

RESEARCH PROTOCOL

Title of project: Transcutaneous Vagus Nerve Stimulation for the treatment of SLE: (The TRUST study)

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Background and significance

Systemic lupus erythematosus is a heterogeneous autoimmune syndrome characterized by loss of tolerance to nucleic self-antigens due to aberrant innate and adaptive immune responses. Pro-inflammatory cytokines, such as the TNF superfamily ligands (BlyS, TNF- α), INF α and IL-6, are abundantly produced in lupus and can serve as a bridge between innate and adaptive immunity, contributing to the overall inflammatory burden of the disease [1, 2].

It has been recently recognized that production of certain cytokines in the spleen, particularly TNF α , IL1 and IL-6, can be physiologically inhibited by vagus nerve stimulation, representing the efferent limb of the cholinergic anti-inflammatory pathway [3, 4]. Efferent action potentials generated in the brainstem are relayed through the vagus nerve to adrenergic neurons in the celiac ganglion, which travel to the splenic white pulp [4]. Norepinephrine released by celiac inter-neurons binds through adrenergic receptors on memory T cells with intrinsic ability to synthesize acetylcholine (ACh) [5]. In contrast to classic parasympathetic signaling through muscarinic receptors, T cell-derived ACh binds to $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChR) on mononuclear cells and macrophages in the red pulp and marginal zone inhibiting cytokine production [6, 7]. Instead of ion channel activation, $\alpha 7$ nAChR ligation inhibits nuclear factor (NF)- κ B translocation resulting in decreased transcription of cytokine genes [3]. In addition to regulating cytokine production, vagus nerve stimulation down regulates neutrophil adhesion and chemotaxis [8] and can arrest B-cell migration to the spleen and T cell-independent antibody production [9].

Under basal conditions the cholinergic anti-inflammatory pathway exerts a tonic inhibition on innate immunity, whereas interruption of the vagus or splenic nerves or splenectomy can produce an inflammatory phenotype characterized by exaggerated responses to endotoxins and tissue injury [10]. On the contrary, electrical, mechanical or pharmacological vagus nerve stimulation inhibits inflammatory cytokine production, prevents tissue injury and improves survival in multiple experimental models of systemic inflammation and sepsis [11-14].

Since vagus outflow to the sinoatrial node of the heart controls the heart rate, heart rate variability (HRV) has been widely employed as a measure of general vagal activity. HRV is measured by software calculating the distance between consecutive R waves on the electrocardiogram (ECG) tracing [15, 16]. HRV can be analyzed in the time domain (i.e. the standard deviation of all intervals between consecutive R waves with normal-to-normal conduction) or in the frequency domain. The latter analysis utilizes a mathematical algorithm (Fourier transform) that generates frequency components of the power spectrum, categorized as low frequency (LF; 0.04 to 0.15 Hz) and high frequency (HF; 0.15 to 0.4 Hz) [15]. The HF power component reflects the activity of the efferent vagus nerve to the heart (ACh antagonists or vagotomy down modulate the HF component and electrical vagus nerve stimulation increases HF power), whereas the ratio of the LF to HF power is an index of the sympathetic to parasympathetic balance [15]. It has been postulated that HRV might also be a surrogate of activity in the anti-inflammatory reflex.

Decreased HRV has been associated with increased morbidity and mortality from diverse diseases, including myocardial infarction, sepsis and chronic autoimmune disease like rheumatoid arthritis, inflammatory bowel disease and sarcoidosis [3]. Measures of HRV have also been inversely correlated with serum inflammatory biomarkers (C-reactive protein, IL-6) in the general population [17, 18]. Individuals with rheumatoid arthritis were found to have decreased HRV compared to controls, which correlated with HMGB1 levels and disease activity [19, 20]. Autonomic dysfunction and decreased HRV are also well recognized in SLE [21, 22]. In a series of 54 patients with SLE, sympathetic and parasympathetic autonomic dysfunction measured by the Ewing cardiovascular reflex tests, were significantly more common compared to healthy controls ($p < 0.0001$) [21]. We have previously shown in 58 patients with SLE from the Oklahoma Lupus Cohort that changes in HRV are associated with changes in disease activity measured by the BILAG Index score and the physician global assessment, and with changes in serum BLYS and IL6 levels [23] (see preliminary data).

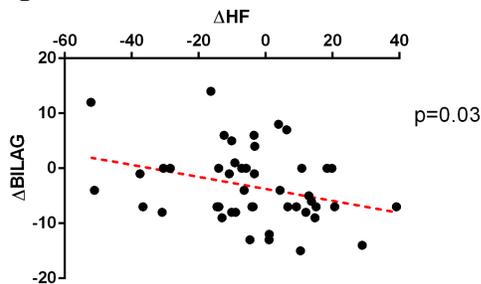
Based on these preliminary data, we hypothesize that electrical stimulation of the vagus nerve could modulate the activity of the cholinergic anti-inflammatory neuroimmune circuit that appears to be pertinent at least in some patients with SLE. Electrical stimulation of the vagus nerve (VNS) by an implantable stimulator is an approved therapy for resistant depression in the US and has been investigated in wide range of conditions including heart failure and epilepsy with promising results [24]. In an open label study of 8 patients with RA inadequately controlled on MTX, significant improvement in signs and symptoms was observed after 42 days of VNS by an electrical implanted device [25]. These results support observations following electrical vagus nerve stimulation in mice with acute collagen-induced arthritis, where significant

improvements in inflammation, pannus formation, cartilage destruction, and bone erosion were noted and were accompanied by numerical reductions in systemic cytokine levels [26]. The strong $\alpha 7nAChR$ staining of macrophages, fibroblasts as well as T and B cells on synovial biopsies from patients with rheumatoid or psoriatic arthritis is in accordance with these findings, suggesting these cells may balance inflammatory mechanisms through feedback cholinergic stimulation by nearby ACh-producing immune cells [27].

Although promising, VNS by means of implantable devices is nonetheless an invasive modality not devoid of surgical and technical complications that hinder its widespread use. In search for alternative non-invasive routes for VNS, electrical stimulation of the auricular branch of the vagus nerve has been investigated, through surface electrodes or acupuncture needles applied to the external ear. Transcutaneous VNS (tvNS) significantly increased HRV in healthy participants indicating a shift in cardiac autonomic function toward parasympathetic predominance [24]. This approach has been studied in patients with coronary artery disease [28] epilepsy [29], tinnitus [30, 31] and chronic pain [32], and could be a novel, noninvasive non-pharmacologic therapy for SLE.

Preliminary Data

Figure 1



58 patients with SLE from the Oklahoma Lupus Cohort were evaluated at two visits with the stipulation that there must be at least mild/moderate disease activity, in the clinician's opinion, at the first visit. Standard of care was rendered to each patient per usual clinic practice. Clinical assessments included the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), the British Isles

Lupus Assessment Group (BILAG 2004) index and the Physician Global Assessment (PGA). A 5-minute ECG was obtained at each visit for HRV analysis using the AliveCor iPhone ECG device. Serum cytokine levels, including interleukin 6 (IL-6) and B Lymphocyte Stimulator (BLyS) were measured with an enzyme-linked immunoassay (ELISA). Linear regression analysis and multivariate linear mixed effect models were used. At baseline, mean SLEDAI was 7.6 ± 0.3 , mean cumulative BILAG 2004 index was 10.0 ± 0.6 and mean PGA was 1.4 ± 0.1 . Baseline HRV measures were negatively associated with baseline disease activity using the BILAG 2004 index ($p=0.01$), but less consistently with the SLEDAI score ($p=0.60$) and the PGA ($p=0.16$). HRV measures were also negatively associated with baseline IL-6 levels ($p=0.02$). At the follow up visit (median 1 month from the baseline visit), there was a significant decrease in SLEDAI (average decrease -2.3 ± 0.5), BILAG 2004 index (average decrease -3.7 ± 1.0) and PGA (average decrease -0.4 ± 0.1) compared to baseline. Change in HRV measures was negatively associated with change in the BILAG 2004 index ($p=0.03$) (Figure 1) and the PGA ($p=0.02$) and there was also a trend with change in SLEDAI ($p=0.12$), indicating that an increase in HRV is associated with a favorable change in disease activity over time. In addition, there was a significant association between the change in HRV measures and decrease in IL-6 ($p=0.04$) and BLyS ($p=0.03$). None of the disease activity indices were correlated with change in IL-6 levels ($p>0.05$ for each); however, the change in BLyS levels was positively associated with change in

SLEDAI ($p=0.03$) and BILAG 2004 index ($p=0.02$). These pilot findings suggest that HRV measures could provide a sensitive marker for lupus disease activity and improvement, and support a role for HRV as an easily measured, non-invasive, safe and inexpensive outpatient procedure.

We have previously examined the antiarrhythmic and anti-inflammatory effects of low-level tVNS (LLTS) in patients with paroxysmal atrial fibrillation (AF), by means of electrical stimulation of the auricular branch of the vagus nerve at the tragus [33]. Forty patients with paroxysmal AF who presented for AF ablation, were randomized to either 1 hour of LLTS ($n=20$) or sham stimulation ($n=20$). LLTS (20Hz) in the right ear, 50% lower than the voltage slowing the sinus rate, was accomplished by attaching a flat metal clip onto the tragus. Under general anesthesia, AF was induced by burst atrial pacing at baseline and after 1 hour of LLTS or sham stimulation. Blood samples from the coronary sinus and the femoral vein were collected at baseline and after 1 hour of LLTS or sham and were analyzed for inflammatory cytokines, including TNF α and CRP, using a multiplex immunoassay. Pacing-induced AF duration decreased significantly by 6.3 ± 1.9 min compared to baseline in the LLTS group, but not in the control ($p=0.002$ for comparison between groups; Figure 2, left panel). AF cycle length increased significantly from baseline by 28.8 ± 6.5 ms in the LLTS group, but not in the control ($p=0.0002$ for comparison between groups). Systemic (femoral vein) but not coronary sinus TNF α and CRP levels decreased significantly only in the LLTS group (Figure 2, right panel). These results support the emerging paradigm of neuromodulation to treat AF and other inflammatory diseases.

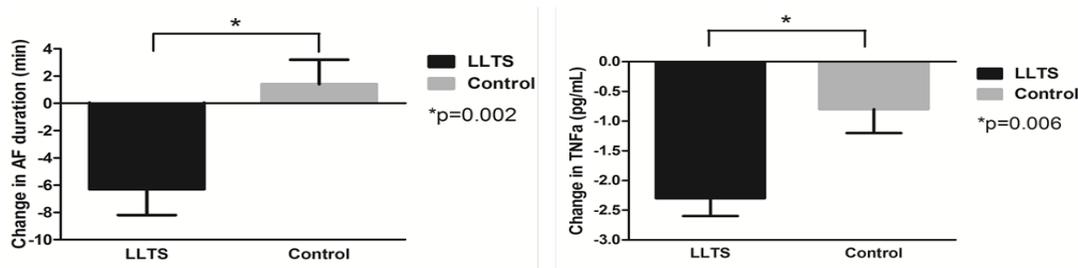


Figure 2

Design and Methods

Patients with SLE and active disease will be eligible to participate in this prospective randomized controlled trial of active or sham tVNS.

Eligibility criteria

Inclusion Criteria:

1. Patients with SLE age 18-70 meeting the ACR Classification Criteria [34]. Patients need to meet a minimum of 4 out of 11 criteria simultaneously or serially on two separate occasions.
2. Evidence of positive ANA or anti-dsDNA within one year of screening
3. Non-serological SLEDAI ≥ 4 or ≥ 1 BILAG B or A and with presence inflammatory arthritis (defined by at least 3 swollen and 3 tender joints) at screening.
4. Patients may receive one or more of the following immune suppressive therapies: hydroxychloroquine, quinacrine, methotrexate, azathioprine, mycophenolate mofetil,

tacrolimus, sirolimus, belimumab, abatacept. Immune suppressive medications should have been administered at stable doses for ≥ 30 days prior to baseline. Patients may also be on prednisone up to 10mg daily or equivalent steroid treatment at the baseline visit.

Exclusion Criteria³

1. Acute lupus nephritis defined as class II,III, IV or V nephritis diagnosed within 6 months or prot/creat > 1.5 gm/gm due to active lupus (by the most recent urinalysis) or in process of receiving induction therapy for nephritis
2. Active CNS lupus affecting mental status
3. Any other organ threatening or life threatening manifestation of SLE as well as those, who, in the opinion of the investigator, have severe multi-organ or refractory lupus
4. Rituximab treatment within 6 months prior to screening and/or without return of B cells to baseline levels
5. Treatment with cyclophosphamide within a month prior to screening
6. Treatment with any investigational drug within 3 months or 5 half-lives whichever is longer
7. Recurrent vaso-vagal syncopal episodes
8. Unilateral or bilateral vagotomy
9. Presence of any evidence of vagus nerve pathology or injury
10. Heart failure (NYHA class III or IV)
11. Known atherosclerotic disease, including severe carotid artery disease, uncontrolled hypertension, uncontrolled diabetes, any-and history of myocardial infarction (MI)-(not just recent or less than 1-year)-or-, cardiomyopathy or history of stroke within the past year. Clinically stable patients with coronary artery disease, but no recent MI (within the past year) and no current symptoms of angina are not however excluded.
12. Valvular or other structural heart disease that is evident by transthoracic echocardiogram and is associated with heart failure (NYHA class III or IV)
13. Prolonged QT interval or abnormal baseline ECG – sick sinus syndrome, Mobitz type 2 second or third degree heart block, atrial fibrillation, atrial flutter, recent history of ventricular tachycardia or ventricular fibrillation or clinically significant premature ventricular contraction
14. Individuals currently implanted with an electrical and/or neurostimulator device, such as cardiac pacemaker or defibrillator, vagal neurostimulator, deep brain stimulator, spinal stimulator, bone growth stimulator, or cochlear implant
15. Known respiratory disease that has decreased any pulmonary function test more than 25% below expected values or has resulted in hospitalization within the past year
16. All diagnosed syndromes affecting the central nervous system (CNS) or autonomic nervous system
17. Major psychiatric disorders including evidence of major depression-depressive disorder (DSM-5 diagnostic criteria) within the past year that is not currently controlled by medications
18. Hemoglobin below 9.0 gm/dL (by the most recent CBC)
19. Pregnancy or breast feeding
20. Inability or unwillingness to understand and/or sign informed consent
21. Any other medical condition, whether or not related to lupus which, in the opinion of the investigator, would render the patient inappropriate or too unstable to complete the study protocol

Study details: Patients meeting the eligibility criteria will be invited to participate in this double-blinded randomized controlled trial. Patients may have one or several active manifestations of

lupus, but with enough disease stability in the opinion of the investigator to allow for this trial design, which includes continuation of background immune suppressive therapies at stable doses and does not allow administration of additional corticosteroids for the duration of this short protocol, despite the possibility of receiving a sham intervention. This will be made clear during the informed consent process.

Patients entering the study will continue background standard of care medications subject to the following restrictions. Immune suppressive therapy is not required per protocol, but patients may receive a combination of one or more of the following immune suppressants: antimalarials (hydroxychloroquine, quinacrine), methotrexate, azathioprine, mycophenolate mofetil, tacrolimus, sirolimus, belimumab, abatacept. DMARDs should be administered at stable doses for ≥ 30 days prior to baseline and no dose increases will be allowed during the study. Patients may also be receiving up to 10mg prednisone or equivalent steroid treatment.

Participants will be screened and enrolled at the same visit (baseline, week 0). Screening procedures will begin with the informed consent process during which patients will review the IRB approved informed consent information, which will include a full description of the study and the procedures involved, patients' rights and responsibilities, and alternative treatments that are available if the patient does not decide to participate in the study.

After informed consent and screening procedures are completed, patients will be randomly assigned (1:1) to active or sham tVNS. Patients will be blinded to the treatment allocation and will be requested to refrain from discussing the details of their treatment with other patients in the clinic, the physicians, and the rest of clinic staff. The clinical coordinators will be unblinded to the treatment allocation and will instruct the patients on the proper use of the device. These study personnel will be designated to address the patients questions and concerns as well as to record any side effects related to the use of the device.



Active tVNS will be performed by use of a transcutaneous electrical nerve stimulation (TENS) device with electrodes attached to the tragus of the ear, which is innervated by auricular branch of the vagus nerve [35]. Stimulation will be limited to the left vagus nerve. **The SaluSTIM® transcutaneous electrical stimulation (TENS) device, will be used for this study. This device is manufactured in China by EASYMED INSTRUMENT CO., LTD, and imported and distributed within the US by US Medical Inc. This device has received a 501k approval by the FDA (EASY MED TN-28 C, K040253, 04/08/2004). The device will be used with an earclip electrode with two contacts of equal size, placed one in contact with the medial side and one in contact with the lateral side of the tragus in the active stimulation group (device manual attached). The earclip**

electrode has not been previously reviewed by the FDA for experimental studies. It has been however studied in Europe and is available in the European market.

The same TENS protocol will be followed in sham tVNS arm, but the electrode pads will be placed on the ear lobe, which is devoid of vagus innervation. The TENS unit will be set at a pulse width of 200 μ s and a pulse frequency of 20 Hz. Amplitude will be titrated to the level of sensory threshold (10–50 mA), i.e. will be gradually increased until the patient experiences mild discomfort, then decreased by 1 mA below that threshold. Patients will be advised to limit the amplitude of stimulation to 13.5 mA or below, since larger currents for prolonged periods of time could result in skin burns. Although this is not a hard limit, warning language will be included in the user manual provided to the patients to avoid stimulation beyond 13.5 mA. Similarly, the study coordinator in charge of determining settings for individual patients will be advised to use caution when programming the stimulator beyond 13.5 mA.

TENS will be applied for at least 60 min and up to 120 min daily as tolerated. After individual training, participants will apply TENS by themselves as part of their daily routine. Participants will be requested to keep a daily log with the time and duration of TENS application, amplitude settings and any comments related to each daily session.

Participants will be evaluated at baseline, week 4, week 8, and at completion of the study (week 12) \pm 5 days. An additional optional visit may be also scheduled at week 1. At the week 1 visit, TENS will be administered continuously for one hour in the clinic under direct supervision. Blood for biomarker analysis will be drawn before and at the end of this session. The patients will be interviewed by the unblinded study coordinator to address any issues or concerns with TENS administration at home, and to assess their adherence to the stimulation protocol. An additional study visit may be scheduled at the time of a clinically significant flare (flare visit, see below).

Patient safety will take precedence over the requirements of the protocol at all times. At the time of a clinically significant flare (defined as at least one BILAG letter grade increase AND clinician determination of clinically significant deterioration) patients may elect treatment with intramuscular methylprednisolone acetate or, if necessary new or changed DMARD therapy, but will continue the study as permanent non-responders in the primary clinical endpoint, even if they subsequently improve with additional treatment.

Evaluation of general health status, adverse events will be performed at each above visit. Evaluation of lupus disease activity (SLEDAI, BILAG, PGA, CLASI, Joint Count, LFA-REAL, SF-36v2, Lupus QoL, FACIT-fatigue), sleep (PROMIS Sleep Disturbance SF-8b, PROMIS Sleep Related Impairment SF-8a), depression (CES-D), caffeine and ethanol consumption and tobacco exposure (by the ECG questionnaire) will be performed at the same visits.

In addition to disease activity assessments and patient reported endpoints, autonomic function including HRV will be assessed at each visit by the ANX 3.0 physiologic monitoring system performed by trained clinic staff. The ANX 3.0 is a non-invasive, real-time, digital monitor of autonomic functioning, which monitors both branches of the autonomic nervous system simultaneously (<http://www.ans-hrv.com/index.htm>). Testing lasts for 15 min after 5 minutes of rest and is performed with the patient initially seated and then standing, while heart rate, blood

pressure and respiration are monitored. All participants will be asked to avoid caffeine for 4 hours and alcohol, smoking and exercise for 12 hours prior to the visit to avoid any interfering with the results of their ECG. Analysis and interpretation of ANX 3.0 results will be performed in a blinded fashion in collaboration with a consultant cardiologist, Dr. Stavros Stavrakis at the Department of Cardiology/Electrophysiology at the University of Oklahoma.

In addition to the ANX 3.0 test, a routine 12-lead ECG will be also performed at the baseline visit. Laboratory Assessments will include CBC with differential, metabolic profile, urinalysis, anti-dsDNA, and complement studies collected at baseline, every 4 weeks through week 12 and the flare visit. If urine dipstick is $\geq 2+$, a protein/creatinine ratio will be performed at the following visit (or sooner at the discretion of the investigator). Whole blood, plasma, serum and RNA PAXgene tubes for cell stimulation, measurement of cytokines and gene expression analysis will be collected at the same visits (described in more detail below). Blood will be drawn only after completion of ANX 3.0 for HRV analysis. Patients will also donate a sample of urine for biomarker analysis.

The primary endpoint will be improvement in disease activity at 12 weeks compared to baseline as measured by the BICLA (BILAG-based Combined Lupus Assessment) in patients on active vs sham tNVS. The BICLA scoring system incorporates improvement by the BILAG (British Isles Lupus Assessment Group) index, with requirements for no worsening in any organ by BILAG or SLEDAI (SLE Disease Activity Index) and no more than a 10% worsening in PGA (Physicians Global Assessment) as well as no initiation of off-protocol treatments.

Secondary clinical endpoints will be changes in HRV parameters at week 12 and each monthly visit, the Systemic lupus erythematosus Responder Index (SRI) 4/5 and the BICLA at each monthly visit, and correlations between HRV, SRI and BICLA over three months (baseline and three follow up visits). Prespecified exploratory endpoints will be: 1. BICLA and SRI component analyses, CLASI responses, flare rate and time to first flare 2. Patient reported outcomes: health related quality of life [measured by the Lupus QoL and the Short Form 36v2 and a visual analogue scale (VAS)] 3. Correlations between these other endpoints, HRV assessments and other parameters of autonomic nervous system functioning. All clinical assessment instruments to be tested are included in Appendix 1 (page 17).

Biologic Profiling: Whole blood, plasma, serum and PAXgene tubes for cell stimulation, cytokine and gene expression analysis will be collected at baseline, at week 1 (optional visit) and at each monthly visit (Refer to Appendix 1). In addition to direct measurements of IL6, TNF alpha and IL1 a panel of B and T cell cytokine and gene expression arrays developed at the OMRF by Drs. Judith James and Joel Guthridge, will provide sensitive markers for changes in the TNF α , IL1, IL6, IL17, and BLYS, pathways as well as comprehensive data for exploratory analysis. Patients will be assigned to specific pathway subgroups based on their dominant gene expression and cytokine signature at the baseline visit (presence or absence of Type I interferon signature) in order to determine relationships between clinical and critical pathway responses to vagal modulation and discrete biologic phenotypes of lupus.

Statistical considerations

The primary endpoint (the proportion of placebo vs treatment subjects who meet the BICLA response criteria using Fisher's Exact Test at week 12) will be determined by an intention to treat analysis. Thus all randomized subjects will be included in this analysis. Subjects who drop out prematurely for any reason or are treated with off protocol medications are considered non responders. Statistical significance will be determined when $\alpha < .05$. In an exploratory expansion of the primary endpoint, given the small size of this trial, potential confounders will be integrated into a propensity score in order to perform a multivariate evaluation with few variables. **For secondary analyses**, in order to evaluate time to flare and time to severe flare in treatment vs placebo patients, a log rank analysis will be performed.

Power analysis for the primary endpoint: The analysis plan is powered on the assumption that the active tVNS arm will have a 35 to 50% response rate at week 12, equivalent to the response to pharmacologic treatments in most clinical trials of moderate lupus. A sample size of 50 patients will provide a power of approximately 80% at a significance level of 0.05 at week 12. By these settings and assumptions, the following power estimates are obtained.

Power calculations for a 50 patient study, if $\alpha=0.05$ with response rates:

	Rate 1	Rate 2	Rate 3	Rate 4	Rate 5	Rate 6	Rate 7
Active tVNS n=25	60%	55%	50%	45%	40%	35%	35%
Sham tVNS n=25	20%	15%	10%	10%	5%	5%	3%
Power	0.84	0.86	0.89	0.80	0.86	0.77	0.84

Given the small size of this study no stratification scheme based on demographics and baseline characteristics are proposed, but variables of age, race, gender, steroid use, autoantibody positivity, complement consumption, and BILAG score at baseline will be compared in order to identify any glaring imbalances in group assignments.

Pitfalls to Sample Size Calculation: For this pilot study the sample size was calculated on the basis of an assumption that the cholinergic anti-inflammatory reflex is relevant to a cross section of lupus patients. As discussed above, this may not be the case. However in order not to bias this study the selection of patients will not be restricted to those with known propensity for elevations of IL6 or other probable candidate cytokines previously implicated in vagal mediation. An interim analysis will be performed after 20 patients will have completed the study, and will be used to assess the quality of the data collected and strength of associations observed. Consideration of the necessity and/or utility of sample size changes or will be made at that time. At the end of the study a similar analysis will be performed to determine the utility or futility of a future, significantly larger study.

Analysis of Biologic Endpoints: Changes in biomarkers will be evaluated by repeated measures analysis of variance, adjusted for background medications (ANCOVA) and will be

correlated with clinical responses and changes in HRV parameters. This work will be largely exploratory. Given the complex array of data we are likely to generate, principal component analysis will be applied to identify directions (principal components) along which variation of data is maximal. It should be considered that even though these analyses will be exploratory and probably underpowered, a large enough treatment effect size might still be encouraging enough to continue explorations of this intervention, particularly if sub analyses provide insight into the responder profile.

Pitfalls and alternative methods relevant HRV analysis: In addition to cardiovascular diseases several other pathologic conditions, like diabetes, renal failure and obstruction sleep apnea, have been associated with decreased HRV, which can be further influenced by exposure to tobacco, caffeine and several cardiovascular drugs [16, 36, 37]. Both acute and chronic smoking appear to decrease HRV and increase cardiac vulnerability and arrhythmia susceptibility, an effect mediated by nicotine-induced peripheral sympathetic activation and possibly exposure of particulate matter. Furthermore, neuropsychiatric conditions like fibromyalgia, epilepsy, depression and anxiety are associated with decreased HRV [38-40], whereas interventions like weight loss, exercise and behavior therapies were shown to increase vagus nerve activity in clinical studies [41-43].

It is obvious that HRV is influenced by a wide array of covariates, not all of which are directly related to lupus disease activity. Some of these comorbidities are considered exclusion criteria. Some others (e.g. cardiovascular and psychiatric comorbidities, pulmonary diseases, sleep disturbances and medications) are recorded, though physician inquiry or patient-filled questionnaires. These parameters will be included in our multivariate or propensity scoring models. Associations with patient reported outcomes will be additionally examined as secondary endpoints.

Human subjects

1. Study population

The study population will include male/female patients with SLE, of age 18 or older. Race, minority status and gender will not affect enrollment. This study will be performed at the Department of Clinical Pharmacology at the Oklahoma Medical Research Foundation.

2. Recruitment and Consent Procedures

Patients will be recruited at the lupus clinic at the Oklahoma Medical Research Foundation. Candidate subjects will have the purpose of the study explained to them, including the benefits, risks and options, will be provided with the consent form, and after questions have been answered, will be invited to participate. No study specific procedures will be performed until a participant completes the informed consent process and signs the IFC form. Participants will be allowed adequate time to review the consent and encouraged to discuss the study with other caregivers or family. An important element of the process will be the understanding by the patient that their decision to participate or not will not affect their access to medical care in the clinic. Employees and clinic staff will be excluded from participation.

3. Potential risks and their likelihood and seriousness

TENS is a noninvasive well tolerated modality that has been extensively used for treatment of pain in various settings. TENS has not been associated with side effects more than minimal discomfort at the area of application. These are usually regarded as inherent effects of the tVNS therapy and not rated as adverse events except they were described as painful [31]. Other side effects previously reported include dizziness, mild dyspnea, chest pain, headaches, hoarseness, numbness and neck pain [44, 45] [31]. Cognitive testing has not revealed a hint for cognitive side effects of tVNS, which fits with the literature of implanted VNS [31].: Previous studies examining TENS for vagus nerve stimulation in healthy individuals [24] and in patients with chronic pain [32] and epilepsy [29] showed that this was safe and well tolerated.

Cardiovascular safety of SaluSTIM

Based on communication of the PI with the FDA (see attached letter of correspondence), the proposed clinical investigation presents a significant risk, in accordance with the definition for a significant risk device in section 812.3(m) of the investigational device exemptions (IDE) regulation. This determination was attributed to the risk of cardiac side effects that Vagus Nerve Stimulation may have on the SLE population. Therefore, the investigators are required submit an IDE application to FDA, and receive both FDA and institutional review board (IRB) approval before initiating this study. The IDE application was approved by the FDA on 7.10.2015 (see attached letter of response), pending submission of the User's Manual of the SaluSTIM TENS device and approval by the institutional IRB(s).

Since efferent fibers of the vagus nerve modulate cardiac function, cardiac safety has always been a concern in the therapeutic use of vagus nerve stimulation [46]. Efferent vagal fibers to the heart are supposed to be located on the right side. In order to avoid cardiac side effects, electrode placement is usually performed on the left side in treatment of central nervous diseases [47]. **No significant effects on heart rate, blood pressure, or peripheral microcirculation could be detected during short term tVNS in the 10 patients with tinnitus after short term treatment with SaluSTIM [30]. tVNS for 60min daily for 5 days using the SaluSTIM was also examined in 30 patients with moderate or severe tinnitus. Heart rate monitoring during tVNS treatments showed no cardiac or circulatory effects (e.g. bradycardia) and no other adverse effects were observed (Lehtimäki J. et al. Efficacy and Safety of Transcutaneous Vagus Nerve Stimulation (TVNS) in Tinnitus; a case control study. 7th International TRI Conference on Tinnitus, Tinnitus: A Treatable Disease. May 15 - 18, 2013, Valencia, Spain. In another series of 27 patients with tinnitus treated with tVNS for 60min with HRV monitoring before and after stimulation, no cardiac or circulatory effects (e.g. bradycardia) were observed (Ylikoski J. et al. Acute effects of transcutaneous vagus nerve stimulation (tVNS) on tinnitus related mental stress (TRMS). 11th International Tinnitus Conference. May 21-24.2014. Berlin, Germany.**

Cardiovascular safety of tVNS using other devices

Studies of tVNS using different protocols and stimulation devices although support the cardiovascular safety of this modality. In a pilot study of 24 patients with tinnitus treated with tVNS using a wired neurostimulating device (CM02, Cerbomed, Erlangen, Germany) applied as much as possible and for at least 6 hours per day over 3-10 weeks (30 seconds on-180 seconds off), 2 adverse cardiac events (one classified as a severe adverse event) were registered leading to early study termination, but were eventually considered very unlikely to

have been caused by tVNS as other explanations were evident [46]. In particular, one patient had experienced sinus arrhythmic episodes already in the past, and in the other patient comorbid hypertension had caused concentric cardiac hypertrophy which might have contributed to the described temporary left bundle branch block. Retrospective analyses of ECG parameters revealed a trend toward shortening of the QRS complex by tVNS. This was observed after the 2 patients with cardiac adverse events were excluded from the analysis, but not when the whole sample of patients was analyzed. There was definitely no prolongation of the QRS complex which is a known predictor of cardiac morbidity and mortality. This study was extended to a 2nd phase that used an improved stimulation device (NEMOS, Cerbomed, Erlangen, Germany) and intensified cardiac monitoring in a different sample of 26 patients [31]. Stimulation was applied 4 hours daily in a 30 seconds on-30 seconds off paradigm. An immediate significant (uncorrected) reduction of the heart rate after 30-60 min of tVNS was noted in phase 2. During the time course of tVNS treatment pulse frequency tended to increase and diminished again at follow-up 4 weeks after end of treatment. ANOVA for the parameter QTc for all time points and for contrast screening/baseline to W24 visit (end of treatment) displayed near-significant effects with regard to a prolongation of the QTc time ($p=0.081$ and 0.063 respectively). No such trend was observed in the initial phase 1 study. Notably, the QTc range remained always below critical levels. **In conclusion, in subjects with no known pre-existing cardiac pathology, there has been no indication of arrhythmogenic effects of tVNS [30, 31, 46].** This is in line with the low incidence of adverse cardiac reactions during the long-term experience in more than 50,000 patients with implanted left VNS for treatment of epilepsy and depression [48]. Nonetheless, in individuals with preexisting cardiac disease the potential for tVNS to cause cardiac conduction adverse events has not been studied. Cardiac disease is more common in patients with SLE than in the overall population and may be asymptomatic. In this population, risk will be mitigated by inclusion/exclusion criteria specific to cardiac concerns and pre-stimulation cardiac testing.

The ANX 3.0 procedure for recording of HRV is painless and overall very safe and well tolerated. Certain procedures however performed during testing like deep breathing, standing from a seated position and the Valsalva maneuver (bearing down while holding your breath) have been associated with side effects in some individuals. These side effects may include lightheadedness, dizziness, flushing, palpitations, sweating and GI upset with nausea. Rarely people have fainted during testing. People with significant cardiovascular disease or arrhythmias that may be more prone to develop such symptoms during testing will be excluded from the study.

In the course of the background treatments that patients receive, standard clinical and laboratory monitoring will be performed as appropriate. Minimally, this will involve blood drawing, urine collection and clinical assessment at each protocol visit. Blood will be drawn using standard sterile techniques.

Blood draw is usually well tolerated, except for mild discomfort at the venipuncture site and occasionally lightheadedness, nausea and fainting. Blood draw might occasionally be associated with localized bleeding and bruising and more rarely with superficial thrombophlebitis or other localized soft tissue infections. Up to 60-120 ml will be collected at all visits. This amount of blood draw is usually not associated with significant side effects, except for patients with severe preexisting anemia (hemoglobin <9 gm/dL), who will be excluded from the study.

4. Procedures for protecting against or minimizing any potential risks

Patients will be told about potential side effects during the informed consent process and will be provided with contact information for the clinic and the investigators.

5. Potential benefits to be gained by the subjects and the society in general

Immune modulating therapies in current use for SLE are either extremely expensive (belimumab) or have significant side effects (prednisone and other immune suppressants). There is a definite unmet need for safer treatments for use alone or in combination to control this difficult illness as well as for the insights into pathophysiology of the disease that can be obtained by studying treatments with known effects on immunity.

6. Relation of risks to anticipated benefits

The risks are minimal and acceptable compared to alternative treatments for SLE. The potential benefits in knowledge and determining whether there may be potential usefulness of this treatment therefore justify these risks.

Data and Safety Monitoring Plan

Information on adverse events will be collected throughout the study and reported to the IRB annually. Serious adverse events will be reported to the IRB within 24 hours of the site becoming aware of them. All study related protocol procedures will be stopped in patients who develop sinus bradycardia (below 50 bpm) or atrioventricular block, until these adverse events have been reviewed by the DSMB and the FDA. Patients will be followed closely for the resolution of all such “events and for their general safety and/or treatment of any and all adverse events in the interim. Adverse events will also be adjudicated quarterly by an independent data safety monitoring board (DSMB) consisting of one electrophysiologist, Paul Garabelli, MD, and two physicians with expertise in lupus who will submit a report to the OMRF IRB every annually and at any additional time deemed appropriate. Dr. Garabelli is an Assistant Professor of Medicine at the Department of Cardiology at the University of Oklahoma Health Sciences Center. The other 2 members will be selected from our Department’s rotating DSMB group, which includes: Dr. Diane Kamen, Assistant Professor of Medicine at the Division of Rheumatology at the Medical University of South Carolina in Charleston, Dr. Maria Dall’Era, Associate Professor of Medicine and Director of the Lupus Clinic and Rheumatology Clinical Research Center at the University of California in San Francisco, Dr. Amr Sawalha, Associate Professor of Medicine at the Division of Rheumatology at the University of Michigan in Ann Arbor and Dr. John Harley, Director at the Division of Rheumatology at the Cincinnati Children’s Hospital Medical Center in Cincinnati. Data monitoring will be performed by a trained study coordinator or study monitor who is not assigned to this project, but may be an employee of OMRF.

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APPENDIX 1 Schedule of Assessments

Events	Treatment Period				
	Screening/ Baseline	Week1 ^a	Week 4	Week 8	Week 12 (EOT/Flare)
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Medical History	X				
Concomitant Medications	X	X	X	X	X
Adverse Events	X	X	X	X	X
ANX 3.0 ^b	X	X	X	X	X
Baseline ECG	X				
Patient TENS Unit Daily Diary ^c	X	X	X	X	X
TENS Administration	X ^d	X ^e			
SLEDAI	X	X	X	X	X
BILAG	X	X	X	X	X
PGA	X	X	X	X	X
CLASI	X	X	X	X	X
LFA-REAL	X	X	X	X	X
SF-36v2	X	X	X	X	X
Lupus QoL	X	X	X	X	X
FACIT-fatigue	X	X	X	X	X
PROMIS Sleep Related Impairment SF-8a	X	X	X	X	X
PROMIS Sleep Disturbance SF-8b	X	X	X	X	X
Depression CES-D	X	X	X	X	X
ECG Questionnaire	X	X	X	X	X
Physical Exam	X	X	X	X	X
Joint Count	X	X	X	X	X
Vital Signs	X	X	X	X	X
CBC w/diff	X		X	X	X
CMP	X		X	X	X
anti-dsDNA	X		X	X	X
Complements (C3, C4)	X		X	X	X
RNA Paxgene	X	X ^f	X	X	X
Whole blood stimulation assays	X	X	X	X	X
Cytokines	X		X	X	X
Urinalysis/Urine biomarkers	X		X	X	X
Urine Pregnancy Test	X		X	X	X
Protein/Creatinine Ratio ^g					

Notes: Patients, their providers and the rest of the study team are blinded to TENS treatment allocation. Only the study coordinators are unblinded to treatment. During assessments and administration of tests and/or TENS treatment, please use extra caution not to unblind the patient and the blinded study team.

a) Week 1 is an optional visit.

b) Performed prior to labs and TENS treatment.

c) Review Diary for home compliance at each visit.

d) Instruct patient on proper use and treatment protocol with return demonstration. Please use caution when programming the TENS above 13.5 mA although this is not a hard limit and may vary from patient to patient. Please also warn patients to avoid [stimulating above 13.5mA since the chance for burning may be higher at these levels](#).

e) Administer continuously for 1 hour by unblinded coordinator, after ANX 3.0 is complete.

f) Drawn pre and post TENS treatment, at the week 1 (optional) visit.

g) If urine dipstick is $\geq 2+$, perform at following visit (or sooner at the discretion of the investigator).