CLINICAL INVESTIGATION PLAN

Title:	Non-Invasive Vagal Neurostimulation to Mitigate Traumatic Brain Injury-Induced Acute Respiratory Distress and Acute Lung Injury
Clinical Investigation Number:	N/A
Clinical Investigation Medical Device:	gammaCore® Sapphire (non-invasive vagus nerve stimulator)
Indication:	Acute Respiratory Distress Syndrome
Device Class:	IIa
Protocol Date:	07 November 2024
Protocol Version:	Version 11
IRB Protocol Number:	2021-090-AGH

Sponsor
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ABBREVIATIONS AND DEFINITIONS OF TERMS

6MWT	6 minute walk test
AHN	Allegheny Health Network
AE	Adverse event
ALI	Acute lung injury
APACHE II	Acute Physiology and Chronic Health Evaluation II
ARDS	Acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
BBB	Blood-brain barrier
BIPAP	Bi-level positive airway pressure
BMI	Body Mass Index
CAP	Cholinergic Anti-inflammatory Pathway
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CNS	Central nervous system
CRF	Case Report Form
CSR	Clinical Study Report
DAMPS	Danger-associated molecular patterns
DSMB	Data and Safety Monitoring Board
EMRs	Electronic Medical Records
FAS	Full analysis set
FDA	U.S. Food and Drug Administration
FEV_1	Forced Expiratory Volume
GEE	General estimating equations
GCS	Glasgow Coma Scale
HFOV	High-frequency oscillatory ventilation
HMGB1	High mobility group box 1
ICF	Informed consent form
ICP	Intracranial pressure
ICU	Intensive Care Unit
LIPS	Lung Injury Prediction Score
MIP	Macrophage inflammatory protein
ORs	Odds ratios
nVNS	Non-invasive vagus neurostimulation
PCSS	Post-Concussion Symptom Scale
PEEP	Positive end-expiratory pressure

PP	Per-protocol
QOL	Quality of Life
RAGE	Receptor for advanced glycation end-products
SAE	Serious Adverse Event
SIRS	Systemic inflammatory response syndrome
SOC	Standard of Care
SOFA	Sequential Organ Failure Assessment
TBI	Traumatic brain injury
TLRs	Toll-like receptors
VNS	Vagus nerve stimulation
WOB	Work of breathing

PROTOCOL SYNOPSIS

Study Title	Non-Invasive Vagal Neurostimulation to Mitigate Traumatic Brain Injury-Induced Acute Respiratory Distress and Acute Lung Injury
Funding	Chuck Noll Foundation
Clinical Phase	Phase I Pilot Study
Study Rationale	We propose to use non-invasive vagus neurostimulation (nVNS) as a drug-free "bioelectronics medicine" in patients who experience mild-to-moderate traumatic brain injury (TBI) in order to prevent the development of a systemic inflammatory response syndrome (SIRS) that fuels severe respiratory distress/acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation. Towards this objective, we propose a prospective, randomized, two-arm, controlled 30-day investigational pilot trial using a nVNS device to prevent immunokine storms and acute lung injury (ALI) in order to delay/prevent the need for invasive mechanical ventilation in recently-hospitalized or outpatient traumatic brain injury (TBI) patients. Outpatients will be recruited ≤14 days from TBI event. We plan to use nVNS early in the course of TBI, prior to the onset of respiratory distress and the need for invasive mechanical ventilation. We propose to use the gammaCore Sapphire S nVNS device in newly-hospitalized patients who are diagnosed with mild-to-moderate TBI, limited to the head/neck region, before the catecholamine and immunokine storm surge begins to cause ARDS. Initiation of nVNS treatment as soon as possible following head/neck trauma may prevent the worsening inflammatory response and ALI, thus decreasing ICU and/or ventilator dependence and thereby reducing patient mortality. Given the lack of simple, rapidly-deployable, non-pharmacologic options for TBI-ALI/ARDS and the demands on healthcare systems, nVNS is a transformative, novel, and safe approach.
Study Objective(s)	**Primary** To seek 76 subjects to provide pilot data for the following purposes to determine whether nVNS in mild-to-moderate TBI patients reduces the rate of all-cause 90-day hospital admission or ED encounter when compared to the control arm • To provide preliminary evidence for a larger efficacy study of nVNS in mild-to-moderate TBI patients • To perform more reliable sample size calculations for a larger efficacy study • To evaluate the feasibility of enrollment for a larger efficacy study **Secondary** • The incidence and severity of Adverse Events (AEs) up to day 30 from baseline • Clinical outcomes up to day 30 **Tertiary** • All-cause mortality assessed at day 30

	• Morbidity of extrapulmonary organ dysfunction (coagulation, liver, circulatory system, consciousness, or kidney dysfunction • Length of hospitalization • FEV1, neurological functioning (6MWT) and QOL/Cognitive Assessments (PROMIS CAT and PCSS) assessed at 30, 60, and 90 days **Exploratory** • To ascertain correlations of circulating cytokine profile to clinical outcomes specified in the primary and secondary objectives • To ascertain the correlations of circulating catecholamine levels to clinical outcomes specified in the primary and secondary objectives
Medical Device	gammaCore Sapphire® (non-invasive vagus nerve stimulator)
Study Design	The trial design is illustrated in **Figure 1**. This is prospective, randomized, two-arm, controlled 30-day investigational pilot trial using the gammaCore Sapphire S nVNS device + standard of care (SOC) in newly-hospitalized patients or outpatients with mild-to-moderate TBI to prevent the progression towards immunokine storms, SIRS, severe respiratory distress, and requirement for invasive mechanical ventilation, and death, when compared to SOC alone (the control arm). We plan to use nVNS early in the course of TBI; prior to respiratory distress and the need for mechanical ventilation (**See Figure 1**). A pilot population of 76 individuals between 12 -80 years of age inclusive, will be enrolled, with an in-trial monitoring period of up to 30 days following baseline measurements. The primary outcome is all-cause 90-day admission to any hospital or ED encounter following presentation to an outpatient clinic or the ED, or hospitalization for mild-to-moderate TBI Study subjects will remain in-trial until one of the following occurs: **a)** 30 days without any progression to respiratory distress requiring mechanical ventilation; **b)** requirement for mechanical ventilation prior to 30 days; or **c)** death prior to 30 days. In addition to the screening, a total of up to 5 additional in-trial events plus one additional clinical site visit at the end of the follow-up period will comprise the study events schedule of this trial (**See Figure 1**). With the inclusion/exclusion criteria proposed herein and based on our experience with TBI intervention trials at Allegheny Health Network (AHN), we estimate that we can enroll one subject for every 2 screened. Therefore, to enroll the complete study population, we anticipate screening 152 patients. Once the patient is enrolled in the trial, ***the patient will be treated according to the institutional*** SOC. A study physician or delegated study team member will administer the informed consent form (ICF), assent form for those under the age of 18, and following the screening – if study eligibility is met, will randomize the patient 1:1 into one of the two study arms (**See Figure 1**) (nVNS+SOC or SOC only) prior to baseline measurements. Once qualified, the patient, their designated legal representative, a study nurse, or an institutional nurse involved in the clinical care of the subject shall be trained on the use of the nVNS

	technology and provided an initial treatment by the study staff. The nVNS administration will be conducted using nVNS settings informed by previous clinical experience where the outcomes demonstrated improvement in pulmonary function, airway performance, and disease-associated inflammation. A non-zero treatment effect observed in the hypothesized direction (ie. reduced all-cause 90-day admission or ED encounter in the nVNS arm as compared to the control arm) will serve as evidence for a larger efficacy trial evaluating nVNS for the prevention of SIRS that results in ARDS in TBI patients.
Subject Population	Study physicians and research study staff will screen patients in the hospital and outpatient clinics for eligibility and a delegated study team member will obtain written informed consent. Study staff and physicians will actively monitor the electronic medical records (EMRs) of all patients in order to identify potential participants.
Key Criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Patient is between 12-80 years, inclusive 2. Outpatient with a diagnosis of a TBI event ≤ 14 days from event OR patient that has been admitted to the hospital for mild-to-moderate TBI that is restricted to the head and/or neck region 3. Patient is not on invasive mechanical ventilation 4. Patient has a mild-to-moderate TBI based on a non-resuscitated or post-resuscitated Glasgow Coma Scale (GCS) sum score of =>12 [1] 5. Patient has a Lung Injury Prediction Score (LIPS) of ≥ 2 [2,3] 6. Administration of the first nVNS treatment must be planned to take place within 24 hours of enrolment 7. A signed written informed consent form from the patient or legally authorized representative and assent from those under the age of 18 **Exclusion Criteria** 1. Patient has a diagnosis of ***moderate or greater grade of respiratory distress/ARDS*** according to the Berlin definition of ARDS [4,5]: PaO2 /FiO2 > 100 mmHg (>13.3 kPa) to ≤ 200 mmHg (≤ 26.6 kPa) with positive end-expiratory pressure (PEEP) ≥ 5 cmH_2O) 2. Woman known to be pregnant, lactating or with a positive (urine or serum test) or indeterminate (urine or serum test) pregnancy test 3. Patient simultaneously taking part in another clinical trial 4. Patient is not expected to survive for 24 hours 5. Patient has an underlying clinical condition where, in the opinion of the study physicians and the institutional health provider physician, it would be extremely unlikely that the patient would not progress to invasive mechanical ventilation within 48 hours or any other condition that might require immediate invasive mechanical ventilation (e.g. motor neuron disease, Duchenne muscular dystrophy, or rapidly-progressive interstitial pulmonary fibrosis 6. Patient has severe chronic obstructive pulmonary disease (COPD) requiring long-term home oxygen therapy or mechanical ventilation (non-invasive ventilation or via tracheotomy) except for

	continuous positive airway pressure (CPAP) or bi-level positive airway pressure (BIPAP) used solely for sleep-disorder breathing 7. Patient has congestive heart failure 8. Patient has acute left ventricular failure 9. Patient has liver failure (Child-Pugh grade C) 10. Patient is receiving renal dialysis therapy for chronic renal failure 11. Patient is receiving extracorporeal membrane oxygenation, high-frequency oscillatory ventilation (HFOV) or any form of extracorporeal lung support 12. Patient has had any form of mechanical ventilation (invasive or non-invasive, excluding CPAP alone) for longer than 48 hours prior to the diagnosis of mild-to-moderate respiratory distress/ARDS 13. Patient has burns to ≥ 15% of their total body surface area
Number Of Subjects	A pilot population of 76 individuals between 12-80 years of age inclusive, will be enrolled.
Study Duration	Each subject's participation will last up to 95 days following baseline measurements.
Safety Evaluations	The primary endpoint is all-cause 90-day admission to any hospital or ED encounter following presentation to ED or an outpatient clinic or discharge for initial hospitalization for TBI. This includes emergency department (ED) encounters followed by discharge or ED encounter followed by hospitalization. Hospital admission is defined as hospitalization greater than 24 hours.
Statistical And Analytic Plan	Statistical analyses for the primary and secondary endpoints will be performed on both the full analysis set and per-protocol set. The safety analysis set will consist of all patients who receive at least one nVNS treatment. The safety analyses will be based on this analysis set. The primary endpoint, all-cause 90-day admission or ED encounter, will be analyzed as a difference in proportions between the control and intervention arms of the study. As this is a pilot study sized using the method of Cocks and Torgerson [85], any non-zero treatment effect observed in the hypothesized direction will warrant proceeding to the main study (a larger efficacy trial).
Data And Safety Monitoring Plan	The PI will continuously monitor the general oversite of the trial. Annual reports (enrollment, withdraws, etc.) will be submitted during annual renewal with the AHN IRB. An independent Data and Safety Monitoring Board (DSMB) consisting of five members who possess significant relevant experience and who are not involved in the study. The DSMB will review accumulating safety data until the last enrolled subject has been discharged from the study.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

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Study Phase	**Screening /Baseline**	**Treatment Period**					**Follow-up Period (telephone follow-up)**	**Follow-up Visit**	**Follow-up Visit/Study Termination**
Visit Number	**1**	**2**	**3[7]**	**4**			**FU**	**5**	**6**
Study Days	**Day 0**	**Day 3 (±2 days)**	**Day 7 (±2 days)**	**Day 30 (±5 days)**			**Days 31-59**	**Day 60 (±5 days)**	**Day 90 (±5 days)**
Informed Consent/Assent[10]	X								
Review Inclusion/Exclusion Criteria	X								
Data Collection: Physical Exam, Demographics- baseline visit only Medical History- baseline visit only Concomitant medications (inpatient)	X	X	X						
Physical Exam, Demographics- baseline visit only, Medical History - baseline visit only, Concomitant medications (all outpatient visits)	X	X	X	X				X	X
Vital Signs[1] (height baseline visit only)	X	X	X	X				X	X
Adverse Event Assessement[2]	X	X	X	X			X	X	X
Research labs[3]	X	X	X	X				X	X

Tests results: Hematology/Metabolic/Hepatic, pregnancy (inpatients)	X	X	X					
Hematology/Metabolic/Hepatic panels[4] (outpatient visits)	X	X	X	X			X	X
Urine Pregnancy Test- (women of child-bearing potential) during active treatment outpatients	X	X	X	X				
Randomization	X							
BORG Survey	X	X	X	X			X	X
Daily Treatment Log[5]	X	X	X	X				
6MWT[6]	X	X	X	X			X	X
QOL/Cognitive Testing (PROMIS CAT and PCSS)	X	X	X	X			X	X
Dispense Study Device & Device Training	X							
nVNS Treatment	X	X	X	X				
Investigative device returned to research staff[8]				X				
Follow-up Period (telephone follow-up)[9] Review of concomitant medications and AE/SAE assessment						X		

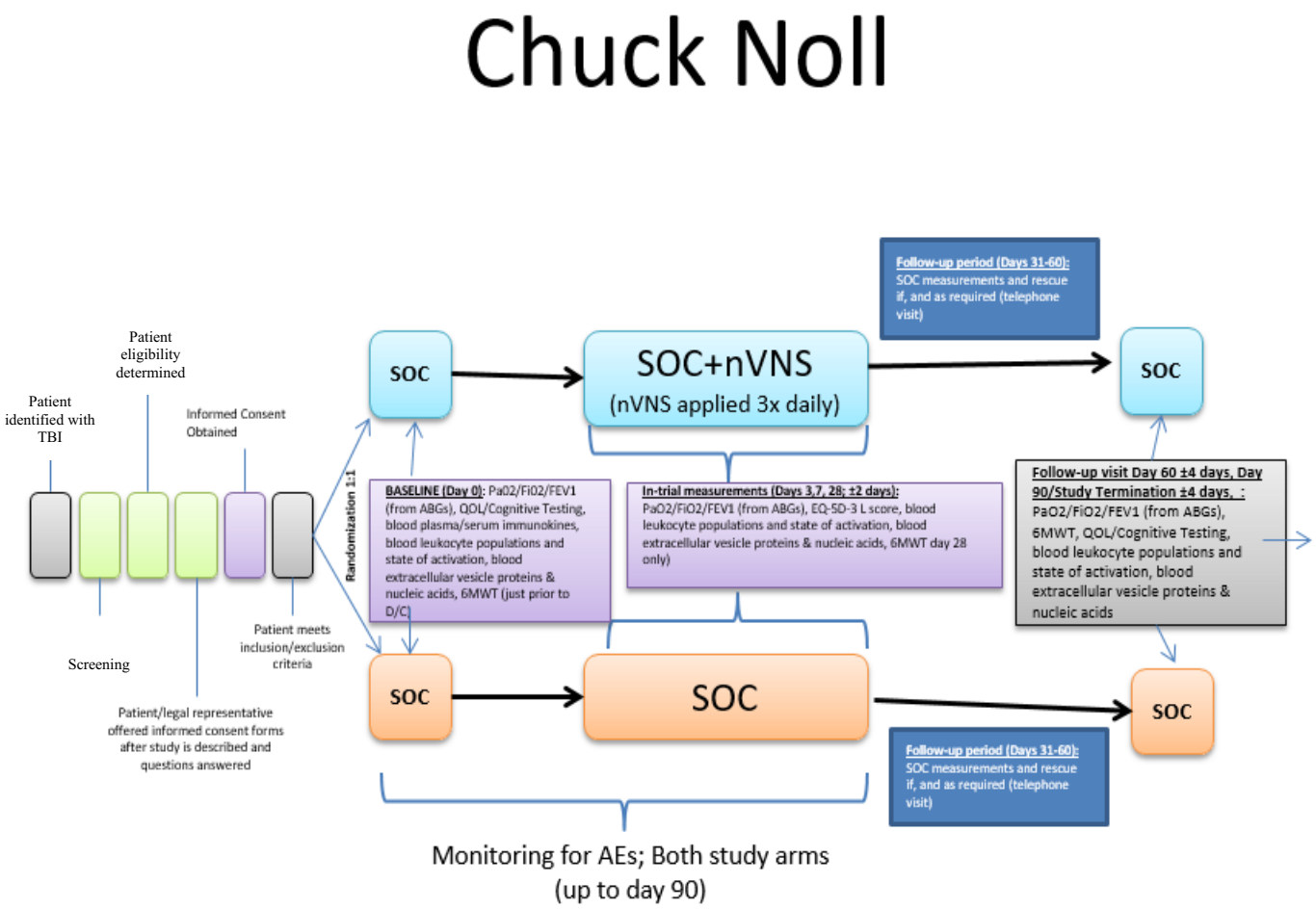
[1] Height, weight, Body Mass Index (BMI), temperature, blood pressure, PaO_2/FiO_2, FEV1 (reviewed and recorded from routine Arterial Blood Gas (ABG) collection)

[2] Incidence/severity/persistence/resolution of medical problems in recovered patients (including acute infections)

[3] Serum/plasma immunokine panel (multi-analyte TH1, TH2 22-PLEX Luminex panel; must include TNFα, IL-1β, IL-6), pro-calcitonin, CRP, ferritin, D-dimer; Absolute count and % of: B-cells: CD19+, CD20+, IL-10+; CD19+ CD24+, CD138+, T-cells: CD4+, CD69+; CD8+ CD69+; Granzyme B+ FasL+; CD4+ CD25+ Foxp3+ $CD127^{low}$, NK cells: CD3+ CD56+, Macrophages: (M1-HLA-DR+ CD68+ CD86+ $CD80^{low}$ $CD206^{-/low}$ and M2-HLA-DR+ CD68+ $CD80^{low}$ $CD206^{+/high}$), Neutrophils: CD177+, CD15+, CD16+, CD62L+; CD15+ PSGL-1+, CD66b+ CD68+, Neutrophil NETosis: SYTOX+ MPO+ CitH3+ ***(Blood must be collected prior to 10 AM in the fasted state)***

[4] Complete hematology panel (CBC with differential), Comprehensive metabolic and hepatic panels
[5] nVNS treatment (date, time of, and # of treatments), oxygen requirements, assisted ventilation dependence (device type, date and time and initiation/cessation), oxygen level (%, based on device type), patient discharge (date and time), Death (date and time)
[6] If subject is able to complete baseline 6MWT during hospitalization (just prior to discharge), they will also complete at Visits 4, 5, and 6. If subject is unable to complete during their hospitalization, they will not complete during Visits 4, 5, and 6.
[7] Visit 3 will only be completed if the subject remains hospitalized (inpatients only)
[8] Investigative device returned to research staff (Day 30, end of treatment phase)
[9] Follow-up monitoring will be conducted via telephone once a week between days 31-59. Review of concomitant medications and AE/SAE assessments will occur
10 Patients who turn 18 during the course of the study will be re-consented with the main consent form.

FIGURE 1: STUDY DIAGRAM



1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Traumatic brain injury (TBI) remains a major public health concern and a leading cause of mortality and morbidity throughout the world [6]. In the US, ~ 1.7 million individuals sustain a TBI annually which results in more than 5.3 million individuals suffering from TBI-related disabilities [7]. The leading causes of non-fatal TBI in the US are falls, motor-vehicle accidents, sports injuries, and strikes or blows to the head [8]. TBI triggers a neuroinflammatory Central Nervous System (CNS) response [9-11] that initiates secondary injury mechanisms in the periphery [10]. Patients with severe TBI exhibit a high incidence of systemic complications [12] including pneumonia, sepsis, and multiple organ dysfunction syndrome which are the principal drivers of TBI-associated morbidity and mortality [13]. More importantly, even in the absence of extracranial organ injuries (i.e. TBI is limited to the head and neck region), 89% of severe TBI patients exhibit peripheral organ dysfunction [14]. The most commonly-affected organ systems in severe TBI include are the respiratory (81%), cardiovascular (52%), coagulation (17%), renal (8%), and hepatic (7%) [15]. Following TBI, the systemic vasoconstriction can lead to non-neurological organ complications in the cardiorespiratory system, including cardiogenic pulmonary edema, cardiac injury, and neurogenic pulmonary edema [16,17]. Furthermore, TBI can promote increased intracranial pressure (ICP), which then increases sympathetic activation-driven cardiorespiratory complications [18]. The lungs appear to be among the most vulnerable organs and very susceptible to TBI-associated peripheral organ dysfunction. In the ICU setting, TBI can lead to a respiratory distress condition referred to as “acute lung injury (ALI)”[19].

The worldwide incidence of ALI/ARDS in patients with severe TBI ranges from 20 to 30% with mortality rates from 28%- to 38%[20]. In the US, the reported incidence of TBI-associated ALI, whether it progresses to ARDS or not, is ~79/100,000 patients[21]. Two main mechanisms appear to underlie the development of extracranial organ dysfunction and failure after TBI. The first involves the “catecholamine surge”. Driven by the hypothalamic pituitary axis [17], this sympathetic-excited catecholamine release causes vasoconstriction of peripheral vessels, elevating systemic arterial pressure [13]. The second pathway is the systemic inflammatory response syndrome (SIRS) observed in the acute period following TBI [22]. SIRS results in peripheral organ dysfunction and damage [22]. Examples of post-TBI circulating inflammatory mediators are danger-associated molecular patterns (DAMPs), proinflammaoty cytokines, chemokines, coagulation factors, growth factors, and nitric oxide [16,22]. Post-TBI SIRS leads to cardiorespiratory complications such as myocardial tissue injury and ALI which can progress to Acute Respiratory Distress Syndrome (ARDS)[16]. Within 3 hours post-TBI, a systemic inflammation is triggered, which is similar to that induced by direct injury through high tidal volume ventilation [23]. Clinical studies in brain-injured patients suggest that there is an increased intracranial production and eventual release of pro-inflammatory mediators into the systemic circulation, which allows for the activation of various inflammatory cascades [24]. In the brain, these pro-inflammatory mediators are most likely produced in microglia and astrocytes. Once the blood-brain barrier (BBB) is disrupted, these mediators may reach peripheral organs, such as the lungs [24]. Pro-inflammatory cytokines have been found in the bronchoalveolar lavage fluid (BALF) in patients that suffered fatal TBI [25]. Significant migration of neutrophils and macrophages into

the lungs has been observed as early as 24 hours post-TBI [26]. In rodent and porcine models of TBI, including in studies where lung injury was probed, cytokines like TNFα, IL-1β, IL-6, and proapoptotic proteins like caspase-1 and S-100B, have been found at very high concentrations inside the lungs [24]. Furthermore, a major DAMP, high mobility group box 1 (HMGB1), was found to be in high concentrations in the circulation of TBI in a relevant mouse model which then progressed to ALI [27]. HMGB1 is thought to be released into the circulation following TBI stress and CNS injury [28]. Once bound to toll-like receptors (TLRs), or the receptor for advanced glycation end-products (RAGE) on leukocytes of the innate arm of the immune system, what ensues is a systemic hypoxia, ALI, and decreased lung compliance compared to control animals. This systemic response was attenuated in RAGE-deficient transgenic mice [27] as well as by the administration of an HMGB1 neutralizing antibody [27].

Currently, mechanical ventilation is the only treatment for TBI patients who progress to ALI/ARDS, even though it may have a detrimental effect on the lungs [29] and the brain [30]. Nevertheless, a variety of therapeutic strategies have been attempted to block and/or reverse TBI secondary injury cascade [31]. These include: calcium channel blockers, corticosteroids, excitatory amino acids inhibitors, NMDA receptor antagonists, anti-inflammatory drugs, free radical scavengers, magnesium sulfate, and growth factors [32]. Because of the complexity of secondary injury mechanisms, treatment options must be multi-faceted and have the ability to simultaneously modulate different cellular changes [33]. While critical care management of TBI patients has improved, there are still no U.S. Food and Drug Administration (FDA) approved pharmaceutical agents that target multiple secondary injury mechanisms.

1.2 Non-invasive vagal neurostimulation to prevent or mitigate TBI-ALI

Vagus nerve stimulation (VNS) has an established history of reducing airway distress by at least two mechanisms of action. First, VNS modulates bronchoconstriction [34], where stimulation has demonstrated a marked improvement in Work of Breathing (WOB) as well as Forced Expiratory Volume (FEV_1) in patients with severe respiratory distress due to airway reactivity [35,36]. This effect appears to occur via an afferent response to stimulation of the vagus nerve [35,36]. Second, and perhaps more importantly, VNS has been shown to be a potent moderator of pathologic immune reactions, specifically suppressing proinflammatory cytokine levels via activation of the Cholinergic Anti-inflammatory Pathway (CAP)[37-42]. VNS is

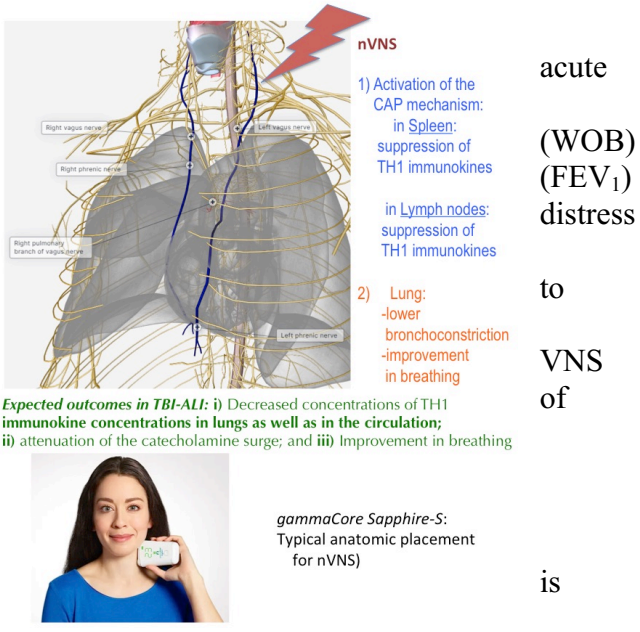


Figure 2: Know actions of VNS/nVNS on the immune system and pulmonary function and expected outcomes in TBI-ALI

currently being studied to modulate proinflammatory cytokines patterns and concentrations in a variety of acute and progressive inflammatory conditions, ranging from septic shock and asthma to stroke, rheumatoid arthritis and inflammatory bowel disease [37]. VNS has been studied in animal models of acute septic shock, consistently demonstrating life-saving potential. In one such study, cecal ligation and puncture was used to induce a septic state in an animal model and VNS reduced the expression of cytokines which was tightly associated with survival [43,44]. Also, in animals and humans, nVNS has the capacity to reduce the expression of inflammatory mediators, including TNF α, IL-6 and IL-1β [43]. These are precisely the same cytokines which are elevated in ARDS and other acute airway inflammatory disorders [45,46]. Very recently, VNS was shown to be neuroprotective following TBI, suppressing NLRP3 inflammasome-mediated CNS inflammation and apoptosis [47]. Intriguingly, vagus nerve signaling appears to promote lung stem cell expansion, accelerating repair processes in a CAP and FGF10-dependent manner following acute lung injury in a mouse model [48].

Historically, VNS is delivered using implanted electrodes that wrap around the vagus nerve. The implantation surgery is complicated with inherent risks, particularly in the critically ill. More recently, non-invasive approaches to VNS have been developed for a number of health syndromes [39,40,44,49-52]. These non-invasive VNS devices (***nVNS***) require no surgery or implants. They are simple to use and readily-applied by healthcare providers or patients to, depending on the design, different anatomic regions of the head or neck in order to deliver periodic doses of VNS non-invasively. nVNS can also be administered to patients inside the ICU setting and even to anesthetized patients. With respect to bronchoconstriction, some nVNS approaches demonstrated modulation of airway reactivity in hospitalized asthmatic patients, improving various measures of airway patency [35,36,53]. For these reasons, we propose that nVNS may ameliorate the over-activity of the proinflammatory immune condition in mild-to-moderate TBI patients, thus conferring a superior therapeutic option especially for elderly patients and those presenting with respiratory illness in setting of comorbid conditions who experience severe symptoms. These groups are at particularly high risk of developing ARDS requiring mechanical ventilation, experiencing severe cytokine storm and have a higher mortality rate.

1.3 Proposal to use gammaCore® nVNS treatment

We propose to use the gammaCore Sapphire S portable nVNS (conceptually-illustrated in **Figure 2**) which provides VNS by self-administration or by a health care practitioner, gammaCore exhibits an excellent safety profile [54-56]. In terms of its broader impact, nVNS represents a drug-free, quick, and "personal" method easily adaptable to treat other acute systemic inborn and acute environmentally-triggered inflammatory conditions. It has never before been considered for prevention or early treatment of TBI-ALI, even though its rationale, as summarized above, is based on a compelling body of experimental and clinical evidence.

We summarize some data in support of one of the pillars underlying the scientific premise of the study: 1) nVNS Safety: the gammaCore device as a "bioelectronics medicine" has demonstrated an excellent safety profile in conditions ranging from their use in treating and

preventing the symptoms of cluster headaches and migraine to respiratory symptoms in acute bronchoconstriction due to asthma [35] and respiratory distress associated even with COVID-19 (ClinicalTrials.gov identifier NCT01679314); 2) Clinical experience with gammaCore in pulmonary function and inflammatory conditions: Clinical data suggest that nVNS might provide benefits in patients who have respiratory symptoms that are sometimes associated with COVID-19, such as acute bronchoconstriction due to asthma [35,53] and respiratory distress associated with Chronic Obstructive Pulmonary Disease (COPD) [55]. With respect to bronchoconstriction, some nVNS approaches demonstrated modulation of airway reactivity in hospitalized asthmatic patients, improving various measures of airway patency [35,53]. Preliminary evidence from multiple clinical trials suggests that gammaCore could ease the breathing and confer symptomatic relief in patients with asthma or COPD[35] (ClinicalTrials.gov identifier: NCT01532817 and NCT01679314); 3) More recently, two case reports were presented whose data suggest that gammaCore may improve COVID-19 outcomes [55]; 4) Two studies demonstrate the ability of gammaCore to decrease the level of inflammatory cytokines. The first, conducted in healthy individuals, showed a greater percentage reduction in IL-1 , TNF , IL-6, IL-8, macrophage inflammatory protein (MIP)-1α, and macrophage inflammatory protein (MCP-1) levels in nVNS-treated subjects than in sham-treated control subjects [40]. In patients with Sjögren's syndrome, levels of MIP-1α, IL-1β, TNF α, IL-6, and IFN –induced protein (IP)-10 were significantly reduced 90 minutes after stimulation as well as at day 7 and 28 of the study [39].

1.4 Compliance Statement

This study will be conducted in full accordance all applicable Allegheny Health Network Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonization (ICH). All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with the AHN IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary study objective will be to determine whether nVNS in mild-to-moderate TBI patients reduces the rate of all-cause 90-day hospital admission or ED encounter when compared to the control arm. A non-zero treatment effect will serve as evidence for conducting a larger efficacy trial.

2.2 Secondary Objectives

The secondary objectives are:

- The incidence and severity of AEs up to day 30 from baseline
- Clinical outcomes up to day 30

2.3 Tertiary Objectives

The tertiary objectives are to:

- All-cause mortality assessed at day 30
- Morbidity of extrapulmonary organ dysfunction (coagulation, liver, circulatory system, consciousness, or kidney dysfunction
- Length of hospitalization
- FEV1, neurological functioning (6MWT) and QOL/Cognitive Assessments (PROMIS CAT and PCSS) assessed at 30, 60, and 90 days

2.4 Exploratory Objectives

The exploratory objectives are to:

- Ascertain correlations of circulating cytokine profile to clinical outcomes specified in the primary and secondary objectives
- Ascertain the correlations of circulating catecholamine levels to clinical outcomes specified in the primary and secondary objectives

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

The trial design is illustrated in **Figure 1**. This is prospective, randomized, two-arm, controlled 30-day investigational pilot trial using the gammaCore Sapphire S nVNS device + standard of care (SOC) in newly-hospitalized mild-to-moderate TBI patients to prevent the progression towards immunokine storms, SIRS, severe respiratory distress, and requirement for invasive mechanical ventilation, and death, when compared to SOC alone (the control arm). We plan to use nVNS early in the course of TBI; prior to respiratory distress and the need for mechanical ventilation (**See Figure 1**). A pilot population of 76 individuals between 12-80 years of age inclusive, will be enrolled, with an in-trial monitoring period of up to 30 days following baseline measurements. The primary outcome, all-cause 90-day admission to any hospital or ED encounter following presentation to an outpatient clinic, the emergency department (ED) or hospitalization for mild-to-moderate TBI. This includes ED encounters followed by discharge or ED encounter followed by hospitalization. Hospital admission is defined as a hospitalization greater than 24 hours.

TBI study subjects will remain in-trial until one of the following occurs: **a)** 30 days without any progression to respiratory distress requiring mechanical ventilation; **b)** requirement for

mechanical ventilation prior to 30 days; or **c)** death prior to 30 days. In addition to the screening, a total of up to 5 additional in-trial events plus one additional clinical site visit at the end of the follow-up period will comprise the study events schedule of this trial (**See Figure 1**). With the inclusion/exclusion criteria proposed herein and based on our experience with TBI intervention trials at Allegheny Health Network (AHN), we estimate that we can enroll one subject for every 2 screened. Therefore, to enroll the complete study population, we anticipate screening 152 patients.

3.1.1 Screening Phase

Study physicians and research study staff will screen EMRs of inpatients and outpatients for potential subject eligibility using the inclusion and exclusion criteria. Written informed consent and assent will be obtained prior to any study related procedures being performed.

If a subject is screened and found to be potentially eligible, a study physician assigned to the patient will discuss the study and present the informed consent form (ICF) to the patient and parent, giving them time to consider participation. Once the subject is enrolled in the trial, the subject will be treated according to the institutional SOC. If study eligibility is met, the subject will be randomized 1:1 into each of the two study arms (nVNS+SOC or SOC only) (See figure 1) prior to baseline measurements.

Outpatients are eligible for participation ≤14 days from TBI event. Depending on the date of TBI event and study enrollment date, visits 2 and 3 may not occur.

3.1.2 Study Treatment Phase (start of the study intervention)

Once qualified, the subject, their designated legal representative, a study nurse, or an institutional nurse involved in the clinical care of the subject shall be trained on the use of the nVNS technology and provided an initial treatment by the study staff. The nVNS administration will be conducted using nVNS settings informed by previous clinical experience where the outcomes demonstrated improvement in pulmonary function, airway performance, and disease-associated inflammation.

3.2 Allocation to Treatment Groups and Blinding

If the subject is found to be eligible for enrollment, they will be randomized on a 1:1 basis into the nVNS+SOC or SOC alone [control] groups. Randomized study subjects will be assigned a study randomization number to which a treatment group assignment has been made. Randomization will be conducted via a central web-based system. The study principal investigator, the laboratory personnel will be masked to the treatment assignment of each participant. Given the nature of the nVNS, the study physician, the patient, and the study staff interacting with the patient cannot be masked to the treatment. Only the study physician and the trial coordinators will know the arm to which the subject has been randomized. The patient's TBI will be classified as moderate or severe and any respiratory distress/ARDS will be classified based on age, co-morbidities, and mild or moderate oxygen saturation. To prevent allocation bias, the patient will be assigned to the test or control arm of the study based on severity of these classifications in a 1:1 ratio in blocks of four.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

The study duration per subject will be up to 90 (+/- 5 days) days

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at Allegheny General Hospital, Federal North in Pittsburgh, PA, AHN Orthopedic Institute locations at Wexford Health + Wellness Pavilion, Cranberry Shoppes, and Wise Road in Harmony, PA the AHN Montour Health and Sports Medicine Center, and AHN Pediatric Orthopaedic Institute (POI). Recruitment will stop when approximately 76 subjects are enrolled. It is expected that approximately 152 subjects will be screened to produce 76 evaluable subjects.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Males or females age 12 to 80 years.

2) Patient has been admitted to the hospital for mild-to-moderate TBI that is restricted to the head and/or neck region OR outpatients with a diagnosis of a TBI event ≤ 14 days from event

3) Patient is not on invasive mechanical ventilation

4) Patient has a mild-to-moderate TBI based on a non-resuscitated or post-resuscitated Glasgow Coma Scale (GCS) sum score of =>12 [1]

5) Patient has a Lung Injury Prediction Score (LIPS) of ≥ 2 [2,3]

6) Administration of the first nVNS treatment must be planned to take place within 24 hours of intake

7) A signed written informed consent form, witnessed by a study physician, from the patient, patient's parent, or an assent from the patient's personal legal representative or a professional legal representative, as well as an assent form from the patient if they are under the age of 18

3.4.2 Exclusion Criteria

1) Patient has a diagnosis of ***moderate or greater grade of respiratory distress/ARDS*** according to the Berlin definition of ARDS [4,5]: PaO2 /FiO2 > 100 mmHg (>13.3 kPa) to ≤ 200 mmHg (≤ 26.6 kPa) with positive end-expiratory pressure (PEEP) ≥ 5 cmH_2O)

2) Woman known to be pregnant, lactating or with a positive (urine or serum test) or indeterminate (serum test) pregnancy test

3) Patient simultaneously taking part in another clinical trial

4) Patient is not expected to survive for 24 hours

5) Patient has an underlying clinical condition where, in the opinion of the study physicians and the institutional health provider physician, it would be extremely unlikely that the patient would not progress to invasive mechanical ventilation within 48 hours or any other condition that might require immediate invasive mechanical ventilation (e.g. motor neuron disease, Duchenne muscular dystrophy, or rapidly-progressive interstitial pulmonary fibrosis

6) Patient has severe chronic obstructive pulmonary disease (COPD) requiring long-term home oxygen therapy or mechanical ventilation (non-invasive ventilation or via tracheotomy) except for continuous positive airway pressure (CPAP) or bi-level positive airway pressure (BIPAP) used solely for sleep-disorder breathing

7) Patient has congestive heart failure

8) Patient has acute left ventricular failure

9) Patient has liver failure (Child-Pugh grade C)

10) Patient is receiving renal dialysis therapy for chronic renal failure

11) Patient is receiving extracorporeal membrane oxygenation, high-frequency oscillatory ventilation (HFOV) or any form of extracorporeal lung support

12) Patient has had any form of mechanical ventilation (invasive or non-invasive, excluding CPAP alone) for longer than 48 h prior to the diagnosis of mild-to-moderate respiratory distress/ARDS

13) Patient has burns to ≥ 15% of their total body surface area

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

3.4.3 Inclusion of Minors

Over the past several decades, the rate of concussion detection and diagnosis among adolescent athletes has increased at an alarming rate.[109,110] It is thus important to evaluate concussion interventions in this population. Excluding this population from concussion research could result in delays to the development of effective treatment plans for this vulnerable population. In light of this, patients who are 12-18 years of age will be considered for inclusion in this study (as long as all eligibility criteria described previously are met).

Listed below are select references that detail the prevalence of concussions in adolescent athletes and that thus highlight the need to include this patient population in our study.

1) A large cross-sectional study (N=52,949 8th, 10th and 12th-graders) published in the Journal of the American Medical Association (JAMA) found that lifetime prevalence of at least one self-reported concussion increased from 19.5% in 2016 to 24.6% in 2020. Increases in any self-reported concussion were found across both sexes and race/ethnicity categories, among respondents whose parents had a high school diploma or less, and among respondents who participated in competitive sports during the past year.[109]

2) TBI is the leading cause of death and disability in children in the United States. Over the last two to three decades, these rates have doubled. Rates of TBI are highest in the very young (1-4 years), adolescents and young adults (15-24 years), and the elderly (>65 years).[110]

3) Overall age-adjusted rates of pediatric TBI-related emergency department visits increased from 2006 to 2013 (largely caused by mild TBIs). Each year, an estimated 50,000 to 60,000 US children are hospitalized for TBI, at a rate of 70-75 cases per 100,000 children.[111]

4 STUDY PROCEDURES

Male and female individuals between the ages of 12-80 inclusive, without any restrictions based on race, ethnicity or any other demographic criteria, and who meet all the eligibility criteria will be candidates for study enrollment. Potential study subjects will be those individuals who arrive at Allegheny Health Network hospitals and outpatient clinics, who exhibit moderate-to-severe TBI that is limited to the head and neck region. Following the witnessed signing of informed consent the study subject will be randomized 1:1 into one of the two study arms, nVNS+SOC (n=38) or SOC only (control arm, n=38).

4.1 Pre-Screening

Newly hospitalized patients or outpatients diagnosed with recent mild-to-moderate TBI and exhibiting symptoms of mild to moderate respiratory distress/ARDS, but not requiring mechanical ventilation, will be identified by the study staff via the EMR database.

4.2 Screening and Baseline Visit (Day 0)

Once identified by pre-screening, patients will undergo the following/data collection:

- Informed Consent/Assent
- Randomization: 1:1 ratio nVNS + SOC verses SOC treatment (control group)

The following data will be collected at baseline:

- Demographics

- Height (baseline visit only), weight, BMI, temperature, blood pressure, *PaO2/FiO2, *FEV1 (*if Arterial Blood Gas (ABG) is obtained per Standard of Care)
- Physical examination
- Concomitant Medications
- Co-morbidities
- Hematology/Metabolic/Hepatic panels. Results from inpatient records may be used.
- Urine or serum hCG (for females of child-bearing potential). Results from inpatient records may be used
- Research Labs ***(Blood must be collected prior to 10 AM in the fasted state)***
    - Serum/plasma immunokine panel (multi-analyte TH1, TH2 22-PLEX Luminex panel; must include TNFα, IL-1β, IL-6), pro-calcitonin, CRP, ferritin, D-dimer, complete hematology panel (CBC with differential
    - Multiparameter flow cytometric profiling: Absolute count and % of:
      B-cells: CD19+, CD20+, IL-10+; CD19+ CD24+, CD138+
      T-cells: CD4+, CD69+; CD8+ CD69+; Granzyme B+ FasL+; CD4+ CD25+ Foxp3+ $CD127^{low}$
      NK cells: CD3+ CD56+
      Macrophages: (M1-HLA-DR+ CD68+ CD86+ $CD80^{low}$ $CD206-/^{low}$ and M2-HLA-DR+ CD68+ $CD80^{low}$ $CD206+/^{high}$)
      Neutrophils: CD177+, CD15+, CD16+, CD62L+; CD15+ PSGL-1+, CD66b+ CD68+
      Neutrophil NETosis: SYTOX+ MPO+ CitH3+
- Dispense Study Device and device training by study team
- Daily Treatment Log
- nVNS treatment (date, time of, and # of treatments)
- Oxygen requirements, assisted ventilation dependence (device type, date and time of initiation/cessation), oxygen level (%, based on device type), patient discharge (Date and time), Death (date and time)
- 6MWT score (to occur once, just prior to Discharge for inpatients; should patient be immobile or incapable of completing the test for any physical reasons, the 6MWT will be deferred and documented in the research record)
- BORG Survey

- Quality of Life (QOL)/Cognitive Assessments
- PROMIS CAT score
- Post-Concussion Symptom Scale (PCSS)
- Adverse Events

4.3 Treatment period (Day 3, 7, and 30 data)

4.3.1 In-treatment period, Visit 2-4 (Day 3*, 7*, and 30: ±5 day window)

*Visit 2 and 3 may not occur for outpatients, depending on date of TBI and the date of enrollment (≤ 14 days from TBI occurrence)
*Visit 3 (Day 7) will only occur if the subject remains hospitalized.

The following data will be collected at each visit during the in-trial period. Data will be collected from patient medical records for inpatients on Days 3 and 7.

- Weight, BMI, temperature, blood pressure, *PaO2/FiO2, *FEV1 (*if ABG is obtained per Standard of Care))
- Concomitant Medications
- Research Labs ***(Blood must be collected prior to 10 AM in the fasted state)***
    - Hematology/Metabolic/Hepatic panels
    - Serum/plasma immunokine panel (multi-analyte TH1, TH2 22-PLEX Luminex panel; must include TNFα, IL-1β, IL-6), pro-calcitonin, CRP, ferritin, D-dimer
    - Multiparameter flow cytometric profiling: Absolute count and % of:

      B-cells: CD19+, CD20+, IL-10+; CD19+ CD24+, CD138+
      T-cells: CD4+, CD69+; CD8+ CD69+; Granzyme B+ FasL+; CD4+ CD25+ Foxp3+ $CD127^{low}$
      NK cells: CD3+ CD56+
      Macrophages: (M1-HLA-DR+ CD68+ CD86+ $CD80^{low}$ $CD206^{-/low}$ and M2-HLA-DR+ CD68+ $CD80^{low}$ $CD206^{+/high}$)
      Neutrophils: CD177+, CD15+, CD16+, CD62L+; CD15+ PSGL-1+, CD66b+ CD68+
      Neutrophil NETosis: SYTOX+ MPO+ CitH3+
- Daily Treatment Log

- nVNS treatment (date, time of, and # of treatments)
- Oxygen requirements, assisted ventilation dependence (device type, date and time of initiation/cessation), oxygen level (%, based on device type), patient discharge (Date and time), Death (date and time)

- 6MWT score (Baseline, day 30, day 60, and day 90; should patient be immobile or incapable of completing the test for any physical reasons, the 6MWT will be deferred and documented in the research record). If Baseline 6MWT not assessed, none will be assessed during in-office visits.
- BORG Survey
- QOL/Cognitive Assessments
  - PROMIS CAT score
  - Post-Concussion Symptom Scale (PCSS)
- Adverse Events Assessment
- Investigative device returned to research staff (Day 30, end of treatment phase)

4.4 Follow-up Period (Days 31-59)

We will monitor the study subjects for post-TBI complications up to 90 days irrespective of whether they remain hospitalized or have been discharged. Inpatient monitoring will be conducted according to SOC. Monitoring will be conducted via telephone once a week between days 31-59.

The following data will be collected during the follow-up period:

- Concomitant Medications
- Adverse Event Assessment- Incidence/severity/persistence/resolution of medical problems in recovered patients (including acute infections)

4.5 Follow-up Visits (Day 60 ± 5 days and Day 90 ± 5 days)

At 90 days from baseline; Subjects should not have any debilitations that affect respiration or the need for any device or assistance in regards to ventilation requirements.

4.5.1 Visit 5 (Day 60±5 days)

The following data will be collected at visit 5 during the in-trial period:

- Demographics (Height, weight, BMI, temperature, blood pressure, *PaO2/FiO2, *FEV1 (*if ABG is obtained per Standard of Care))

- Concomitant Medications
- Research Labs ***(Blood must be collected prior to 10 AM in the fasted state)***
    - +Hematology, Metabolic and Hepatic panels
    - Serum/plasma immunokine panel (multi-analyte TH1, TH2 22-PLEX Luminex panel; must include TNFα, IL-1β, IL-6), pro-calcitonin, CRP, ferritin, D-dimer, complete hematology panel (CBC with differential)
    - Multiparameter flow cytometric profiling: Absolute count and % of:

      B-cells: CD19+, CD20+, IL-10+; CD19+ CD24+, CD138+
      T-cells: CD4+, CD69+; CD8+ CD69+; Granzyme B+ FasL+; CD4+ CD25+ Foxp3+ $CD127^{low}$
      NK cells: CD3+ CD56+
      Macrophages: (M1-HLA-DR+ CD68+ CD86+ $CD80^{low}$ $CD206^{-/low}$ and M2-HLA-DR+ CD68+ $CD80^{low}$ $CD206^{+/high}$)
      Neutrophils: CD177+, CD15+, CD16+, CD62L+; CD15+ PSGL-1+, CD66b+ CD68+
      Neutrophil NETosis: SYTOX+ MPO+ CitH3+
- 6MWT score (should patient be immobile or incapable of completing the test for any physical reasons, the 6MWT will be deferred and documented in the research record)
- BORG Survey
- QOL/Cognitive Assessments
    - PROMIS CAT score
    - Post-Concussion Symptom Scale
- Adverse Events Assessment

4.5.2 Visit 6 (Day 90±5 days)

The following data will be collected at visit 6 during the in-trial period:

- Demographics (Height, weight, BMI, temperature, blood pressure, *PaO2/FiO2, *FEV1 (*if ABG is obtained per Standard of Care)
- Concomitant Medications
- Research Labs ***(Blood must be collected prior to 10 AM in the fasted state)***
    - Hematology, Metabolic and Hepatic panels

- Serum/plasma immunokine panel (multi-analyte TH1, TH2 22-PLEX Luminex panel; must include TNFα, IL-1β, IL-6), pro-calcitonin, CRP, ferritin, D-dimer

- Multiparameter flow cytometric profiling: Absolute count and % of:

  B-cells: CD19+, CD20+, IL-10+; CD19+ CD24+, CD138+
  T-cells: CD4+, CD69+; CD8+ CD69+; Granzyme B+ FasL+; CD4+ CD25+ Foxp3+ $CD127^{low}$
  NK cells: CD3+ CD56+
  Macrophages: (M1-HLA-DR+ CD68+ CD86+ $CD80^{low}$ $CD206^{-/low}$ and M2-HLA-DR+ CD68+ $CD80^{low}$ $CD206^{+/high}$)
  Neutrophils: CD177+, CD15+, CD16+, CD62L+; CD15+ PSGL-1+, CD66b+ CD68+
  Neutrophil NETosis: SYTOX+ MPO+ CitH3+

- 6MWT score (should patient be immobile or incapable of completing the test for any physical reasons, the 6MWT will be deferred and documented in the research record)

- BORG Survey

- QOL/Cognitive Assessments

  - PROMIS CAT score

  - Post-Concussion Symptom Scale

- Adverse Events Assessment

4.6 Concomitant Medication

Medication, which is considered necessary for the patient's safety and well-being, may be given at the discretion of the study physicians together with the patient's institutional health provider physicians. Study sites will document all medication usage during the study in the specific CRF and will monitor the CRF accordingly.

4.7 Subject Completion/Withdrawal

Patients may withdraw from the study at any time and for any reason and such a decision will not affect the ongoing care given to the patient. Data recorded up to the point of withdrawal will be included in the study analyses, unless consent for use of the data has also been withdrawn. If a patient requests termination of the administration of the study agent during the treatment period, then the administration of the study agent will be stopped but the patient will continue in the study and all follow-up assessments will be performed. If a patient withdraws consent during or after the treatment period then no further active study assessments will be performed from that time point. However, permission will be sought to

access the patient's medical records to obtain data relevant to the study (e.g., outcome status). Subjects may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules or AEs. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

4.8 Biospecimen Collection

Once consented, patients will be asked to provide up to 46 mL of blood for purposes outlined in this study protocol. Should any residual sample remain after the study-specific testing is complete, the study team will preserve and store the de-identified sample for potential biomarker testing that may be developed in the future.

Residual samples will be stored long-term in a secure space at AHN Allegheny General Hospital, 10th floor ICT lab. Any future research on the residual samples must go under IRB review and approval and will only be utilized for purposes consistent with patient informed consent.

All residual samples will be stored indefinitely, until depletion, or until study is closed by the Principal Investigator, whichever occurs first. If the study is closed, any remaining samples and all data will be destroyed per institutional policy. A code number will be assigned to the specimen and stored without identifiers. The records linking the code number to the corresponding participant's identities will be kept in a password protected database stored on an AHN Network server. These records will be accessible only to key study personnel.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Screening

Once identified by pre-screening, patients, or their legally authorized representative, will then be offered the opportunity to participate in the trial. An informed consent form (ICF) will be offered and the study will be explained to the patient, parent, or their legal representative. The patient, so long as they do not require mechanical ventilation, or their legal representative, will have up to 24 hours to sign the ICF. The patient's medical record and history will then be reviewed to confirm study eligibility. Screening tests will include: PaO2/FiO2, FEV1 (if ABG is obtained per standard of care), complete hematology, and blood hepatic, metabolic panels as well as a urine/serum hCG assessment for pregnancy (females only). The results will constitute the screening and the findings will be recorded on

CRFs, to be subsequently entered into the study database. Study staff will determine eligibility within 8 hours.

5.1.2 Baseline

We will conduct the tests and measurements shown in **Table 1**. The day these tests are conducted is considered “baseline”.

From the day of baseline measurements, study subjects will remain in-trial until one of the following occurs: **a)** 30 days without any progression to severe respiratory distress/ARDS; **b)** requirement for invasive mechanical ventilation prior to 30 days; or **c)** death prior to 30 days. Patients in the nVNS study arm will receive three treatments daily as described below for a period of 30 consecutive days or until the time at onset of invasive mechanical ventilation, or death, whichever occurs first. Study subjects in the nVNS and SOC only arm will remain on the prescribed SOC. All subjects on the nVNS arm will continue the treatment until Day 30 from baseline, except if they require mechanical ventilation. If a change is required in the SOC of individual patients it must be noted in the appropriate CRF. If the institution directs a change in SOC, it must also be noted in the CRF. **Table 1** lists the tests and measurements to be conducted over the trial. Blood must be collected prior to 10 AM in the fasted state to account for the known diurnal variation in circulating levels of immunokines.

5.1.3 SOC and General Patient Management

Apart from administration of nVNS, patients will be managed according to institutional best practices and SOC for TBI. We aim for early detection and treatment of secondary brain injury such as space-occupying intracerebral hematomas and brain edema. These patients are managed as per advanced Trauma Life Support and Brain Trauma Foundation Guidelines. Following diagnostic procedures and resuscitation/damage control surgery (if required), patients are transferred to an intensive care unit. The main goal of intensive care management is prevention of secondary brain injury by optimizing cerebral perfusion, oxygenation, nutritional supply, and homeostasis. Sedation is one of the mainstays in early TBI management. Hyperosmolar therapy with mannitol or hypertonic saline is an additional important medical treatment option to reduce intracranial pressure. If the condition evolves to moderate to severe respiratory distress/ARDS, we will manage this with supportive care, low tidal volume, prone positioning, fluid balance, sedation, mechanical ventilation and avoidance of volutrauma and barotrauma. nVNS will not be administered at any time to these patients. SOC is at the discretion of the study physician and in line with the SOC for TBI and/or ARDS in place, and as directed by the institution.

5.1.4 Study Agent Administration

Beginning at the baseline visit and up to and including Day 30, nVNS will be applied three times daily to one side of the neck as illustrated in **Figure 1**. Patients will administer their own treatment unless unable to do so safely. Treatment can be administered by the health care provider with patient permission and appropriate training.

It is posible that children may experience difficulty operating the interventional device. To reduce the risk of improper use due to this reason, a patient/legal guardian will be trained on properly operating the device, and will be present when the child is being trained. This

parent/legal guardian will be present for self-administration of the device by the child over the course of the study.

nVNS will continue until one of the following occurs: a) 30 days without any progression to severe respiratory distress; b) requirement for invasive mechanical ventilation prior to 30 days; or c) death prior to 30 days.

Treatment schedule (3 times/day): The patient will self-administer one treatment of gammaCore® three times a day. Patients who are 12 to 18 years of age will self-administer the intervention under the supervision of a trained parent/legal guardian. The parent/legal guardian may assist the child in operating the device if the child is having difficulty doing so them self.

If a patient is unable to self-administer the device while hospitalized, a research staff member who has been trained on operating the device can assist the patient.

The treatment schedule is as follows:

- **One treatment is defined as 2 consecutive stimulations:** one, 2-minute stimulation on the side of the neck followed by a second, 2-minute stimulation on the *same* side of the neck
- **The treatment will be done 3 times per day (morning, mid-day and 1 hour before bed at night),** every day, for 30 days or requires mechanical ventilation.
- The patient (or their parent/legal guardian, in the case of patients who are 12 to 18 years old) will **record the time treatment was administered.**

5.1.5 Peripheral Venous Blood Collection

Blood will be collected at the screening, baseline and then days 3, 7, 30, 60, and 90. Blood may additionally be collected based on SOC or emergency requirements as directed by the health care provider physician. For the trial, up to 46 mL of blood will be collected. We will use Vacutainer tubes particular for the kind of measurements and analyses indicated (e.g. different cap-colored tubes are specific for blood destined for nucleic acid purification, serum/plasma purification, cell enrichment for flow cytometry and molecular analyses, ELISA/Luminex analyses for immunokines, and for purification of extracellular vesicles). Flow cytometry, Luminex-based immunokine profiling, characterization of circulating extracellular vesicle proteins and nucleic acids will be conducted using methods described in [65,66] and in [62-64].

Should any residual sample remain after the study-specific testing is complete, the study team will preserve and store the de-identified sample for potential biomarker testing that may be developed in the future *(see section 4.8)*.

5.1.6 Concomitant Medication

Medication, which is considered necessary for the patient's safety and well-being, may be given at the discretion of the study physicians together with the patient's institutional health provider physicians. Study sites will document all medication usage during the study in the specific CRF and will monitor the CRF accordingly.

5.1.7 End of in-trial Period

From the day of baseline measurements, study subjects will remain in-trial until one of the following occurs: **a)** 30 days without any progression to severe respiratory distress; **b)** requirement for invasive mechanical ventilation prior to 30 days; or **c)** death prior to 30 days. With the exception of death, subjects in the nVNS arm will stop the treatment at the end of the day when either of **a,b** occurs. All recovered study subjects must continue in the follow-up period as explained in section 5.1.8.

5.1.7 Follow-up Period

We will monitor the study subjects for post-TBI complications up to 95 days irrespective of whether they remain hospitalized or have been discharged. Inpatient monitoring will be conducted according to SOC. Post-discharge monitoring will be conducted by telephone once every two days in the first two weeks following discharge and then once weekly until 60 days. Pulmonary complications are common after TBI [67]. Neurogenic pulmonary edema, epileptic seizures, subarachnoid hemorrhage, intracerebral hemorrhage, stroke, or an abrupt rise in intracranial pressure can occur up to 14 days from the TBI [68]. Intense pulmonary vasoconstriction, increased intravascular hydrostatic pressure and transudation of plasma fluid into the extravascular space are hallmarks [69]. Catecholamine-associated myocardial damage may cause left heart failure and resultant pulmonary edema [70]. Pneumonia is the most common non-neurological complication, occurring in 40–65% of patients [71,12,67] and most commonly occurring within the first 5 days post- TBI [12,67,72]. Late onset pneumonia developing after 5 days is a typical ventilator-associated pneumonia and associated with gram-negative and multi-resistant bacteria [73].

5.1.8 Follow-up Study Visit

At 90 days from baseline, subjects should not have any debilitations that affect respiration or the need for any device or assistance in regards to ventilation requirements. At this visit, blood will be collected, QOL/Cognitive Assessments (PROMIS CAT and PCSS), the forced expiratory volume in 1 s (FEV1) and the 6-minute Walk Test (6MWT) will be assessed. The Clinical Study Report (CSR) will be completed once all the data up to day 90 from baseline have been collected, verified and analyzed according to the statistical plan discussed in section 6. The study will be completed when the final patient completes their day 90 follow-up study assessment. No interim analyses are planned.

STATISTICAL CONSIDERATIONS

5.2 Primary Endpoint

The primary endpoint is all-cause 90-day admission to any hospital or ED encounter following presentation to the ED or hospitalization for mild-to-moderate TBI. This includes emergency department (ED) encounters followed by discharge or ED encounter followed by hospitalization. Hospital admission is defined as a hospitalization greater than 24 hours.

5.3 Secondary Endpoints

Secondary safety endpoints include outpatient visits

- Incidence, rate and severity of AEs/SAEs up to day 30 from baseline
- Physical examination, vital signs and laboratory results up to day 30 (or last day in hospital if patient leaves the hospital earlier)

5.4 Tertiary endpoints will include the following which may apply to the few inpatients, if any, that may be enrolled.

- All-cause mortality assessed at 30 days
- Days free of organ failure (assessed using the SOFA score), days free of renal support, days free of vasoactive support, days free of invasive mechanical ventilation, number of ICU-free days assessed at day 30 (or on the last day in the ICU if the patient leaves the ICU before day 30)
- Length of hospital stay
- FEV1, neurological functioning (6MWT) and QOL/Cognitive Assessments (PROMIS CAT and PCSS) assessed at 30, 60, and 90 days

Subjects are considered as mechanical ventilation-free after two consecutive calendar days of unassisted breathing, defined as breathing spontaneously with a face mask, nasal prong oxygen or room air; T-piece breathing; tracheostomy mask breathing; CPAP $\leq$5 cmH_2O without pressure support or intermittent mandatory ventilation assistance; or use of CPAP or BIPAP solely for sleep apnea management.

5.5 Exploratory Endpoints

Exploratory endpoints include: *Immunology/Inflammatory* e.g., change in the concentration of immunokines in the circulation using Luminex multianalyte methods, change in leukocyte populations using multiparameter flow cytometry, and characterization of pro-inflammatory proteins and nucleic acids found in circulating extracellular vesicles.

5.6 Statistical Methods

The full analysis set (FAS) will consist of all randomized and treated patients. The per-protocol set (PPS) will consist of patients in the FAS excluding those with major protocol violations. We will perform statistical analyses for the primary and secondary endpoints on both the FAS and PPS. The safety analysis set will consist of all patients who receive at least one nVNS treatment. The safety analyses will be based on this analysis set.

The primary endpoint, all-cause 90-day admission or ED encounter, will be primarily analyzed as a difference in proportions between the control and intervention arms of the study. As this is a pilot study sized using the method of Cocks and Torgerson [85] (see the following section), any non-zero treatment effect in the expected direction will serve as evidence for conducting a larger efficacy study.

Additional analyses for this study may include statistical testing of differences in proportions, Kaplan-Meier survival analysis, Poisson regression or proportional hazards regression.

5.7 Sample Size and Power

A total of 750 patients (n=375 nVNS+SOC arm vs. n=375 SOC alone, control arm) are required to be enrolled in order to detect a difference in primary outcome between both arms under the following assumptions: 19% of patients in the control arm present to the ED or are admitted to the hospital for any reason within 90 days as compared to 11.4% of patients in the nVNS arm (a difference of 7.6% between arms). This sample size is based on a power analysis that we conducted for a two-sample Z test with a significance level of 0.05 and for 80% power.

The assumption for an all-cause 90-day admission rate of 19% for the SOC arm is based on a feasibility study where we analyzed patient data from Allegheny General Hospital between 1/11/2022 and 7/27/2022. A total of 100 patients were found who had presented to the ED with a mild to moderate TBI and who met the inclusion criteria for the proposed trial. Of these patients, 19% presented to the ED or were admitted to the hospital within 90 days. The assumption of an all-cause 90-day admission rate of 11.4% in the nVNS+SOC arm is based on a study that showed readmission *rate* (strictly hospitalization) following index hospitalization for mild TBI was reduced by 70% using a care-based intervention.[75] For this sample size analysis, a conservative reduction of 40% in admission *rate* was assumed.

Assuming that 20% of patients will drop out and a further 10% of the remaining patients will not be evaluable for efficacy analysis, the actual number of patients that need to be randomized to reach the sample size of 750 is 1042. However, given that the available program budget cannot accommodate a multi-site trial that would be able to enroll the study sample indicated, we instead propose a pilot study design that, in addition to demonstrating feasibility in recruitment and retention, can also detect an effect. Such an outcome would then serve as compelling preliminary data to obtain support elsewhere with a budget and timeline that would make feasible the main trial sample size and required power. It is important to note that effect sizes from any pilot will be bounded by a high degree of

uncertainty. Consequently, in planning for a definitive trial, sample sizes based on estimates from pilots almost always result in main trials that are underpowered as treatment effects are overestimated in the pilot [77]. Nevertheless, in the last decade, a number of approaches have been developed [78-83] and have withstood statistical rigor in determining a pilot sample size driven by the proposed sample size of the main trial. In these approaches, objective criteria to establish pilot sample size use a confidence interval (CI) approach [84] instead of the usual power and statistical significance methods.

We therefore follow the CI approach of Cocks and Torgerson [85] to propose a pilot sample size that can allow us to proceed to the main trial in the future if we obtain some evidence of effectiveness. Based on their approach, we calculate that a sample size of 76 (n=38 nVNS+SOC vs. n=38 SOC alone) would result in a one-sided 80% confidence limit for difference in proportions (0.075 upper 80% one-sided confidence limit) that would exclude the 7.6% difference that would be expected in the main trial.

We thus seek 76 subjects to provide pilot data for the following purposes:

- To provide preliminary evidence for a larger efficacy study of nVNS in TBI patients
- To perform more reliable sample size calculations for a larger efficacy study
- To evaluate the feasibility of enrollment for a larger efficacy study

Following the approach of Cocks and Torgerson, a non-zero treatment effect in the expected direction observed in this study would serve as preliminary evidence of effectiveness for conducting a larger efficacy trial.[85]

6 CLINICAL INVESTIGATIONAL MEDICAL DEVICE

6.1 Description

gammaCore Sapphire (non-invasive vagus nerve stimulator) is a multi-use, handheld, rechargeable, portable device consisting of a rechargeable battery, signal-generating and amplifying electronics, and a control button for the patient to control the signal amplitude. The device provides visible (display) and audible (beep) feedback on the device and stimulation status. A pair of stainless-steel surfaces, which are the skin contact surfaces ("stimulation surfaces"), allows the delivery of a proprietary electrical signal. The patient will apply VNS-appropriate gel to the stimulation surfaces to maintain an uninterrupted conductive path from the stimulation surfaces to the skin on the neck. Tubes of VNS-appropriate gel will be provided with each unit. The stimulation surfaces will be capped when not in use. gammaCore® produces a low-voltage electric signal consisting of five 5000-Hz pulses that are repeated at a rate of 25 Hz. The waveform of the electric pulses is approximately a sine wave with a peak voltage limited to 24 Volts when placed on the skin and a maximum output current of 60mA.

The signal is transmitted through the skin of the neck to the vagus nerve. gammaCore® allows for the patient to appropriately position and adjust the stimulation intensity level as

instructed by the research team. Each stimulation is designed to be applied for 2 minutes, after which the device automatically stops delivering the stimulation. Each device allows for multiple treatments.

gammaCore® can deliver up to thirty 120–second stimulations within a 24-hour period. Once the maximum daily number of treatments has been reached, the device will not deliver any more stimulations until the following 24-hour period. (The stimulation automatically stops 120 seconds after the device is powered on). The gammaCore® Sapphire device includes a charging station to hold and charge the device.

Figure 1. gammaCore® Sapphire

Figure 2. gammaCore® Sapphire with Charging Station

The patient will apply conductive gel (supplied with the device) to the stimulation surfaces and then hold the nVNS device on the skin over the vagus nerve on the neck (between the trachea and the sternocleidomastoid muscle, over the carotid pulse). Details of device placement and operation will be provided in the Instructions for Use (IFU).

The gammaCore Sapphire device produces a proprietary, low voltage electric signal that generates an electric field in the vicinity of the vagus nerve when the device is placed in the intended location.

6.1.1 Packaging and Labeling of the Clinical Investigational Medical Device

The commercial packages will be used in this Clinical Investigation, all device packaging will be marked “For Clinical Investigation Only”.

6.1.2 Installation and Use of the Clinical Investigational Medical Device

A qualified research personnel (i.e. device trained) will train the study patients before they can start self-administration of stimulations. The gammaCore® Sapphire device will be used following the treatment assigned in this protocol.

6.1.3 Compliance with Device Usage

Patient will record all use of the device in a study log, trained study staff can assist the patient, as necessary. Compliance will be assessed in the hospital setting by the research staff.

Study patients will be encouraged to ask study staff for obtaining re-training on the device, at any time during the hospitalization. A trained study team member will be available for assistance with stimulations or device re-training 24 hours/7days per week during patient's hospitalization.

6.1.4 Potential Risks and Benefits of the Investigational Device

The anticipated benefits include:

- A preliminary understanding of whether or not VNS can prevent serious respiratory outcomes in TBI patients

There are no significant risks identified with the participation in this study however study patients can rarely experience transient symptoms such as:

- Muscle twitching, discomfort, or pain during stimulations
- Tingling, pricking or a feeling of "pins and needles" on the skin where the device is applied (paraesthesia or dysaesthesia) lasting beyond the treatment period
- Skin irritation/inflammation
- Dizziness

Study subjects will be exposed to potential risks and complications associated with gammaCore. These are anticipated to resolve shortly after discontinuation of the stimulation procedure without medical intervention or clinical sequelae. Occurrence of several of these events can be mitigated by the user repositioning the device on the neck and/or decreasing the stimulation intensity. Training on the positioning of the device and controlling the stimulation intensity is conducted at the time the device is provided to the subjects. In addition, the device is provided with detailed Instruction for Use. These potential anticipated adverse events include, but are not limited to:

- Application site discomfort (4.85%)
- Application site irritation/redness (3.74%)
- Local pain, face/head/neck area (including toothache) (3.00%)
- Muscle twitching and/or contractions, face/head/neck area (including facial droop and/or lip pull) (3.47%)
- Headache/migraine (2.56%)
- Dizziness (2.00%)
- Tingling, pricking or a feeling of "pins and needles" on the skin where the device is applied (paresthesia/dysesthesia) (1.63%)

NOTE: When all available safety data was pooled from all studies, the following AEs occurred in >1% of patients, but less than < 5% (there were no reported side effects that occurred in >5% of study patients) All adverse events occurred in at least 1 in 100 (1%) patients and are therefore "common".

Risks associated with blood draws

- Pain or discomfort from needle
- Bleeding
- Infection

Risks associated with 6MWT

- When performed appropriately, the complication rate with the 6MWT is very low as patients determine their own pace during the test. Large scale studies have confirmed this.
- The test should be terminated if a patient complains of excessive fatigue, angina, or light-headedness during the test.

Pregnancy:

It is unknown whether gammaCore can harm an unborn child if used during pregnancy. If you are pregnant, you will not be allowed to participate in this study.

Each device is for single patient use. No device sharing will be done. Every patient will have an individual device throughout the study. It will be kept with the patient throughout the duration of the study and at completion will be discarded by study staff, according to institutional policy and following all regulations for disposal of infectious waste and electrical equipment.

Site will be instructed to contact the manufacturer immediately if the product malfunctions and does not work as expected or if the device have been damaged or is suspected to have been damaged in some way. If patients are using it without clinical supervision, patients will

report any product malfunctions to the PI and/or research staff. Should any type of malfunction occur, the patient would discontinue use of the device and the research staff will assess for any adverse events. Any device deficiencies/malfunctions will be reported to the PI, the IRB via a prompt report form and the device manufacturer. The device will be quarantined by the research staff and a new device will be provided to the patient.

The benefits of this study are estimated to outweigh the risks as the patient's improvement is expected to be considerably greater than any expected side effects.

7 SAFETY MANAGEMENT

7.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

7.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with ASRI-WPAHS IRB SOP 011: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

All serious adverse events (SAEs) that occur between the signing of informed consent and day 30 will be recorded. Events occurring after day 30 will be reported only if they are considered to be causally related to the investigational study agent; however, all deaths up to day 30 will be reported as SAEs.

7.3 Adverse Events/Adverse Device Effects and Device Deficiencies

The definitions and procedures for reporting adverse events (AE), adverse device effects (ADE), serious adverse events (SAE), serious adverse device effects (SADE) and are presented in the sections below. It is of utmost importance that all staff involved in the investigation are familiar with the definitions and procedures and it is the responsibility of the Clinical Investigator to ensure this.

7.3.1 Adverse Event/Adverse Device Effect Definitions

Adverse Event

Any untoward or unfavourable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or

disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Adverse Device Effect

Adverse event related to the use of an investigational medical device.

Note 1: This definition includes any event resulting from insufficiencies or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.

Note 2: This definition includes any event that is a resulting from a user error or from intentional misuse of the investigational medical device.

7.3.2 Serious Adverse Event/Serious Adverse Device Effect/Unanticipated Adverse Device Effect Definitions

Serious Adverse Event

An adverse event is considered serious and should be reported to IRB when the patient outcome is:

- **Death**
- **Life-threatening** - the patient was at substantial risk of dying at the time of the adverse event, or use or continued use of the device or other medical product might have resulted in the death of the patient.
- **Hospitalization (initial or prolonged) -** admission to the hospital or prolongation of hospitalization was a result of the adverse event. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).
- **Disability or Permanent Damage -** if the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.
- **Congenital Anomaly/Birth Defect -** suspected exposure to a medical product prior to conception or during pregnancy may have resulted in an adverse outcome in the child.
- **Required Intervention to Prevent Permanent Impairment or Damage (Devices)** - medical or surgical intervention was necessary to preclude permanent impairment

of a body function, or prevent permanent damage to a body structure, either situation suspected to be due to the use of a medical product.

- **Other Serious (Important Medical Events)** - the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

Serious Adverse Device Effect

A SADE is an ADE that results in any of the consequences characteristic of an SAE or that might lead to any of these consequences if suitable action is not taken, if intervention is not made or if circumstances are less opportune.

7.3.3 Unanticipated serious adverse device effect (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

Note: Anticipated serious adverse device effect (ASADE) is an effect which by nature, incidence, severity or outcome has been identified in the risk analysis report.

7.3.4 Reporting of Adverse Events/Adverse Device Effects

7.3.4.1 Methods for Eliciting Adverse Events/Adverse Device Effects

All patients will be carefully monitored for the occurrence of AEs during the investigation period from the run-in to the completion of follow up. The Clinical Investigator will collect AE information using non-leading questions such as “have you experienced any new health problems or worsening of existing conditions”. Events directly observed or spontaneously volunteered by patients will also be recorded.

Clearly related signs, symptoms and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible.

All AEs including but not limited to events reported by the patient or reported in response to an open question by the Clinical Investigator or member of this team, which fall into any of the above definitions must be recorded as an AE in the CRF and should include the following information.

- Brief description of the event (diagnosis)
- Start date (and time, if relevant)

- Stop date (and time, if relevant) (or resolution)
- Severity
- Action taken regarding the medical device
- Opinion on causality
- Seriousness
- Outcome

Severity describes the intensity of an event and will be assessed as:

Mild - The AE does not interfere in a significant manner with the patient's normal functioning level. It may be an annoyance.

Moderate- The AE produces some impairment of function but not hazardous to health. It is uncomfortable and/or an embarrassment.

Severe- The AE produces significant impairment of functioning or incapacitation and/or it is a hazard to the patient.

If an AE changes in severity, it should be reported as an AE of new severity but with the same description.

Causality (Adverse Device Effects/ADE)

Causality will be assessed as:

- Related (definitely, possible or probable) - A causal relationship between the clinical investigational medical device and the AE is at least a reasonable possibility, i.e. there is evidence or argument suggesting a causal relationship.
- Not related - There is no indication that the AE was caused by the clinical investigational medical device.

7.3.4.2 Follow-up of Patients with Adverse Events

Any AE that is ongoing when the patient is withdrawn from the investigation should be followed-up until the AE is resolved or the Clinical Investigator decides that the AE is stable and needs no further follow-up. The date when the Clinical Investigator considers one of these outcomes to have occurred for the last ongoing AE for a patient will be considered the last visit for this patient, and the outcome should be recorded in the CRF.

7.3.5 Reporting of Events

INTERNAL: REPORTABLE EVENTS THAT OCCUR AT THE PI's RESEARCH SITE or are ASSOCIATED WITH THE PI			
Event	**Examples** (not all-inclusive)	**How to Report**	**Report Timeframe**
Serious Adverse Event (SAE)	○ Pneumonia resulting from study drug administration ○ Significant allergic reaction resulting from study drug(s) ○ Cardiovascular event induced by study drug(s)	*Prompt Report Form* Copy of SAE Report to Sponsor Copy of subject's signed ICF	5 days
Unanticipated Adverse Device Event (UADE)	○ Guide wire breaks during insertion and cannot be retrieved ○ Revision surgery to replace component ○ Cardiovascular event induced by study device	*Prompt Report Form* Copy of UADE (Device SAE) Report to Sponsor Copy of subject's signed ICF	5 days
SAE or UDAE resulting in death	○ Any death related or possibly related to use of investigational drug or device	*Prompt Report Form* Copy of SAE Report to Sponsor Copy of subject's signed ICF	24 hour phone notice 5 days: written report
Deaths within 30 days of study treatment (all others reported at continuing review)	○ Any death that occurs within 30 days of receiving study drug/device/intervention, regardless if PI determined death is "unrelated" and/or "expected" (this excludes MINIMAL RISK STUDIES such as registries or observational studies).	*Prompt Report Form* Copy of SAE Report to Sponsor	5 days
Major Protocol Deviation/ Violation	○ Failure to obtain informed consent ○ Omitting study procedure(s) required by-approved protocol ○ Drug dispensing/dosing error ○ Failure to securely control the study product ○ Deviation necessary to eliminate an apparent immediate hazard to a participant	*Prompt Report Form*	5 days
Research Complaint	○ Complaint from a participant regarding a research-related injury or study activities ○ Complaint from study personnel regarding fabrication of data or research misconduct	*Prompt Report Form*	5 days
Adverse Monitoring or Audit Reports, or Enforcement Action	○ Reports of study monitor visits or sponsor audits with findings that require action at this site to address potential risks to participants or others. ○ Suspension or restriction of medical license ○ FDA Form 483 or Warning Letter	*Prompt Report Form* Copy of reports, audits, etc.	24 hour phone notice: suspensions or FDA 483 5 days: monitoring or audit reports
Other Unanticipated Problem	○ Participant becomes incarcerated ○ Breach of participant confidentiality (e.g., breach of secured database) ○ Study personnel misconduct that adversely affects the study	*Prompt Report Form*	5 days
Recalls / Withdrawals / Clinical Holds	○ Correspondence communicating a Regulatory Agency or Sponsor mandated marketing recall, withdrawal, or clinical hold	*Prompt Report Form* Copy of the correspondence	5 days
Reports, publications, or interim results or findings	○ DSMB reports and recommendations ○ Regulatory Agency Public Health Advisory ○ "Dear Healthcare Professional" Letter	*Prompt Report Form* Copy of report, publication, interim finding, etc.	10 days
All Adverse Events (regardless if serious, related or expected) **that do not meet Prompt Reporting criteria as described above**	○ If applicable, a summary report of all adverse events should be provided to the IRB at least annually. ○ The specific format of this report should be described in the Local Data Safety Monitoring Plan at the time of initial review. Many protocols will require only written verification on the *Protocol Renewal Form* (e.g. All adverse events have been reviewed by [the PI & study coord] and appear to be occurring at the frequency and intensity as is expected in this subject population)	*Protocol Renewal Form* (if applicable)	Continuing review (if applicable)

7.4 Medical Emergencies

Site will be instructed to contact the manufacturer immediately if the product malfunctions and does not work as expected or if the device have been damaged or is suspected to have been damaged in some way. If patients are using it without clinical supervision, patients will report any product malfunctions to the PI and/or research staff. Should any type of malfunction occur, the patient would discontinue use of the device and the research staff will

assess for any adverse events. Any device deficiencies/malfunctions will be reported to the PI, the IRB via a prompt report form and the device manufacturer. The device will be quarantined by the research staff and a new device will be provided to the patient.

8 STUDY ADMINISTRATION

8.1 Treatment Assignment Methods

8.1.1 Randomization

If the subject is found to be eligible for enrollment, they will be randomized on a 1:1 basis into the nVNS+SOC or SOC alone [control] groups. Randomized study subjects will be assigned a study randomization number to which a treatment group assignment has been made. Randomization will be conducted via a central web-based system. The study principal investigator, the laboratory personnel will be masked to the treatment assignment of each participant. Given the nature of the nVNS, the study physician, the patient, and the study staff interacting with the patient cannot be masked to the treatment. Only the study physician and the trial coordinators will know the arm to which the subject has been randomized. The patient's TBI will be classified as moderate or severe and any respiratory distress/ARDS will be classified based on age, co-morbidities, and mild or moderate oxygen saturation. To prevent allocation bias, the patient will be assigned to the test or control arm of the study based on severity of these classifications in a 1:1 ratio in blocks of four.

8.2 Data Collection and Management

The sponsor or sponsor's designee will conduct a site visit to each study center to verify the qualifications of each investigator, inspect the site facilities and inform the investigator of their responsibilities and the procedures for ensuring adequate and correct documentation. The investigators will be given access to an online, web-based, electronic data-capture system that is compliant with US Food and Drug Administration Title 21 Code of Federal Regulations Part 11. Access rights to the electronic data-capture system will be carefully controlled and configured according to each individual's role throughout the study. Computerized data-check programs and manual checks will identify any data discrepancies for resolution. All discrepancies must be resolved online directly by the investigator. Only the investigator and study coordinators will be able to enter and correct data in the CRF. All study findings and documents will be regarded as confidential. Patients will be identified on the CRF by the randomly-generated alphanumeric code. Only the study physicians and the designated study staff that need to communicate with the patient will know their identity and has access to PHI.

8.2.1 Patient Data Protection

Minimal necessary data will be collected and stored on a password protected server only accessible to the research staff. Risk of breach of confidentiality of this data has been minimized as much as possible. We have used the "minimum necessary" standard in choosing specific and pertinent data parameters for this research. All key research team

members that have access to clinical data (containing PHI) being collected and analyzed, currently work within our system. Data containing PHI will be stored on secure, password-protected servers and when necessary, will be securely emailed to other team members using only secure institutional email accounts (as per institutional IT Security Policy). Team members will use secure servers and systems to access and work with study spreadsheet data/files. When any information is shared with the sponsor, this will be de-identified. PHI will not be shared with the sponsor. At the conclusion of data analysis for the protocol, identifiers will be stripped from the compiled datasheet to anonymize the dataset by the study staff, with the goal that the data will be presented formally. Patient identifiers will be deleted and the dataset anonymized as soon as possible. We will comply with the institutional IRB "Records Retention" plan which states "…for research involving PHI, the records will be retained for 10 years after the study is completed."

8.3 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers (including others at AHN) before sharing a limited dataset (PHI limited to dates and zip codes).

8.4 Regulatory and Ethical Considerations

The trial will be in full conformity with the principles of the Belmont Report (April 18, 1979) and codified in 45 CFR 46, 21 CFR 312, 21 CFR 50, 56, ICH E6; 62 Federal Regulations 25691 (1997). Prior to trial start, the clinical protocol, informed consent forms, study advertisements, and subject visit/event reimbursement plan will be reviewed by the study IRBs. The trial will be registered at clinicaltrials.gov. No person will be enrolled without offering willing, witnessed and documented informed consent. The overarching safety objective is to ensure that the study and the study agent is safe and does not put the study population at risk. Our minimum goal is to meet a potential benefit: risk ratio no different than the accepted institutional SOC for TBI.

8.4.1 Data and Safety Monitoring Plan

The study design incorporates an independent Data and Safety Monitoring Board (DSMB) consisting of five members: one independent biostatistician and four physicians (one neurologist, two trauma physicians, and one pulmonologist) who possess significant experience in TBI, ALI, and ARDS, and who are not involved in the study. The DSMB will review accumulating safety data every 14 days until the last enrolled subject has been discharged from the study (at 90 days post-baseline). At each meeting, the DSMB will make

recommendations to the study staff and the study sponsor regarding the study to continue without change, modify study or enrollment to be placed on hold, or study termination.

In addition to the DSMB, the PI will continuously monitor the general oversite of the trial. Annual reports of the PI oversite will be submitted during annual renewal with the AHN IRB in the IRB manager renewal application.

8.4.2 Study Risks and Potential Benefits

For hospitalized subjects, TBI can rapidly develop into a life-threatening condition. This is a both a disease- and study-related risk. Patient SOC includes 24-hour monitoring, supportive and symptomatic care and a disease treatment regimen that is evolving, informed by the latest clinical findings and treatment options.

In terms of the nVNS, the following have been identified as technology use-associated risks: a) Muscle twitching, discomfort, or pain during stimulations; b) Tingling, pricking or a feeling of "pins and needles" on the skin where the nVNS is applied (paresthesia or dysesthesia) lasting beyond the treatment period; c) Skin irritation/inflammation; d) Dizziness. These rare events are anticipated to resolve shortly after discontinuation of the stimulation procedure without medical intervention or clinical sequelae. Occurrence of several of these events can be mitigated by the user repositioning the nVNS technology on the neck and/or decreasing the stimulation intensity. Training on the positioning of the device and controlling the stimulation intensity is conducted at the time the device is provided to the subjects.

Other in-trial and follow-up risks include those associated with peripheral venous blood collection: a) Pain or discomfort from needle; b) Bleeding; c) Bruising at and around the needle insertion point; and d) Infection of the needle insertion site. When performed appropriately, the complication rate with the 6 MWT is very low as patients determine their own pace during the test. Large scale studies have confirmed this. The test should be terminated if a patient complains of excessive fatigue, angina, or light-headedness during the test. Although pregnancy is an exclusion criterion, we cannot control for this in the follow-up period, even though nVNS will not be used. Also the effects of nVNS on the embryo and fetus in recovering patients who become pregnant in the follow-up period is currently unknown.

The potential benefits based on the known effects of nVNS in airway disease and inflammation could include: a) prevention of assisted ventilation need; b) prevention of death; c) decreased hospitalization/earlier discharge; d) mitigation of immunokine storms and SIRS; e) prevention of disease-associated non-airway organ pathology; f) suppression of airway inflammation-driven damage; and g) improved airway function.

8.5 Recruitment Strategy

Suggestion:

We will pre-screen the EMR looking at patients who had a TBI using EPIC, Slicer Dicer and Trinetx tools to identify potential subjects. Once identified, the treating physician will be offered to distribute a flyer to potential subjects and/or the treating physician (KSP) will approach the subject to enroll possibly with study staff to assist with the consenting process. The consenting will take place either in the hospital inpatient room or in clinic offices

The flyers will also be attached to emails sent by Dr. Snell to colleagues who see concussion patients to let them know about the study in hopes of referral.

8.6 Informed Consent/Assent and HIPAA Authorization

All patients will receive written and verbal information regarding the investigation prior to any investigation-related procedures. This information will emphasize that participation in the clinical trial is voluntary and that the subject may withdraw from the study at any time for any reason. All patients will be given the opportunity to ask questions about the investigation and will be given sufficient time to decide whether to participate in the study.

Before any study-related procedures occur, the informed consent form will be signed and dated by the patient, parent, or legally authorized representative (LAR), a witness to signature, and by the Clinical Investigator who gave the patient the verbal and written information. For patients under the age of 18, assent will be obtained.

The consent specifies that data will be recorded, collected, processed and used in accordance with patient consent and applicable law.

8.7 Payment to Subjects/Families

8.7.1 Reimbursement for parking

Participants will be reimbursed for parking for each in-clinic visit during the study.

8.7.2 Payments to subject for time, effort and inconvenience (i.e. compensation)

Participants will be paid $50.00 per visit for completion of the Day 30 visit, the Day 60 follow-up visit, and the Day 90 follow-up visit.

9 PUBLICATION

All publications and presentations must be based upon the clinical investigation report.

10 REFERENCES

1. Pavlovic D, Pekic S, Stojanovic M, Popovic V. Traumatic brain injury: neuropathological, neurocognitive and neurobehavioral sequelae. Pituitary 2019;22:270-82.
2. Gajic O, Dabbagh O, Park PK, et al. Early identification of patients at risk of acute lung injury: evaluation of lung injury prediction score in a multicenter cohort study. American journal of respiratory and critical care medicine 2011;183:462-70.
3. Soto GJ, Kor DJ, Park PK, et al. Lung Injury Prediction Score in Hospitalized Patients at Risk of Acute Respiratory Distress Syndrome. Critical care medicine 2016;44:2182-91.
4. Camporota L, Ranieri VM. What's new in the "Berlin" definition of acute respiratory distress syndrome? Minerva anestesiologica 2012;78:1162-6.
5. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. Jama 2012;307:2526-33.
6. Summers CR, Ivins B, Schwab KA. Traumatic brain injury in the United States: an epidemiologic overview. The Mount Sinai journal of medicine, New York 2009;76:105-10.
7. Kokiko-Cochran ON, Godbout JP. The Inflammatory Continuum of Traumatic Brain Injury and Alzheimer's Disease. Frontiers in immunology 2018;9:672.
8. Nizamutdinov D, Shapiro LA. Overview of Traumatic Brain Injury: An Immunological Context. Brain sciences 2017;7.
9. Corps KN, Roth TL, McGavern DB. Inflammation and neuroprotection in traumatic brain injury. JAMA neurology 2015;72:355-62.
10. Simon DW, McGeachy MJ, Bayir H, Clark RSB, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. Nature reviews Neurology 2017;13:572.
11. Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 2010;7:22-30.
12. Piek J, Chesnut RM, Marshall LF, et al. Extracranial complications of severe head injury. J Neurosurg 1992;77:901-7.
13. Kinoshita K. Traumatic brain injury: pathophysiology for neurocritical care. Journal of intensive care 2016;4:29.
14. Kemp CD, Johnson JC, Riordan WP, Cotton BA. How we die: the impact of nonneurologic organ dysfunction after severe traumatic brain injury. The American surgeon 2008;74:866-72.
15. Zygun D. Non-neurological organ dysfunction in neurocritical care: impact on outcome and etiological considerations. Current opinion in critical care 2005;11:139-43.
16. Hu PJ, Pittet JF, Kerby JD, Bosarge PL, Wagener BM. Acute brain trauma, lung injury, and pneumonia: more than just altered mental status and decreased airway protection. American journal of physiology Lung cellular and molecular physiology 2017;313:L1-L15.
17. Nguyen H, Zaroff JG. Neurogenic stunned myocardium. Current neurology and neuroscience reports 2009;9:486-91.
18. Wijayatilake DS, Sherren PB, Jigajinni SV. Systemic complications of traumatic brain injury. Current opinion in anaesthesiology 2015;28:525-31.
19. Bratton SL, Davis RL. Acute lung injury in isolated traumatic brain injury. Neurosurgery 1997;40:707-12; discussion 12.
20. Aisiku IP, Yamal JM, Doshi P, et al. The incidence of ARDS and associated mortality in severe TBI using the Berlin definition. The journal of trauma and acute care surgery 2016;80:308-12.
21. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. The New England journal of medicine 2005;353:1685-93.
22. Lu J, Goh SJ, Tng PY, Deng YY, Ling EA, Moochhala S. Systemic inflammatory response following acute traumatic brain injury. Frontiers in bioscience 2009;14:3795-813.
23. Lopez-Aguilar J, Blanch L. Brain injury requires lung protection. Annals of translational medicine 2015;3:S5.

24. Koutsoukou A, Katsiari M, Orfanos SE, et al. Respiratory mechanics in brain injury: A review. World journal of critical care medicine 2016;5:65-73.
25. Fisher AJ, Donnelly SC, Hirani N, et al. Enhanced pulmonary inflammation in organ donors following fatal non-traumatic brain injury. Lancet 1999;353:1412-3.
26. Kalsotra A, Zhao J, Anakk S, Dash PK, Strobel HW. Brain trauma leads to enhanced lung inflammation and injury: evidence for role of P4504Fs in resolution. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2007;27:963-74.
27. Weber DJ, Gracon AS, Ripsch MS, et al. The HMGB1-RAGE axis mediates traumatic brain injury-induced pulmonary dysfunction in lung transplantation. Science translational medicine 2014;6:252ra124.
28. Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. Journal of leukocyte biology 2013;93:865-73.
29. Kallet RH, Matthay MA. Hyperoxic acute lung injury. Respiratory care 2013;58:123-41.
30. Della Torre V, Badenes R, Corradi F, et al. Acute respiratory distress syndrome in traumatic brain injury: how do we manage it? Journal of thoracic disease 2017;9:5368-81.
31. McKee AC, Daneshvar DH. The neuropathology of traumatic brain injury. Handbook of clinical neurology 2015;127:45-66.
32. Narayan RK, Michel ME, Ansell B, et al. Clinical trials in head injury. Journal of neurotrauma 2002;19:503-57.
33. Scheff SW, Ansari MA. Natural Compounds as a Therapeutic Intervention following Traumatic Brain Injury: The Role of Phytochemicals. Journal of neurotrauma 2017;34:1491-510.
34. Hoffmann TJ, Mendez S, Staats P, Emala CW, Guo P. Inhibition of histamine-induced bronchoconstriction in Guinea pig and Swine by pulsed electrical vagus nerve stimulation. Neuromodulation : journal of the International Neuromodulation Society 2009;12:261-9.
35. Steyn E, Mohamed Z, Husselman C. Non-invasive vagus nerve stimulation for the treatment of acute asthma exacerbations-results from an initial case series. International journal of emergency medicine 2013;6:7.
36. Miner JR, Lewis LM, Mosnaim GS, Varon J, Theodoro D, Hoffmann TJ. Feasibility of percutaneous vagus nerve stimulation for the treatment of acute asthma exacerbations. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine 2012;19:421-9.
37. Zila I, Mokra D, Kopincova J, Kolomaznik M, Javorka M, Calkovska A. Vagal-immune interactions involved in cholinergic anti-inflammatory pathway. Physiological research 2017;66:S139-S45.
38. Zhao M, He X, Bi XY, Yu XJ, Gil Wier W, Zang WJ. Vagal stimulation triggers peripheral vascular protection through the cholinergic anti-inflammatory pathway in a rat model of myocardial ischemia/reperfusion. Basic research in cardiology 2013;108:345.
39. Tarn J, Legg S, Mitchell S, Simon B, Ng WF. The Effects of Noninvasive Vagus Nerve Stimulation on Fatigue and Immune Responses in Patients With Primary Sjogren's Syndrome. Neuromodulation : journal of the International Neuromodulation Society 2019;22:580-5.
40. Lerman I, Hauger R, Sorkin L, et al. Noninvasive Transcutaneous Vagus Nerve Stimulation Decreases Whole Blood Culture-Derived Cytokines and Chemokines: A Randomized, Blinded, Healthy Control Pilot Trial. Neuromodulation : journal of the International Neuromodulation Society 2016;19:283-90.
41. Koopman FA, Chavan SS, Miljko S, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. Proceedings of the National Academy of Sciences of the United States of America 2016;113:8284-9.

42. Bonaz B, Picq C, Sinniger V, Mayol JF, Clarencon D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 2013;25:208-21.
43. Zi S, Li J, Liu L, Liu F. Cholinergic anti-inflammatory pathway and its role in treatment of sepsis. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences 2020;45:68-73.
44. Huston JM, Gallowitsch-Puerta M, Ochani M, et al. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. Critical care medicine 2007;35:2762-8.
45. Conti P, Ronconi G, Caraffa A, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. Journal of biological regulators and homeostatic agents 2020;34.
46. Wang CH, Liu CY, Wan YL, et al. Persistence of lung inflammation and lung cytokines with high-resolution CT abnormalities during recovery from SARS. Respiratory research 2005;6:42.
47. Tang Y, Dong X, Chen G, et al. Vagus Nerve Stimulation Attenuates Early Traumatic Brain Injury by Regulating the NF-kappaB/NLRP3 Signaling Pathway. Neurorehabilitation and neural repair 2020:1545968320948065.
48. Chen X, Zhao C, Zhang C, et al. Vagal-alpha7nAChR signaling promotes lung stem cells regeneration via fibroblast growth factor 10 during lung injury repair. Stem cell research & therapy 2020;11:230.
49. Brock C, Brock B, Aziz Q, et al. Transcutaneous cervical vagal nerve stimulation modulates cardiac vagal tone and tumor necrosis factor-alpha. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 2017;29.
50. Kamourieh S, Lagrata S, Matharu MS. Non-invasive vagus nerve stimulation is beneficial in chronic paroxysmal hemicrania. Journal of neurology, neurosurgery, and psychiatry 2019;90:1072-4.
51. Diener HC, Goadsby PJ, Ashina M, et al. Non-invasive vagus nerve stimulation (nVNS) for the preventive treatment of episodic migraine: The multicentre, double-blind, randomised, sham-controlled PREMIUM trial. Cephalalgia : an international journal of headache 2019;39:1475-87.
52. Mwamburi M, Tenaglia AT, Leibler EJ, Staats PS. Review of evidence on noninvasive vagus nerve stimulation for treatment of migraine: efficacy, safety, and implications. The American journal of managed care 2018;24:S507-S16.
53. Staats P, al e. Neurostimulation for asthma. In: Krames E, al e, eds. Neuromodulation: Comprehensive Textbook of Principles, Technologies and Therapies. 2nd ed. London, UK: Academic Press, Elsevier; 2018:1339-45.
54. Mwamburi M, Liebler EJ, Staats PS. Patient experience with non-invasive vagus nerve stimulator: gammaCore patient registry. The American journal of managed care 2020;26:S15-S9.
55. Polson M, Williams TD, Speicher LC, Mwamburi M, Staats PS, Tenaglia AT. Concomitant medical conditions and total cost of care in patients with migraine: a real-world claims analysis. The American journal of managed care 2020;26:S3-S7.
56. Staats P, Giannakopoulos G, Blake J, Liebler E, Levy RM. The Use of Non-invasive Vagus Nerve Stimulation to Treat Respiratory Symptoms Associated With COVID-19: A Theoretical Hypothesis and Early Clinical Experience. Neuromodulation : journal of the International Neuromodulation Society 2020.
57. Camporota L, Hart N. Lung protective ventilation. BMJ 2012;344:e2491.
58. Kato H, Fujimoto A, Okanishi T, Sugiura R, Ijima K, Enoki H. New onset syncopal events following vagus nerve stimulator implantation might be key to preventing vagus nerve stimulation-induced symptomatic bradycardia - A case report and review. Epilepsy & behavior case reports 2018;10:57-60.
59. Tsaava T, Datta-Chaudhuri T, Addorisio ME, et al. Specific vagus nerve stimulation parameters alter serum cytokine levels in the absence of inflammation. Bioelectronic medicine 2020;6:8.

60. Nik A, Sheikh Andalibi MS, Ehsaei MR, Zarifian A, Ghayoor Karimiani E, Bahadoorkhan G. The Efficacy of Glasgow Coma Scale (GCS) Score and Acute Physiology and Chronic Health Evaluation (APACHE) II for Predicting Hospital Mortality of ICU Patients with Acute Traumatic Brain Injury. Bulletin of emergency and trauma 2018;6:141-5.
61. Ramtinfar S, Chabok SY, Chari AJ, Reihanian Z, Leili EK, Alizadeh A. Early detection of nonneurologic organ failure in patients with severe traumatic brain injury: Multiple organ dysfunction score or sequential organ failure assessment? Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine 2016;20:575-80.
62. Ghai V, Fallen S, Baxter D, et al. Alterations in Plasma microRNA and Protein Levels in War Veterans with Chronic Mild Traumatic Brain Injury. J Neurotrauma 2020;37:1418-30.
63. Martin GE, Pugh AM, Moran R, et al. Microvesicles generated following traumatic brain injury induce platelet dysfunction via adenosine diphosphate receptor. J Trauma Acute Care Surg 2019;86:592-600.
64. Hazelton I, Yates A, Dale A, et al. Exacerbation of Acute Traumatic Brain Injury by Circulating Extracellular Vesicles. J Neurotrauma 2018;35:639-51.
65. Finak G, Langweiler M, Jaimes M, et al. Standardizing Flow Cytometry Immunophenotyping Analysis from the Human ImmunoPhenotyping Consortium. Sci Rep 2016;6:20686.
66. Staples E, Ingram RJ, Atherton JC, Robinson K. Optimising the quantification of cytokines present at low concentrations in small human mucosal tissue samples using Luminex assays. J Immunol Methods 2013;394:1-9.
67. Zygun DA, Kortbeek JB, Fick GH, Laupland KB, Doig CJ. Non-neurologic organ dysfunction in severe traumatic brain injury. Crit Care Med 2005;33:654-60.
68. Simon RP. Neurogenic pulmonary edema. Neurol Clin 1993;11:309-23.
69. Smith WS, Matthay MA. Evidence for a hydrostatic mechanism in human neurogenic pulmonary edema. Chest 1997;111:1326-33.
70. Mann DL, Kent RL, Parsons B, Cooper Gt. Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation 1992;85:790-804.
71. Helling TS, Evans LL, Fowler DL, Hays LV, Kennedy FR. Infectious complications in patients with severe head injury. J Trauma 1988;28:1575-7.
72. Bronchard R, Albaladejo P, Brezac G, et al. Early onset pneumonia: risk factors and consequences in head trauma patients. Anesthesiology 2004;100:234-9.
73. Croce MA, Fabian TC, Waddle-Smith L, et al. Utility of Gram's stain and efficacy of quantitative cultures for posttraumatic pneumonia: a prospective study. Ann Surg 1998;227:743-51; discussion 51-5.
74. Kost RG, Corregano LM, Rainer TL, Melendez C, Coller BS. A data-rich recruitment core to support translational clinical research. Clinical and translational science 2015;8:91-9.
75. Ashworth S, Conrad M, Laloo M. Impact of direct access to outpatient physical therapy post mild traumatic brain injury in reduction of hospital readmissions. Archives of Physical Medicine and Rehabilitation. https://www.archives-pmr.org/article/S0003-9993(21)00050-2/fulltext#relatedArticles. Published April 1, 2021. Accessed November 7, 2022.
77. Kraemer HC, Mintz J, Noda A, Tinklenberg J, Yesavage JA. Caution regarding the use of pilot studies to guide power calculations for study proposals. Arch Gen Psychiatry 2006;63:484-9.
78. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Methods Med Res 2016;25:1057-73.
79. Sim J, Lewis M. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. J Clin Epidemiol 2012;65:301-8.
80. Julious SA, Owen RJ. Sample size calculations for clinical studies allowing for uncertainty about the variance. Pharm Stat 2006;5:29-37.

81. Browne RH. On the use of a pilot sample for sample size determination. Stat Med 1995;14:1933-40.
82. Birkett MA, Day SJ. Internal pilot studies for estimating sample size. Stat Med 1994;13:2455-63.
83. Wittes J, Brittain E. The role of internal pilot studies in increasing the efficiency of clinical trials. Stat Med 1990;9:65-71; discussion -2.
84. Bland JM. The tyranny of power: is there a better way to calculate sample size? BMJ 2009;339:b3985.
85. Cocks K, Torgerson DJ. Sample size calculations for pilot randomized trials: a confidence interval approach. J Clin Epidemiol 2013;66:197-201.
86. Finkelstein DM, Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. Statistics in medicine 1999;18:1341-54.
87. Bell ML, Horton NJ, Dhillon HM, Bray VJ, Vardy J. Using generalized estimating equations and extensions in randomized trials with missing longitudinal patient reported outcome data. Psycho-oncology 2018;27:2125-31.
88. Birhanu T, Molenberghs G, Sotto C, Kenward MG. Doubly robust and multiple-imputation-based generalized estimating equations. Journal of biopharmaceutical statistics 2011;21:202-25.
89. Carey VJ, Wang YG. Working covariance model selection for generalized estimating equations. Statistics in medicine 2011;30:3117-24.
90. Duenas M, Salazar A, Ojeda B, Arana R, Failde I. Generalized Estimating Equations (GEE) to handle missing data and time-dependent variables in longitudinal studies: an application to assess the evolution of Health Related Quality of Life in coronary patients. Epidemiologia e prevenzione 2016;40:116-23.
91. Evans BA, Feng Z, Peterson AV. A comparison of generalized linear mixed model procedures with estimating equations for variance and covariance parameter estimation in longitudinal studies and group randomized trials. Statistics in medicine 2001;20:3353-73.
92. Hanley JA, Negassa A, Edwardes MD, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation. American journal of epidemiology 2003;157:364-75.
93. May WL, Johnson WD. Generalized estimating equations for multivariate response with the variates having different distributions. Journal of biopharmaceutical statistics 1996;6:139-53.
94. Mehrotra DV. A comparison of generalized linear mixed model procedures with estimating equations for variance and covariance parameter estimation in longitudinal studies and group randomized trials. Statistics in medicine 2002;21:3745-7;author reply 7-8.
95. Molenberghs G. Generalized estimating equations. Notes on the choice of the working correlation matrix. Methods of information in medicine 2010;49:419-20.
96. Muth C, Bales KL, Hinde K, Maninger N, Mendoza SP, Ferrer E. Alternative Models for Small Samples in Psychological Research: Applying Linear Mixed Effects Models and Generalized Estimating Equations to Repeated Measures Data. Educational and psychological measurement 2016;76:64-87.
97. Salazar A, Ojeda B, Duenas M, Fernandez F, Failde I. Simple generalized estimating equations (GEEs) and weighted generalized estimating equations (WGEEs) in longitudinal studies with dropouts: guidelines and implementation in R. Statistics in medicine 2016;35:3424-48.
98. Seaman S, Copas A. Doubly robust generalized estimating equations for longitudinal data. Statistics in medicine 2009;28:937-55.
99. Shen CW, Chen YH. Model selection for generalized estimating equations accommodating dropout missingness. Biometrics 2012;68:1046-54.
100. Shults J, Whitt MC, Kumanyika S. Analysis of data with multiple sources of correlation in the framework of generalized estimating equations. Statistics in medicine 2004;23:3209-26.
101. Wang M, Long Q. Modified robust variance estimator for generalized estimating equations with improved small-sample performance. Statistics in medicine 2011;30:1278-91.

102. Westgate PM, Burchett WW. Improving power in small-sample longitudinal studies when using generalized estimating equations. Statistics in medicine 2016;35:3733-44.
103. Hin LY, Wang YG. Working-correlation-structure identification in generalized estimating equations. Statistics in medicine 2009;28:642-58.
104. Lipsitz SR, Fitzmaurice GM, Orav EJ, Laird NM. Performance of generalized estimating equations in practical situations. Biometrics 1994;50:270-8.
105. Wang M, Kong L, Li Z, Zhang L. Covariance estimators for Generalized Estimating Equations (GEE) in longitudinal analysis with small samples. Statistics in medicine 2016;35:5318-9.
106. Wang YG, Fu L. Selection of working correlation structure in generalized estimating equations. Statistics in medicine 2017;36:2206-19.
107. Ziegler A, Vens M. Generalized estimating equations. Notes on the choice of the working correlation matrix. Methods of information in medicine 2010;49:421-5; discussion 6-32.
108. Hosmer D, Lemeshow S. Applied Logistic Regression New Yorkj: John Wiley & Sons; 2000.
109. Veliz P, McCabe SE, Eckner JT, Schulenberg JE. Trends in the Prevalence of Concussion Reported by US Adolescents, 2016-2020. JAMA. 2021;325(17):1789-1791. doi:10.1001/jama.2021.1538
110. Kazl C, Torres A. Definition, Classification, and Epidemiology of Concussion. Semin Pediatr Neurol. 2019;30:9-13. doi:10.1016/j.spen.2019.03.003
111. Chen C, Peng J, Sribnick EA, Zhu M, Xiang H. Trend of Age-Adjusted Rates of Pediatric Traumatic Brain Injury in U.S. Emergency Departments from 2006 to 2013. Int J Environ Res Public Health. 2018;15(6):1171. Published 2018 Jun 5. doi:10.3390/ijerph15061171