Transcutaneous Auricular Vagus Nerve Stimulation Reduces Inflammatory Biomarkers and May Improve Outcomes after Large Vessel Occlusion Strokes: Results of the Randomized Clinical Trial NUVISTA

Osvaldo J. Laurido-Soto MD[a]; Gansheng Tan MEng[*b, c, d]; Susan Searles Nielsen PhD[*a]; Anna L. Huguenard MD[b, c]; Kara M. Donovan MS[b, c, d]; Isabella Xu[e]; James Giles MD, DPhil[f]; Rajat Dhar MD[a]; Opeolu Adeoye MD[g]; Jin-Moo Lee MD, PhD[** a, d, h]; Eric Leuthardt, MD[** b, c, d]

[a]Department of Neurology, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA
[b]Department of Neurosurgery, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA
[c]Division of Neurotechnology, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA
[d]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA
[e]Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA
[f] Department of Neurology, Yale University School of Medicine, New Haven, Connecticut, USA
[g]Department of Emergency Medicine, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA
[h]Department of Radiology, Washington University School of Medicine in St. Louis, St. Louis, Missouri, USA

*Authors contributed equally to the manuscript.

**Co-Principal Investigators contributed equally to the trial performance and manuscript.

Short Title: VNS reduces inflammation after ischemic stroke

NCT number: NCT05390580, **Date:** 8/6/2025

Corresponding Author:

Osvaldo J. Laurido-Soto MD
Department of Neurology, Vascular Neurology and Neurocritical Care section
Washington University School of Medicine
Campus Box 8111
660 South Euclid Avenue
St. Louis, MO 63110
Phone: 314-296-0016
E-mail: ojlaurido-soto@wustl.edu

Authors' ORCID IDs: 0000-0002-7724-1772

Word count: Abstract 248, main text 4338

Tables/figures: 3 tables, 3 figures

Supplemental files: 6

References: 48

Abstract

Background: Inflammation plays a critical role in brain injury following acute ischemic stroke (AIS). Transcutaneous auricular vagus nerve stimulation (taVNS) has shown anti-inflammatory properties, but its efficacy in AIS remains unexplored. We investigated if taVNS could mitigate post-AIS inflammation and its safety.

Methods: In this randomized, sham-controlled trial with blinded outcomes assessment, patients with anterior circulation large vessel occlusion (LVO) AIS were assigned to twice-daily taVNS or sham stimulation for five days or until discharge. Inclusion criteria: age ≥18 years, NIHSS ≥6, anterior circulation LVO, and enrollment within 36-hours of last known normal. Primary endpoints were changes in inflammatory biomarkers (interleukins (IL)-1β, 6, 10, 17α, and tumor necrosis factor alpha (TNFα) on Days 0, 1, 3, 5, and 7, and taVNS safety). Secondary exploratory endpoints included change in NIHSS, 90 day modified Rankin scale (mRS), and safety (bradycardia, hypotension, infection, and death).

Results: Thirty-five patients (17 taVNS, 18 sham) were enrolled. The taVNS group showed a significant rate of change in normalized aggregate pro-inflammatory cytokines and IL-6 levels compared to sham ($p=0.04$ and $p<0.001$, respectively). Each 1 pg/mL reduction in IL-6 correlated with a 0.798-point improvement in NIHSS in the taVNS group (95% confidence interval [0.077, 1.518], $p = 0.031$]). IL-1β, 10, 17α, and TNFα showed reduction in cytokine levels, but did not reach statistical significance. There were no statistically significant differences amongst mRS and safety outcomes between groups.

Conclusions: taVNS safely reduced post-AIS inflammation in anterior circulation LVO patients with potential NIHSS improvements. These findings warrant further investigation in larger trials.

ClinicalTrials.gov ID: NCT05390580, https://clinicaltrials.gov/study/NCT05390580

MeSH Keywords: Ischemic stroke, large vessel occlusion, neuromodulation, vagus nerve stimulation, inflammation

Nonstandard abbreviations and acronyms

ANOVA: Analysis of Variance

AIS: Acute Ischemic Stroke

CRP: C-Reactive Protein

CT: Computerized Tomography

Hz: Hertz

ID: Identification Number

IFN: Interferon

IL: Interleukin

LKN: Last Known Normal

LVO: Large Vessel Occlusion

mA: milliampere

mRS: Modified Rankin Scale

MRI: Magnetic Resonance Imaging

NIHSS: National Institutes of Health Stroke Scale

taVNS: Transcutaneous Auricular Vagus Nerve Stimulator

TGF: Transforming growth factor

TICI: Thrombolysis in Cerebral Infarction Score

TNF: Tumor Necrosis Factor

μs: microseconds

VNS: Vagus Nerve Stimulation

WBC: White Blood Cell

Introduction

Acute ischemic stroke (AIS) due to large vessel occlusions (LVOs) lead to disproportionate morbidity and mortality without emergency treatment [1]. Unfortunately, even after successful recanalization with mechanical thrombectomy, i.e., reperfusion of the primary vessel occluded [Thrombolysis In Cerebral Infarction score (TICI) 2B, 2C, or 3] [2], infarcts can continue to increase in size or develop hemorrhagic transformation [3, 4], leading to poor outcomes, highlighting the need for adjunct therapies to minimize ischemia progression and morbidity for both recanalized and non-recanalized patients. Neuroinflammation has been recognized as an important contributor to ischemic brain injury [5]. In the acute phase of AIS, inflammatory biomarkers elevation, such as pro-inflammatory cytokines [e.g., interleukin (IL) 6 (IL-6)] [6-10], are associated with exacerbating brain injury. Although ongoing trials are occurring in AIS, most immunomodulatory drug trials so far have been disappointing due to limited efficacy and/or medication side effects [11-14]. Thus, finding adjunct treatments that are easy to administer and safe is of the utmost importance.

Vagal nerve stimulation (VNS) has been established to have anti-inflammatory effects in several inflammatory conditions in animal models, including aneurysmal subarachnoid hemorrhage and inflammatory bowel disease [15-18]. In animal models of cerebral ischemia and reperfusion injury, VNS not only reduced infarct size, but also improved neurological outcomes [19-21]. In practice, most FDA-approved VNS are currently administered through surgical implantation of an electrode placed directly on the vagus nerve in the carotid sheath in the neck. While appropriate for chronic conditions such as depression, epilepsy, and chronic stroke recovery [22], implantable solutions are impractical in the emergent setting of AIS. In this context, noninvasive

transcutaneous auricular VNS (taVNS) offers a possible solution given its immunological effects and extremely low morbidity [23, 24]. Based on encouraging animal studies in AIS, demonstrated safety of taVNS in the critical care settings [25], and evidence of efficacy in subarachnoid hemorrhage [26], we conducted the "Neuromodulation Using Vagus Nerve Stimulation Following Ischemic Stroke as Therapeutic Adjunct" (NUVISTA) trial. This prospective randomized sham-controlled with blinded outcomes clinical study aimed to quantify the effect of taVNS on plasma inflammatory biomarkers in patients with LVO and explore its impact on post-stroke patient outcomes.

Methods

Study population.

All patients with incident AIS due to LVO who were admitted to Barnes Jewish Hospital in St. Louis, Missouri, between November 2022 and May 2024 were screened for eligibility. Inclusion criteria included: age≥18 years, acute anterior circulation LVO [Internal Carotid Artery (ICA), Middle Cerebral Artery first branch (M1), Middle Cerebral Artery second branch (M2)], National Institutes of Health stroke scale (NIHSS) score ≥6, pre-morbid modified Rankin scale (mRS) ≤2, randomization <36 hours (hrs) from symptom discovery/last known normal (LKN), expected life expectancy >3 months, and no active cancer or immunosuppressive/modulating therapy, concomitant infections or inflammatory states (i.e. chronic autoimmune diseases), hypotension, or bradycardia on arrival. The patient/family, the medical team, and the outcomes assessor were blinded to which arm the patient was enrolled (**Figure 1).** Full *Inclusion* and *Exclusion* criteria shown in **Online Resource 1.**

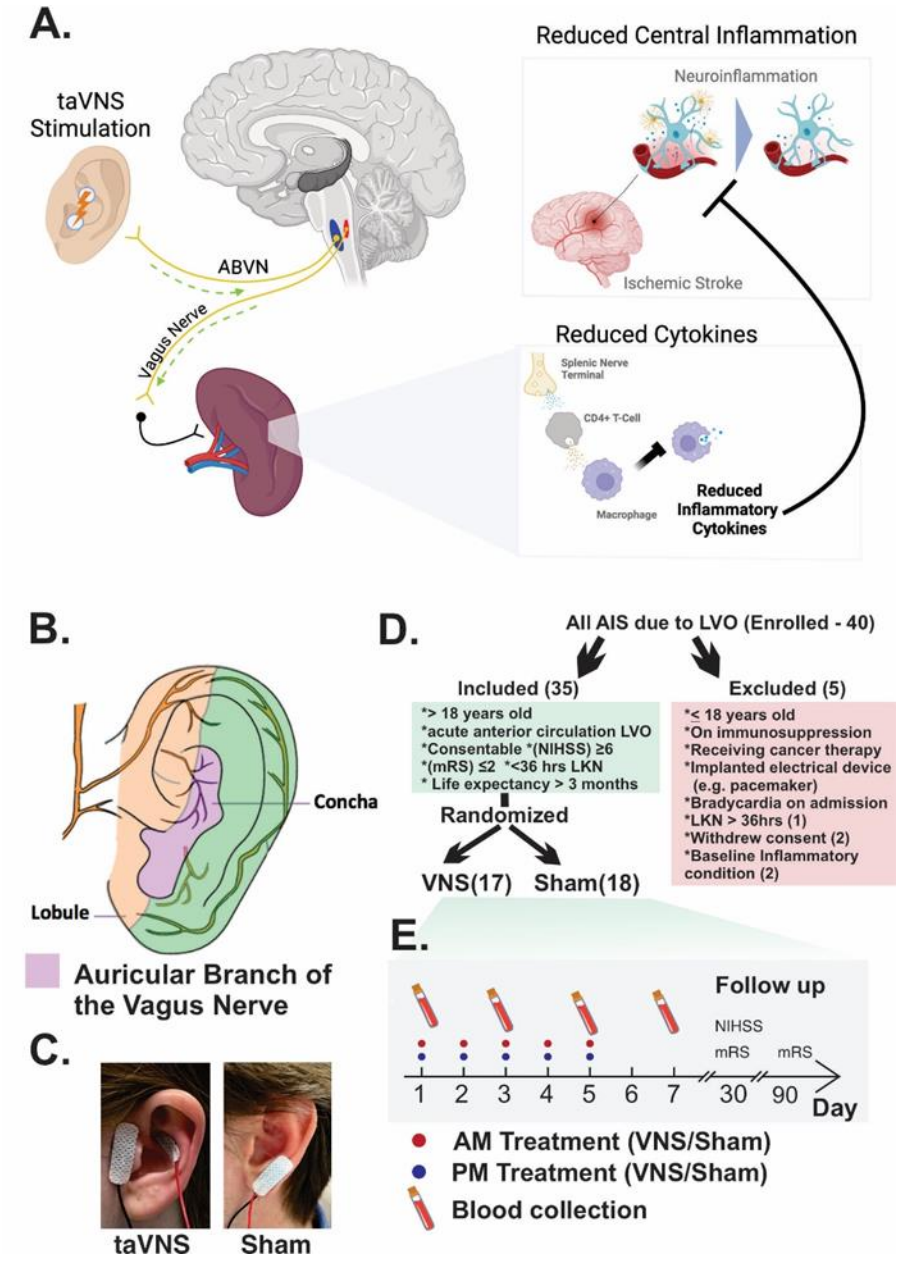

Fig 1 Rationale and trial protocol. **A**. The proposed mechanism of taVNS in acute ischemic stroke patients. Electrical stimulation of the auricular branch of the vagus nerve activates the cholinergic anti-inflammatory pathway. When the dorsal vagal complex receives input from the auricular branch of the vagus nerve, it activates efferent vagal fibers that project to the spleen. This process suppresses the production and release of pro-inflammatory cytokines like TNF-alpha, IL-1beta, and IL-6 and reduces central inflammation. **B**. Distribution of the auricular

branch of the vagus nerve on the outer ear [27]. **C**. Electrode placement for taVNS stimulation and sham group stimulation. **D**. Inclusion and exclusion criteria. **E**. Timeline for treatment and measurement of primary outcomes.

Protocol.

Patients were randomized to taVNS stimulation for 20 minutes every 12 hrs vs. sham electrical stimulation according to the same schedule. Our basic stimulation parameters were: intensity – 0.5 mA, frequency – 25 Hz, and pulse width - 200 μs (**Figure 1)** [27]. Patients continued left ear taVNS or left ear sham stimulation for five days (10 total stimulations), discharge, or death, whichever came first, and received standard procedural and medical care. Full protocol and device information shown in **Online Resource 2a** and CONSORT checklist [28] adherence as per **Online Resource 2b**.

Assessment of patient characteristics and standard treatment.

Baseline patient characteristics were documented, including age, sex, race, smoking history, comorbidities, admission baseline NIHSS, and pre-stroke modified Rankin Scale (mRS) score. Each stroke event was characterized by laterality, occlusion type, and acute interventions. Treatment documentation included thrombolytic administration and thrombectomy details. In thrombectomy cases, reperfusion outcomes were assessed using the TICI score at the end of thrombectomy [2]. This score was analyzed both as a semi-continuous measure of reperfusion percentage and as a dichotomized outcome (successful reperfusion defined as TICI ≥2B) [2].

Brain imaging and assessment of biomarkers of cerebral edema and infarct size as a baseline variable.

Head CT scans were obtained at baseline (within 12 hrs of last known normal) and follow-up, including at 24 +/-12 hrs post-intervention. Baseline scans were available for 13 (76.5%) treatment and 13 (72.2%) sham patients. All patients had at least one follow-up scan. Following established protocols [29, 30], we analyzed the scans for measures of cerebral edema and infarct size, including cerebral spinal fluid volume ratio and relative hemispheric volume to utilize as baseline measures. We also assessed for hemorrhagic transformation and malignant edema.

Assessment of blood biomarkers of inflammation.

Assessment of blood biomarkers of inflammation was the primary outcome. Total blood levels (pg/ml) of five cytokines were assessed: four ILs, specifically IL-1β, IL-6, IL-10, and IL-17α, and tumor necrosis factor alpha (TNFα). White Blood Cell (WBC) counts (K/cumm) were also measured. The full blood draw protocol can be found in **Online Resource 2c**. All five cytokines were measured at baseline (Day zero/<24 hrs) and every other day for the week thereafter (Day 1, Day 3, Day 5, and Day 7) or until hospital discharge. Protein concentrations were reported as pg/mL. As a summary measure, the mean of the z-score for each of the five cytokines (hereafter "cytokine score") was calculated. Z-scores were used to give similar weight to each cytokine, as IL-6 pg/ml levels were much greater than the other cytokines and therefore dominated a summary measure based on absolute values. All five cytokines were included together, rather than excluding IL-10 due to its anti-inflammatory characteristics, based on preliminary analyses that indicated this cytokine generally rises similarly to the other cytokines [31]; and we also

analyzed pro-inflammatory cytokines in isolation. Cytokine quantification methods can be found in **Online Resource 2d** [32].

Assessment of potential adverse effects.

Due to the potential cardiovascular effects of VNS [33], safety outcomes were monitored, including hypotension (systolic blood pressure <80 mmHg) and bradycardia (heart rate <50 beats per minute). Vital signs were recorded at multiple times around each stimulation session: 5 minutes before, every 5 minutes during, and 5 minutes after stimulation. Additionally, routine vital sign monitoring was performed every 12 hrs throughout the hospital stay.

Assessment of neurological outcomes.

The NIHSS total score and subscores were assessed at presentation/admission and daily thereafter until discharge or death, as well as on Day 30 +/- 7 days from LKN. Clinical assessments were performed by NIHSS certified stroke coordinators, nurses, and providers and documented in the medical record. The mRS was evaluated on Days 30 and 90 +/- 15 days from LKN over the phone, utilizing the Joint Commission National Quality Measures [34]. Additional clinical outcomes included hospital length of stay, while discharge disposition was categorized as either favorable (home, home with health services, or inpatient rehabilitation) or unfavorable (skilled nursing facility or long-term acute care hospital), or death before discharge. Patients were followed for 105 days after admission or until death [34], with readmissions to the hospital system and deaths at any location documented during this period. All vital status information and death dates were verified through electronic medical records.

Statistical analysis.

Statistical analyses were performed using Stata/MP version 18 (StataCorp LLC 2023, Statistical Software, College Station, TX) and Python packages Scipy and Statsmodels. All continuous variables were retained as continuous, except where noted. Multivariable regression was used to examine treatment group associations with outcomes, except for rare secondary outcomes/adverse effects. Following FDA recommendations [35], an intention-to-treat (ITT) approach was implemented. Primary ITT analyses were conducted without transformation of continuous outcome variables because ≥35 observations were available.

Statistical analysis of rate of change in WBC, cytokines, and neurological outcomes in taVNS vs. sham treatment.

Longitudinal data were analyzed using mixed effects models per the FDA [36], with person as a random effect, given multiple records per person. For cytokine models, assay plate was included as an additional random effect. This approach accommodated irregularly timed points, less restrictive missing data assumptions, and time-varying variables. To complement our primary ITT approach, treatment also was handled as time-varying when noted, with assignment occurring at randomization. All longitudinal models included treatment, time since LKN, and their interaction term(s) as primary independent variables to test whether the pattern of change over time differed between the taVNS and sham groups. Time was modeled quadratically for WBC and cytokine outcomes to capture the known U-shaped associations with time [37] when modeling these outcomes and linearly for in-hospital NIHSS scores based on panel data plots. For NIHSS, a single model was constructed to estimate the rate of change (change in NIHSS per day) in each of the two treatment groups and to formally test whether these rates were different.

For 30-day NIHSS changes (vs. baseline) and 90-day mRS changes, we used the parallel model, with actual assessment days scaled to standardize the evaluation period to 30 or 90 days, respectively. We characterized the daily change in each WBC or cytokine outcome using a similar model expanded to include the quadratic terms. Treatment-time interactions were tested using likelihood ratio tests comparing models with and without interaction term (NIHSS, mRS) or terms (WBC, cytokines), with significance at two-sided $\alpha=0.05$. These analyses were adjusted for baseline covariates [38], including baseline NIHSS (except in NIHSS models, which included this observation), time from LKN to thrombus removal time or recanalization attempt (groin puncture time), and percent reperfusion (0-100%) based on TICI score [2] to obtain a single semi-continuous measure. In sensitivity analyses, we also performed the analysis with a dichotomized TICI scale (reperfused ≥2B, not reperfused <2B) or restricted data to the first five days post-LKN (when most stimulations and hospitalizations had ended). Finally, we explored whether any difference in longitudinal patterns between taVNS and sham depended on laterality, occlusion type, or any of the *a priori* adjustment variables, i.e., whether any time-treatment interaction depended on one of these three variables. Laterality was of particular interest due to previous research suggesting differential VNS effects based on the side of stimulation [39, 40] and its influence on NIHSS subscore patterns. Full model descriptions can be found in **Online Resource 2d**.

Statistical analysis of difference in cytokine levels between taVNS and sham groups.

We conducted post-hoc t-tests to compare cytokine levels between groups on each day. We chose to structure further analysis utilizing IL-6 based on prior literature suggesting IL-6 is a key

cytokine associated with stroke outcomes and prior reduction of IL-6 with taVNS in aneurysmal subarachnoid patients [26, 41].

Statistical analysis of the association between change in cytokines and change in clinical outcomes.

We investigated the association between IL-6 levels and clinical outcomes, or their respective changes, to understand the short- and long-term functional implications of reducing inflammatory cytokines. We first verified if the reduced pro-inflammatory cytokine was associated with improving neurological outcomes using regression with changes in NIHSS scores as the dependent variable and changes in IL-6 as the independent variable. In this analysis, we paired Day 3 and Day 5 NIHSS and IL-6 measurements based on the measured time and then calculated the change by subtracting the baseline (first) measurement. To explore if this relationship is treatment-dependent, we conducted the analysis separately for each treatment group. Given that recanalization status following thrombectomy significantly influences neurological outcomes, we verified if the relationship between inflammation and neurological outcomes depends on recanalization status. To this end, a linear mixed model was used, including IL-6 levels, treatment, the interaction between treatment and IL-6 levels, recanalization status, and the interaction between recanalization status and IL-6 levels as predictors. We also explored the relation between IL-6 and NIHSS overall, as well as change in IL-6 in relation to mRS90 overall.

Statistical analysis of the Stroke Complications and Poor Outcomes (SCPO) in relation to treatment.

To test the hypothesis that taVNS reduces the incidence of complications and poor outcomes, the total number of select complications and poor outcomes was modeled as a function of stroke severity, quantified by the average NIHSS score over the first three days. SCPO were defined as: hemorrhagic transformation, hemi-craniectomy, bradycardia, hypotension, infection, readmission, and hospital poor disposition (i.e. nursing home or long-term acute care hospital). In-hospital deaths were excluded from these analyses as all three patients who passed inpatient (in the treatment group) were due to comfort measures. Given the ordinal nature of total complications and poor outcomes, a generalized linear regression model with a Poisson link function was employed. The model includes total complications and poor outcomes as dependent variables and treatment and admission disability as predictors.

Results

Characteristics of participants.

We randomized 40 participants; after applying all study eligibility criteria, we excluded five participants post-hoc and included 35 participants (17 treated, 18 sham) (**Figure 1).** Our cohort was biracial: Caucasian (77.1%) and Black (22.9%); had a mean age of 67.7 years (SD 13.7), and was 48.6% female. The baseline NIHSS was 16.4 (SD 6.6) for the treatment group and 15.7 (SD 4.4) for the sham group. The treated group had a higher burden of left-sided stroke (64.7% vs. 44.4%), ICA occlusions (47.1% vs. 27.8%), and greater time from LKN to recanalization (mean 13.2 hrs vs. 9.8 hrs) when compared to the sham group, but these differences were not statistically significant. Thrombolytics were administered to just under half the cohort and thrombectomy was attempted in the majority (94%), with 82% of the treated group and 89% of the sham group achieving recanalization after thrombectomy (TICI ≥2B). The treated group had

greater early edema at baseline, prior to the effects of treatment, as compared to the sham group based on cerebrospinal fluid (CSF) changes, Δ CSF -26% vs. -13%, p=0.05. The mean time to first stimulation from LKN was 22.8 hrs (SD 6.5) in the treated vs. 24.8 hrs (SD 6.2) in the sham group (**Table 1)**.

Longitudinal change in cytokines in relation to treatment group.

Across individual cytokines and the z score-based summary measures, differences in the pattern of change over time between the treatment and sham groups were suggested in both our primary analysis and the sensitivity analysis utilizing a time-varying exposure (treatment) variable (**Table 2, Figure 2, and Online Resource 3)**. The greatest difference between the groups was observed for IL-6. Without accounting for the lack of exposure prior to randomization, the treatment and sham groups appeared much more similar than in the time-varying model. In this latter model, the two groups diverged, with the groups having U-shaped associations similar in magnitude but opposite in direction (upward U-shape for the treatment group and inverted U-shape for the sham group, $p_{interacton} = 0.0001$). All other cytokines studied (i.e. IL-1β, IL-10, IL-17α, and TNFα) and WBC, despite having different trajectories, did not reach statistical significance. The pattern of change in normalized pro-inflammatory cytokines over time was also different according to both assigned treatment group ($p_{interacton}=0.01$) or time-varying treatment ($p_{interacton}=0.04$) (**Table 2 and Figure 2)**. Looking at IL-6 specifically, levels were significantly lower in the taVNS treatment group on Day 3 compared with the sham group [t = 2.083, p = 0.045, N(taVNS)=16, N(sham) = 17, t-test, Cohen's d = -0.726] (**Figure 2B)**. Furthermore, normalized pro-inflammatory cytokines were significantly lower in the taVNS treatment groups on Day 3, compared to the

sham treatment group [t = -2.632, p = 0.013, N(taVNS)=16, N(sham) = 17, t-test, Cohen's d = -0.917] (**Figure 2F)**, but were not significantly different on Days 1, 5, and 7.

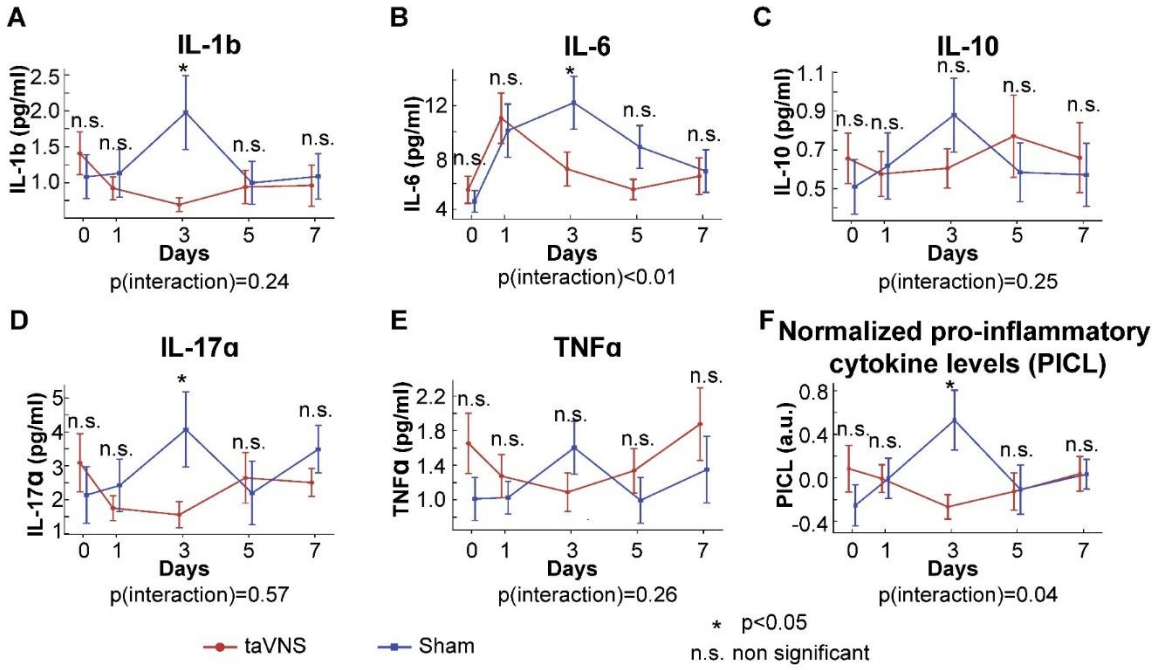

Fig 2 Trajectory of the different cytokine levels individually and as a composite normalized value by treatment vs sham group. P(interaction) is from the time-varying model and tests whether there is a statistically significant difference in the cytokine level trajectory longitudinally according to randomized treatment (sham before randomization and assigned treatment group for all points after randomization). The P(interaction) in the time varying model showed a significant difference amongst the trajectory IL-6 cytokine levels over days 0-7 when comparing treatment vs sham. **A,B, D**. IL-1b, IL-6, and IL-17a levels for taVNS and sham treatment groups were significantly lower in the taVNS treatment group on day 3 compared with the sham treatment group, e.g. IL-6 [t = 2.083, p = 0.045, N(taVNS)=16, N(sham) = 17, t-test]. The error bars in **A-F** represent standard errors. **F**. Normalized pro-inflammatory cytokine (TNF-a, IL-1β, IL-17a, and

IL-6) score over days 0-7 for taVNS and sham treatment groups (randomized on approximately day 1 and treated through day 5, death, or discharge, whichever came earliest). Normalized pro-inflammatory cytokine levels were significantly lower in the taVNS treatment group on day 3 compared with the sham treatment group [t = 2.632, p = 0.013, N(taVNS)=16, N(sham) = 17, t-test]. The P(interaction) in the time varying model showed a significant difference amongst the trajectory of the normalized pro-inflammatory cytokine levels when comparing treatment vs sham.

IL-6 and clinical outcomes.

Across combined treatment groups, changes in IL-6 demonstrated a correlation with Day 90 mRS scores (95% confidence interval (CI) for β_(IL-6) = [0.061, 0.177], p <0.001, z-test). For the acute inpatient recovery period, a statistically significant relationship was also identified between IL-6 and NIHSS scores (F-statistic = 5.276, p = 0.023, $R^2 = 0.040$), with an IL-6 coefficient of 0.252 (p = 0.023, t-test, n = 130).

Change in IL-6 and change in NIHSS by treatment group.

Changes in NIHSS scores were positively associated with changes in IL-6 levels, indicating reduced pro-inflammatory cytokine levels were associated with improving neurological outcomes (slope = 0.358, 95% CI = [0.055, 0.661], t = 2.370, p = 0.021, n = 57, t-test). The regression performed separately for the two treatment groups showed significant positive correlation between changes in IL-6 levels and changes in NIHSS scores in the taVNS group (slope = 0.798, 95% CI = [0.077, 1.518], t = 2.286, p = 0.031, n = 26, t-test) and borderline significant relationship in the sham group (slope = 0.330, 95% CI = [-0.029, 0.690], t = 1.881, p

= 0.070, n = 31, t-test). That is, for the taVNS treatment group, a 1 pg/mL reduction in IL-6 over 3 to 5 days was associated with a reduction of 0.80 points in NIHSS (**Figure 3A)**. The model incorporating subject random effect largely confirmed this finding; the association was significant in the taVNS treatment group and non-significant in the sham treatment group (**Online Resource 4).**

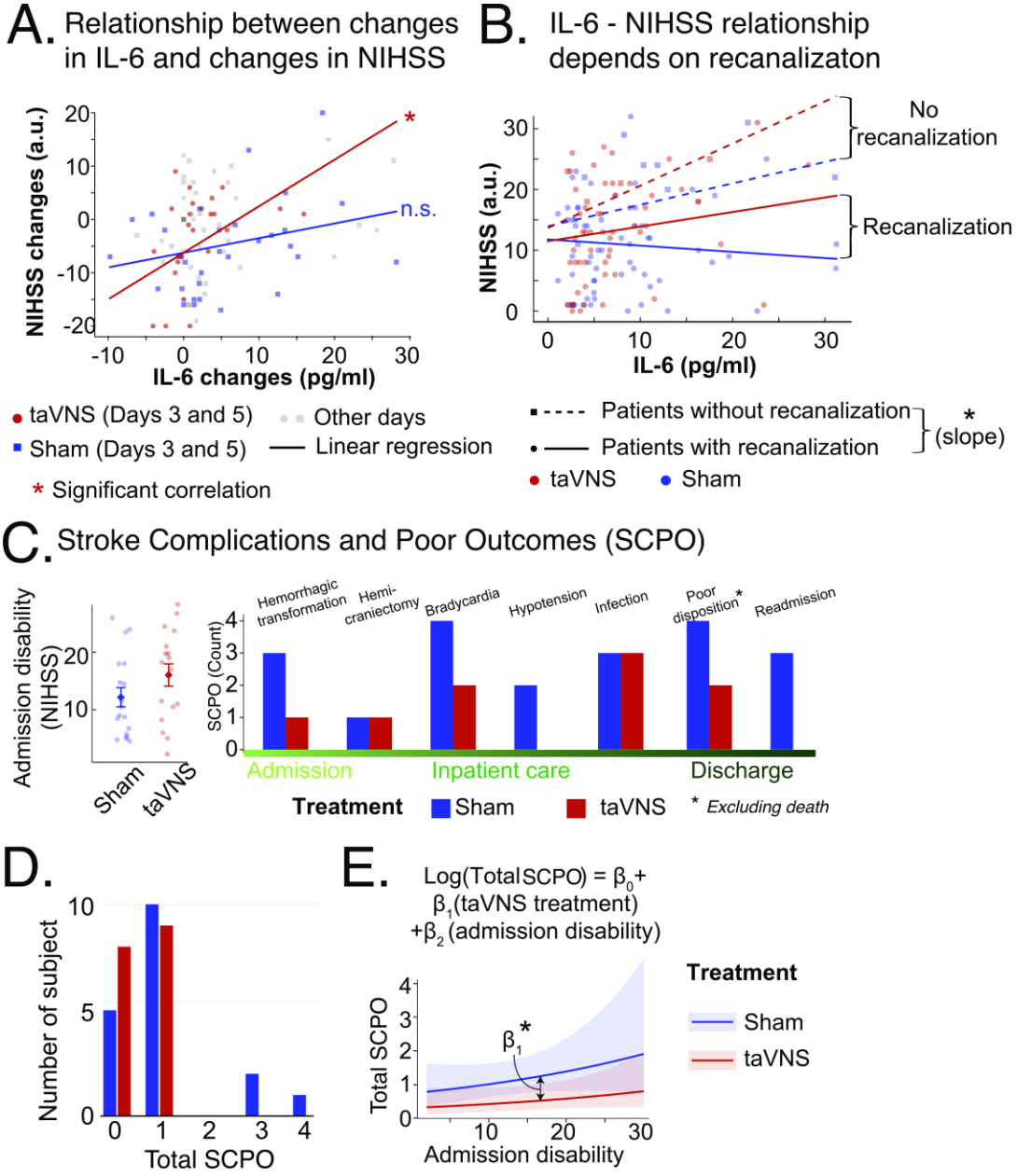

Fig 3 A. Changes in NIHSS scores were significantly positively associated with changes in IL-6 levels in the taVNS treatment group, suggesting that reduced pro-inflammatory cytokine levels were associated with improving neurological outcomes (slope = 0.798, 95% CI = [0.077, 1.518], t = 2.286, p = 0.031, n = 26, t-test). The relationship between changes in NIHSS scores and changes in IL-6 levels was not significant in the sham group (slope = 0.330, 95% CI = [-0.029, 0.690], t = 1.881, p = 0.070, n = 31, t-test). **B**. The positive association between change in IL-6 and change in NIHSS was greater among patients without recanalization. A 1 pg/mL increase in IL-6 was associated with an additional 0.46-point increase in NIHSS scores in patients who did not achieve recanalization compared with patients who did (z=-2.015, p=0.044, 95% CI = [0.012, 0.898]). **C-E**. Stroke complications and poor outcomes (SCPO) in the two treatment groups. **C**. The mean initial stroke severity was higher in the taVNS group measured by average NIHSS for Days 0 – 3. The right figure shows the occurrence of stroke complications and poor outcomes for the two treatment groups, while excluding patients who died inpatient due to comfort measures as they did not have time to develop inflammation related complications. **D**. Distribution of total stroke complications and poor outcomes by treatment group. **E**. The relationship between total SCPO and initial stroke severity for the two treatment groups. The 95% CI for the slope β_2 is [-0.018, 0.081] (z = 1.264, p = 0.206). Total SCPO was significantly higher in the sham treatment group (95% CI for the difference in intercept between the two treatment groups = [-1.680, -0,058], z = -2.097, p = 0.036).

The relationship between IL-6 and NIHSS by recanalization status.

When we analyzed how recanalization status modulates the relationship between pro-inflammatory cytokine IL-6 and NIHSS scores by including recanalization status and its

interaction with IL-6, our analysis revealed that the positive association between change in IL-6 and change in NIHSS was greater among patients without recanalization. A 1 pg/mL increase in IL-6 was associated with an additional 0.46-point increase in NIHSS scores in patients who did not achieve recanalization compared with patients who did ($z = -2.015$, $p = 0.044$, 95% CI = [0.012, 0.898], z-test (**Figure 3B**). In this model, i.e., while accounting for a possible interaction between IL-6 and recanalization status, patients in the taVNS treatment group showed a trend toward a stronger IL-6/NIHSS relationship, although this difference did not reach statistical significance (95% CI of the slope difference = [0.002, 0.685], $p = 0.051$, z-test, $t = 1.951$).

Change in NIHSS by laterality

During hospitalization, NIHSS improvement rates were initially similar between treatment groups, even when restricting to the first five days in sensitivity analysis, as shown in **Online Resource 5**. A significant three-way interaction between treatment group, time, and laterality was observed, with more rapid improvement noted in treated vs. sham patients with left sided strokes, i.e., ipsilateral to the site of stimulation, but not right sided strokes. This three-way interaction remained significant when we explored the effect of adding a parallel three-way interaction for occlusion type. The laterality effect noted above was also seen in cytokines generally and IL-6 most clearly demonstrated a difference between treatment groups for left sided strokes ($p_{interaction} = 0.0005$) (**Online Resource 6)**.

Stroke Complications and Poor Outcomes in relation to treatment

No adverse events directly attributable to taVNS were noted. Primary cardiac events were observed more frequently in the sham group, including bradycardia (22.2% vs. 11.8%) and

hypotension (11.1% vs. 0%). Hemorrhagic transformation was documented more frequently in the sham group (16.7% vs. 5.9%). Other conditions that could be associated with inflammation occurred at similar rates between groups (sham 27.8% vs. treatment 23.5%), including infections (three in each group) and hemicraniectomy (one patient in each group), but none of these were statistically significant. While in-hospital mortality was higher in the treatment group (17.6% vs. 0%), at the mRS 90-day mark, the treatment group had three deaths (17.6%), and the sham group had two deaths (11.1%), and none of these findings were statistically significant. Those patients who passed in the treatment group were statistically significantly older than those patients who did not die [passed age 84.7 (SD 7.7) vs. alive age 64.5 (SD 3.6), $p = 0.03$], were on the trial on average only 2.17 days, and all passed due to the family/patient's wishes being congruent with comfort measures only. In addition, overall poor dispositions were similar in the two groups and good discharge dispositions were achieved in comparable proportions (treatment 70.6% vs. sham 77.8%). Hospital readmissions were recorded only in the sham group (16.7%). Although none of these individual differences reached statistical significance (all $p \geq 0.60$), we noted a trend when taken in aggregate. Despite having higher stroke severity and larger infarct volumes, patients treated with taVNS appeared to have less morbidity when excluding patients who died early after admission (**Figure 3 C-E).** The generalized linear regression modeling total stroke complications and poor outcomes as a function of treatment and admission disability showed that surviving patients in the taVNS treatment group experienced significantly fewer complications and poor outcomes than the surviving sham group (incidence rate ratio = 0.42, 95% CI = [0.19, 0.94], $z = -2.097$, $p = 0.036$) (**Figure 3 and Table 3).** When deaths were included in the analysis, surviving patients in the taVNS treatment group experienced 51% fewer complications and poor outcomes

than the surviving sham group, but the difference was non-significant (95% CI of the incidence rate ratio = [0.24, 1.01], z = -1.921, p = 0.055).

Discussion

The NUVISTA trial demonstrated that taVNS, when added to standard medical care, safely and effectively modulated the post-AIS inflammatory response in patients with anterior circulation LVO strokes. Patients receiving taVNS within 36 hrs of symptom onset showed significantly faster reductions in inflammatory cytokines, most notably in IL-6, in the first days following the stroke onset after initiation of treatment. In addition, a reduction in IL-6 significantly correlated with a reduction in neurologic deficit and may be more pronounced in patients with persistent LVO or unsuccessful recanalization via thrombectomy. This randomized pilot trial demonstrated that taVNS may aid in mitigating the inflammatory sequelae associated with AIS and may reduce its associated neurologic complications and morbidity.

Although the study was not powered to detect differences in functional neurologic outcomes, we have confirmed that elevated pro-inflammatory cytokines, in particular IL-6, are associated with worsened functional outcomes, as previously reported in the literature [6-10, 42-44]. Furthermore, the taVNS treatment and sham groups were not balanced: the taVNS treatment group had more proximal LVO locations, larger infarct sizes, longer times from LKN to recanalization, worse reperfusion scores, and more early edema development than the sham group. Despite these baseline imbalances between groups, we still saw an effect on inflammatory biomarkers, highlighting the potential robustness of this intervention. Further, when clinical outcomes were assessed relative to the change in IL-6, there were indeed differences between the

taVNS-treated and sham groups, with treated surviving patients experiencing significantly fewer complications and poor outcomes compared to the sham group.

Our findings demonstrate that taVNS treatment was associated with a greater correlation between reduced IL-6 levels and improved NIHSS scores, with every 1 pg/mL decrease in IL-6 corresponding to a reduction of 0.798 points in NIHSS scores (95% CI = [0.077, 1.518]), as compared to 0.330 (95% CI = [-0.029, 0.690]) reduction in the sham group. In addition, the overall relationship between change in IL-6 and change in NIHSS may be stronger in patients who did not have successful recanalization, suggesting that taVNS may offer additional therapeutic benefit in cases where mechanical thrombectomy fails to restore blood flow. Taken together, these findings suggest a potential role for taVNS as an adjunctive therapy and further exploration in patients where standard recanalization strategies are unsuccessful or only partially effective may be valuable.

The mechanisms of action of VNS and its impact on clinical outcomes may be pleiotropic. It is postulated that taVNS leads to immunomodulation through neural pathways associated with the cholinergic anti-inflammatory pathway, parasympathetic nervous system, and hypothalamic-pituitary-adrenal (HPA) axis. Immunogenic stimuli activate vagal afferents terminating primarily in the dorsal vagal complex. Ascending projections from the dorsal vagal complex reach the paraventricular nucleus (PVN) and rostral ventromedial medulla (RVM). The arrival of these incoming signals generates action potentials that travel from the brainstem to the spleen and other organs. This culminates in T cell release of acetylcholine, which interacts with α7 nicotinic acetylcholine receptors (α7 nAChR) on immunocompetent cells to inhibit cytokine release in

macrophages [45]. This is likely the mechanism explaining this study's reduction in inflammatory cytokines. Transcutaneous auricular VNS can also positively impact cardiovascular function through the sympathetic nervous system or efferent vagus nerve [46]. Furthermore, it can reduce blood-brain barrier permeability, cortical spreading depressions, and improve regionalized plasticity [46, 47]. In aggregate, these pleiotropic effects may play a role in reducing stroke complications and improving outcomes.

Interestingly, we saw differential effects of the stimulation depending on the laterality of the stroke. In this study, we exclusively stimulated the left vagus nerve, which is a widely accepted side, to avoid stimulation of cardiac centers [48]. Despite this practice, prior reports suggest that stimulation may induce a more robust brain activation in the ipsilateral hemisphere [39, 40]. Although the differences we saw may be due to a limited-size study group, this should be explored further in future trials.

This trial has notable strengths, including its randomized, blinded design and comprehensive biomarker analysis. However, several important limitations warrant discussion. First, the small sample size introduces the possibility that our findings, particularly the clinical outcomes, may be subject to chance variation, though the consistency of cytokine changes provides some reassurance. Second, as a single-center study not powered to detect clinical differences, these results require validation through larger, multicenter trials. Third, fundamental questions remain about optimal therapeutic parameters, including stimulation settings, treatment duration, and most relevant physiologic and biomarker endpoints when utilizing taVNS. This is highlighted in our trial by the Day 5 to 7 rises in some cytokine levels, which may be a rebound effect of

stopping treatment, although an additional explanation could be that our sample size decremented at those time points and stroke patients who were still in the hospital at that time tended to be sicker. Finally, the focus on large vessel occlusion strokes limits the generalizability of these findings to other stroke subtypes. These limitations highlight critical areas for future investigation while providing a foundation for larger-scale studies of taVNS in acute ischemic stroke.

In conclusion, the NUVISTA trial demonstrates that taVNS safely modulates post-AIS inflammation in anterior circulation large vessel occlusion, with reductions in IL-6 correlating with improved NIHSS in the days following stroke. These findings support further investigation of taVNS as a potential adjunctive therapy through larger, multicenter trials.

Statements and Declarations:

Funding. This trial was supported by a Clinical and Translational Research Funding Program (CTRFP) award UL1TR002345 (for OJLS); NIH/NINDS R37 NS110699 (for JML); NIH/NINDS R01NS121218 (for RD); and NIH/NINDS K23NS136843 (for JG).

Competing interests. Dr. Leuthardt reports stock ownership in Neurolutions, Face to Face Biometrics, Caeli Vascular, Acera, Sora Neuroscience, Inner Cosmos, Inflexion Vascular, Aurenar, Petal Surgical, Cordance Medical, and Silent Surgical. They serve as a consultant for Monteris Medical, E15, and Neurolutions. They have received licensing fees from intellectual property for Neurolutions, Caeli Vascular, and Inner Cosmos. Additionally, they have licensing/product development agreements or receive royalties for inventions/IP from Intellectual Ventures, Sora Neuroscience, Inner Cosmos, Neurolutions, and Aurenar. Washington University

owns equity in Neurolutions. Dr. Anna Huguenard reports stock ownership in Aurenar and may receive royalties for interventions/IP from Aurenar. Kara Donovan reports the option of stock ownership in Aurenar and may receive royalties for interventions/IP from Aurenar. Dr. Opeolu Adeoye reports stock ownership in Sense Diagnostics, Inc (Founder) and may receive royalties for interventions/IP. Dr. Jin-Moo Lee, Dr. Osvaldo J. Laurido-Soto, Dr. Susane Searles Nielsen, Dr. James Giles, Dr. Rajat Dhar, and Gansheng Tan do not have related financial disclosures. At no time was this trial funded, designed, nor supported by industry.

Ethics approval. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Washington University School of Medicine in St. Louis institutional review board.

Consent to participate. Informed consent was obtained from all individual participants, or their legal guardian, included in the study.

Data availability. Data requests will be considered within reasonable request after agreement with the Steering Committee, provided data transfer complies with general data protection regulation and is approved by the respective ethical review board.

Author contributions.

Table 1. Baseline characteristics of participants and adherence to randomized treatment, by treatment group, NUVISTA

Demographics	Treatment N=17 n (%)	Sham N=18 n (%)
Age, years		
Mean (SD)	68.1 (SD 15.2)	67.3 (SD 12.5)
Range	37-96	45-90
Sex (female)	7 (41.2)	10 (55.6)
Race		
Caucasian	15 (88.2)	12 (66.7)
Black	2 (11.8)	6 (33.3)
Comorbidities/functioning at LKN		
Smoking		
Never	4 (23.5)	6 (33.3)
Former	4 (23.5)	7 (38.9)
Current	9 (52.9)	5 (27.8)
Baseline mRS		
0	13 (76.5)	15 (83.3)
1	2 (11.8)	1 (5.6)
2	2 (11.8)	2 (11.1)
Comorbidities		
Hypertension	14 (82.4)	14 (77.8)
Hyperlipidemia	11 (64.7)	13 (72.2)
Type 2 diabetes	7 (41.2)	7 (38.9)
Carotid disease	4 (23.5)	3 (16.7)
Intracranial atherosclerosis	2 (11.8)	0 (0)
Atrial arrythmia (Atrial fibrillation or flutter)	6 (35.3)	9 (50.0)
Coronary artery disease	3 (17.7)	3 (16.7)
Congestive heart failure	2 (11.8)	5 (27.8)
Kidney issues	3 (17.7)	8 (44.4)
Cerebral ischemia	1 (5.9)	3 (16.7)
Intracranial hemorrhage	0 (0)	1 (5.6)
Other[a]	6 (35.3)	3 (16.7)
Stroke characteristics and severity		
Laterality		
Right	6 (35.3)	10 (55.6)
Left	11 (64.7)	8 (44.4)
Maximum severity occlusion		

M2	4 (23.5)	5 (27.8)
M1	5 (29.4)	8 (44.4)
ICA	8 (47.1)	5 (27.8)
Baseline (first) NIHSS, mean (SD)	16.4 (SD 6.6)	15.7 (SD 4.4)
Standard stroke treatments		
Lytics (tPA)	7 (41.2)	8 (44.4)
Thrombectomy	16 (94.1)	17 (94.4)
Time from LKN to recanalization attempt, hours, mean (SD)	13.2 (SD 11.1)	9.8 (SD 10.6)
TICI score		
N/A (no thrombectomy)	1 (5.9)	1 (5.6)
0	0 (0)	1 (5.6)
2a	2 (11.8)	0 (0)
2b	7 (41.2)	3 (16.7)
2c	5 (29.4)	5 (27.8)
3	2 (11.8)	8 (44.4)
Percent reperfusion, mean (SD)[b]	76.0 (SD 27.1)	83.8 (SD 31.5)
Imaging-assessed infarct characteristics		
Δ CSF (edema) in the first 36 hrs[c]	-0.26 (-26%)	-0.13 (-13%)
Cytokine levels, mean (SD)		
IL1β	1.3 (SD 1.1)	1.4 (SD 1.6)
IL6	5.4 (SD 3.9)	5.5 (SD 3.7)
IL10	0.63 (SD 0.49)	0.66 (SD 0.80)
IL17α	2.8 (SD 3.2)	2.8 (SD 3.9)
TNFα	1.5 (SD 1.3)	1.1 (SD 1.0)
Mean z-score[d]	0.003 (SD 0.79)	-0.04 (SD 0.99)
Mean z-score[b] without IL-10 (SD)	0.0007 (SD 0.81)	-0.07 (SD 0.90)
Experimental taVNS		
Time between LKN and first intervention (hours), mean (SD)	22.8 (SD 6.5)	24.8 (SD 6.2)
Number of stimulations, mean (SD)	8.24 (SD 2.77)	7.33 (SD 3.20)
<7	5 (SD 29.4)	7 (SD 38.9)
7 to 9	2 (SD 11.8)	3 (SD 16.7)
All 10	10 (SD 58.8)	8 (SD 44.4)
Reason stimulation incomplete		
Died in hospital/hospice	3 (17.6)	0 (0)
Transitioned to comfort measures only (alive)	1 (5.9)	1 (5.6)
Rapid improvement/early discharge	2 (11.8)	3 (16.7)
Potential side effect	0 (0)	3 (16.7)
Device malfunction	0 (0)	2 (11.1)
Other	1 (5.9)	1 (5.6)
N/A (completed all 10 stimulations)	10 (--)	8 (--)

[a] Other comorbidities not categorized, include the following among the treatment groups (sham, taVNS): aortic thrombus (0, 1); protein C deficiency (0, 1); poly substance abuse (1, 0); essential

thrombocythemia (1, 0); methamphetamine use (1; 0); seizure (1, 0); obstructive sleep apnea (1, 0); lung cancer (1, 0); peripheral arterial disease, poly substance abuse, history of ventricular thrombus (0, 1).

[b] We estimated the approximate percent reperfusion based on the TICI score (0% for TICI 0, 25% for TICI 1, 37.5% for TICI 2a, 77.5% for TICI 2b, 95% for TICI 2c, and 100% for TICI 3) [2] to obtain a single semi-continuous measure.

[c] Mean of the z-score for each of the five cytokines.

[d] Baseline scans were available for 13 (76.5%) treatment and 13 (72.2%) sham patients, with all subjects having a follow up 24 +/- 12 hrs scans. ΔCSF was defined as difference in CSF in the affected hemisphere when comparing the follow up scan to the baseline and is a marker of cerebral edema [29, 30].

Abbreviations: CSF = Cerebrospinal fluid; hrs = hours; IL = interleukin; LKN = last known normal; mRS = modified Rankin scale; NIHSS = National Institutes of Health Stroke Scale; SD = standard deviation; TICI = thrombolysis in cerebral infarction; TNF = tumor necrosis factor.

Table 2. Blood biomarkers of inflammation in relation to time from last known normal, by treatment

			Adjusted[a] difference in cytokine per specified time unit (95% CI)	
Biomarker	**Treatment**	**Time unit**	**Primary model**	**Time-varying treatment**
WBC count[b]				
	Treatment	Days	-0.025 (-0.494, 0.444)	-0.914 (-1.687, -0.141)
		Days squared	-0.002 (-0.060, 0.056)	0.082 (0.0004, 0.163)
	Sham	Days	-0.062 (-0.482, 0.358)	-0.050 (-0.455, 0.356)
		Days squared	-0.011 (-0.061, 0.039)	-0.014 (-0.062, 0.035)
	Interaction p-value[d]		0.60	0.14
IL1β[c]				
	Treatment	Days	-0.390 (-0.821, 0.041)	-0.222 (-0.903 ,0.460)
		Days squared	0.048 (-0.016, 0.111)	0.030 (-0.057, 0.116)
	Sham	Days	0.379 (-0.031, 0.788)	0.327 (-0.047, 0.702)
		Days squared	-0.060 (-0.119, -0.001)	-0.055 (-0.111, 0.002)
	Interaction p-value[d]		0.04*	0.24
IL6[c]				
	Treatment	Days	0.956 (-1.412, 3.323)	-4.099 (-7.655, -0.543)
		Days squared	-0.189 (-0.535, 0.158)	0.372 (-0.078, 0.823)
	Sham	Days	4.102 (1.848, 6.355)	4.323 (2.369, 6.277)
		Days squared	-0.560 (-0.887, -0.234)	-0.585 (-0.878, -0.292)
	Interaction p-value[d]		0.11	0.0001*
IL10[c]				
	Treatment	Days	-0.098 (-0.308, 0.112)	-0.078 (-0.412, 0.255)
		Days squared	0.016 (-0.014, 0.047)	0.014 (-0.028, 0.057)
	Sham	Days	0.180 (-0.019, 0.380)	0.150 (-0.034, 0.333)
		Days squared	-0.026 (-0.055, 0.002)	-0.023 (-0.050, 0.005)
	Interaction p-value[d]		0.14	0.25
IL17α[c]				
	Treatment	Days	-0.795 (-1.874, 0.284)	-0.158 (-1.864, 1.547)
		Days squared	0.110 (-0.048, 0.268)	0.041 (-0.175, 0.257)
	Sham	Days	0.553 (-0.473, 1.578)	0.432 (-0.506, 1.370)
		Days squared	-0.078 (-0.226, 0.071)	-0.065 (-0.206, 0.076)
	Interaction p-value[d]		0.21	0.57
TNF-α[c]				
	Treatment	Days	-0.244 (-0.545, 0.058)	-0.120 (-0.597, 0.357)
		Days squared	0.031 (-0.014, 0.075)	0.018 (-0.043, 0.078)
	Sham	Days	0.302 (0.014, 0.590)	0.225 (-0.038, 0.488)
		Days squared	-0.046 (-0.088, -0.004)	-0.037 (-0.077, 0.002)
	Interaction p-value[d]		0.04*	0.26
Cytokine z-score[e]				
	Treatment	Days	-0.168 (-0.433, 0.096)	-0.240 (-0.661, 0.181)

		Days squared	0.021 (-0.018, 0.060)	0.030 (-0.024, 0.083)
	Sham	Days	0.339 (0.088, 0.590)	0.304 (0.072, 0.536)
		Days squared	-0.049 (-0.086, -0.013)	-0.046 (-0.080, -0.011)
	Interaction p-value[d]		0.03*	0.07
Cytokine z-score w/o IL10				
	Treatment	Days	-0.165 (-0.416, 0.086)	-0.259 (-0.659, 0.140)
		Days squared	0.018 (-0.019, 0.055)	0.029 (-0.021, 0.080)
	Sham	Days	0.356 (0.117, 0.595)	0.323 (0.103, 0.544)
		Days squared	-0.052 (-0.087, -0.017)	-0.049 (-0.082, -0.016)
	Interaction p-value[d]		0.01*	0.04*

[a] Adjusted for time from LKN to recanalization (or similar reference time), percent reperfusion, and baseline NIHSS as fixed effects, in addition to plate and patient as random effects in a mixed model with the specified cytokine as the outcome, or patient only as a random effect with WBC count as the outcome.

[b] Based on 279 measurements: 136 WBC measurements from 17 patients in the treatment group (42 before randomization and 94 after randomization) and 143 measurements from 18 patients in the sham group, with treatment as randomly assigned (primary model) or as time-varying variable prior to randomization and per assignment after randomization (time-varying treatment model).

[c] Based on 132 measurements for each cytokine: 63 cytokine measurements from 17 patients in the treatment group (14 before randomization and 49 after randomization) and 69 measurements from 18 patients in the sham group, with treatment as randomly assigned (primary model) or as time-varying variable prior to randomization and per assignment after randomization (time-varying treatment model).

[d] Interaction between treatment and time from LKN, to test whether the pattern of association between time and the biomarker (coefficients for days and days squared) differs according to treatment. Obtained from likelihood ratio test comparing the model with two interaction terms (days and treatment; and days squared and treatment) and all main effects terms to a model with all main effects terms.

[e] Mean of the z-score for each of the five cytokines, as an overall summary measure.

* Significant value

Abbreviations: CI = confidence interval; IL = interleukin; LKN = last known normal (prior to stroke); NIHSS = National Institutes of Health Stroke Scale; TNF = tumor necrosis factor; WBC = white blood cell

Table 3. Adverse Events and Secondary Outcomes

	Treatment N=17 n (%)	Sham N=18 n (%)	Unadjusted p-value
Cardiac Events			
Bradycardia	2 (11.8)	4 (22.2)	$p_{exact} = 0.66$
Hypotension	0 (0.0)	2 (11.1)	$p_{exact} = 0.49$
Adverse Events			
Infection	3 (17.7)	3 (16.7)	$p_{exact} = 1.00$
Hemicraniectomy	1 (5.9)	1 (5.6)	$p_{exact} = 1.00$
Hemorrhagic transformation	1 (5.9)	3 (16.7)	$p_{exact} = 0.60$
Other secondary outcomes			
Early discharge due to improvement	2 (11.8)	3 (16.7)	$p_{exact} = 1.00$
Discharge disposition[a]			$p_{trend} = 0.24$
Good	12 (70.6)	14 (77.8)	
Poor	2 (11.8)	4 (22.2)	
Died before discharge[b]	3 (17.7)	0 (0)	
Readmitted to hospital	0 (0)	3 (16.7)	$p_{exact} = 0.23$
mRS at 30 days,[c] mean (SD)	3.63 (1.82)	2.83 (1.92)	$p_{trend} = 0.21$
0	1 (6.3)	2 (11.1)	
1	1 (6.3)	5 (27.8)	
2	3 (18.8)	0 (0)	
3	1 (6.3)	3 (16.7)	
4	5 (31.3)	4 (22.2)	
5	2 (12.5)	3 (16.7)	
6	3 (18.8)	1 (5.6)	
Missing	1 (--)	0 (--)	
mRS at 90 days,[c] mean (SD)	3.27 (2.15)	2.78 (2.07)	$p_{trend} = 0.50$
0	2 (13.3)	4 (22.2)	
1	2 (13.3)	2 (11.1)	
2	2 (13.3)	1 (5.6)	
3	1 (6.7)	4 (22.2)	
4	3 (20.0)	3 (16.7)	
5	2 (13.3)	2 (11.1)	
6	3 (20.0)	2 (11.1)	
Missing	2 (--)	0 (--)	
Length of stay among patients discharged alive and not to hospice, days, mean (SD)	8.01 (4.16)	8.91 (5.34)	$p_{trend} = 0.59$

[a] Good disposition includes home or inpatient rehabilitation facility; poor disposition includes long term care hospital, skilled nursing facility.

[b] We excluded inpatient death from poor disposition to restrict to those patients who would have had time for an inflammatory response to be the cause of the death. Those patients who passed in the treatment group were statistically significantly older than those patients who did not die [passed age 84.7 (SD 7.7) vs alive 64.5 (SD 3.6), p = 0.03], were on the trial on average only 2.17 days, and all passed due to the family/patient's wishes being congruent with comfort measures only.

[c] Percentages shown among those without missing data. mRS at 30 days excludes one participant from the treatment group lost to follow-up; mRS at 90 days also excludes one additional participant from the treatment group. The two patients who died in the sham group, one bounced back due to atrial fibrillation with rapid ventricular response leading to cardiac arrest and the other patient's cause of death was not able to be identified due to lack of access to their external medical record.

Abbreviations: mRS = modified Rankin scale; SD = standard deviation

References

1. Malhotra K, Gornbein J, Saver JL (2017) Ischemic Strokes Due to Large-Vessel Occlusions Contribute Disproportionately to Stroke-Related Dependence and Death: A Review. Front Neurol 8:651. https://doi.org/10.3389/fneur.2017.00651
2. LeCouffe NE, Kappelhof M, Treurniet KM, Lingsma HF, Zhang G, van den Wijngaard IR, van Es A, Emmer BJ, Majoie C, Roos Y, Coutinho JM, MR CLEAN Registry Investigators (2020) 2B, 2C, or 3: What Should Be the Angiographic Target for Endovascular Treatment in Ischemic Stroke? Stroke 51:1790-1796. https://doi.org/10.1161/STROKEAHA.119.028891
3. Nour M, Scalzo F, Liebeskind DS (2013) Ischemia-reperfusion injury in stroke. Interv Neurol 1:185-199. https://doi.org/10.1159/000353125
4. Mizuma A, You JS, Yenari MA (2018) Targeting Reperfusion Injury in the Age of Mechanical Thrombectomy. Stroke 49:1796-1802. https://doi.org/10.1161/STROKEAHA.117.017286
5. Stoll G, Nieswandt B (2019) Thrombo-inflammation in acute ischaemic stroke - implications for treatment. Nat Rev Neurol 15:473-481. https://doi.org/10.1038/s41582-019-0221-1
6. Ridker PM, Luscher TF (2014) Anti-inflammatory therapies for cardiovascular disease. Eur Heart J 35:1782-1791. https://doi.org/10.1093/eurheartj/ehu203
7. Guruswamy R, ElAli A (2017) Complex Roles of Microglial Cells in Ischemic Stroke Pathobiology: New Insights and Future Directions. Int J Mol Sci 18:3. https://doi.org/10.3390/ijms18030496
8. Yang C, Hawkins KE, Dore S, Candelario-Jalil E (2019) Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. Am J Physiol Cell Physiol 316:C135-C153. https://doi.org/10.1152/ajpcell.00136.2018
9. Ramiro L, Simats A, Garcia-Berrocoso T, Montaner J (2018) Inflammatory molecules might become both biomarkers and therapeutic targets for stroke management. Ther Adv Neurol Disord 11:1756286418789340. https://doi.org/10.1177/1756286418789340
10. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, Iwaki T, Okada Y, Iida M, Cua DJ, Iwakura Y, Yoshimura A (2009) Pivotal role of cerebral interleukin17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. Nat Med 15:946-950. https://doi.org/10.1038/nm.1999
11. Chou SH (2018) Inflammation, Cerebral Vasospasm, and Brain Injury in Subarachnoid Hemorrhage-A Shifting Paradigm and a New Beginning. Crit Care Med 46:1883-1885. https://doi.org/10.1097/CCM.0000000000003373
12. Lucke-Wold BP, Logsdon AF, Manoranjan B, Turner RC, McConnell E, Vates GE, Huber JD, Rosen CL, Simard JM (2016) Aneurysmal Subarachnoid Hemorrhage and Neuroinflammation: A Comprehensive Review. Int J Mol Sci 17:497. https://doi.org/10.3390/ijms17040497

13. Jurcau A, Simion A (2021) Neuroinflammation in Cerebral Ischemia and Ischemia/Reperfusion Injuries: From Pathophysiology to Therapeutic Strategies. Int J Mol Sci 23:14. https://doi.org/10.3390/ijms23010014

14. Lee Y, Lee SR, Choi SS, Yeo HG, Chang KT, Lee HJ (2014) Therapeutically targeting neuroinflammation and microglia after acute ischemic stroke. Biomed Res Int 2014:297241. https://doi.org/10.1155/2014/297241

15. Meneses G, Bautista M, Florentino A, Diaz G, Acero G, Besedovsky H, Meneses D, Fleury A, Del Rey A, Gevorkian G, Fragoso G, Sciutto E (2016) Electric stimulation of the vagus nerve reduced mouse neuroinflammation induced by lipopolysaccharide. J Inflamm (Lond) 13:33. https://doi.org/10.1186/s12950-016-0140-5

16. Suzuki T, Takizawa T, Kamio Y, Qin T, Hashimoto T, Fujii Y, Murayama Y, Patel AB, Ayata C (2019) Noninvasive Vagus Nerve Stimulation Prevents Ruptures and Improves Outcomes in a Model of Intracranial Aneurysm in Mice. Stroke 50:1216-1223. https://doi.org/10.1161/STROKEAHA.118.023928

17. Marshall R, Taylor I, Lahr C, Abell TL, Espinoza I, Gupta NK, Gomez CR (2015) Bioelectrical Stimulation for the Reduction of Inflammation in Inflammatory Bowel Disease. Clin Med Insights Gastroenterol 8:55-59. https://doi.org/10.4137/CGast.S31779

18. Chen X, He X, Luo S, Feng Y, Liang F, Shi T, Huang R, Pei Z, Li Z (2018) Vagus Nerve Stimulation Attenuates Cerebral Microinfarct and Colitis-induced Cerebral Microinfarct Aggravation in Mice. Front Neurol 9:798. https://doi.org/10.3389/fneur.2018.00798

19. Zhao X-P ZY, Qin X-Y, Wan L-Y, Fan X-X (2019) Non-invasive Vagus Nerve Stimulation Protects Against Cerebral Ischemia/Reperfusion Injury and Promotes Microglial M2 Polarization Via Interleukin-17A Inhibition. J Mol Neurosci 67:217-226. https://doi.org/10.1007/s12031-018-1227-7

20. Sun Z, Baker W, Hiraki T, Greenberg JH (2012) The effect of right vagus nerve stimulation on focal cerebral ischemia: an experimental study in the rat. Brain Stimul 5:1-10. https://doi.org/10.1016/j.brs.2011.01.009

21. Hiraki T, Baker W, Greenberg JH (2012) Effect of vagus nerve stimulation during transient focal cerebral ischemia on chronic outcome in rats. J Neurosci Res 90:887-894. https://doi.org/10.1002/jnr.22812

22. FDA (2017) SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED): FDA approval for VNS Therapy System - Depression, Seizures, and Chronic Stroke Rehabiliation. PMA P970003/S207. https://www.accessdata.fda.gov/cdrh_docs/pdf/p970003s207b.pdf. Accessed 8/31/2022

23. Kaniusas E, Kampusch S, Tittgemeyer M, Panetsos F, Gines RF, Papa M, Kiss A, Podesser B, Cassara AM, Tanghe E, Samoudi AM, Tarnaud T, Joseph W, Marozas V, Lukosevicius A, Istuk N, Sarolic A, Lechner S, Klonowski W, Varoneckas G, Szeles JC (2019) Current Directions in the Auricular Vagus Nerve Stimulation I - A Physiological Perspective. Front Neurosci 13:854. https://doi.org/10.3389/fnins.2019.00854

24. Kaniusas E, Kampusch S, Tittgemeyer M, Panetsos F, Gines RF, Papa M, Kiss A, Podesser B, Cassara AM, Tanghe E, Samoudi AM, Tarnaud T, Joseph W, Marozas V, Lukosevicius A, Istuk N, Lechner S, Klonowski W, Varoneckas G, Szeles JC, Sarolic A

(2019) Current Directions in the Auricular Vagus Nerve Stimulation II - An Engineering Perspective. Front Neurosci 13:772. https://doi.org/10.3389/fnins.2019.00772

25. Kim AY, Marduy A, de Melo PS, Gianlorenco AC, Kim CK, Choi H, Song JJ, Fregni F (2022) Safety of transcutaneous auricular vagus nerve stimulation (taVNS): a systematic review and meta-analysis. Sci Rep 12:22055. https://doi.org/10.1038/s41598-022-25864-1

26. Huguenard AL, Tan G, Rivet DJ, Gao F, Johnson GW, Adamek M, Coxon AT, Kummer TT, Osbun JW, Vellimana AK, Limbrick DD, Zipfel GJ, Brunner P, Leuthardt EC (2025) Auricular vagus nerve stimulation for mitigation of inflammation and vasospasm in subarachnoid hemorrhage: a single-institution randomized controlled trial. J Neurosurg Jan 24:1-12. https://doi.org/10.3171/2024.10.Jns241643

27. Huguenard A, Tan G, Johnson G, Adamek M, Coxon A, Kummer T, Osbun J, Vellimana A, Limbrick D, Jr., Zipfel G, Brunner P, Leuthardt E (2024) Non-invasive Auricular Vagus nerve stimulation for Subarachnoid Hemorrhage (NAVSaH): Protocol for a prospective, triple-blinded, randomized controlled trial. PLoS One 19:e0301154. https://doi.org/10.1371/journal.pone.0301154

28. Schulz KF, Altman DG, Moher D, Consort Group (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332. https://doi.org/10.1136/bmj.c332

29. Dhar R, Kumar A, Chen Y, Begunova Y, Olexa M, Prasad A, Carey G, Gonzalez I, Bhatia K, Hamed M, Heitsch L, Mainali S, Petersen N, Lee JM (2023) Imaging biomarkers of cerebral edema automatically extracted from routine CT scans of large vessel occlusion strokes. J Neuroimaging 33:606-616. https://doi.org/10.1111/jon.13109

30. Bui Q, Kumar A, Chen Y, Hamzehloo A, Heitsch L, Slowik A, Strbian D, Lee JM, Dhar R (2024) CSF-Based Volumetric Imaging Biomarkers Highlight Incidence and Risk Factors for Cerebral Edema After Ischemic Stroke. Neurocrit Care 40:303-313. https://doi.org/10.1007/s12028-023-01742-0

31. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, Albini A (2023) The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front Immunol 14:1161067. https://doi.org/10.3389/fimmu.2023.1161067

32. Pfister IB, Zandi S, Gerhardt C, Spindler J, Reichen N, Garweg JG (2020) Risks and Challenges in Interpreting Simultaneous Analyses of Multiple Cytokines. Transl Vis Sci Technol 9:27. https://doi.org/10.1167/tvst.9.7.27

33. Yap JYY, Keatch C, Lambert E, Woods W, Stoddart PR, Kameneva T (2020) Critical Review of Transcutaneous Vagus Nerve Stimulation: Challenges for Translation to Clinical Practice. Front Neurosci 14:284. https://doi.org/10.3389/fnins.2020.00284

34. The Joint Commission (2017) Specifications Manual for Joint Commission National Quality Measures (v2016B). https://manual.jointcommission.org/releases/TJC2016B/DataElem0569.html. Accessed 9/22/2024

35. U.S. Food and Drug Administration (1998) International Conference on Harmonisation; Guidance on Statistical Principles for Clinical Trials. Federal Register, Department of Health and Human Services, Washington D.C., Report # 98-24754; (63)179:49583-49598. https://www.govinfo.gov/content/pkg/FR-1998-09-16/html/98-24754.htm. Accessed 12/15/2024

36. Garcia TP, Marder K (2017) Statistical Approaches to Longitudinal Data Analysis in Neurodegenerative Diseases: Huntington's Disease as a Model. Curr Neurol Neurosci Rep 17:14. https://doi.org/10.1007/s11910-017-0723-4

37. Audebert HJ, Rott MM, Eck T, Haberl RL (2004) Systemic inflammatory response depends on initial stroke severity but is attenuated by successful thrombolysis. Stroke 35:2128-2133. https://doi.org/10.1161/01.STR.0000137607.61697.77

38. U.S. Food and Drug Administration (2023) Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products Guidance for Industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/adjusting-covariates-randomized-clinical-trials-drugs-and-biological-products. Accessed 12/15/2024

39. Konakoglu G, Ozden AV, Solmaz H, Bildik C (2023) The effect of auricular vagus nerve stimulation on electroencephalography and electromyography measurements in healthy persons. Front Physiol 14:1215757. https://doi.org/10.3389/fphys.2023.1215757

40. Peng X, Baker-Vogel B, Sarhan M, Short EB, Zhu W, Liu H, Kautz S, Badran BW (2023) Left or right ear? A neuroimaging study using combined taVNS/fMRI to understand the interaction between ear stimulation target and lesion location in chronic stroke. Brain Stimul 16:1144-1153. https://doi.org/10.1016/j.brs.2023.07.050

41. Bustamante A, Sobrino T, Giralt D, Garcia-Berrocoso T, Llombart V, Ugarriza I, Espadaler M, Rodriguez N, Sudlow C, Castellanos M, Smith CJ, Rodriguez-Yanez M, Waje-Andreassen U, Tanne D, Oto J, Barber M, Worthmann H, Wartenberg KE, Becker KJ, Chakraborty B, Oh SH, Whiteley WN, Castillo J, Montaner J (2014) Prognostic value of blood interleukin-6 in the prediction of functional outcome after stroke: a systematic review and meta-analysis. J Neuroimmunol 274:215-224. https://doi.org/10.1016/j.jneuroim.2014.07.015

42. Li M, Li Z, Yao Y, Jin WN, Wood K, Liu Q, Shi FD, Hao J (2017) Astrocyte-derived interleukin-15 exacerbates ischemic brain injury via propagation of cellular immunity. Proc Natl Acad Sci U S A 114:E396-E405. https://doi.org/10.1073/pnas.1612930114

43. Yilmaz G, Arumugam TV, Stokes KY, Granger DN (2006) Role of T lymphocytes and interferon-gamma in ischemic stroke. Circulation 113:2105-2112. https://doi.org/10.1161/CIRCULATIONAHA.105.593046

44. McCabe JJ, Walsh C, Gorey S, Arnold M, DeMarchis GM, Harris K, Hervella P, Iglesias-Rey R, Jern C, Katan M, Li L, Miyamoto N, Montaner J, Purroy F, Rothwell PM, Stanne TM, Sudlow C, Ueno Y, Vicente-Pascual M, Whiteley W, Woodward M, Kelly PJ (2024) Interleukin-6, C-Reactive Protein, and Recurrence After Stroke: A Time-Course Analysis of Individual-Participant Data. Stroke 55:2825-2834. https://doi.org/10.1161/STROKEAHA.124.047820

45. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421:384-388. https://doi.org/10.1038/nature01339

46. Tan G, Huguenard AL, Donovan KM, Demarest P, Liu X, Li Z, Adamek M, Lavine K, Vellimana AK, Kummer TT, Osbun JW, Zipfel GJ, Brunner P, Leuthardt EC (2024) The effect of transcutaneous auricular vagus nerve stimulation on cardiovascular function in subarachnoid hemorrhage patients: a safety study. eLife 13:RP100088. https://doi.org/10.7554/elife.100088.1

47. Baig SS, Kamarova M, Bell SM, Ali AN, Su L, Dimairo M, Dawson J, Redgrave JN, Majid A (2023) tVNS in Stroke: A Narrative Review on the Current State and the Future. Stroke 54:2676-2687. https://doi.org/10.1161/STROKEAHA.123.043414

48. Mandalaneni K, Rayi A (2024) Vagus Nerve Stimulator. Treasure Island (FL), StatPearls Publishing

Washington University in St. Louis

FOR IRB USE ONLY
IRB ID #: 202203086
APPROVAL DATE: 02/04/25
RELEASED DATE: 02/05/25
EXPIRATION DATE: 02/03/26

INFORMED CONSENT DOCUMENT

Project Title: Neuromodulation Using Vagus Nerve Stimulation Following Ischemic Stroke as Therapeutic Adjunct (NUVISTA)

Principal Investigator: Osvaldo Laurido-Soto, MD

Research Team Contact: Osvaldo Laurido-Soto, MD
Email: ojlaurido-soto@wustl.edu
Work Phone: 314-273-3294

If you are the legally authorized representative providing consent the word "you" in this document refers to the person you represent.

This consent form describes the research study and helps you decide if you want to participate. It provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights and responsibilities as a research participant. By signing this form you are agreeing to participate in this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We invite you to participate in this research study because you have presented to the hospital with an acute ischemic stroke due to a large vessel occlusion. An acute ischemic stroke due to a large vessel occlusion is a clot inside the head in one of the main vessels/arteries in the brain. It can occur for many reasons, which include: age, high blood pressure, diabetes, bad cholesterol, and potential heart irregular rhythms, among others.

The purpose of this research study is to better understand the role of inflammation in recovery from an acute ischemic stroke, and to see if electrical stimulation to the ear can improve outcomes. Previous research has shown that the inflammation markers in our blood increase after an acute ischemic stroke, and that this inflammation may lead to worse outcomes. Stimulating the vagus nerve, which has a branch that runs in the outer ear, has been shown to reduce inflammation in the body. Our team wants to measure your inflammatory markers throughout your hospital stay, and provide either the current standard medical therapy or daily ear stimulation in addition to current standard medical therapy to determine if there is a difference in recovery. If you are assigned to the stimulation arm of this study, you will have a daily electrical stimulation applied to your vagus nerve through a device placed on your ear for five days. You may also be assigned to a "control" group. If you are assigned to the "control" group, you will still be fitted with the device, but no electrical current will be applied through the device.

Auricular transcutaneous vagus nerve stimulation is approved by the U.S. Food and Drug Administration for epilepsy, refractory depression, and obesity. However, the use of auricular transcutaneous vagus nerve stimulation is considered investigational in this study.

WHAT WILL HAPPEN DURING THIS STUDY?

Following enrollment in the study, your medical record will be reviewed to obtain some important details about your acute ischemic stroke, and your medical history prior to this hospitalization. The details recorded by the research team will include the severity of your stroke (both based on your imaging and your physical exam on arrival to the hospital). It will also include information about the location of the occlusion that is identified on your imaging, how much brain tissue is damaged and at risk, and how you are treated during your hospital stay. With regards to your medical history, the research team will review the medical record and document your medical history. The research team will also review your medications you take at home.

Laboratory Studies

Initial lab work already obtained by the intensive care or progressive care unit team will be reviewed. Additional blood samples will also be taken to measure the level of several inflammatory markers on the first day of enrollment. This blood work will be repeated approximately every 2 days during the course of your hospital stay. This blood work may require a needle stick, similar to how other blood samples are drawn, or may be sampled from a line already in place without a new stick. Each set of lab draws will require approximately 2 teaspoon of volume, and the total volume required will depend on the duration of your hospital stay. For example, if you are in the hospital for 5 days, a total of 24 cc blood will be collected over this time period. For some patients, additional blood tests may be drawn a second time on these days after treatment with ear stimulation. Therefore, the total volume over the duration of hospitalization would be higher but less than 10 to 12 teaspoons of blood over 2 weeks. There is a possibility that you may also be enrolled on an observational study called “MAESTRO”, IRB# 202110028. If blood was already drawn for this study we might share the blood samples instead of drawing new blood.

Genetic Research

Genes are a unique combination of molecules (called DNA) that we inherit from our parents. There are millions of tiny differences in our genes. These differences may make us more or less likely to develop certain diseases or conditions or to have certain characteristics. Genetic research involves studying the differences in genes and DNA between individuals. This type of testing creates information that is as unique to you as your fingerprint.

We will study the relationship of your genes to your response to the intervention with the auricular transcutaneous vagus nerve stimulation and see if this is related to a difference in stroke size and functional recovery.

Ear Stimulation

You will be randomly assigned to treatment with electrical stimulation to different parts of the ear. Electrical stimulation to the part of the Ear that has the branch of the vagus nerve (intervention) or the part that has the great auricular nerve (sham).. This means that the part of the ear to be treated will be determined purely by chance, like flipping a coin. You will have a 50/50 chance of being in any one of the study groups. You will be “blinded”, which means you will not know which of these groups you are

assigned to.

The device that will provide stimulation is the transcutaneous auricular Vagus Nerve Stimulation (taVNS) device by Sotarix Medical©. It is a portable transcutaneous electrical nerve stimulation unit. This small device is connected to the ear with adhesives, which will be applied to the left ear during the treatment periods. The treatment period with the device will last 20 minutes, and will occur twice each day during your stay in the hospital. The most common negative side effect of the stimulation is redness or irritation at the site, although this is uncommon with the length and amount of stimulation we will use. Our team will be trained to provide the stimulation and inspect the area of stimulation daily to ensure there is no redness or irritation. Further stimulation will not be applied if irritation is noted.

Before, during, and after the ear stimulation your vital signs (like your heart rate, and your blood pressure) will be monitored and recorded. Monitoring devices will already be in place while you are in the Intensive Care Unit or Neurological Care Unit, and this study will not require any new monitoring devices to collect this information.

This stimulation device is FDA approved for the treatment of epilepsy (seizures), depression, and obesity, but is not approved for the treatment of subarachnoid hemorrhage.

Follow-up

You will be required to attend a follow up appointment at 30 days following your discharge from the hospital (or longer depending on physician availability). This follow up appointment will be either in-person, by phone, or via telemedicine. This will be either your normal scheduled visit if it is at 30 days or a scheduled appointment coordinated by us. If it is a scheduled appointment coordinated by us outside standard of care, you will be eligible for $25 compensation in the form of a gift card. You are eligible for a $50 check payment upon completion of this initial follow up visit. The check will be mailed to your home address upon completion of the visit. You will also be required to participate in a brief telephone call at 90 days to assess your functional status. The research team will review the normally scheduled visits you attend following your hospitalization to better understand the long-term impact of the stimulation on outcomes. The information obtained will include disability assessment results, and physical exams performed. The study team will continue to access these records for up to two years following your initial hospitalization.

Will you save my research information to use in future research studies?

We would like to use the data we are obtaining in this study for studies going on right now as well as studies that are conducted in the future. These studies may provide additional information that will be helpful in understanding acute ischemic strokes due to large vessel occlusion, or other diseases or conditions, including research to develop investigational tests, treatments, drugs or devices that are not yet approved by the U.S. Food and Drug Administration. It is unlikely that what we learn from these studies will have a direct benefit to you. There are no plans to provide financial compensation to you should this occur. By allowing us to use your data you give up any property rights you may have in the data.

Your data will be stored without your name or any other kind of link that would enable us to identify

which sample(s) or data are yours. Therefore, it will be available for use in future research studies indefinitely and cannot be removed.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 80 people will take part in this study conducted by investigators at Washington University

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for the duration of your hospitalization, and any follow-up appointments that occur in the 2 years following your hospitalization for your acute ischemic stroke.

WHAT ARE THE RISKS OF THIS STUDY?

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study.

Likely / Common

- None

Less Likely / Less Common

Life Threatening

- None

Serious

- None

Mild

- Skin irritation/redness at the site of stimulation

Rare

Life Threatening

- None

Serious

- Bradycardia (slow heart rate)
- Hypotension (low blood pressure)

Mild

- Discomfort or pain during the stimulation period

Blood Drawing
The blood draw may cause bleeding, bruising, or pain. Some people become dizzy or feel faint. There is also a rare risk of infection.

Risks of Genetic Research

There may be information obtained from the genetic testing that indicates that you, or potentially a family member (since we inherit genes from our parents, and pass genes on to our children) are at risk for a particular disease or condition. For example, genetic sequencing may indicate that an individual is more prone to develop certain types of cancer or other types of diseases, (e.g. Alzheimer's or other inherited diseases).

If made available to persons or agencies outside of our research group, information about genetic test results could affect your employment or insurance. For instance, employers, insurers, or others may use this information when making decisions about you or your family members regarding employment, insurance, or other benefits.

While the data developed for this study is being stored without traditional identifiers (stored only with coded ID numbers, no names), there may be ways of linking the genetic materials back to you. Because your DNA is unique to you, it is possible that someone could look at the information in the DNA database and compare it to information in another database, and use that to identify you. This is difficult to do and is very unlikely to happen.

There is a federal law called the Genetic Information Nondiscrimination Act (GINA). In general, this law makes it illegal for health insurance companies, group health plans and employers with greater than 15 employees to discriminate against you based on your genetic information. However, it does not protect you against discrimination by companies that sell life insurance, disability insurance or long term-care insurance.

Breach of Confidentiality

One risk of participating in this study is that confidential information about you may be accidentally disclosed. We will use our best efforts to keep the information about you secure, and we think the risk of accidental disclosure is very small. Please see the section in this consent form titled "*How will you keep my information confidential?"* for more information.

WHAT ARE THE BENEFITS OF THIS STUDY?

You may or may not benefit from being in this study.

However, we hope that, in the future, other people might benefit from this study because it could give us new treatment strategies for acute ischemic strokes due to large vessel occlusion.

WHAT OTHER TREATMENT OPTIONS ARE THERE?

Before you decide whether or not to be in this study, your doctor will discuss the other options that are available to you. Instead of being in this study, you could receive the currently accepted standard care for acute ischemic stroke alone. Please note, the addition of treatment with the vagus nerve stimulator

does not prevent you from receiving all of the normally available and recommended medications and therapies currently used for subarachnoid hemorrhage.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will not have any additional costs for being in this research study. You and/or your medical/hospital insurance provider will remain responsible for your regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

You may be paid for being in this research study. You are eligible for a $50 check payment upon completion of in-hospital interventions. This check will be mailed to your home address after the in-hospital interventions have been completed. You are also eligible for $50 check payment upon completion of your first follow up visit after your discharge from the hospital. This check will be mailed to your home address after the first follow up visit after your discharge has been completed.

You will be asked to provide your social security number (SSN). You may also need to provide your address if a check will be mailed to you. You should receive payment between 3 and 6 weeks upon completion of the follow-up study visit If a follow-up appointment is scheduled by us outside standard of care, you will be eligible for $25 compensation in the form of a gift card.

WHO IS FUNDING THIS STUDY?

The University and the research team are not receiving payment from other agencies, organizations, or companies to conduct this research study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

Washington University investigators and staff will try to reduce, control, and treat any complications from this research. If you feel you are injured because of the study, please contact the investigator at 314-296-0016 and/or the Human Research Protection Office at 1-(800)-438-0445.

Decisions about whether payment for medical treatment for injuries relating to your participation in research will be made by Washington University**.** If you need to seek medical care for a research-related injury, please notify the investigator as soon as possible.

HOW WILL YOU KEEP MY INFORMATION CONFIDENTIAL?

Other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you. We will keep your participation in this research study confidential to the extent permitted by law.

- Government representatives (including the Office for Human Research Protections) to complete federal or state responsibilities
- The U.S. Food and Drug Administration

- Hospital or University representatives to complete Hospital or University responsibilities
- Information about your participation in this study may be documented in your health care records and will be available to anyone with access to your health care record, including your health insurance company. This information may also be released as part of a release of information request.
- The last four digits of your social security number may be used in hospital or University systems to track billing information for research procedures.
- Washington University's Institutional Review Board (a committee that oversees the conduct of research involving human participants) and the Human Research Protection Office. The Institutional Review Board has reviewed and approved this study.
- Any report or article that we write will not include information that can directly identify you. The journals that publish these reports or articles require that we share your information that was collected for this study with others to make sure the results of this study are correct and help develop new ideas for research. Your information will be shared in a way that cannot directly identify you.

To help protect your confidentiality, we will limit access to your personal health information to the fewest number of necessary researchers. We will use a de-identified method of recording your data, so that your name or other identifiers are not linked with the collected data. We will also implement the use of encrypted devices for storage of all data.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Are there additional protections for my health information?

Protected Health Information (PHI) is health information that identifies you. PHI is protected by federal law under HIPAA (the Health Insurance Portability and Accountability Act). To take part in this research, you must give the research team permission to use and disclose (share) your PHI for the study as explained in this consent form. The research team will follow state and federal laws and may share your health information with the agencies and people listed under the previous section titled, "How will you keep my information confidential?"

Once your health information is shared with someone outside of the research team, it may no longer be protected by HIPAA.

The research team will only use and share your information as talked about in this form or as permitted or required by law. When possible, the research team will make sure information cannot be linked to you (de-identified). Once information is de-identified, it may be used and shared for other purposes not discussed in this consent form. If you have questions or concerns about your privacy and the use of your PHI, please contact the University's Privacy Officer at 866-747-4975.

Although you will not be allowed to see the study information, you may be given access to your health care records by contacting your health care provider.

If you decide not to sign this form, it will not affect

- Your treatment or the care given by your health provider.
- Your insurance payment or enrollment in any health plans.
- Any benefits to which you are entitled.

However, it will not be possible for you to take part in the study.

If you sign this form:

- You authorize the use of your PHI for this research
- This authorization does not expire.
- You may later change your mind and not let the research team use or share your information (you may revoke your authorization).
  - To revoke your authorization, complete the withdrawal letter, found in the Participant section of the Human Research Protection Office website at https://hrpo.wustl.edu/participants/withdrawing-from-a-study/ or you may request that the investigator send you a copy of the letter.
    - **If you revoke your authorization:**
      - The research team may only use and share information already collected for the study.
      - Your information may still be used and shared as necessary to maintain the integrity of the research, for example, to account for a participant's withdrawal from the research study or for safety reasons.
      - You will not be allowed to continue to participate in the study.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. Any data that was collected as part of your participation in the study will remain as part of the study records and cannot be removed.

If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

What if I decide to withdraw from the study?

You may withdraw by telling the study team you are no longer interested in participating in the study or you may send in a withdrawal letter. A sample withdrawal letter can be found at https://hrpo.wustl.edu/participants/withdrawing-from-a-study/ under Withdrawing from a Research Study.

Will I receive new information about the study while participating?

If we obtain any new information during this study that might affect your willingness to continue participating in the study, we'll promptly provide you with that information.

Can someone else end my participation in this study?

Under certain circumstances, the investigator might decide to end your participation in this research study earlier than planned. This might happen for no reason or because it is considered unsafe for you to continue in the study, because funding for the study has ended, or because the sponsor has decided to stop the research project.

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: Osvaldo Laurido-Soto, MD at 314-273-3294. If you experience a research-related injury, please contact: Osvaldo Laurido-Soto, MD at 314-273-3294.

If you have questions, concerns, or complaints about your rights as a research participant, please contact the Human Research Protection Office at 1-(800)-438-0445, or email hrpo@wustl.edu. General information about being a research participant can be found on the Human Research Protection Office web site, http://hrpo.wustl.edu. To offer input about your experiences as a research participant or to speak to someone other than the research staff, call the Human Research Protection Office at the number above.

This consent form is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by agreeing to participate in this study. As a participant you have rights and responsibilities as described in this document and including:

- To be given enough time before signing below to weigh the risks and potential benefits and decide if you want to participate without any pressure from the research team or others.
- To understand all of the information included in the document, have your questions answered, and receive an explanation of anything you do not understand.
- To follow the procedures described in this document and the instructions of the research team to the best of your ability unless you choose to stop your participation in the research study.
- To give the research team accurate and complete information.
- To tell the research team promptly about any problems you have related to your participation, or if you are unable to continue and wish to stop participating in the research study.

Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a signed and dated copy of this form.

Do not sign this form if today's date is after EXPIRATION DATE: 02/03/26.

_____ _____

(Signature of Participant) (Date)

_____
(Participant's name – printed)

Legally Authorized Representative's Name and Relationship to Participant:

Do not sign this form if today's date is after **EXPIRATION DATE: 02/03/26.**

_____
(Participant's name – printed)

_____ _____
(Signature of Legally Authorized Representative) (Date)

_____ _____
(Name of Legally Authorized Representative – printed) (Relationship to Participant – printed)

Who should sign as the Legally Authorized Representative (LAR)?
If the participant has a legal guardian or attorney-in-fact this individual must sign as the LAR.

If there is no legal guardian or attorney-in-fact the individuals listed below may sign in order of priority.

(1) Spouse unless the participant has no spouse, or is separated, or the spouse is physically or mentally incapable of giving consent, or the spouse's whereabouts is unknown or the spouse is overseas;
(2) Adult child;
(3) Parent;
(4) Brother or sister;
(5) Relative by blood or marriage.

Statement of Person Who Obtained Consent

The information in this document has been discussed with the participant or, where appropriate, with the participant's legally authorized representative. The participant has indicated that they understand the risks, benefits, and procedures involved with participation in this research study.

_____ _____
(Signature of Person who Obtained Consent) (Date)

_____
(Name of Person who Obtained Consent - printed)