immune marker. Administered at a 75-gram dose twice weekly over 12 weeks, high dose intravenous vitamin C was shown to be both well tolerated and safe. The secondary and exploratory aims of the study provide insight into the potential efficacy of this protocol, as both subjective measures of symptom severity and objective assessment of Lyme biomarkers showed marked improvement. Ultimately, this study paves the way for future research using high dose intravenous vitamin C in Lyme patents either as a stand-alone treatment or in synergy with other therapies. It is this author's hope that employing an integrative medical approach such as this will eventually see chronic Lyme disease as a rare rather than common occurrence.

Introduction

Lyme disease (LD), caused by the intracellular bacteria *Borrelia burgdorferi sensu lato*, is the most common tick-borne disease in the United States[1]. Transmission arises primarily from bites of infected blacklegged ticks, *Ixodes scapularis and Ixodes pacificus*[2]. Clinical manifestations of LD are divided into various stages including early localized, early disseminated, and late LD. Multiple organ systems may be affected, depending on the stage of disease, such as skin, neurological, musculoskeletal, and cardiovascular[3].

Current CDC guidelines for the treatment of LD involves a 14 to 28 day antibiotic regimen of Doxycycline, Amoxicillin, Cefuroxime or IV Ceftriaxone, depending on risk, disease stage and clinical manifestations[4,5]. This protocol has been shown to be far more effective when treating early LD as seen in the first 3-30 days of infection; whereas, delay in treatment beyond 30 days was statistically correlated with an increased likelihood of developing chronic symptoms[6,7]. This outcome has been substantiated by several retrospective studies of LD done at least 1 year post-treatment which concluded that delaying treatment beyond 4 weeks of symptom presentation led to 50% or more of participants reporting persistent and/or recurrent cognitive and musculoskeletal symptoms for more than 6 months after completion of the recommended antibiotic therapy[8,9]. Estimated to affect 20% or more of treated LD patients, a recent report from 2020 suggests that nearly 2 million people are currently suffering from these chronic post-treatment LD (PTLD) symptoms[10].

While controversial, three primary mechanisms are commonly discussed to explain the etiology of PTLD. These include autoimmunity/immune dysregulation, sequelae of previous active infection, and persistent *Borrelia* infection. The complexity of this topic and the numerous papers both in support of and refuting each of these postulates makes it likely that it is a combination of mechanisms underlying the heterogeneity and complexity of the signs and symptoms associated with PTLD. For the purposes of this study, evidence to support antibiotic persistence is the most meaningful, as it belies the fact that *B. burgdorferi* is capable of surviving multiple different antibiotic regimens to establish persistence in the host[11,12]. Not surprisingly, the ability to establish persistence is also multifactorial and involves a combination of factors which include immune evasion, biofilm formation, the ability to survive in poorly vascularized and low oxygenated tissues, a slow growth rate, formation of metabolically inactive persister cells, differential gene regulation and protein expression and it's pleomorphic capacity to transform from a spirochete into atypical, non-motile shapes such as L-form, blebs and round bodies or cystic forms[14].

Solving the problem of how to both prevent and effectively treat PTLD has been both the focal as well as another source of disagreement amongst doctors and researchers. The primary discussion continues around whether further antibiotic therapy is helpful, harmful or benign[14]. Data can be found to support as well as refute longer courses of antibiotic treatment for both initial infection as well as those patients who continue to suffer with chronic symptoms in the presence of positive IgG/IgM serology[15,16,17,18]. Ultimately, the only apparent

agreement is that current treatment protocols lack clear, lasting and predictable results, and, in some cases, have been shown to be no more effective than placebo. This lends support to explore and identify alternative treatments that may offer better outcomes or synergistic benefit to current models of care, while remaining safe and tolerable for those with LD[19].

High-dose intravenous vitamin C (HDIVC) therapy is already used in complementary and alternative medicine for infectious disease, immune deficiency, and cancer[20,21]. Vitamin C (ascorbic acid) is a water-soluble nutrient typically known for its antioxidant and reducing capacity. In higher doses, however, it exerts both prooxidative and immune stimulating effects.

In vitro and animal studies investigating pharmacological concentrations of ascorbic acid have found that, via the Fenton reaction, it reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) to produce hydrogen peroxide (H_2O_2) and Dehydroascorbic Acid (DHA)[22]. Fe^{2+} is subsequently transported across the endosomal membrane by divalent metal transporter 1 (DMT1) where it becomes part of the cellular labile iron pool (LIP)[23]. High concentrations of intracellular H_2O_2 interact with Fe^{2+} to catalyze the formation of hydroxyl radicals (OH^-) which can deplete adenosine triphosphate (ATP), and lead to direct cell death through hydroxyl induced lipid, protein and DNA oxidation[25,25,26]. Through an elevation of the intracellular labile iron pool (LIP), HDIVC may also induce cellular death through ferroptosis, which is an iron-dependent type of controlled cell death, characterized by an excess of lipid peroxides on the cell membrane[27].

In addition to the direct cytotoxic effects of HDIVC, it has been found that indirectly, Vitamin C enhances microbicidal activity through enhanced immune function. Clinical studies have found that it upregulates neutrophil function, enhances natural killer cell activities and induces lymphocyte proliferation[28]. Furthermore, in murine studies, Vitamin C also stimulated dendritic cells to the more distinct interleukin (IL)-12 secretion which may activate T and B cell function[29].

No mechanism of action of HDIVC administration effects on *B. burgdorferi has been identified; however, an in vitro study has shown the spirochete is susceptible to killing by* H_2O_2, as well as other cytotoxic components produced by human polymorphonuclear leukocytes (PMNL) which include neutrophils and basophils[30].

It is necessary to administer vitamin C intravenously (IV) to achieve supraphysiologic serum concentrations, because oral supplementation of vitamin C has limited bioavailability. Three factors impact plasma vitamin C concentration: intestinal absorption, tissue transport, and renal absorption[31]. As doses increase, absorption decreases, with 100% absorption at 200mg dose but only 33% absorption at 1,250mg dose[32]. The intestinal transport proteins responsible for the absorption of vitamin C, sodium-vitamin c transporters 1 and 2, downregulate as plasma and tissue stores are saturated[31]. Intestinal regulation of vitamin C absorption limits its pharmacologic potential, reaching peak plasma concentrations of <0.25mmol/L[31]. Maximum tolerated doses range from 2-4 g with saturation of intestinal absorption resulting in osmotic diarrhea.

Intravenous administration of vitamin C in larger doses than orally tolerated have been shown to result in plasma concentrations 70-fold higher than oral doses[33]. A study evaluating safety, tolerability and pharmacokinetics of HDIVC administration in patients with cancer resulted in only mild side effects, i.e., nausea and headache, at doses less than 90g/m^2 [34]. In doses greater than 90g/m^2, few adverse events were reported, but these did include moderate to severe hypernatremia and hypokalemia. The authors also determined that tissue saturation, kidney clearance, and half-life of vitamin C increased with doses from 30 to 110g/m^2, and while all doses were well-tolerated, maximal concentration in plasma was reached at 70g/m^2. They concluded future studies should utilize a dose between 70-80 g/m^2.

Currently, HDIVC is not approved by the FDA for the treatment of any medical condition. There is a growing population in the Complementary and Alternative Medicine (CAM) community willing to administer HDIVC to treat a myriad number of ailments. HDIVC is primarily used for the treatment of infection, cancer, and fatigue; however, it is unclear what the true extent of its use is within the functional medical community.

Assessment of clinical use for HDIVC has been shown to be safe and tolerable when individuals are properly screened prior to and monitored during treatment. In data collected on over 9,000 patients who received HDIVC therapy, only 101 adverse effects were reported, which included fatigue, change in mental status, and phlebitis[35]. Unfortunately, safety and tolerability of HDIVC has never been properly evaluated in people with LD.

The primary purpose of this study is to determine if HDIVC administration twice weekly for 12 weeks is safe and tolerable for individuals with LD. This is determined by routinely surveying a 91-point patient-reported adverse event monitoring form (Appendix 1) as well as measuring blood biomarkers of safety. The secondary aim of the study is to assess the effect of HDIVC on changes in patient-reported symptoms associated with LD. This is accomplished with the PROMIS-29[44] (Patient-Reported Outcomes Measurement Information System-29) for quality of life (physical function subscale is the primary measure) and the Horowitz-Lyme-MSIDS questionnaire[45] specific to Lyme infection and symptomology. The exploratory aim of the study is to measure changes in biomarkers associated with LD before and after the course of HDIVC administration. These tests include the *B. burgdorferi* IgM and IgG Immunoblot, CD57+ NK Cells and antigen-specific T-cell response to *B. burgdorferi* measured via IFN-gamma production.

Study Design

The study is a 12-week single-arm, open-label clinical trial to assess safety and tolerability of HDIVC by adverse event questionnaire reporting and routine biomarker assessment in adults with LD. Secondary and tertiary aims are to look at both subjective and objective improvement in symptoms and lab markers associated with LD.

The study population are men and women aged 18-65 years old that have tested CDC positive for *B. burgdorferi* (LD) by two-tier criteria including positive IFA/ELISA and Immunoblot within the past 3 months. Additionally, each participant was required to

have normal glucose-6-phosphate dehydrogenase (G6PD) activity, be willing to have five fasting blood draws, receive twenty-seven 150–180-minute intravenous vitamin C infusions over 14 weeks and be able to speak, read and understand English.

Exclusion criteria include antibiotic treatment at any time following the established Lyme diagnosis, changes to other medications or supplements within 30 days of starting the first vitamin C infusion, pregnancy/breast feeding, abnormal GFR, electrolyte or AST/ALT levels outside the laboratory reference range, prior diagnosis of cardiovascular disease, diabetes mellitus, chronic kidney disease or lead encephalopathy and an inability to safely and reliably access veins in the hand/arm for angiocatheter instillation.

High Dose Intravenous Vitamin C Protocol

Formulation includes the following ingredients: ascorbic acid, calcium chloride, dimethyl sulfoxide (DMSO), edetate calcium disodium (CaEDTA), magnesium sulfate, potassium chloride, and sodium bicarbonate. [Table 1] Calcium, magnesium, and potassium are included to avoid any electrolyte depleting effects from the diuresis associated with HDIVC. Sodium bicarbonate is used to balance pH and to reduce potential for phlebitis. *B. burgdorferi is an obligate anaerobe that preferentially establishes itself in microaerophilic conditions such as joint spaces, extracellular matrix, fibrotic tissue and within biofilm*[36]. DMSO is included in an effort to increase vascular/tissue permeability; thereby, facilitating delivery of a higher concentration of ascorbic acid into these less vascularized regions of the body[37]. Previous clinical trials have validated DMSO safe for intravenous use at dosages and administration frequency higher than the current proposed administration schedule[38,39]. *Borrelia* is also capable of producing prolific biofilm to shield itself from immune detection, support quorum sensing and reduce antimicrobial efficacy[40]. Part of the composition of this biofilm is characterized by calcium binding to bacterial alginate to create a highly cross-linked and rigid biofilm[41]. By chelating calcium, EDTA inhibits formation of, and destabilizes, microbial biofilms allowing for greater efficacy of the antimicrobial agent[42,43].

The initial infusion begins at 30 grams of ascorbic acid and titrates up to 75 grams over the course of 4 study visits (30, 45, 50, 75grams). The titration schedule is important to assess tolerability to the treatment and minimize potential side effects. Administration of HDIV will occur at least 24 hours apart to evaluate for adverse events related to treatment and for proper infusion rate to maximize plasma concentration. This time frame is appropriate to determine if an adverse event occurs, if it is attributable to HDIVC, as the half-life of vitamin C is 2 hours with a clearance of 21 hours[34].

The IV infusion rate is set at 0.5 grams per minute to minimize discomfort. Once a participant has been titrated to 75g/m^2, they continue to receive that dose twice per week for 12 weeks (24 total HDIVC infusions), with a minimum of 24 hours between infusions.

	Milligrams per mL (mg/ml)	Volume in ML(s)	Milliosmoles per mL	Total Milliosmoles
Sterile Water	0	600	0	0
Ascorbic Acid	500	150	5.94	891
Calcium Chloride	100	3	2.04	6.12
DMSO		5	0.02	0.10
EDTA Calcium Disodium	150	5	2.14	10.70
Magnesium Sulfate	500	9	4.06	36.54
Potassium Chloride	2 mEq	9	4.00	36.00
Sodium Bicarbonate 8.4%		40	2.00	80.00
		Total Volume of Solution		Total Milliosmoles
		821		1,060.46
FINAL OSMOLARITY		1290		

Study Schedule:

Potential participants were initially screened over the phone. After determining initial eligibility, the participant is required to attend an in-person screening visit as outlined below. Eligible participants would then attend a total of 27 additional in-person study visits as outlined below.

SCREENING VISIT

This visit includes the following:

- Signed consent.
- Medical record and current medication/ supplement review
- Vital signs which are defined by blood pressure, heart rate, respiration rate, temporal temperature, height, and weight.
- Lab assessment: G-6-PD, Complete Blood Count (CBC), Comprehensive Metabolic Profile (CMP)
- Assess venous access.

BASELINE VISIT (STUDY VISIT #1)

- Vital Signs
- Completion of PROMIS-29 and Horowitz-Lyme-MSIDS questionnaires
- Completion of Adverse Event (AE) Monitoring Form
- Comprehensive lab assessment: CD57 (HNK1) Lymphocyte Profile, *Borrelia* Elispot
- HDIVC: 30g/infusion

ROUTINE IV VISIT (STUDY VISITS #2-11, 13-20, 22-26)

This visit includes the following:

- Vital signs
- Review of current medications/supplements
- Completion of the AE Monitoring Form
- HDIVC administered at 75g unless otherwise indicated within Figure 2

SAFETY CHECK VISIT (STUDY VISITS #12, 21)

This is a Routine Visit, plus the following:

- CBC and CMP will be drawn at the beginning of the visit to assess electrolytes (sodium and potassium), liver function enzymes (AST and ALT), kidney function (eGFR), blood markers and CD57 Lymphocyte Profile.
- Completion of PROMIS-29 and Horowitz-Lyme-MSIDS questionnaires

FINAL VISIT (STUDY VISIT #27)

This is a Safety Check Visit, plus the following:

- A blood draw will be performed, and sample processed to analyze for *B. burgdorferi* infection associated biomarkers (Lyme IFA and Immunoblot, Borrelia Elispot)

FOLLOW-UP SURVEYS

Participants complete the PROMIS-29 and Horowitz-Lyme-MSIDS questionnaires 12 weeks after completing the final visit to assess long-term impacts of HDIVC.

Data Analysis

SAFETY AND TOLERABILITY MEASURES:

The safety and tolerability of HDIVC is examined by determining the proportion of participants who experience adverse events defined as the following:

- Multi-system symptom(s) graded as 3 or higher (using the AE Monitoring form) which is attributable to the study supplement.
- Dropped out of the study citing side effects.

If a participant does not complete at least 80% of the study visits, they are excluded from the study and not included in data analysis.

STOPPING GUIDELINES:

Participants are withdrawn from the study for the following specific reasons:

- Multi-system symptom(s) graded as 3 or higher (using AE Monitoring form) which is attributable to the study supplement.
- Electrolyte levels that are outside the laboratory reference range
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) that are outside the normal laboratory reference range.
- Estimated glomerular filtration rate (eGFR) values that have decreased by 15 mL/min or more from baseline values.

ADVERSE EVENT DEFINITIONS:

Any untoward medical occurrence in a clinical investigation subject that may or may not have a causal relationship with a study product, occurring up to 48 hours after administration of HDIVC. An AE can therefore be an unfavorable and unintended sign (including abnormal laboratory findings), symptoms (nausea and loose stools), or disease temporally associated with the study, whether-or-not related to the study intervention. Any worsening of a pre-existing condition or illness is also considered an AE.

Primary Outcomes – In order to meet the primary aim measuring safety and tolerability of HDIVC, the study examines the data sources outlined in Table 2. If applicable, these measurements are also tracked on the AE Monitoring Form.

Table 2. Primary Outcome Measures – Safety and Tolerability

MEASURE	DESCRIPTION	PERTINENT EXCLUSION CRITERIA*	INDIVIDUAL LEVEL OF CHANGE UPON ANALYSIS TO BE CONSIDERED EVIDENCE OF POTENTIAL NON-SAFETY/NON-TOLERABILITY	COMPOSITE LEVEL OF CHANGE UPON ANALYSIS TO DETERMINE OVERALL SAFETY AND TOLERABILITY
Adverse Event (AE) Monitoring form	Standardized monitoring form that requires asking 91 questions pertaining to the following organ systems: eyes/ears/nose/throat, gastrointestinal, neurological/musculoskeletal, psychological/general, cardiopulmonary, skin, genitourinary and whole-body systems.	Sign or symptom of Grade 3 (severe or medically significant but not immediately life-threatening) or higher is reported at screening.	Sign or symptom of Grade 3 or higher attributable to the study supplement	Frequency and proportion of all participants who report signs or symptoms of Grade 3 or higher attributable to the study supplement
Liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT)	AST is an enzyme present in many tissues that is released in the blood when tissue is damaged. Blood levels are most commonly measured to evaluate liver and heart disease. ALT is an enzyme present in several tissues with its highest concentration in the liver. Assessment of blood levels are most commonly used to diagnose and monitor liver disease[25].	Elevated AST or ALT above normal reference range upon screening	Elevated AST or ALT above normal reference range	Frequency and proportion of all participants with Elevated AST or ALT above normal reference range

Electrolytes : sodium, potassium, calcium, magnesium	Electrolytes are essential nutrients involved in normal cellular function, including neurological and cardiovascular systems.	Electrolyte levels outside of laboratory reference ranges and confirmed on re-test	Electrolyte levels outside the normal lab reference range	Frequency and proportion of all participants with electrolyte levels outside of laboratory reference ranges
Estimated glomerular filtration rate (eGFR)	Blood levels are measured to detect early kidney damage and monitor kidney status. Calculated based on blood creatinine levels[25].	eGFR $\leq$60 mL/min upon screening	Decrease in eGFR of 15 ml/min or more	Frequency and proportion of all participants with a decrease in eGFR of 15 ml/min or more

Secondary Aim – In order to meet the secondary aim, the study utilizes the data sources outlined in Table 3.

Table 3. Secondary Aim Outcome Measures

MEASURE	DESCRIPTION
Changes in Horowitz-Lyme-MSIDS	A survey measuring Lyme disease symptomology severity. The Horowitz-Lyme-MSIDS questionnaire consists of 55 questions divided into 4 sections. Each section is totaled. All sections are then added together to determine the final score. A score of 46 or higher indicates the probability of a tick-borne disorder. A score of 21 to 45 is a possible infection of a tick-borne illness. A score under 21 indicates a tick-borne illness is unlikely. Although, values are not expected to shift categorically during the study, the values of this assessment are to establish and track Lyme specific symptom status.[45]
Changes in PROMIS-29 (v. 2)	A collection of 4-item short forms assessing anxiety, depression, fatigue, pain interference, physical function, sleep disturbance, and ability to participate in social roles and activities as well as a single pain intensity item. Each domain is rated on a 5-point rating scale, along with a one question Pain intensity section rated on an 11-point scale. The assessments of each domain are anchored to the past 7 days, except for the Physical Function domain, which is not anchored to a specific time frame. PROMIS-29 provides a continuous severity score for each domain. The maximum score per domain (other than Pain Intensity) is 20. The maximum score for Pain Intensity is 10. It is considered a standardized, reliable, and valid measure of health-related quality of life.[44]

E.1.3. **Exploratory Aims** – In order to meet the exploratory aim of tracking infection rate associated with safety and tolerability, the study utilizes the data sources outlined in Table 4 to assess infection status.

Table 4. Exploratory Aim Outcome Measures

MEASURE	DESCRIPTION
Immunoblot test	Similar to a Western Blot, the modern Immunoblot is both more sensitive and more specific at detecting antibody reactions to all *Borrelia burgdorferi sensu lato* antigens[46].
Elispot	The Elispot is an enzyme-linked immunospot assay that detects human T cell reactivity to *B. burgdorferi* specific antigens by measuring the quantifiable production of gamma interferon. Excluding memory T cells, the vast majority of circulating T (effector) Cells only remain in circulation for 45 to 60 days[49]. This principle makes this test ideal for tracking effectiveness of treatment[47].
CD57 Lymphocyte Profile	A CD57 lymphocyte is a selective subset of natural killer cells (CD56+ Cells) that can be decreased in patients with chronic Lyme disease. Its value can provide insight into severity of illness and success of therapy[48].

Results

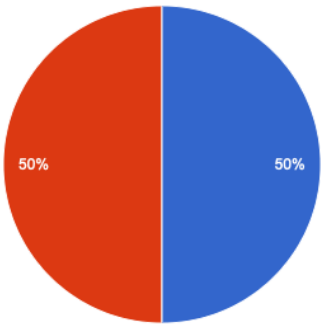
At baseline, all participants (n=6) reported a current diagnosis of *B. burgdorferi* (i.e., Lyme) infection via ELISA or IFA and Western or Immunoblot within the past 3 months and had not received any antibiotic therapy following the diagnosis. Participants did not change or start any new medications or nutraceutical supplementation within the 30 days prior to starting the study.

At baseline, all participants were free of abnormal liver or kidney function, a diagnosis of cardiovascular disease, diabetes (I or II), chronic kidney disease, G6PD deficiency or cancer in the past 5 years, per self-report.

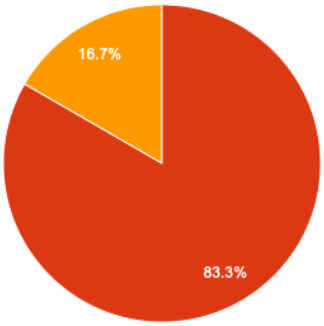
None of the women were pregnant or breastfeeding at baseline. Three participants reported current alcohol use.

One participant was unable to fully complete the study due to an unrelated medical incident that occurred prior to the final visit.

Demographics:



Counts/frequency: Female (3, 50.0%), Male (3, 50.0%), Non-binary (0, 0.0%), Prefer not to say (0, 0.0%)



Counts/frequency: Hispanic or Latino (0, 0.0%), Not Hispanic or Latino (5, 83.3%), Unknown/Not reported (1, 16.7%)

SAFETY & TOLERABILITY

Adverse events were reported using the Adverse Event (AE) monitoring form (Appendix 1). Grade 3 (severe or medically significant, but not immediately life-threatening) or higher adverse events were rare. Table 5 reflects which symptoms and the number of participants that reported Grade 3 adverse events. No Grade 3 adverse event was upgraded to a higher grade at any subsequent visit.

Table 5 – Grade 3 (Severe) Adverse Events

Adverse Events	Number of Participants Reporting AE (n)
Ringing in ears	2
Nasal congestion	1
Allergy symptoms	1
Abdominal cramps	1
Decreased appetite	2
Diarrhea	1
Dry mouth	2
Drowsiness	2
Weakness	3
Pain	2

Lab surveillance shown in Table 6 revealed no worsening of lab values relating to electrolytes, liver function tests, renal function or blood markers. Of note, the white blood count (WBC) actually increased by 41% between the initial baseline (5.1 thousand/uL) and three-month follow-up visit (8.0 thousand/uL) [ref range 3.8-10.8].

Table 6 – Objective Measures

	Baseline (n=6)		Final (n=5)		Absolute Change	% Change	Follow-up (n=6)		Absolute Change	% Change
	Mean	SD	Mean	SD			Mean	SD		
Anthropometric Measures										
Systolic Blood Pressure	120.0	8.0	113.2	5.9	-6.8	-5.7%	120.0	8.1	6.8	6.0%
Diastolic Blood Pressure	70.7	4.7	67.2	8.6	-3.5	-4.9%	70.3	6.7	3.1	4.7%
Clinical Lab Measures										
White Blood Cell Count	5.8	0.5	5.7	1.1	-0.2	-2.7%	8.0	1.8	2.3	41.0%
Red Blood Cell Count	4.5	0.4	4.4	0.3	-0.1	-2.3%	4.6	0.2	0.2	5.3%
Hematocrit	40.8	3.3	39.9	3.3	-0.8	-2.0%	42.4	2.0	2.5	6.1%
Hemoglobin	13.6	1.4	13.5	1.3	-0.1	-0.5%	14.1	0.8	0.6	4.3%
Glucose	109.0	47.1	81.8	17.3	-27.3	-25.0%	--	--	--	--
Blood Urea Nitrogen	16.8	6.2	14.0	6.9	-2.8	-16.8%	--	--	--	--
Creatine	0.9	0.2	0.9	0.2	0.0	3.8%	--	--	--	--
Sodium	139.7	1.4	140.8	1.3	1.1	0.8%	--	--	--	--
Potassium	4.4	0.4	4.4	0.3	0.0	1.0%	--	--	--	--
Chloride	102.8	2.7	102.3	1.9	-0.6	-0.6%	--	--	--	--
Carbon Dioxide	24.8	1.5	25.8	3.0	0.9	3.7%	--	--	--	--
Calcium	9.7	0.3	9.4	0.3	-0.4	-3.9%	--	--	--	--
Protein	7.2	0.5	6.8	0.3	-0.4	-5.8%	--	--	--	--
Albumen	4.8	0.3	4.6	0.2	-0.2	-5.0%	--	--	--	--
Globulin	2.4	0.4	2.2	0.2	-0.2	-7.3%	--	--	--	--
Albumen/Globulin Ratio	2.1	0.4	2.1	0.2	0.0	0.4%	--	--	--	--

	Baseline (n=6)		Final (n=5)		Absolute Change	% Change	Follow-up (n=6)		Absolute Change	% Change
Billirubin	0.5	0.2	0.4	0.2	-0.1	-11.1%	--	--	--	--
Alkaline Phosphatase	69.8	32.6	53.5	22.2	-16.3	-23.4%	--	--	--	--
Aspartate Transaminase	19.3	5.4	24.0	12.1	4.7	24.1%	--	--	--	--
Alanine Transaminase	18.8	12.5	19.3	14.5	0.4	2.2%	--	--	--	--
Estimated Glomerular Filtration Rate (eGFR)	97.8	16.8	105.0	21.6	7.2	7.3%	--	--	--	--

Notes:

- Absolute change and % change for Follow-up as compared to Final.
- No Final visit recorded for participant #3

Clinical labs ordered at the Screening Visit are reported as Baseline if baseline values are not available.

Secondary aim: patient reported symptom severity

PROMIS-29

The PROMIS-29 provides a measurement of participant symptom severity relating to physical function, anxiety, depression, fatigue, sleep disturbance, ability to participate in social roles and activities, pain interference, and pain intensity. While the mean physical function score did not decrease over the course of the study (Baseline: 17.0 (SD: 2.4); Final: 17.6 (SD: 2.9); Follow-up: 18.0 (SD: 1.9), decreases in scores from Baseline to Final were seen in the following domains: Anxiety (14%), Depression (20.8%), Sleep Disturbance (4.3%), Pain Interference (16.5%) and Pain Intensity (14.3%). At the 3-month follow-up visit, there was a significant further reduction in fatigue (17.8%) and pain interference (13.2) compared to the final visit assessment (See Table 7).

HOROWITZ LYME-MSIDS

The mean Horowitz-Lyme-MSIDS score decreased from 69.5 (SD: 31.9) at baseline to 52.0 (SD: 33.8) at the final visit, and then decreased again to 46.3 (SD: 25.7) at the follow-up visit (see Table 7). Additionally, 4 out of the 5 participants reported improvement at the final visit, and, at the 3-month follow-up survey, 5 out of 6 participants reported ongoing improvement.

Table 7 – Subjective Measures

	Baseline (n=6)		Final (n=5)		Absolute Change	% Change	Follow-up (n=6)		Absolute Change	% Change
	Mean	SD	Mean	SD			Mean	SD		
PROMIS-29										
Physical Function	17.0	2.4	17.6	2.9	0.6	3.5%	18.0	1.9	0.4	2.3%
Improvement, proportion			1/5				0/5,			
%			20.0%				0.0%			
Anxiety	10.5	3.5	9.0	3.5	-1.5	-14.3%	9.2	2.6	0.2	1.9%
Depression	8.3	2.9	6.6	3.7	-1.7	-20.8%	7.2	1.8	0.6	8.6%
Fatigue	14.0	4.5	14.2	4.7	0.2	1.4%	11.7	3.3	-2.5	-17.8%
Sleep Disturbance	12.3	4.1	11.8	1.9	-0.5	-4.3%	12.2	1.8	0.4	3.1%
Social Roles and Activities	11.8	2.9	14.2	4.3	2.4	20.0%	16.0	3.4	1.8	12.7%
Pain Interference	11.5	5.5	9.6	6.1	-1.9	-16.5%	8.3	3.2	-1.3	-13.2%
Pain Intensity	3.5	2.4	3.0	2.5	-0.5	-14.3%	3.0	1.9	0.0	0.0%
Horowitz Lyme-MSIDS										
Score	69.5	31.9	52.0	33.8	-17.5	-25.2%	46.3	25.7	-5.7	-10.9%
Improvement, proportion			4/5				5/6			
Percentage			80.0%				83.3%			

Exploratory aim: lyme associated biomarkers

LYME IMMUNOBLOT

Following 12 weeks of treatment, the Lyme Immunoblot results still reflect a 40% positive result for IgM antibody criteria and a 20% positive for IgG antibody recognition (table 8). Taken together, three out of the five participants (60%) who tested antibody mediated serology at the final visit remained positive for LD by CDC criteria. This result suggests that more than half of the participants still retained humoral immune recognition to *Borrelia*.

BORRELIA ELISPOT

In contrast to the Immunoblot results, T-cell testing for *Borrelia* recognition revealed no gamma interferon production on exposure to either the full antigen mix or the OSP (outer surface protein [23, 31 & 34kDa]) mix in all 5 participants at the final visit assessment (table 8). These results suggest that there were no remaining or newly activated T effector (CD4+) cells to *Borrelia burgdorferi SL* following 12 weeks of high dose IV vitamin C treatment.

Table 8 – Results of Lyme Biomarkers at Final Visit

Lyme ImmunoBlot IgM	Counts	Percentage*
Positive	2	40.0%
Negative	3	60.0%
Missing	1	
Lyme ImmunoBlot IgG		
Positive	1	20.0%
Negative	4	80.0%
Missing	1	
Borrelia Elispot - Full Antigen		
Positive	0	
Negative	5	100%
Missing	1	
Borrelia Elispot - OSP-Mix		
Positive	0	
Negative	5	100%
Missing	1	

*% based on non-missing results, not total sample

CD57+ Lymphocyte Profile

$CD57^+$ T cells are NK (Natural Killer) subsets that are regarded as terminally differentiated, oligoclonal populations of cytotoxic cells generated in response to chronic antigen stimulation[50]. Retaining distinct cytolytic properties, CD57+ NK cells are recruited in chronic viral infections but contrastingly show a reduced population in chronic LD compared to those with acute disease and uninfected controls[48]. It is theorized that this may be due to either progressive depletion of these cells in response to infection or due to *Borrelia* specific inhibition of NK cell differentiation in an effort to reduce cytotoxic capacity. Results from baseline to final visit showed a 37.9% increase in CD57+ cell numbers and a 51.8% increase in CD57+ cell percent. At the 3-month follow-up visit, these numbers further increased by 12.1% and 4.2% respectively as shown in Table 9.

Table 9 – Objective Measures

	Baseline (n=6)		Final (n=5)		Absolute Change	% Change	Follow-up (n=6)		Absolute Change	% Change
	Mean	SD	Mean	SD			Mean	SD		
CD57+: Count	3.5	1.5	4.8	NA	1.3	37.9%	5.4	2.4	0.6	12.1%
CD57+: %	69.8	48.0	106.0	NA	36.2	51.8%	110.5	105.4	4.5	4.2%

Discussion

Twice weekly high-dose intravenous vitamin C (HDIVC) therapy for 12 weeks appears to be safe and tolerable for individuals with LD. While this dose did precipitate reporting of 10 different symptoms as grade 3 adverse events, none of the participants withdrew from the study, upgraded their symptom severity, or missed any IV infusions. Routine lab surveillance showed no concerns with electrolyte abnormalities, renal or hepatic stress or blood dyscrasia. Treatment, in fact, increased white blood cell count over 40% supporting a possible secondary immune benefit to HDIVC treatment in people suffering from LD.

The secondary aim of the study to assess changes in symptom severity showed an approximate 25% average improvement from baseline to final visit as measured by the Horowitz Lyme-MSIDS questionnaire. Participants reported a further 11% improvement at the follow-up assessment three months after completing the final IV infusion. The results of the PROMIS-29 survey corroborate these findings with participants reporting improvements in multiple subdomains including Anxiety, Depression, Sleep Disturbance, Pain Interference and Pain Intensity on completing the study. Similarly, compared to the final visit assessment, participants also reported further improvement in both fatigue and pain interference at the follow-up visit. The results of both subjective surveys implicate a potential delayed benefit from treatment. This may be due to either one or a combined reduction in the antigenic load over time and a waning of the immune-inflammatory response.

Measuring Lyme specific biomarkers between the baseline visit and the final visit showed some variation between outcomes. The combined Lyme IgG and IgM Immunoblot results remained positive in 3 of the 5 participants. The author postulates that due to the prolonged nature of IgG persistence due to B-memory cells, this antibody can remain elevated for months or even years following treatment of infection. IgM antibodies, on the other hand, have been shown to remain elevated in Lyme infected hosts contrary to what is observed for most infections that only show increased levels during the first 1-2 months of illness. Since this antibody does not mitigate bacterial dissemination into solid tissue or affect *Borrelia* tissue burden, it has been theorized that *B. burgdorferi* infection itself drives production of IgM preferentially over the more tissue-penetrable IgG antibody[57]. Had this test been done at the 3 month follow-up visit instead of the final visit, IgM results in particular may have been different.

Contrastingly, T-cell assessment (Elispot) via antigen induced gamma-interferon production

was negative in all 5 participants at study completion. CD57+ Lymphocyte measurement showed an over 50% rise compared to baseline. This result further supports the potential immune based benefit of HDIVC which was also evidenced by the increased total WBC count. The CD57+ Lymphocyte measurement was the only exploratory lab drawn at the 3-month follow-up visit. These values showed further improvement resulting in a 46.2% improvement in CD57 count and a 47.9% improvement in CD57+ percent compared to measurements taken at the baseline visit prior to treatment. When considered along with the negative Elispot results and the ongoing improvement in reported symptoms, these findings lend strong support to the theory that HDIVC may be effective at reducing total infectious burden in the treatment of LD.

Given the disparity between antibody-based lab data and the ongoing improvement seen in both immune function and subjective symptoms, it is the author's recommendation that future studies obtain post-study data collection for a longer period than 90 days. Consistent with the predominance of symptom relapse in treated Lyme patients (PTLD) after 6 months, it would also be useful to have reporting of the PROMIS-29 and the Horowitz Lyme-MSIDS questionnaire at this time as well as at 9, 12 and 18 months post-HDIVC completion. Additional lab data at these same points in time would show how long and sustained the improved immune response (CD57+ and WBC) lasts, whether and when IgM immunoblot results become negative, and if IgG titers to *Borrelia*-specific proteins resolve or slowly fade following treatment.

Conclusion

The CDC has categorized Lyme as the most prolific and fastest spreading vector-borne illness in the country with over 30,000 cases reported each year. Due to long-standing problems with under-reporting, poor surveillance data, restrictive and misunderstood diagnostic criteria, reluctance to seek care, and misdiagnosis, estimates suggest that this number is grossly underrepresented, and the actual rate of infectivity in the US population more closely approximates 476,000 cases of LD per year[51,52,53]. The scale of this epidemic along with the recognition that delayed or inadequately treated cases frequently relapse or fail to fully resolve symptoms suggests an urgent need to explore synergistic or alternative therapies, as we collectively re-examine how LD is being treated[54,55].

These results support the primary objective of validating HDIVC with EDTA and DMSO as both safe and well tolerated in people suffering with LD. Additional research with a larger sample size to support more robust statistical significance is needed to better investigate this potential. The secondary and exploratory aims of this study further suggest that HDIVC may be effective not only as an antimicrobial therapy to treat *Borrelia burgdorferi SL* but also as an ancillary means to simultaneously enhance immune function. This study has paved a path forward for follow-up research to examine treatment efficacy and long-term outcomes more robustly. The ability of HDIVC to selectively eradicate pathogens without harming the host while also boosting immune competency makes it an ideal candidate for consideration

in the treatment of chronic infectious illness such as LD.

While unproven, it can be speculated that the more generalized cytotoxic capability of high dose ascorbic acid, via the Fenton reaction, effectively treats persister forms and other morphological variants that traditionally resist the targeted mechanism of action for currently accepted antibiotic therapies. Additionally, the unique and previously unexplored inclusion of EDTA and DMSO in this formulation may enhance the antimicrobial potential of HDIVC by theoretically improving tissue perfusion and reducing the burden of *Borrelia* generated biofilm. Coupled with an upregulation of antigen presenting cell function and T and B-cell activation, this unique IV formula appears to address the two-pronged dilemma of entrenched infection and immune dysfunction found in chronic LD.

Although this study was done in antibiotic naive Lyme patients, it is also possible that the combination of HDIVC with established treatment guidelines[56] would provide even better outcomes. Additional research to first examine the safety and potential interactions of HDIVC with various antibiotics would need to be done before considering this type of integrative treatment. Given the difficulty in successfully treating chronic Lyme, it is, however, exactly these out-of-the-box therapies that will need to be considered if we are to eventually resolve this growing worldwide epidemic.

Conflicts of Interest Statement:

The authors have no conflicts of interest to declare.

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Appendix 1

ADVERSE EVENTS MONITORING SYSTEM

Grade	Definition*
0=Not Present	No AE, Function within normal limits
1=Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention	No intervention; asymptomatic lab or radiographic findings; marginal clinicalsignificance
2=Moderate; minimal, local or noninvasiveintervention indicated	AE limited activities of daily living (ADLS)
3=Severe or medically significant but notimmediately life-threatening	AE significantly limited basic self-care, AErequired initial hospitalization** or prolongation of hospitalization
4=Life-threatening	Life-threatening consequences AE**
5=Fatal	Fatal AE**

*Defined per NCI Common Terminology Criteria for Adverse Events v4.0 2009

** FDA-defined "Serious" AE

Ask: "Do you currently have any…" OR "Since the last visit, have you had any…" (rate any symptom, even ifsince resolved):

EYES/EARS/NOSE/THROAT

1. Blurred Vision	0	1	2	3	4	5	NA
2. Double Vision	0	1	2	3	4	5	NA
3. Eyes rolled up	0	1	2	3	4	5	NA
4. Movement: rapid vertical/horizontal nystagmus	0	1	2	3	4	5	NA
5. Ringing in ears/tinnitus	0	1	2	3	4	5	NA
6. Nasal congestion/sinusitis	0	1	2	3	4	5	NA
7. Allergy symptoms	0	1	2	3	4	5	NA
8. Other EENT	0	1	2	3	4	5	NA

GASTROINTESTINAL, INCLUDING MOUTH

9. Abdominal pain/cramps	0	1	2	3	4	5	NA
10. Appetite: decreased	0	1	2	3	4	5	NA
11. Appetite: increased	0	1	2	3	4	5	NA
12. Constipation	0	1	2	3	4	5	NA
13. Diarrhea	0	1	2	3	4	5	NA
14. Drooling/salivation	0	1	2	3	4	5	NA
15. Dry mouth	0	1	2	3	4	5	NA
16. Gas/indigestion	0	1	2	3	4	5	NA
17. Gum growth	0	1	2	3	4	5	NA
18. Nausea	0	1	2	3	4	5	NA
19. Taste: abnormal/metallic	0	1	2	3	4	5	NA
20.Thirst: increased or decreased (specify)	0	1	2	3	4	5	NA
21. Vomiting	0	1	2	3	4	5	NA
22. Other GI	0	1	2	3	4	5	NA

NEUROLOGICAL/MUSCLE

23. Contortions/spasticity	0	1	2	3	4	5	NA
24. Gait: imbalance/unsteady	0	1	2	3	4	5	NA
25. Gait: shuffling	0	1	2	3	4	5	NA
26. Musculoskeletal pain	0	1	2	3	4	5	NA
27. Headache	0	1	2	3	4	5	NA
28. Inability to sit still/pacing/restlessness	0	1	2	3	4	5	NA
29. Limb jerking/writhing	0	1	2	3	4	5	NA
30. Lip smacking/chewing/tongue movements/grimacing	0	1	2	3	4	5	NA
31. Mask-like, expressionless face	0	1	2	3	4	5	NA
32. Neck/back arching	0	1	2	3	4	5	NA
33. Pill rolling	0	1	2	3	4	5	NA
34. Rigidity	0	1	2	3	4	5	NA
35. Slurred speech	0	1	2	3	4	5	NA
36. Tremor	0	1	2	3	4	5	NA
37. Other Neurological	0	1	2	3	4	5	NA

PSYCHOLOGICAL/GENERAL

38. Agitation/jitters	0	1	2	3	4	5	NA
39. Anxiety	0	1	2	3	4	5	NA
40. Attention/concentration: decrease	0	1	2	3	4	5	NA
41. Confusion	0	1	2	3	4	5	NA
42. Depression	0	1	2	3	4	5	NA
43. Drowsiness/sedation	0	1	2	3	4	5	NA
44. Irritability	0	1	2	3	4	5	NA
45. Lethargy/no movement	0	1	2	3	4	5	NA
46. Sleep: excessive	0	1	2	3	4	5	NA
47. Sleep: insomnia, restless	0	1	2	3	4	5	NA
48. Weakness/fatigue	0	1	2	3	4	5	NA
49. Hyperactivity	0	1	2	3	4	5	NA
50. Other Psychological	0	1	2	3	4	5	NA

CARDIOPULMONARY

51. Difficult/labored breathing SOBE	0	1	2	3	4	5	NA
52. Rapid breathing	0	1	2	3	4	5	NA
53. Palpitations/cardiac arrhythmia	0	1	2	3	4	5	NA
54. Hypertension	0	1	2	3	4	5	NA
55. Hypotension	0	1	2	3	4	5	NA
56. Chest pain	0	1	2	3	4	5	NA
57. Peripheral edema or swelling	0	1	2	3	4	5	NA
58. Other Cardiopulmonary	0	1	2	3	4	5	NA

SKIN

59. Acne	0	1	2	3	4	5	NA
60. Abnormal hair growth	0	1	2	3	4	5	NA
61. Color (circle): pale – yellow – other (specify):	0	1	2	3	4	5	NA
62. Color: redness/erythema	0	1	2	3	4	5	NA
63. Itching/dry	0	1	2	3	4	5	NA
64. Puffy/tissue fluid	0	1	2	3	4	5	NA
65. Rash/hives	0	1	2	3	4	5	NA
66. Sunburn/photosensitivity	0	1	2	3	4	5	NA
67. Sweating: increasing	0	1	2	3	4	5	NA
68. Other skin	0	1	2	3	4	5	NA

GENITOURINARY

69. Breast: swelling/growth/lumps	0	1	2	3	4	5	NA
70. Breast: discharge	0	1	2	3	4	5	NA
71. Menstrual: absence	0	1	2	3	4	5	NA
72. Menstrual: irregularities	0	1	2	3	4	5	NA
73. Sexual: decreased/impotence	0	1	2	3	4	5	NA
74. Sexual: increased interest/ priapism	0	1	2	3	4	5	NA
75. Urinary: decreased/retention	0	1	2	3	4	5	NA
76. Urinary: difficult/painful	0	1	2	3	4	5	NA
77. Urinary: enuresis/ nocturesis	0	1	2	3	4	5	NA
78. Urinary: increased	0	1	2	3	4	5	NA
79. Other Genitourinary	0	1	2	3	4	5	NA

WHOLE BODY

80. Convulsions/seizures	0	1	2	3	4	5	NA
81. Fainting/dizziness	0	1	2	3	4	5	NA
82. Vertigo	0	1	2	3	4	5	NA
83. Fever	0	1	2	3	4	5	NA
84. Sore throat	0	1	2	3	4	5	NA
85. Weight: gain	0	1	2	3	4	5	NA
86. Weight: loss	0	1	2	3	4	5	NA
87. Pain, generalized	0	1	2	3	4	5	NA
88. Trauma (Specify type):	0	1	2	3	4	5	NA
89. Other Whole Body	0	1	2	3	4	5	NA

ADVERSE EVENT

90. Did an FDA labeled "Serious Adverse Event" occur? ◯N=0 ◯Y=1

(If yes, notify IRB within 24 hours of PI notification)

91. If yes, please check what type of event occurred:

FDA labeled "Serious" AE's:

___ Death
___ Potentially life threatening medical problem
___ Congenital deformity
___ Hospitalization
_ Long-term disability