Smell in Covid-19 and Efficacy of Nasal Theophylline (SCENT 3)

Principal Investigator:

Theresa Tharakan

Version Date/Number:

3.6.2023 (5)

A Introduction

A1 Study Abstract

Olfactory and gustatory disorders (OGDs) are hallmarks of COVID-19 infection. The prevalence of COVID-related OGD symptoms ranges from 17-80% depending on viral variant, and the prevalence using objective chemosensory testing is likely even higher. The median recovery time for olfactory dysfunction is approximately 15 days after infection, but these symptoms persist beyond 90 days and 6 months in 15% and 5% of patients, respectively. OGDs have numerous adverse effects such as loss of cortical gray matter and decrease in quality of life. Intranasal and oral corticosteroids as well as olfactory training are currently used to treat post-viral OD; however they have demonstrated limited efficacy and there is no current gold standard of care.
There is no current consensus on the pathogenesis of COVID-related anosmia; however evidence for post-viral olfactory dysfunction suggests sensory axonal regeneration and olfactory signaling may rely on elevated levels of secondary messengers *cAMP* and *cGMP*. Elevation of *cAMP* and *cGMP* is a known effect of theophylline, a phosphodiesterase inhibitor used to treat asthma. In our pilot studies of COVID-19 related OD, a 6-week course of intranasal theophylline led to modest subjective improvement in olfactory symptoms compared to placebo. However, olfactory neuroepithelium regeneration takes a minimum of 4 weeks and is prolonged in inflammatory settings, therefore we believe a longer course of treatment is necessary to observe olfactory improvement. Therefore, we propose a phase II single-site, double-blinded, placebo-controlled randomized clinical trial to test a 12-week course of nasal irrigation with intranasal theophylline for patients with COVID-related anosmia.

A2 Primary Hypothesis

We hypothesize that theophylline irrigation will be more effective than placebo saline irrigation for COVID-19 related OD treatment, and that intranasal theophylline use will have minimal adverse effects due to limited systemic absorption.

A3 Purpose of the Study Protocol

To elucidate the efficacy and safety of intranasal theophylline irrigation in the treatment of COVID-19 related OD in comparison to placebo saline irrigation.

B Background

B1 Prior Literature and Studies

COVID-19 Pandemic. In December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in association with pneumonia in Wuhan China.[1-3] As of August 1st, 2020, over 17.5 million people around the world have been infected with the resulting illness, termed coronavirus disease 2019 (COVID-19). The United States has more cases than any other country, at over 5 million. The

numbers continue to grow exponentially.[4] The CDC has listed the following key symptoms suggestive of illness with COVID-19: cough, shortness of breath or difficulty breathing, fever, chills, muscle pain, sore throat, and new loss of smell or taste.[5]

Anosmia Prevalence in COVID-19. Subjective olfactory dysfunction (OD) has been reported in up to 80% of patients with COVID-19, with varying incidence depending on viral variant.[6,7] Prevalence of OD using objective olfactory testing is likely higher.[8] One early study in Iran showed that 59 of 60 COVID-19 patients reported some degree of objective olfactory dysfunction, as reported by the validated University of Pennsylvania Smell Identification Test (UPSIT).[9] Using a smartphone-based app (COVID Symptom Tracker), another study gathered self-reported symptoms from patients in the UK and the US. Their study reported 65% of patients who tested positive for COVID-19 had loss of smell or taste, and for 16%, there was no associated fever or cough. Using data from over 70,000 patients, they concluded the predictive ability of OD for COVID-19 was higher than the predictive ability of either cough or fever, and that the median duration of anosmia was about 5 days.[10] Preliminary results from the COVID-19 Anosmia Reporting tool developed by the American Academy of Otolaryngology-Head and Neck Surgery suggests that, out of 237 initial entries, 27% reported OD as their initial symptom, and 27% of patients had some improvement in OD with a mean of 7.2 days.[1] A study of 143 patients in Italy assessed persistent symptoms of COVID-19 after about 60 days since onset of a patient's first COVID-19 symptom. About 45% of patients presented with anosmia in the acute setting, and about 17% of patients continued to report anosmia 60 days later.[11] This data suggests anosmia can persist beyond two months in over a third of cases. A meta-analysis of 18 studies with 3699 patients with COVID-related chemosensory dysfunction found that the median recovery time for OD is approximately 15 days, but these symptoms persist beyond 90 days and 6 months in 15% and 5% of patients, respectively.[12]

Based on an estimate in which 25% of new daily cases will experience anosmia (average of 111,000 new daily cases in the first week of August 2022 in the US), and that 5% will experience permanent anosmia, we predict that over 500,000 patients will suffer from chronic OD by August 2023.[4] This estimate does not take into account those that already suffer from long-term OD currently.

Anosmia and Dysgeusia in COVID-19. Current research studies report both anosmia (loss of smell) and dysgeusia (loss of taste) related to COVID-19 interchangeably. In rare cases SARS-CoV-2 may lead to isolated taste symptoms through direct involvement of the gustatory system, but often dysgeusia is related to impaired retronasal olfaction (flavor) rather than gustatory sensations (sweet, salty, sour, bitter).[13,14] Therefore, primarily focusing on treatment for olfactory dysfunction may treat both anosmia and dysgeusia.

It is well established that the nasal cavity and the nasolacrimal duct are key entry points for SARS-CoV-2;[15-17] however there is yet to be a consensus on the exact mechanism that leads to COVID-related anosmia.[2] It is believed that the virus enters a cell using spike protein S1 to adhere to a host ACE2 receptor. ACE2 receptors are expressed throughout the nervous system, which may explain the virus's neurological manifestations.[2,18] A bulk sequencing study showed expression of both ACE2 and TMPRSS2 genes, both thought to facilitate SARS-CoV-2 viral entry, in the olfactory mucosa of humans. A single cell sequencing study, confirmed by immunostaining,

further suggested higher expression of ACE2 in sustentacular cells, basal stem cells, and in pericytes as opposed to olfactory neural cells.[19] There is, however, ultrastructural evidence that SARS-CoV-2 enters the central nervous system via direct viral damage to the olfactory complex as seen via nasal endoscopic dissection at autopsy.[20] A case study using MRI in a 25-year-old female with COVID-associated anosmia and dysgeusia demonstrated signal alteration, suggesting viral brain invasion into the posterior gyrus rectus, which is associated with olfaction. The resolution of the anosmia correlated with resolution in that signal alteration as well.[21] Therefore, preliminary evidence exists for COVID-related anosmia affecting nasal epithelial cells, the olfactory neural complex, and/or the cortex directly. The resolution of COVID-related anosmia in the majority of patients may be related to the unique neuroplasticity the olfactory system exhibits both centrally and peripherally.[22-24] However for those in whom symptoms persist, it may be beneficial to rely on our current understanding of non-COVID-19 post-viral olfactory dysfunction to treat COVID-related anosmia. This research into treatments could retroactively support our understanding of the exact pathogenesis of the disease as well.

Significance and Management of Olfactory Dysfunction. As of 2016 before COVID-19, about 13 million or 1 in 8 Americans over the age of 40 suffered from a measurable smell dysfunction. In total, 3% of all Americans had anosmia or hyposmia, with post-viral olfactory dysfunction (PVOD) being the leading cause.[25] While often considered a benign or innocuous condition, in recent years olfactory loss has in fact been associated with an increased 5-year mortality rate, even after accounting for neurologic disease or weight loss. Anosmia has even been shown to be more predictive of 5-year mortality than cardiovascular disease, cerebrovascular accident, diabetes, heart failure, or cancer in older adults.[26-29] Olfactory dysfunction is known to cause loss of cortical gray matter as well as significantly decreased quality of life due to its inherent importance in taste and flavor, memory, and emotion.[22,30] OD affected up to 25% of persons over the age of 50, and evidence also suggests it may be associated with depression and contribute to the anorexia of aging.[31-33] While spontaneous recovery is possible in up to one-third of patients with post-viral OD, the recovery is often incomplete.[34,35]

Because of our limited understanding of the exact pathophysiology of permanent anosmia, treatment options have been difficult to develop. In the past, oral and intranasal corticosteroids were considered a possible treatment to target nasal mucosal inflammation; however they are not currently recommended due to lack of clear benefit and a number of adverse effects.[3,13] Moreover, systemic corticosteroids (particularly dexamethasone) are only recommended for acute COVID-19 infection if supplemental oxygen is needed, and it is unclear what the effect of steroids would be for COVID-related anosmia.[36] Other medications such as intranasal sodium citrate, intranasal vitamin A, or systemic omega-3 fatty acids have shown potential benefit and negligible adverse effects in treating olfactory dysfunction; however more research is needed to ascertain their use.[3]

It is well established that the olfactory system exhibits unique neuroplasticity both centrally and peripherally. Neurogenesis in the olfactory tract continues throughout our lifetime, leading experts to conduct research on olfactory training to modulate neural olfactory function. The current first-line treatment for post-viral OD is olfactory training, in which a patient is repeatedly exposed to four different odors in an attempt to regenerate olfactory receptor cells and recreate the signaling pathway to the olfactory cortex.[22] Olfactory training has been shown to improve odor discrimination and identification score

as measured by Sniffin Sticks in comparison to controls.[22,37] In non-randomized placebo-controlled trials of post-viral OD, olfactory training was observed to improve subjective smell ratings at 8-16 weeks of treatment, with additional incremental improvements with continued treatment up to 24-56 weeks.[38,39] However, studies of olfactory training have shown varying effectiveness depending on the duration of olfactory dysfunction, the concentration and molecular weight of odors used, the duration of therapy, and addition of steroid nasal irrigation.[40] Olfactory training is currently under investigation for use in COVID, but it has not been standardized or recommended for routine clinical practice. Therefore, investigation of pharmacologic therapies for COVID related olfactory dysfunction is warranted.

The current approach to treating COVID-related anosmia relies on this past knowledge of post-viral OD, and has been outlined by Whitcroft, et al. (**Figure 1**).[13] This approach, however, is continually evolving, and randomized control trials (RCT) on treatments for COVID-related anosmia are ongoing. One three-arm RCT for COVID-related anosmia treatments has been approved in which participants are randomized to olfactory training, budesonide nasal irrigation, or training with smelling household items (NCT04374474).[41] Other trials are testing omega-3 fatty acids vs placebo (NCT04495816),[42] mometasone nasal spray vs placebo (NCT04484493)[43] , and cerebrolycin injections (NCT04830943).[44]

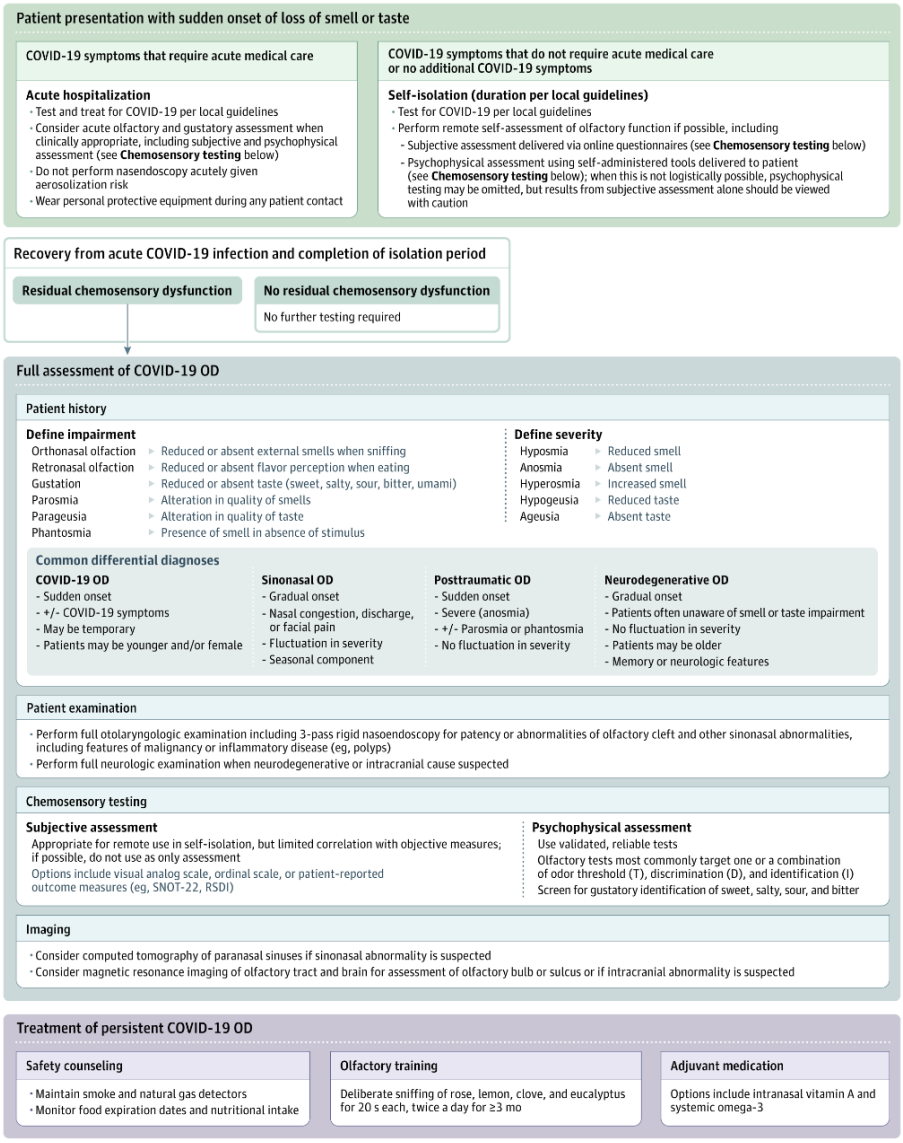

Figure 1. Possible Approach for the Assessment and Management of Suspected Coronavirus Disease 2019 (COVID-19)–Related Olfactory Dysfunction[13]

Use of Theophylline in OD and COVID-related anosmia. Prior *in vivo* and *in vitro* studies have demonstrated the importance of secondary messengers cAMP and cGMP in both olfactory signaling and sensory axonal regeneration.[45-47] When odorants attach to olfactory receptors, downward signal amplification leads to increases in cAMP and cGMP, which then instigate action potentials via sodium and calcium channels.[48] This results in our sense of olfaction.[49] cAMP promotes sensory axonal regeneration by blocking the inhibition of axonal regeneration by myelin and MAG, found in most adult axons.[50] Moreover, cAMP and cGMP levels in nasal mucus were significantly lower in hyposmic participants compared to normosmic controls,[51] and a stepwise increase in olfactory dysfunction was associated with a stepwise decrease in nasal mucus cAMP and cGMP levels.[52] The use of a phosphodiesterase inhibitor, such as theophylline, that prevents the breakdown of intracellular cAMP and cGMP, therefore, has been theorized to improve olfaction.[53,54] Moreover, two preliminary molecular docking studies showed

potential affinity of theophylline derivatives to inhibit two important SARS-CoV-2 proteins, papain-like protease protein (PLpro) and 3-chymotrypsin-like protease (3CLpro), offering promise as a treatment of COVID-19. Further *in vivo* and *in vitro* studies are needed to evaluate clinical utility.[55,56]

Clinically, theophylline is an inexpensive drug with FDA approval for the treatment of both asthma and chronic obstructive pulmonary disease (COPD).[47] Theophylline is a methylxanthine that works by smooth muscle relaxation (bronchodilation) and suppression of tissue response to stimuli (anti-inflammatory).[57] Oral theophylline for OD showed subjective improvement starting at 4-6 weeks but with continuing improvement for 6-72 months of treatment (Henkin 2008). While systemic theophylline has a narrow therapeutic index, the use of intranasal theophylline has recently been studied as a potential treatment for post-viral OD at doses that do not increase serum theophylline levels.[58] A study on OD of various etiologies demonstrated improved smell detection and recognition thresholds after 2 months of 20 ug intranasal theophylline spray (Henkin 2022). Two pilot studies of participants who had post-viral OD refractory to multiple treatments reported statistically significant improvement in quantitative subjective scores of smell.[58,59]

A study at our institution attempted to evaluate the role of intranasal theophylline nasal irrigation, as opposed to nasal spray, on post-viral OD. Delivery via nasal irrigation may improve penetration into the middle meatus and olfactory cleft compared to the nasal spray. This study was conducted at a particularly low dose of 12 mg twice a day and reported no clinically or statistically significant differences in olfactory function improvement between theophylline nasal irrigation and placebo.

A follow-up dose escalation trial tested higher doses up to 400mg twice daily for safety and reported minor side effects in 2 out of 10 participants at the maximum dose thus far. One patient reported pre-existing atrial fibrillation for all four weeks of the trial. 4 out of 10 participants also reported improvements in their sense of smell.

A subsequent trial tested a 6-week course of 400mg theophylline in saline irrigations twice daily compared to placebo (lactose in saline irrigation).[60] Systemic absorption as measured by serum theophylline after 1 week at this dose was negligible. Of 45 participants who completed the study, Eleven (50%) participants in the theophylline arm compared to 6 (26%) in the placebo arm had a clinically meaningful change on objective olfactory testing.13 (59%) in the theophylline arm had subjective improvement compared to 10 (43%) in the placebo arm, for a difference in response rate of 15.6% (95% CI -13.2 to 44.5%). This wide confidence interval precludes definitive conclusions but the upper bound of 44.5% is much larger than the MCID of 25%, suggesting that the observed effect of theophylline on both subjective and objective outcomes warrants a larger trial to investigate the efficacy of this treatment more fully.

B2 Rationale for this Study

COVID-related anosmia is a major symptom of infection with SARS-CoV-2, affecting up to 80% of those with COVID-19. While research on the pathogenesis is ongoing, a significant subset is expected to suffer from permanent olfactory dysfunction. We seek to test intranasal theophylline nasal irrigation as a potential therapeutic option for treatment of COVID-related anosmia lasting over 2 months. Theophylline has been shown to

improve outcomes in post-viral OD in pilot studies, and our initial data suggests therapeutic benefit in patients with post-COVID OD. This phase II placebo-controlled, double-blinded RCT will provide us with the needed observed OD treatment effect for future phase III RCTs.

C Study Objectives

C1 Primary Aim

Evaluate the efficacy of intranasal theophylline irrigation on olfactory recovery and health-related quality of life in patients with COVID-19 related chronic olfactory dysfunction. Participants will receive 12 weeks of either theophylline or placebo nasal saline irrigation. The primary outcome will be within- and between-subject changes in the subjective ratings of smell via the Clinical Global Impression Scale (CGI). A secondary measure will be the Olfactory Dysfunction Outcomes Rating (ODOR) survey.

C2 Rationale for the Selection of Outcome Measures

In accordance with virtual research protocols, all measurement tests at baseline and at the 12-week mark will be administered via online HIPAA-compliant survey. The following outcome measures were chosen to evaluate our hypothesis.

Primary Outcome:

1. **Clinical Global Impression Scale (CGI)-** We will measure the response rate defined as the number of participants self-reporting minimal change or larger in the Clinical Global Impression Scale (CGI) scale, divided by the number of participants in each group. The CGI has two components – the CGI-Severity and the CGI-Improvement. The CGI-Severity Scale from 1-7 (1 is Normal, 7 is Complete loss of smell) will provide us with subjective data on the patient's perceived severity of their dysfunction at baseline. The CGI-Improvement Scale from 1-7 (1 is Very Much Improved, 7 is Very Much Worsened) will allow us to measure changes at 4 weeks and post-treatment at 8 weeks. Each rating is well defined to minimize variability.[61] Participants reporting 3 as Minimally Improved, 2 as Much Improved, or 1 as Very Much Improved in the CGI-I will be deemed responders to treatment, and the number of responders to non-responders will be compared between the two arms.

Secondary Outcomes:

1. **Olfactory Dysfunction Outcomes Rating (ODOR) –** ODOR is a new disease-specific questionnaire that assesses for physical, functional, and emotional limitations in participants with olfactory dysfunction of any etiology. Based on the recurring impairments for participants with post-viral OD in eating/appetite, environmental safety, interpersonal relationships, hygiene, and mood, 28 items were generated to create the new patient reported outcome measure. This test is expected to be validated in Spring 2021.
2. **COVID-19 related questions** – Questions will be asked about the length of time since COVID-19 infection, severity of symptoms, previously attempted treatments for olfactory dysfunction, and presence of parosmia/phantosmia. This survey will help us determine whether the efficacy of theophylline therapy depends on any of those factors.

D Investigational Agent

D1 Preclinical Data

Theophylline is a methylxanthine that works by smooth muscle relaxation (bronchodilation) and suppression of tissue response to stimuli (anti-inflammatory). It non-selectively inhibits phosphodiesterase (PDE) III and IV, thereby increasing intracellular cAMP and cGMP levels.[47] These secondary messengers then result in bronchodilation, smooth muscle relaxation, and decreased inflammation. It also prevents the translocation of nuclear factor-κB (NF-κB), a pro-inflammatory transcription factor, into the nucleus and thus decreases inflammation even further.[57] We hypothesize that due to these mechanisms, theophylline could improve olfactory dysfunction by reducing inflammation in nasal epithelial cells as well.

There are two preliminary molecular docking studies that used derivatives of theophylline to test high-affinity inhibition of three SARS-CoV-2 proteins. Theophylline derivatives in particular showed potential affinity for inhibiting papain-like protease protein (PLpro) and 3-chymotrypsin-like protease (3CLpro); however *in vivo* and *in vitro* studies are needed to evaluate future clinical use.[55,56]

D2 Clinical Data to Date

Clinically, theophylline is an inexpensive drug with FDA approval for the treatment of both asthma and chronic obstructive pulmonary disease (COPD). Clinical studies show conflicting data for its use in acute asthma exacerbations; however in acute COPD exacerbations, theophylline may decrease dyspnea, air trapping, and the work of breathing.[62] It may also improve contractility of diaphragmatic muscles, but it does not improve pulmonary function measurements.[63,64] Theophylline may be efficacious in chronic asthma and exercise-induced bronchospasm, and is an alternative, but not preferred, treatment for mild persistent, moderate, and severe asthma.[47] Theophylline improves respiratory function in COPD in multiple ways and is recommended as daily maintenance therapy with beta2-agonists and anticholinergics. It has been shown to reduce hematocrit and improve symptoms from chronic hypoxemia and allow for greater bronchodilation and reduced diaphragmatic fatigue.[47,64] At serum concentrations approaching 17 mcg/mL, theophylline has been shown to improve peak flow, trapped gas volumes, vital capacity, distances walked, and breathlessness and fatigue.[65] Recent data suggests a goal serum concentration of 10-15 mcg/mL to achieve clinical efficacy without side effects commonly seen at concentrations >20 mcg/mL.[25,47] Non-FDA approved uses may include nocturnal asthma, newborn apnea, and post-dural punctural headache. It has been studied as prophylaxis for acute renal failure, seasonal allergic rhinitis, cerebral vasospasm, and more.[25]

Oral theophylline has also been studied in a single-arm longitudinal trial for the treatment of hyposmia in which 50% of patients (157/312) reported subjective improvement in smell and taste, and 11% of patients (34/312) even reported return of normal function of smell. Mean odor detection and recognition thresholds were also significantly improved.[53] Oral theophylline use has also been associated with increased brain activation signals on fMRI in response to odors.[54]

Systemic effects of theophylline suggest a relatively narrow therapeutic index.[47] These effects may be related to phosphodiesterase III inhibition in particular. Common adverse

effects of theophylline known thus far include headache, nausea, vomiting, tremors, insomnia, lightheadedness, and restlessness. Serious adverse effects can include tachyarrhythmias, atrial fibrillation, Stevens Johnson syndrome, intracranial hemorrhage, and seizure.[25,47]

It was hypothesized that topical theophylline may be preferred for certain disease states. A pilot study of 10 participants who previously had submaximal responses or intolerance to oral theophylline were given intranasal theophylline for the treatment of hyposmia and hypogeusia. This study showed improved taste and smell acuity in 8 participants after 4 weeks of intranasal theophylline therapy, reporting a statistically significant mean increase of 28% in quantitative subjective scores for smell improvement in comparison with 14% for the oral theophylline group. Moreover, serum theophylline levels were undetectable in all participants.[66] Four of 8 patients with chronic anosmia and hyposmia who did not respond to prednisone had improvement of olfactory dysfunction in a pilot study with intranasal theophylline spray.[59]

A trial of 39 patients with olfactory disorders of various etiologies demonstrated a statistically significant improvement in smell detection and identification thresholds after 2 months of 20 mcg intranasal theophylline spray (Henkin 2022).[67]

A study by our group evaluated the role of intranasal theophylline nasal irrigation, as opposed to nasal spray, on post-viral OD. We believe delivery via nasal irrigation improves penetration into the middle meatus and olfactory cleft than the nasal spray.[68] Patients with chronic OD were randomized to 12mg twice daily theophylline nasal irrigation (n=12) or placebo saline nasal irrigation (n=10). There was no statistical or clinical significant difference in objective olfaction identification, but there was a greater improvement in QOD-NS in the theophylline group (median difference -10 points, 95% CI -15 to -4). Our group hypothesized that the low effect of theophylline on objective smell may have been due to underdosing, therefore a dose escalation trial was done to test the tolerance of higher doses of intranasal theophylline delivered via nasal irrigation for hyposmia and hypogeusia.[69] 7 out of 10 patients tolerated the maximum dose of 400mg twice daily without any reported side effects. One patient reported transient minor side effects that lasted for one day following increase in dose to 300mg twice daily and again at 400mg twice daily. Another patient reported continued tremors, anxiety, and abdominal pain after increasing dose to 300mg twice daily and discontinued the study. Abdominal pain is not currently a well-known side effect of theophylline use. The third patient reported pre-existing atrial fibrillation throughout the study.

A subsequent trial by our group tested a 6-week course of 400mg theophylline in saline irrigations twice daily compared to placebo (lactose in saline irrigation).[60] Ten patients who underwent serum testing after 1 week of intervention all had serum theophylline concentration <5 ug/mL, reflecting negligible systemic absorption. At week 3, the theophylline arm had 2 participants reporting insomnia and the placebo arm had 3 participants reporting tachycardia. Two participants assigned to the theophylline arm reported parosmia and foul taste with the intervention resulting in 1 withdrawal and 1 with poor compliance (>30% of pills remaining at 6 weeks). Of 45 participants who completed the study, Eleven (50%) participants in the theophylline arm compared to 6 (26%) in the placebo arm had a clinically meaningful change on objective olfactory testing. Thirteen (59%) in the theophylline arm had subjective improvement compared to 10 (43%) in the placebo arm, with a difference in response rate of 15.6% (95% CI -13.2 to 44.5%). This wide confidence interval precludes definitive conclusions but the upper

bound of 44.5% is much larger than the MCID of 25%, suggesting that the observed effect of theophylline on both subjective and objective outcomes warrants a larger trial to investigate the efficacy of this treatment more fully.

D3 Dose Rationale and Risk/Benefits

Previous data suggest a serum concentration between 10-15 mcg/mL will achieve the majority of the drug's potential benefit while minimizing adverse effects for asthma and COPD. Adverse effects significantly increase beyond a serum concentration of 20 mcg/mL, and severe symptoms (seizures, ventricular arrhythmias, and death) has been shown to occur in chronic concentrations of 40 mcg/mL or an acute concentration above 80 mcg/mL.[25,47]

The dose of oral theophylline used in the treatment trial of hyposmia and hypogeusia ranged from 200 mg up to 800 mg a day divided into two equivalent doses (breakfast and lunch).[53] Some patients only responded to theophylline in a dose-dependent manner, at a minimum of 600 to 800 mg, requiring further escalation of dosing and prolonged treatment duration. This escalation also meant exposing patients to further adverse events associated with theophylline such as headaches. Mean serum levels for patients on 800 mg oral theophylline daily was 11.2 +/- 0.8 mg/dL.[53] Our institution's low-dose theophylline nasal irrigation trial used 12 mg capsules of theophylline dissolved in 240 mL saline twice daily for six weeks, which was the equivalent converted dose from the intranasal theophylline spray open-label trial.[53] Our follow-up dose escalation trial used a maximum daily dose of 800 mg, which was increased from 200 mg daily in 200 mg increments like the earlier oral theophylline trial did with minimal side effects. The systemic of theophylline 800 mg daily via nasal irrigation was clinically negligible after 1 week of treatment.

Treatment duration longer than 6 weeks may be necessary to observe olfactory neural recovery in the post-infectious setting. Therefore, we propose an 800 mg daily intranasal dose with a treatment period of 12 weeks with the expectation of minimal side effects but greater treatment efficacy.

E Study Design

E1 Overview or Design Summary

This study will be a single-site, double-blinded, placebo-controlled randomized clinical trial performed at a tertiary academic medical center. The purpose of this Phase II trial is to evaluate the efficacy of intranasal theophylline irrigation in treating COVID-related olfactory dysfunction. This study will also be used to describe adverse effects related to intranasal theophylline irrigation. Below is a diagram of the approach to a randomized control trial for achieving the specific aims (Figure 2):

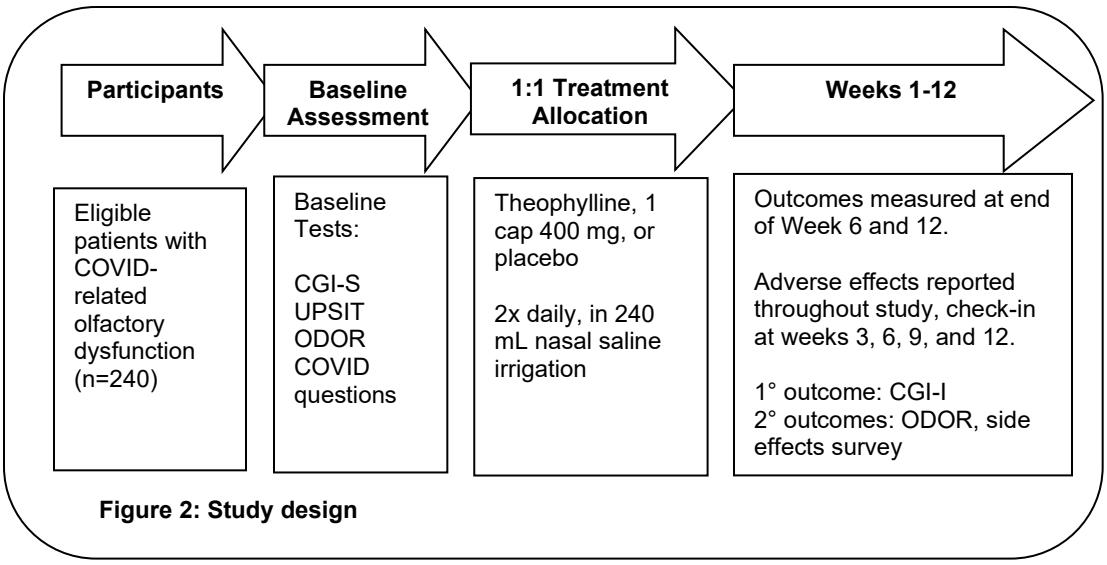

Figure 2: Study design

This study will be conducted via virtual research guidelines and procedures. We will not require in-person patient participation or evaluation. Following initial evaluation to ensure patients meet all inclusion criteria and none of the exclusion criteria, the research study will be explained in full via phone call. Participants will then be randomized in a 1:1 allocation via permuted-block sequencing to the intranasal theophylline irrigation group or the intranasal placebo irrigation group. All assessments will be conducted through a HIPAA-compliant online survey form. Baseline assessments will help us determine subjective and objective rates of olfactory dysfunction. Outcome assessments will be conducted at 6 weeks and at 12 weeks, at which point the study will conclude. The primary objective of this study is to assess the efficacy of intranasal theophylline irrigation in improving COVID-related olfactory dysfunction.

E2 Subject Selection and Withdrawal

2.a Inclusion Criteria

Participants will be recruited based on the following inclusion criteria:

1) males and females ages 18 to 75 years
2) residing within the states of Missouri or Illinois
3) Olfactory dysfunction that has persisted for >3 months following suspected COVID-19 infection
4) Baseline **University of Pennsylvania Smell Identification Test (UPSIT)** consistent with decreased olfactory function (<= 34 in women, <=33 in men)**.** This test is a clinically validated 40-question forced-choice odor identification test where microencapsulated odorants on a strip are released by scratching.[70] This will determine that patients have both subjectively and objectively diagnosed OD prior to undergoing treatment.
5) Ability to read, write, and understand English and have access to email.

2.a Exclusion Criteria

Individuals will not be allowed to participate in this study if they meet one or more of the following exclusion criteria:

1) History of olfactory dysfunction prior to COVID-19 infection
2) Any use of concomitant therapies specifically for the treatment of olfactory dysfunction
3) Use of or participation in previous trials of intranasal theophylline.
4) Known existence of nasal polyps, prior sinonasal, or anterior skull-based surgery
5) Dependence on theophylline for comorbid conditions such as asthma and COPD
6) History of an allergic reaction to theophylline or other methylxanthines
7) History of neurodegenerative disease (ie. Alzheimer's dementia, Parkinson's disease, Lewy body dementia, frontotemporal dementia)
8) Pregnant or breastfeeding mothers.
9) Current use of medications with significant (≥40%) interactions with theophylline, which include cimetidine, ciprofloxacin, disulfiram, enoxacin, fluvoxamine, interferon-alpha, lithium, mexiletine, phenytoin, propafenone, propranolol, tacrine, thiabendazole, ticlopidine, and troleandomycin.

2.b Ethical Considerations

This project relies on the participation of human subjects. To ensure their safety, we will prioritize obtaining informed consent, maximizing benefit and minimizing risk, and maintaining confidentiality. Participants will be evaluated by a medical professional during the first interaction via phone call. Participants will phone in data or submit online surveys at baseline, the 6th week, and the 12th week. Intranasal theophylline has been proven to have a minimal side effect profile and results in no detectable serum theophylline concentrations to date. The study team will be available at all times in case of adverse events, and a participant may remove themselves from the trial at any point throughout the trial. No financial conflicts of interest.

2.c Subject Recruitment Plans and Consent Process

Recruitment will be done at the Washington University Department of Otolaryngology-Head and Neck Surgery outpatient clinics and the Barnes Jewish Hospital Care and Recover After COVID 19 (CARE) Clinic. Advertisements will also be sent to all members of the St. Louis ENT Club, otolaryngologists whose practices are within 150 miles of Washington University Medical Center (WUMC), members of the Washington University Faculty Practice Plan, the BJH Medical Group, and otolaryngologists at academic institutions in the state of Missouri, Illinois, and Kansas City, Kansas. Recruitment of subjects will also be achieved by sending flyers through the Washington University Volunteers for Health Research Participant Registry (VFH), and the Otolaryngology Research Participant Registry. Study posters and flyers may be posted around the medical campus and on Facebook with the help of VFH. This recruitment strategy has been successful for Dr. Jake Lee's Smell Changes & Efficacy of Nasal Theophylline (SCENT) trial (NCT03990766) and SCENT2 trial (NCT04789499). We may also attempt to recruit participants from past studies in our lab, such as Dr. Piccirillo's CODS trial (IRB #202004146), Dr. Piccirillo's NASAL trial (IRB #202010228), and Dr. Piccirillo's GRACE trial (IRB #202110011). Additionally, due to the focus on virtual research at this time, we will also post the flyer on websites and social media, including the WUSTL Clinical Outcomes Research lab website and Facebook groups for olfactory disorders with written permission by Facebook group administrators. We will also work with anosmia

support groups such as AbScent, FifthSense and the Smell and Taste Association of North America (STANA) to share the study flyer. Lastly, we may use ResearchMatch to recruit within Missouri and Illinois.

Contact made by telephone or email from interested individuals to the study team:

- Potentially eligible patients who present to clinic will be given a study flier and instructed to contact the study team or begin online screening as indicated on the flier.
- Potentially eligible individuals who see the approved study flier

The study team member will introduce the study and gauge interest in participation. Interested individuals will be asked to complete an online screening questionnaire to ensure he or she meets all of the inclusion criteria and none of the exclusion criteria. Interested individuals will also have the option to complete the first step of online screening on REDCap via a QR code link from the study flier.

Following eligibility confirmation, an informed econsent will be acquired. Questions will be encouraged from the individual. This may occur during the telephone call or at a mutually agreeable time. If the individual wishes to participate they will proceed with the econsent providing signature and initials where indicated in the form. The signature will be time stamped and the participant will receive a copy of the signed consent (electronic file). Appropriate technical securities will be followed to protect the confidentiality of the participant's information including sending a test email to confirm the email address. A study team member will be on the phone with the individual during the econsent process and available by email or phone following should they have questions. No coercion or undue influence will be exercised. Individuals will be informed that participation is voluntary and that they can withdraw at any time during the study with no consequences.

Any modifications to the project resulting in consent form changes will be tracked by the study team. In the event re-consent is required, the change is made to the econsent form in REDCap and the econsent process is conducted again with the participant as described.

The following privacy protections will be enacted for all email communications involving PHI:

1) emails will be encrypted according HIPAA Privacy Office standards by including [secure] in subject line or
2) PHI will not be included in the email or subject line, but will be sent as a password protected attachment. The password for the attachment will be provided to the subject separately (in a separate email, by phone, or by letter)

Retention of identifiable information on non-consented individuals will be kept for contact purposes for future research opportunities.

2.d Randomization Method and Blinding

Subjects will be randomized in a 1:1 allocation via permuted-block sequencing to the intranasal theophylline irrigation group or the intranasal placebo irrigation group. This

trial will be double-blinded, meaning neither the subjects nor the study team will be aware of the intervention received by any subject.

2.e Risks and Benefits

The potential benefit to the participant is improvement of their smell and taste using this novel therapy. The potential benefit to the society is the use of the results to initiate a large phase III study to definitively determine efficacy of intranasal theophylline use for treatment of COVID-related olfactory dysfunction. If effective, intranasal theophylline would become a mainstay treatment of this disease, which currently has no effective treatment. The potential risk to the participant is an adverse event related to the use of intranasal theophylline. Common adverse effects of systemic theophylline known thus far include headache, nausea, vomiting, tremors, insomnia, lightheadedness, and restlessness. Serious adverse effects can include tachyarrhythmias, atrial fibrillation, Stevens Johnson syndrome, intracranial hemorrhage, and seizure. Intranasal theophylline has shown minimal adverse effects.

2.f Early Withdrawal of Subjects

The consent process informs a volunteer about the study, indicates that participation is voluntary, and that he/she has the right to stop at any time. Risks are enumerated in the informed consent form and described orally during the consent process. Subjects will be allowed to end participation in the study at any point should they desire. If a participant must be withdrawn due to a drug-related serious adverse event, the study drug will be stopped immediately. They will have an exit interview to ascertain any adverse effects and discuss the reason for the ending participation. The study team will follow them for safety reasons up to 30 days after stopping use of the study drug.

If a subject has ended participation for any reason, the only data that will be collected are the data from the exit interview and the data collected prior to withdrawal within the 12-week time period. There will not be any other follow-up or data collected from these subjects.

A participant can withdraw consent for the study at any time. Data collected up to this point will not be used in the analysis, and further data will not be collected from these subjects.

E3 Study Drug

3.a Treatment Regimen

Participants will dissolve the contents of the 400 mg theophylline capsules (experimental) or identical-appearing lactose capsules (control) into the sinus rinse bottle containing nasal saline. All participants will receive an 8-ounce sinus rinse bottle and a 12-week supply of USP Grade Sodium Chloride & Sodium Bicarbonate Mixture (pH balanced, Isotonic & Preservative & Iodine Free) commercially prepared packets. Participants will either need to purchase distilled water or boil tap water for five minutes for use with the saline irrigation. A member of the research team will instruct participants on how to irrigate each nasal cavity with one-half of the contents of the sinus rinse bottle. Written instructions and a video demonstration will also be provided to ensure proper technique. Because the half-life of theophylline in healthy adults (16-60 years) is 8.7

hours and 9.8 hours in the elderly (> 60 year), irrigations will be performed **twice daily** – once in the morning and once at night for all subjects,[71] for a total daily dose of 800mg theophylline nasal irrigation.

3.b Preparation and Administration of Study Drug

After faxing a signed prescription, the compounding pharmacy will formulate and directly ship the study drug regimen to the participant's provided mailing address via FedEx or via the United States Postal Service if the patient only has a PO box. All packages in transit can be monitored via the tracking numbers.

3.c Subject Compliance Monitoring

Participants will be evaluated at 6 weeks and at 12 weeks to ensure compliance with the study drug regimen as a self-reported measure. As a part of the REDCap surveys, patients will be asked whether they have been compliant with twice daily irrigations thus far, and what issues have arisen, if any.

3.d Prior and Concomitant Therapy

Participants receiving concomitant therapy specifically for the treatment of olfactory dysfunction will not be eligible for the trial unless they are able to stop taking those therapies. Participants who have tried previous therapies for their olfactory dysfunction (including over-the-counter treatments such as vitamin A or omega-3 fatty acids) will be eligible for the trial. Information will be gathered on their use of therapies, and if needed, a sensitivity or subgroup analysis may be considered.

F Study Procedures

F1 Screening for Eligibility

Identified patients through chart review as well as interested individuals who reach out after seeing the approved study flier will be contacted via email or phone call to introduce and explain the study. Interested individuals will be asked to complete an online screening questionnaire to ensure he or she meets all of the inclusion criteria and none of the exclusion criteria. Potential participants will also be able to begin the online screening questionnaire in REDcap via a QR code provided on the study flier, in which case they will receive contact from the study team after completing the first part of screening. Following eligibility confirmation, an informed e-consent will be acquired.

F2 Timeline of Measurements

Following the recruitment period, randomization of subjects will occur one week prior to the start of the trial. The timeline of measurements can be seen in Figure 3.

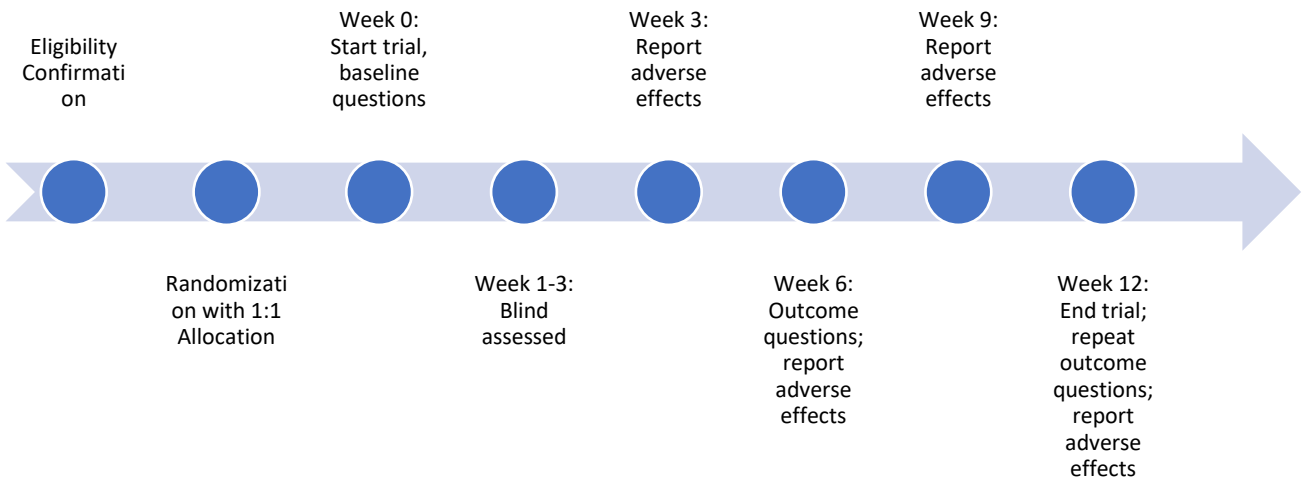

Figure 3. Timeline of measurements for SCENT3.

2.a Start of trial

Throughout the week before the first day of the trial, baseline tests will be administered as described in Figure 1. Participants will be mailed the UPSIT packet and will be required to complete all assessments prior to the first day of the trial through an online secure survey platform. A member of the study team will be available for any questions or concerns. Participants will also be provided instruction on how to properly conduct intranasal saline irrigation with theophylline or placebo. Participants will then receive the study drug by mail and will report their start date of nasal irrigation.

2.b Assessment of blind and adherence

Participants will be contacted via online secure survey platform or telephone within the first 3 weeks of initiating nasal irrigations and asked, “To which arm of the study do you think you were randomized?” Choices will be “theophylline and nasal saline irrigation” or “nasal saline irrigation alone”.

Participants will also be asked, “In the last 7 days, how many rinses have you completed? (For example, if you did 2 rinses every day for 7 days, the total would be 14).”

2.c 3-week and 9-week check-ins

Participants will be contacted via telephone and asked about adverse effects and any difficulties with adherence to medication. Adverse event forms will be filled out if adverse events are reported.

2.d 6-week interaction

At the 6th week of the trial, participants will repeat assessments as according to Figure 2 and submit them via online secure survey platform. Efficacy of theophylline nasal irrigation for 6 weeks can then be compared to efficacy at 12 weeks. They will also be asked to report any adverse events.

2.e End of trial

At the 12th week of the trial, patients will repeat assessments as described in Figure 2 and submit them via online secure survey platform. They will also be asked to report any adverse events. They will also be asked to count how many capsules are left in their bottle as a measure of adherence. This will conclude participation in the clinical trial.

F3 Safety and Adverse Events

3.a Safety and Compliance Monitoring

The specific monitoring plan for this study is based on the potential risk of participation and size and complexity of the planned investigation. Based on these considerations, this study will have a monitoring board comprised of Dr. Piccirillo, Ms. Kukuljan, and Dr. Kallogjeri, the study biostatistician. The monitoring board will meet to review data at least every 6 months. All reports of a Serious Adverse Event (SAE) or an Unexpected Adverse Event (UAE) will be investigated by the monitoring team and reported to Washington University HRPO according to the reporting requirements.

The PI and the study team will be monitoring patients for any safety concerns, such as SAE or UAE, in real time for the duration of the 12-week study. In addition, patients will be specifically asked about any adverse events at the 3-week, 6-week, 9-week, and 12-weeks via phone conversation. Participants will also be able to get in touch with a member of the study team 24/7 via phone or pager for the duration of the 12-week clinical trial. An adverse event form will be completed if a participant indicates an adverse event during phone check-ins or at any other time. The study statistician will hold the randomization codes, and in the event of an SAE or UAE in which the blind needs to be broken, the patient will receive immediate care and will be removed from the trial. A description of the event will be included in the report of adverse events for the clinical study.

Participants who experience serious adverse effects with theophylline therapy will be removed from the study. Participants with serious adverse effects, such as arrhythmia and seizures, will be instructed to call 911, seek immediate medical care and discontinue all further theophylline treatment. If tolerated, all participants will complete a total of 12 weeks of treatment.

G Statistical Plan

G1 Sample Size Determination and Power

In our previous phase II randomized placebo-controlled trial of nasal theophylline irrigation for post-COVID OD, we set a minimal clinically important difference (MCID) of 25% in the rate of patients with subjective improvement within the experimental group compared to the placebo group. We observed subjective improvement rates of 59% (13/22) in the theophylline arm and 43% (10 of 23) in the placebo arm, with a difference in response rate was 15.6% (95% CI -13.2 to 44.5%). The upper bound of 44.5% was much larger than the MCID of 25%, suggesting the potential for a clinically meaningful effect of theophylline despite this statistically insignificant measurement.

For a clinically definitive study with a feasible sample size of 200 participants, the true difference in proportions would need to be at least 29% such that the 95% CI around this difference has a lower bound of at least 15% (upper bound 41%).

Accounting for a 20% dropout rate, we plan to enroll 240 patients. The sample size of 240 patients is feasible based on the August 2022 numbers of new COVID-19 cases in Missouri (average of 1400 daily new cases) and Illinois (average of 4,300 daily new cases) with a conservative estimate of 10% rate of chronic olfactory dysfunction. Using a 20% drop-out and withdrawal rate, we estimate that the sample size of 240 patients will provide us with 200 evaluable cases.

G2 Analysis Plan

An intention-to-treat analysis will be used where all participants will be examined in the groups to which they were initially assigned regardless of the treatment actually received. Standard descriptive statistics will be used to assess the demographics, clinical characteristics, and olfactory test results of the study population. The **difference in rate of responders** at 12 weeks will estimate the effect size of the primary outcome measure, and the 95% CI around that point estimate will measure precision. In each group, the frequency and relative frequency of the participants' response to the global rating of smell change will be reported for each Likert category. Fisher's exact test will be used for comparing the responders' rates between the 2 groups at 6 weeks and at 12 weeks. Histograms and Shapiro-Wilks test will be used to test the normal distribution assumption of the continuously measured **ODOR** scores and the differences pre-post treatment in each of the groups. Independent samples *t*-test or its nonparametric equivalent Mann-Whitney U test will be used to compare the change in **ODOR** scores between the two groups. A mixed model analysis will be used to compare the change in outcomes through different study assessments between the study groups. Effect sizes with 95% CIs will be reported for each analysis. All statistical analyses will be conducted in SPSS 27 (IBM Corp., Armonk, NY).

G3 Missing Outcome Data

Maximum efforts will be made to limit the number of missing values. Missing outcomes will be taken into consideration at the beginning of each analysis. Valid percentages will be reported alongside overall percent for standard descriptive statistics, and data for participants who do not complete the full 12-week trial will be excluded for per-protocol analyses.

H Data Handling and Record Keeping

H1 Confidentiality and Security

Procedures that are in place to curb risks of breaches in confidentiality and privacy are 1) formal training protocols centered on the maintenance of confidentiality for all study team members; 2) secure storage for identified data forms such as completed questionnaires and UPSIT exams; and 3) communication with study team via secure email, phone line, or televideo call.

Only members of the study team will have access to the computer file and password for the master list. All research data files will be stored on secure Washington University servers with computer, network, and database-level passwords that will only be accessible to study team members. Accordingly, these mechanisms intend to limit access to information that can link clinical data to individual subjects. No subject identifying information will be revealed in any publications or presentations.

I Study Monitoring, Auditing, and Inspecting

I1 Study Monitoring Plan

The Principal Investigator (PI) will be responsible for ensuring participants' safety on a daily basis and for reporting Serious Adverse Events and Unanticipated Problems to his or her Institutional Review Board (IRB) as required.

J Study Administration

J1 Subject Stipends or Payments

Subjects will receive $80 at the completion of the 12-week study. If they stop the study early but complete at least 3 weeks of the study, they will be paid $10 for time and effort. If they complete at least 6 weeks, they will be paid an additional $10 ($20 total). If they complete at least 9 weeks, they will be paid an additional $20 ($40 total). If they stop the study earlier than 3 weeks, they will be thanked for their time and effort without monetary payment.

J2 Study Timetable

August-October 2022: IRB approval process and acquisition of resources
November 2022: Rolling recruitment
December-April 2023: 12-week clinical trial
May-June 2023: Statistical analysis and publication of results

K Publication Plan

We plan to analyze the accumulated data throughout the months of March to May 2023 and publish results by the end of June 2023. This data includes an analysis of the results of the CGI-I and ODOR questionnaires as well as a discussion of potential adverse effects related to intranasal theophylline irrigation for treatment of COVID-related olfactory dysfunction.

L Attachments

L1 Informed consent documents

L2 Questionnaires or surveys

- Clinical Global Impression Scale (CGI)
- Olfactory Dysfunction Outcomes Rating (ODOR) survey
- Screening questions, baseline questions, and follow-up questions (attached)

M References

1. Kaye R, Chang CWD, Kazahaya K, Brereton J, Denneny JC, 3rd. COVID-19 Anosmia Reporting Tool: Initial Findings. *Otolaryngol Head Neck Surg.* 2020;163(1):132-134.
2. Meng X, Deng Y, Dai Z, Meng Z. COVID-19 and anosmia: A review based on up-to-date knowledge. *American Journal of Otolaryngology.* 2020;41(5):102581.
3. Whitcroft KL, Hummel T. Clinical Diagnosis and Current Management Strategies for Olfactory Dysfunction: A Review. *JAMA Otolaryngology–Head & Neck Surgery.* 2019;145(9):846-853.
4. COVID-19 Dashboard by the Center for Systems Science and Engineering. Johns Hopkins University.
5. <219741_0_merged_1643772799.pdf>.
6. Menni C, Valdes AM, Polidori L, et al. Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study. *The Lancet.* 2022;399(10335):1618-1624.
7. Chen M, Pekosz A, Villano JS, et al. Evolution of nasal and olfactory infection characteristics of SARS-CoV-2 variants. *bioRxiv.* 2022.

8. Sedaghat AR, Gengler I, Speth MM. Olfactory Dysfunction: A Highly Prevalent Symptom of COVID-19 With Public Health Significance. *Otolaryngology–Head and Neck Surgery.* 2020;163(1):12-15.
9. Moein ST, Hashemian SM, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: a biomarker for COVID-19. *International Forum of Allergy & Rhinology.* 2020;10(8):944-950.
10. Menni C, Sudre CH, Steves CJ, Ourselin S, Spector TD. Quantifying additional COVID-19 symptoms will save lives. *The Lancet.* 2020;395(10241):e107-e108.
11. Carfì A, Bernabei R, Landi F, Group ftGAC-P-ACS. Persistent Symptoms in Patients After Acute COVID-19. *JAMA.* 2020;324(6):603-605.
12. Tan BKJ, Han R, Zhao JJ, et al. Prognosis and persistence of smell and taste dysfunction in patients with covid-19: meta-analysis with parametric cure modelling of recovery curves. *BMJ.* 2022;378:e069503.
13. Whitcroft KL, Hummel T. Olfactory Dysfunction in COVID-19: Diagnosis and Management. *JAMA.* 2020;323(24):2512-2514.
14. Catton G, Gardner A. COVID-19 Induced Taste Dysfunction and Recovery: Association with Smell Dysfunction and Oral Health Behaviour. *Medicina (Kaunas).* 2022;58(6).
15. Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nature Medicine.* 2020;26(5):681-687.
16. Rockx B, Kuiken T, Herfst S, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science.* 2020;368(6494):1012-1015.
17. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *New England Journal of Medicine.* 2020;382(12):1177-1179.
18. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chemical Neuroscience.* 2020;11(7):995-998.
19. Brann DH, Tsukahara T, Weinreb C, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Science Advances.* 2020;6(31):eabc5801.
20. Morbini P, Benazzo M, Verga L, et al. Ultrastructural Evidence of Direct Viral Damage to the Olfactory Complex in Patients Testing Positive for SARS-CoV-2. *JAMA Otolaryngology–Head & Neck Surgery.* 2020;146(10):972-973.
21. Politi LS, Salsano E, Grimaldi M. Magnetic Resonance Imaging Alteration of the Brain in a Patient With Coronavirus Disease 2019 (COVID-19) and Anosmia. *JAMA Neurology.* 2020;77(8):1028-1029.
22. Pekala K, Chandra RK, Turner JH. Efficacy of olfactory training in patients with olfactory loss: a systematic review and meta-analysis. *Int Forum Allergy Rhinol.* 2016;6(3):299-307.
23. Wilson DA, Best AR, Sullivan RM. Plasticity in the olfactory system: lessons for the neurobiology of memory. *Neuroscientist.* 2004;10(6):513-524.
24. Kollndorfer K, Kowalczyk K, Hoche E, et al. Recovery of Olfactory Function Induces Neuroplasticity Effects in Patients with Smell Loss. *Neural Plasticity.* 2014;2014:140419.
25. Hoffman HJ, Rawal S, Li CM, Duffy VB. New chemosensory component in the U.S. National Health and Nutrition Examination Survey (NHANES): first-year results for measured olfactory dysfunction. *Rev Endocr Metab Disord.* 2016;17(2):221-240.

26. Liu B, Luo Z, Pinto JM, et al. Relationship Between Poor Olfaction and Mortality Among Community-Dwelling Older Adults: A Cohort Study. *Ann Intern Med.* 2019;170(10):673-681.
27. Pinto JM, Wroblewski KE, Kern DW, Schumm LP, McClintock MK. Olfactory dysfunction predicts 5-year mortality in older adults. *PLoS One.* 2014;9(10):e107541.
28. Van Regemorter V, Hummel T, Rosenzweig F, Mouraux A, Rombaux P, Huart C. Mechanisms Linking Olfactory Impairment and Risk of Mortality. *Front Neurosci.* 2020;14:140.
29. Soler ZM, Patel ZM, Turner JH, Holbrook EH. A primer on viral-associated olfactory loss in the era of COVID-19. *International Forum of Allergy & Rhinology.* 2020;10(7):814-820.
30. Bitter T, Gudziol H, Burmeister HP, Mentzel HJ, Guntinas-Lichius O, Gaser C. Anosmia leads to a loss of gray matter in cortical brain areas. *Chem Senses.* 2010;35(5):407-415.
31. Murphy C, Schubert CR, Cruickshanks KJ, Klein BE, Klein R, Nondahl DM. Prevalence of olfactory impairment in older adults. *Jama.* 2002;288(18):2307-2312.
32. Cho SH. Clinical Diagnosis and Treatment of Olfactory Dysfunction. *Hanyang Med Rev.* 2014;34(3):107-115.
33. Schiffman SS, Warwick ZS. Flavor enhancement of foods for the elderly can reverse anorexia. *Neurobiol Aging.* 1988;9(1):24-26.
34. Hendriks AP. Olfactory dysfunction. *Rhinology.* 1988;26(4):229-251.
35. Reden J, Mueller A, Mueller C, et al. Recovery of olfactory function following closed head injury or infections of the upper respiratory tract. *Arch Otolaryngol Head Neck Surg.* 2006;132(3):265-269.
36. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. *N Engl J Med.* 2020.
37. Sorokowska A, Drechsler E, Karwowski M, Hummel T. Effects of olfactory training: a meta-analysis. *Rhinology.* 2017;55(1):17-26.
38. Konstantinidis I, Tsakiropoulou E, Bekiaridou P, Kazantzidou C, Constantinidis J. Use of olfactory training in post-traumatic and postinfectious olfactory dysfunction. *Laryngoscope.* 2013;123(12):E85-90.
39. Konstantinidis I, Tsakiropoulou E, Constantinidis J. Long term effects of olfactory training in patients with post-infectious olfactory loss. *Rhinology.* 2016;54(2):170-175.
40. Hura N, Xie DX, Choby GW, et al. Treatment of post-viral olfactory dysfunction: an evidence-based review with recommendations. *Int Forum Allergy Rhinol.* 2020;10(9):1065-1086.
41. Anosmia Rehabilitation in Patients Post Coronavirus Disease (COVID 19). In: https://ClinicalTrials.gov/show/NCT04374474.
42. COVID-19 Anosmia Study. In: https://ClinicalTrials.gov/show/NCT04495816.
43. Corticosteroid Nasal Spray in COVID-19 Anosmia. In: https://ClinicalTrials.gov/show/NCT04484493.
44. *Cerebrolycin for Treatment of Covid-related Anosmia and Ageusia.*
45. Moon C, Simpson PJ, Tu Y, Cho H, Ronnett GV. Regulation of intracellular cyclic GMP levels in olfactory sensory neurons. *J Neurochem.* 2005;95(1):200-209.
46. Pace U, Hanski E, Salomon Y, Lancet D. Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature.* 1985;316:255.
47. Barnes PJ. Theophylline. *Pharmaceuticals (Basel).* 2010;3(3):725-747.

48. Ronnett GV, Parfitt DJ, Hester LD, Snyder SH. Odorant-sensitive adenylate cyclase: rapid, potent activation and desensitization in primary olfactory neuronal cultures. *Proc Natl Acad Sci U S A.* 1991;88(6):2366-2369.
49. Anholt RR. Molecular neurobiology of olfaction. *Crit Rev Neurobiol.* 1993;7(1):1-22.
50. Neumann S, Bradke F, Tessier-Lavigne M, Basbaum AI. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. *Neuron.* 2002;34(6):885-893.
51. Henkin RI, Velicu I. cAMP and cGMP in nasal mucus: relationships to taste and smell dysfunction, gender and age. *Clin Invest Med.* 2008;31(2):E71-77.
52. Henkin RI, Velicu I. cAMP and cGMP in nasal mucus related to severity of smell loss in patients with smell dysfunction. *Clin Invest Med.* 2008;31(2):E78-84.
53. Henkin RI, Velicu I, Schmidt L. An open-label controlled trial of theophylline for treatment of patients with hyposmia. *Am J Med Sci.* 2009;337(6):396-406.
54. Levy LM, Henkin RI, Lin CS, Hutter A, Schellinger D. Increased brain activation in response to odors in patients with hyposmia after theophylline treatment demonstrated by fMRI. *J Comput Assist Tomogr.* 1998;22(5):760-770.
55. AlAjmi MF, Azhar A, Owais M, et al. Antiviral potential of some novel structural analogs of standard drugs repurposed for the treatment of COVID-19. *Journal of Biomolecular Structure and Dynamics.* 2020:1-13.
56. Elzupir AO. Caffeine and caffeine-containing pharmaceuticals as promising inhibitors for 3-chymotrypsin-like protease of SARS-CoV-2. *J Biomol Struct Dyn.* 2020:1-8.
57. Tomita K, Chikumi H, Tokuyasu H, et al. Functional assay of NF-kappaB translocation into nuclei by laser scanning cytometry: inhibitory effect by dexamethasone or theophylline. *Naunyn Schmiedebergs Arch Pharmacol.* 1999;359(4):249-255.
58. Henkin RI, Schultz M, Minnick-Poppe L. Intranasal theophylline treatment of hyposmia and hypogeusia: a pilot study. *Arch Otolaryngol Head Neck Surg.* 2012;138(11):1064-1070.
59. Goldstein MF, Hilditch GJ, Frankel I, Chambers L, Dvorin DJ, Belecanech G. Intra-Nasal Theophylline for the Treatment of Chronic Anosmia and Hyposmia. *Journal of Allergy and Clinical Immunology.* 2017;139(2):AB252.
60. Gupta S, Lee JJ, Perrin A, et al. Efficacy and Safety of Saline Nasal Irrigation Plus Theophylline for Treatment of COVID-19-Related Olfactory Dysfunction: The SCENT2 Phase 2 Randomized Clinical Trial. *JAMA Otolaryngol Head Neck Surg.* 2022.
61. Dunlop BW, Gray J, Rapaport MH. Transdiagnostic Clinical Global Impression Scoring for Routine Clinical Settings. *Behav Sci (Basel).* 2017;7(3).
62. Barnes PJ. Theophylline in Chronic Obstructive Pulmonary Disease. *Proceedings of the American Thoracic Society.* 2005;2(4):334-339.
63. Tzelepis GE, Bascom AT, Safwan Badr M, Goshgarian HG. Effects of theophylline on pulmonary function in patients with traumatic tetraplegia. *J Spinal Cord Med.* 2006;29(3):227-233.
64. Yu TJ, Liu YC, Chu CM, Hu HC, Kao KC. Effects of theophylline therapy on respiratory muscle strength in patients with prolonged mechanical ventilation: A retrospective cohort study. *Medicine (Baltimore).* 2019;98(2):e13982.
65. McKay SE, Howie CA, Thomson AH, Whiting B, Addis GJ. Value of theophylline treatment in patients handicapped by chronic obstructive lung disease. *Thorax.* 1993;48(3):227-232.

66. Henkin RI, Schultz M, Minnick-Poppe L. Intranasal Theophylline Treatment of Hyposmia and Hypogeusia: A Pilot Study. *Archives of Otolaryngology–Head & Neck Surgery.* 2012;138(11):1064-1070.
67. Hosein W, Henkin RI. Therapeutic diminution of Interleukin-10 with intranasal theophylline administration in hyposmic patients. *Am J Otolaryngol.* 2022;43(2):103375.
68. Lee JJ, Peterson AM, Kallogjeri D, et al. Smell Changes and Efficacy of Nasal Theophylline (SCENT) irrigation: A randomized controlled trial for treatment of post-viral olfactory dysfunction. *Am J Otolaryngol.* 2022;43(2):103299.
69. Lee JJ, Gupta S, Kallogjeri D, Piccirillo JF. Safety of High-Dose Nasal Theophylline Irrigation in the Treatment of Postviral Olfactory Dysfunction: a Dose-Escalation Study. *JAMA Otolaryngol Head Neck Surg.* 2022.
70. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol Behav.* 1984;32(3):489-502.
71. *Theophylline in Dextrose [package insert].* Lake Forest, IL: Hospira, INC;2008.