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STUDY PROTOCOL

A Phase 1a/1b Clinical Trial Design to Assess Safety, Acceptability, Pharmacokinetics and Tolerability of Intranasal Q-Griffithsin for COVID-19 Prophylaxis

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Abstract

Background: The COVID-19 pandemic remains an ongoing threat to global public health. Q-Griffithsin (Q-GRFT) is a lectin that has demonstrated potent broad-spectrum inhibitory activity in preclinical studies in models of Nipah virus and the beta coronaviruses SARS-CoV, MERS-CoV, and SARS-CoV-2.

Methods: Here, we propose a clinical trial design to test the safety, pharmacokinetics (PK), and tolerability of intranasally administered Q-GRFT for the prevention of SARS-CoV-2 infection as a prophylaxis strategy. The initial Phase 1a study will assess the safety and PK of a single dose of intranasally administered Q-GRFT. If found safe, the safety, PK, and tolerability of multiple doses of intranasal Q-GRFT will be assessed in a Phase 1b study. Group 1 participants will receive 3 mg of intranasal Q-GRFT (200 μL/nostril) once daily for 7 days. If this dose is tolerated, participants will be enrolled in Group 2 to receive 3 mg twice daily for 7 days. Secondary endpoints of the study will be user perceptions, acceptability, and the impact of product use on participants' olfactory sensation and quality of life.

Discussion: Results from this study will support further development of Q-GRFT as a prophylactic against respiratory viral infections in future clinical trials.

Background

Over the past 2 decades, three coronaviruses of the Betacoronavirus genus have emerged as serious human pathogens. The ongoing COVID-19 pandemic has caused over 620 million infections globally and over 1,060,000 deaths to date in the United States.

The virus that causes COVID-19, SARS-CoV-2, replicates efficiently in the upper respiratory tract—the nasopharynx and oropharynx—as well as in the lungs and gastrointestinal tissue.[1] High viral replication in the nasopharynx in the early stages of infection, prior to symptom onset, accounts for the high transmissibility of SARS-CoV-2. While ocular transmission has been reported [2–5], respiratory aerosol particles are the most frequent sources of human transmission events.[6, 7] Consequently, the development of an intranasal spray that inhibits replication of virus in the upper respiratory tract is likely an effective strategy to curb virus spread. In addition, this strategy will be complementary to vaccine approaches and biomedical interventions, such as personal protective equipment (PPE) and measures such as social distancing and frequent hand washing, in eliminating the pandemic.

The PREVENT-CoV (PRe-Exposure prevention of Viral ENTry of CoronaViruses) study seeks to demonstrate feasibility

for application of an intranasal drug administration approach as a technology to prevent the establishment of upper respiratory infection. This study will be the first-in-human application of Q-GRFT as an intranasal product.

Q-GRFT is an oxidation-resistant variant of Griffithsin (GRFT), a lectin initially extracted from red marine algae: Grifithsia sp.[8] GRFT and the engineered oxidation-resistant variant Q-GRFT, are manufactured using recombinant methods in *Nicotiana benthamiana* plants.[8] GRFT remains remarkably stable in the environment due to its thermal melting temperature of over 78 °C. The lectin resists digestion by human and bacterial proteases.[9, 10] Furthermore, GRFT has demonstrated negligible *in vitro* and *in vivo* host toxicity.[11] With broad-spectrum antiviral activity, GRFT and Q-GRFT are thought to bind oligomannose glycans that represent a significant fraction of the *N*-linked glycan molecules present on the heavily glycosylated coronavirus S protein.[12] GRFT strongly inhibits viral entry by binding to a broad array of coronaviruses, including SARS-CoV [13], MERS-CoV [14, 15], and SARS-CoV-2.[16, 17] GRFT also targets high-mannose glycan structures present on many pathogenic enveloped viruses—including nipah virus [18], hantavirus [19], influenza virus [20], human immunodeficiency viruses (HIV) types 1 and 2, herpes simplex virus type 2 (HSV-2), hepatitis C virus (HCV), Japanese encephalitis virus (JEV), and porcine epidemic diarrhea virus (PEDV)—while exhibiting a remarkable safety profile.[9, 21–28]

Our overarching goal is to develop Q-GRFT, the active pharmaceutical ingredient (API), as a non-vaccine, broad-spectrum pre- and post-exposure prophylactic against respiratory virus infections (including SARS-CoV-2) in the form of a daily administered nasal spray product. While highly effective vaccines against SARS-CoV-2 are available, the pandemic persists due to both breakthrough infections and vaccine hesitancy.[29–31] Additional tools for prevention are needed both to address risk of SARS-CoV-2 infection in highly vulnerable populations, such as organ transplant recipients, and to prepare for the next respiratory viral pandemic. To this end, an intranasal spray product such as Q-GRFT would provide a much-needed strategy for infection prevention, especially in light of the constant evolution of SARS-CoV-2, which will inevitably prolong the pandemic. While other studies have proposed developing intranasal products for utility against SARS-CoV-2 [32–35], our program is the first to evaluate intranasal administration of the Q-GRFT lectin and will establish nasal pharmacokinetic (PK) characteristics for this drug candidate when administered via this route.

The rationale for the development of a Q-GRFT intranasal spray is based upon several criteria. Prophylactic intranasal spray products are designed for the deposition of medications locally in nasal cavities or systemically. Local drug administration, the primary focus of our product, likely prevents or at least significantly reduces the acquisition of respiratory infections with a high concentration of product in the respiratory cavity. Local drug delivery is also a logical choice of product administration for viral respiratory infections, including SARS-CoV-2, whose initial point of entry/infection in the body occurs predominantly via the nasal cavity. Even though Q-GRFT may be subjected to local enzymatic activity in the nasal environment, studies elsewhere have demonstrated the lectin's ability to withstand and avoid degradation in the presence of protease activity.[36] Additionally, nasal administration is a convenient, easy-to-use approach and is widely accepted as a method of drug administration for a variety of disease processes [37, 38], even as chronic therapy.

Against this background, intranasal Q-GRFT delivery is an additive strategy and approach to the prevention of coronaviruses infection and will be synergistic with other proposed interventions, such as vaccines and PPE.

Here, we outline the clinical protocol for the planned PREVENT-CoV Phase 1a/1b clinical study, which will evaluate the safety, tolerability, and pharmacokinetics of intranasally administered Q-GRFT in healthy male and female volunteers. Secondary endpoints for the study will include user perceptions, acceptability, and the impact of product use on participants' olfactory sensation and quality of life. The data generated from this study will support further development of Q-GRFT in future clinical studies as a nasally administered drug candidate prophylactic against coronavirus infection.

Methods

Study objectives

The primary objective of this first-in-human intranasal exposure study is to evaluate the safety, tolerability, and PK of Q-GRFT administered by intranasal spray. Secondary objectives include the assessment of user perceptions and acceptability and the impact of product use on participants' olfactory sensation and quality of life.

Clinical study design

The Phase 1a study will be a randomized, double-blind, single-site trial, with participants assigned to receive a single dose of either placebo or Q-GRFT drug product. A safety evaluation of the single dose exposure will be performed. If the dose is found to be safe and tolerable, additional participants will be enrolled to receive multiple doses of intranasal Q-GRFT daily in a Phase 1b study for 7 days.

In the Phase 1a study (**Figure 1**), 18 participants will be ran-

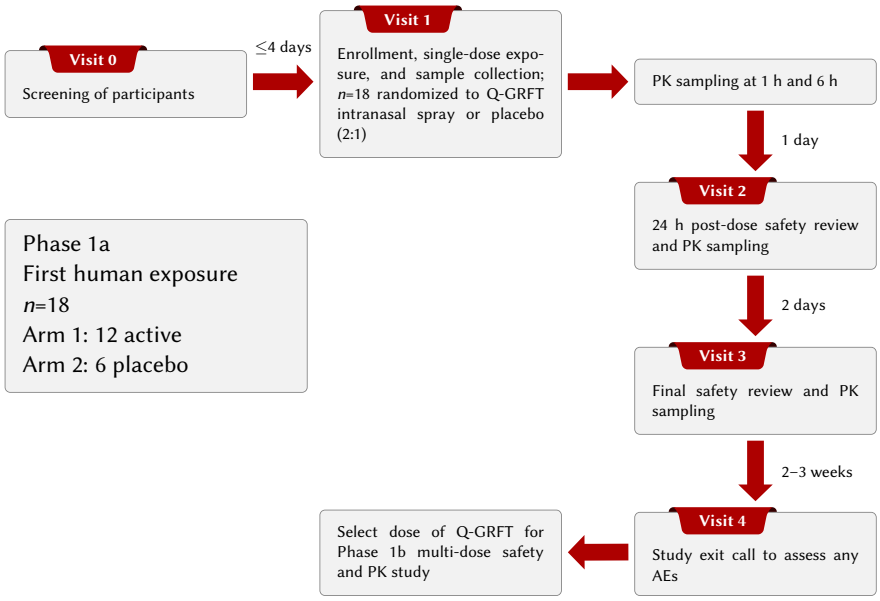

Figure 1. Schematic of phase 1a study design. **Abbreviations:** AE, adverse event; PK, pharmacokinetics.

Table 1. Phase 1a study arms.

Arm	*n*	Method of assignment	Study product	Dosing
1	12	Randomized, double-blind	Q-GRFT intranasal spray (7.5 mg/mL)	Single dose, one time
2	6	Randomized, double-blind	Placebo intranasal spray	Single administration

domly assigned 2:1 to either Study Arm 1 or 2 to receive either a single dose of study product or placebo under direct observation (**Table 1**). This is a small study, and the proposed sample size will allow the detection of any differences following drug administration in both active drug and placebo-treated arms. Due to the 2:1 randomization, stratification was balanced to safeguard for extreme allocation distribution (such as all females being assigned to the placebo arm). Although a "healthy population" will be enrolled, biological sex (male, female) and race (white and other) will be considered as stratification factors. In the placebo arm, the goal is to enroll three males and three females, with the group comprising four white persons (split between male and female) and two others (split between male and female); enrollment for the treatment arm will be double the distribution in the placebo arm. Participants in Arm 1 will receive a single dose of Q-GRFT study product, delivered intranasally as two sprays (200 μL per nostril), for a total dose of 3.0 mg. Participants in Arm 2 will receive a single dose of placebo product, delivered intranasally as two sprays per nostril. This intranasal product contains the same ingredients as the study product minus the active Q-GRFT component. Following administration of the intranasal product in both arms, PK sampling will be performed at multiple time points (1 hour, 6 hours, 24 hours, and 3 days post dose administration), followed by safety reviews after 24 hours, 3 days, and 2–3 weeks later by phone call.*

Phase 1b will be an open-label dose-escalation study assessing the safety, tolerability, and pharmacokinetics (PK) of a multiple-dose schedule of Q-GRFT intranasal spray AIP.

Up to 24 healthy participants will be enrolled and assigned to either of two groups to receive treatment (**Figure 2a** and **2b**). In Group 1, up to 12 participants will receive a dose of 3.0 mg intranasal Q-GRFT, administered once daily as 2 sprays (100 μL/ spray) in each nostril for 7 days (**Table 2**). Group 1 participants will undergo PK sampling (nasal and nasopharyngeal fluids) at baseline (enrollment visit); on days 1, 2, 4, and 7 (pre-dose, 1 hour, 6 hours, and 10 hours after the final dose); day 8, and day 9 following the final dose. Blood for evaluation of systemic exposure will be collected at baseline, on days 1 and 4, and on day 7 upon dose completion. A safety review will be performed after dosing in Group 1 prior to enrolling subjects in Group 2.

In Group 2, up to 12 participants will receive a total of 6.0 mg intranasal Q-GRFT, administered as 3.0 mg twice daily (3.0 mg BID) with 2 sprays (100 μL/ spray) in each nostril

*Trial status update: following determination that the single clinical dose administered was safe and acceptable, the study advanced to Phase 1b for assessment of multiple dosing schedules and strategies for Q-GRFT.

a)

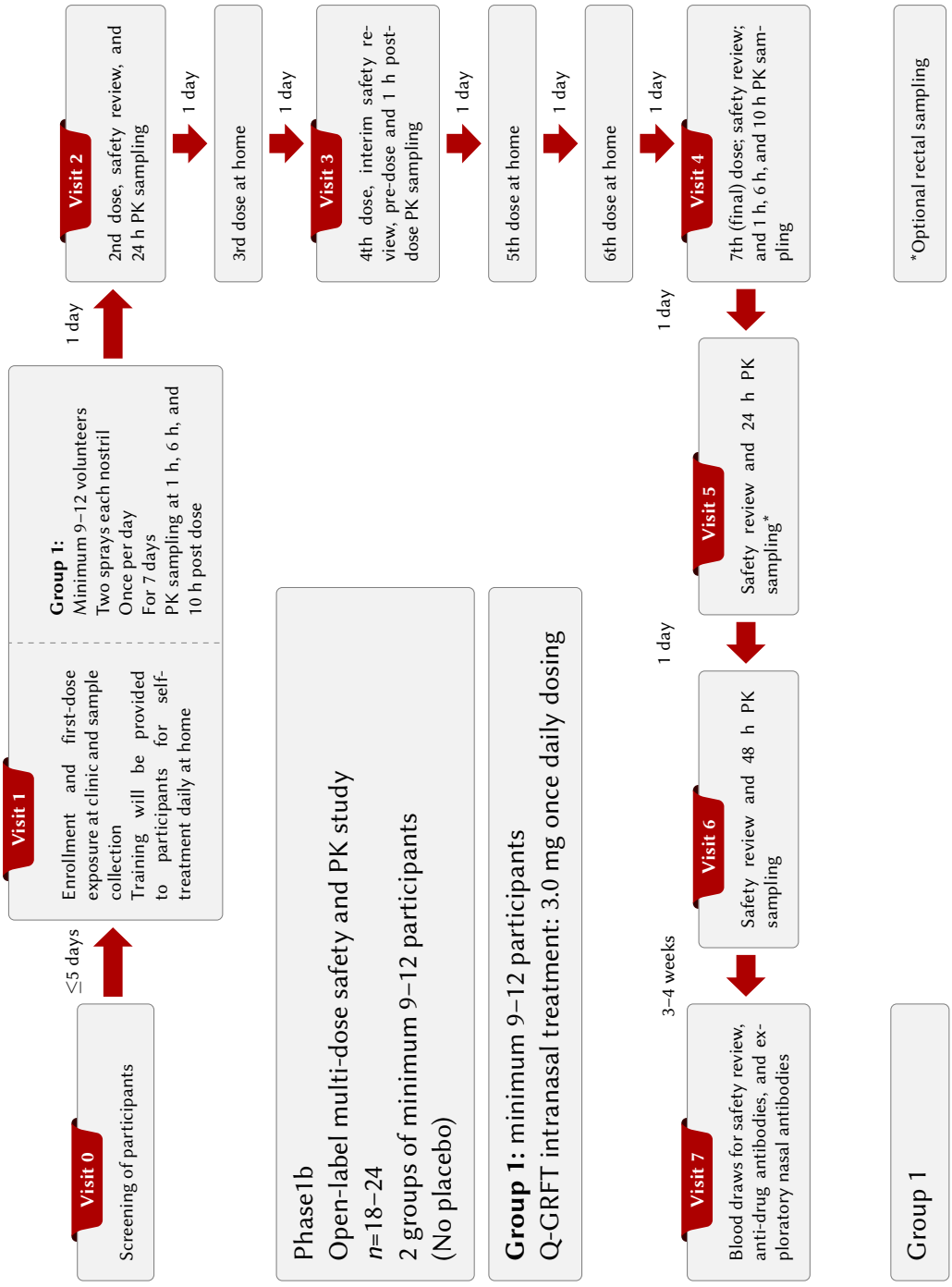

Figure 2. Schematic of phase 1b study design. **Abbreviations:** PK, pharmacokinetics.

b)

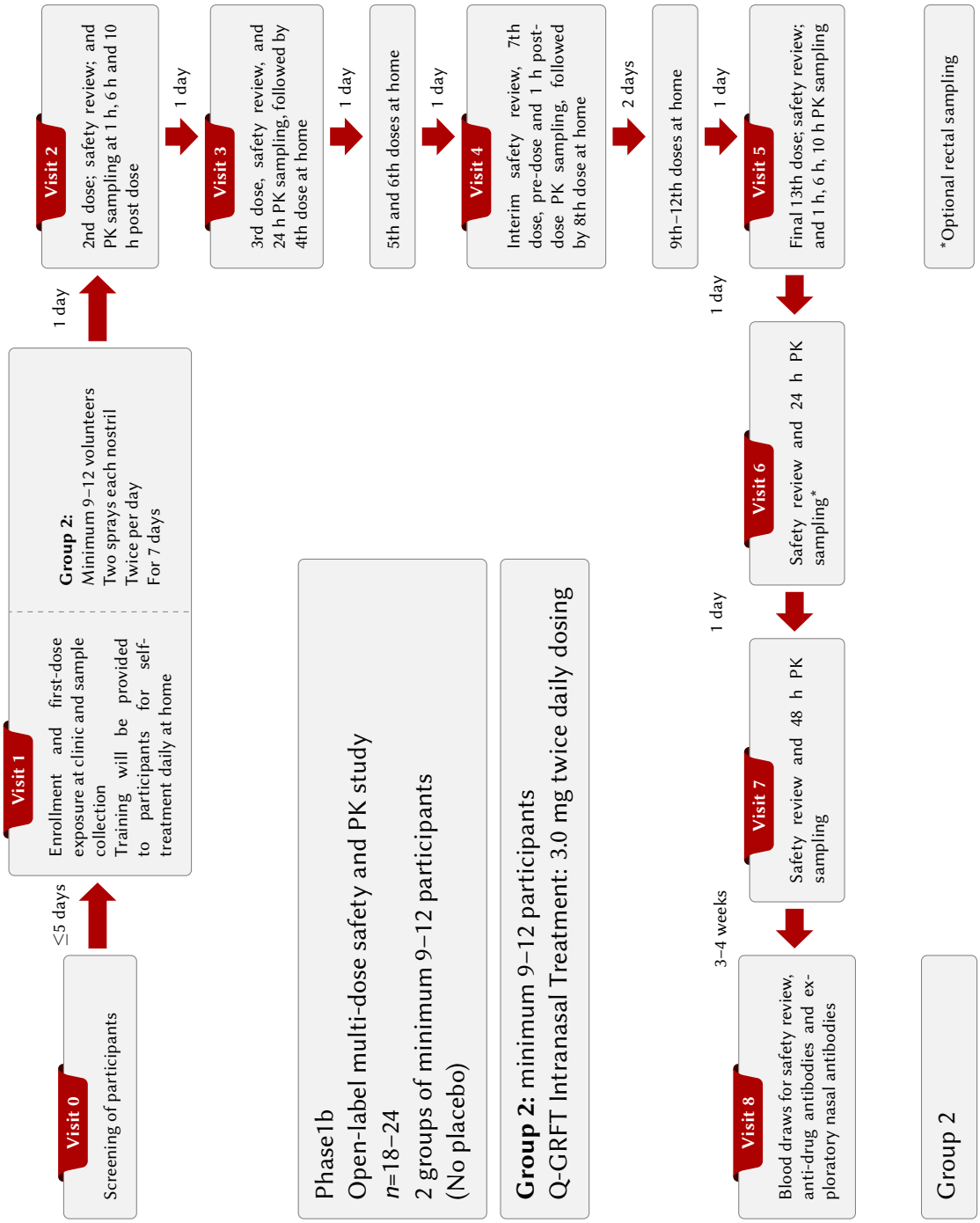

Figure 2 (cont'd).

Table 2. Phase 1b study arms.

Arm	*n*	Method of assignment	Study product	Dosing
1	12	Open-label	Q-GRFT intranasal spray (7.5 mg/ mL)	Multiple doses, two sprays per nostril (200 μL per nostril) once daily, for a total dose of 3.0 mg per day for 7 days
2	12	Open-label	Q-GRFT intranasal spray (7.5 mg/ mL)	Multiple doses, two sprays per nostril (200 μL per nostril) twice daily (12 hours apart), for a total dose of 6.0 mg per day for 7 days

approximately every 12 hours for 7 days (**Table 2**). Administration of the third dose among Group 2 participants will be delayed, enabling investigators to obtain a 24-hour PK timepoint for one completed 6.0 mg BID treatment. Participants in this group will undergo PK sampling (nasal and nasopharyngeal fluids) at baseline (enrollment visit); on day 2 (1 hour, 6 hours, and 10 hours after the second dose); days 3, 5, and 8 (pre-dose, 1 hour, 6 hours, and 10 hours after the final dose); and days 9 and 10. Blood for evaluation of systemic exposure will be collected at baseline, on days 2 and 5, and on day 8 after the final dose completion.

Safety assessment for both groups (Phase 1b) will be conducted after 3 days of dosing, upon completion of the final dose, and within 3–4 weeks of dose completion. An optional rectal fluids sampling procedure using a sponge will be performed 1 day after the final dose, to assess for any study product in the gastrointestinal tract. The body has the potential to generate antibodies against administered drugs. Because of this, anti-drug antibodies/immunogenicity assays will be performed at baseline, day 7, and 3–4 weeks after the final dose administration. All sampling procedures will be performed in the clinic.

Study participants/population

The study population will consist of healthy male or female individuals who will satisfy the following inclusion criteria.

Inclusion criteria

Participants will be aged 18–65 years at screening, have a negative SARS-CoV-2 test at screening, provide written informed consent, provide adequate information for locator purposes, be able to return for subsequent study visits, and agree not to participate in other research studies involving drugs and/or medical devices during the study period. In addition, female participants should not be pregnant at the baseline or enrollment, and not breastfeeding during the study. They will be encouraged to use contraceptive methods while in the study. Participants must be in general good health in the opinion of the investigator.

Exclusion criteria

Participants will be excluded if they have ongoing moderate to severe allergic rhinitis, asthma, chronic obstructive pulmonary disease (COPD), chronic rhinitis or sinusitis. Additionally, participants will not be enrolled if they report ongoing common cold or flu-like symptoms at screening. Participants with known moderate, severe, or higher seasonal allergies; smokers; those with a history of non-therapeutic injection and recreational drug use in the six months prior to screening; and those taking medications such as systemic steroids and intranasal medicines will not be enrolled.

Study product

The Q-GRFT intranasal spray product to be administered to subjects in the Phase 1a/1b studies contains Q-GRFT (7.5 mg/mL) as the API (10 mg/mL Q-GRFT stock solution in phosphate-buffered saline, pH 7.4) and water-soluble preservatives (parabens) in a viscosity modifying agent (lambda carrageenan polymer), along with a stabilizing agent, an acidifying agent, and solvents (PBS and purified water). The placebo intranasal spray used in Phase 1a is identical to the active product formulation minus the API. In Phase 1a, participants will receive two sprays per nostril (100 μL/spray) for a total dose of 3 mg Q-GRFT in the active arm group. The placebo group will receive two sprays per nostril (100 μL/nostril). In Phase 1b, Group 1 participants will receive two sprays per nostril (total dose 3 mg Q-GRFT) once daily for seven days, while Group 2 will receive two sprays per nostril twice daily for seven days.

Study procedures

Participant recruitment, screening, and retention: Participants will be recruited from a variety of sources, using key strategies that will include: clinician-patient referrals, use of existing "study registries" containing the names and phone numbers of individuals who have given informed consent to be reached for future studies for which they may be eligible, participant referrals (participants may refer their friends or partners who meet eligibility criteria), and passive

	Study period				
	Enrollment	Randomization	Follow-up		Completion
Timepoint	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4
Enrollment					
Eligibility screen	×	×			
Informed consent	×				
Medical history	×	×			
COVID-19 testing	×				
Randomization		×			
Interventions					
Study product dosing		×			
Assessments					
Physical exam and vitals	×	×	×	×	
Nose and throat exam	×	×	×	×	
Nasal, nasopharyngeal, oropharyngeal swab	×	×	×	×	
Complete blood count	×			×	
Comprehensive metabolic panel	×			×	
Cytokines evaluation	×			×	
Urinalysis					
Plasma (SARS-CoV-2 antibodies)	×				
Plasma (Q-GRFT detection)		×	×	×	
Olfactory function	×		×	×	
Quality of life	×			×	
Product acceptability questionnaire			×	×	×
Adverse events		×	×	×	×

Figure 3. Schedule of enrolment, interventions, and assessments for the Phase 1a study.

self-referral by interested individuals who see a study poster or brochure. Both male and female participants will be recruited, and strategies will be undertaken to ensure equitable opportunity to enroll for members of both biological sexes to the greatest possible extent. Additionally, members of minority groups that are representative of the diversity of Jefferson County, Louisville, KY, will be sought. Enrollment will be performed at the University of Louisville, Louisville, KY. The schedules of enrolment, intervention, and assessments for these studies are shown in **Figure 3** for Phase 1a and **Figure 4a–b** for Phase 1b.

Volunteers will be prescreened to determine eligibility. Eighteen participants will be enrolled in Phase 1a, while up to 24 will be recruited in the Phase 1b study.

Efforts will be undertaken to ensure that enrolled participants are retained for the duration of follow-up, minimizing loss-to-follow-up bias. This will be done through exhaustive explanation of the study visit schedule and procedural requirements during the informed consent process, both in-person and using a pre-recorded information video, and re-emphasis at each study visit; thorough explanation of the importance of all dosing and sampling phases to the overall success of the study; use of appropriate and timely visit reminder mechanisms (via email and/or telephone); and immediate and multifaceted follow-up on missed visits.

Informed consent, clinic visits, and assessment: All participants will provide written informed consent prior to participation in the study. Participants will have a right to withdraw from the study at any time for any reason.

Participants will be prescreened using online questionnaires and telephone interviews, and selected volunteers will be invited for the screening visit at the clinical trials unit.

In the Phase 1a study, at Visit 0, written informed consent will be obtained, along with locator information, demographics, and medical history. A brief physical examination, including a visual inspection of the nose and throat and an assessment of olfaction, will be conducted. A baseline quality-of-life questionnaire (Short Form-12 item [SF-12]) will also be administered. Blood, urine, nasal, and throat specimens will be collected from eligible participants and a rapid COVID-19 test performed. Baseline specimens (plasma samples; nasal, nasopharyngeal, and oropharyngeal fluids) will be collected using a swab for baseline PK, pharmacodynamics (PD), and cytokines evaluation.

Three to four days after screening (Visit 0), Visit 1 (enrollment and single-dose exposure) will occur. Eligibility will

a) Group 1

	Study period							
	Screening	Enrollment	Follow-up					Completion
Timepoint	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Enrollment								
Eligibility screen	×	×						
Informed consent	×							
Medical history	×	×	×	×	×	×	×	×
COVID-19 testing	×							
Interventions								
Study product dosing		×	×	×	×			
Assessments								
Physical exam and vitals	×	×	×	×	×	×	×	
Nose and throat exam	×	×	×	×	×	×	×	
Nasal, nasopharyngeal swab	×	×	×	×	×	×	×	×
Complete blood count	×			×				
Comprehensive metabolic panel	×			×	×			×
Cytokines evaluation	×			×				
Urinalysis	×			×		×		×
Plasma (SARS-CoV-2 antibodies)	×							
Olfactory function	×			×				
Quality of life	×				×		×	
Product acceptability questionnaire				×	×		×	×
Adverse events		×	×	×	×	×	×	×
Optional rectal sampling						×		

Figure 4. Schedule of enrolment, interventions, and assessments for the Phase 1b study.

be confirmed and a complete clinical assessment performed. Plasma samples will be obtained and stored for future research, and repeat blood tests will be performed where indicated for follow-up of any prior abnormal blood tests. Participants will then receive either the single-dose Q-GRFT API intranasal spray or placebo, two sprays into each nostril, per the randomization assignment, administered by the study clinician. PK sample collection will then occur at 1 hour and 6 hours post dosing.

Visit 2 will occur 24 hours post dosing and include clinical evaluation and PK sampling. Nasal, nasopharyngeal, and oropharyngeal fluids will be collected. Blood will be collected for cytokine evaluation and plasma stored for future research. Olfaction will be assessed using the Brief Smell Identification Test (BSIT), and an acceptability questionnaire will be completed.

Visit 3 will be the final safety evaluation and PK sampling visit, occurring two days after visit 2. Participants will complete a product acceptability questionnaire and the SF-12 and will receive a symptom-directed physical exam, including a nose and throat inspection. Olfaction will also be assessed. Blood will be collected for full hemogram and storage. In addition, nasal, nasopharyngeal, and oropharyngeal fluids will be collected and the product acceptability questionnaire administered.

A final Phase 1a study exit phone call (Visit 4) will be made within 2–3 weeks after visit 3 to inquire about any adverse events that the participant may have experienced as a result of product administration. The product acceptability questionnaire will be administered again in an online format. If the safety and tolerability of the single dose exposure is confirmed in Phase 1a, the study will proceed to Phase 1b.

Participants in Phase 1b will be enrolled and stratified into two groups. In Group 1, up to 12 participants will receive a dose of 3.0 mg intranasal Q-GRFT once daily, administered as two sprays (100 μL/spray) in each nostril, for seven days. The initial dose will be administered in the clinic by a clinician. Participants will be taught how to self-administer the study product at the clinic and receive written instructions for at-home self-administration. Subsequent doses will be self-administered either at the clinic or at home. Sampling will be performed for PK (nasal and nasopharyngeal fluids) at baseline (enrollment visit), on day 1 (1, 6, and 10 hours after the initial dose), day 2 (24$\pm$1 hours after initial dose), day 4 (pre-dose and 1-hour post-dose), day 7 (pre-dose; 1, 6, and 10 hours after the final dose), day 8 (24$\pm$1 hours), and day 9

(48±2 hours) following the final dose. Blood for evaluation of systemic exposure will be collected at baseline, days 1 and 4 (1 hour post-dose), and on day 7 upon dose completion.

Group 2 participants will be enrolled after all participants in Group 1 have been enrolled and at least six participants have received all seven doses, with a safety review done.

In Group 2, up to 12 participants will be enrolled to receive a total of 6.0 mg intranasal Q-GRFT, administered as 3.0 mg twice daily (3.0 mg BID) as two sprays (100 μL/spray) in each nostril approximately every 12 hours for seven days. Administration of the third dose will be delayed so that a 24-hour PK timepoint for one completed 6.0 mg BID treatment dose can be obtained. The initial dose will be administered in the clinic by a clinician. Participants will be taught to self-administer the study product at the clinic and receive written instructions for at-home self-administration. Subsequent doses will be self-administered either at the clinic or at home. Sampling will be performed for PK (nasal and nasopharyngeal fluids) at baseline (enrollment visit), on day 2 (1, 6, and 10 hours after the second dose), day 3 (24±1 hours after the second dose), day 5 (pre-dose after 3 completed doses of 6 mg and 1 hour post-dose), day 8 (pre-dose; 1, 6, and 10 hours after the final dose), day 9 (24±1 hours) and day 10 (48±2 hours) following the final dose. Blood for evaluation of systemic exposure will be collected at baseline, on days 2 and 5 (1 hour post-dose), and on day 8 after the final dose completion.

Safety assessment for both groups will be conducted after three days of dosing, upon completion of the final dose, and within 3–4 weeks of dose completion. An optional rectal fluids sampling procedure using a sponge will be performed one day after the final dose to assess for any study product in the gastrointestinal tract. Blood draws for anti-drug antibodies/immunogenicity assays will be performed at baseline, 6 hours after the last dose, and 3–4 weeks after the final dose administration. All specimen sampling procedures will be performed at the clinical trials unit, University of Louisville Hospital (**Figures 1** and **2**).

Drug and dose selection: Our extensive studies with rectal toxicology in rats and rabbits did not reach a maximum tolerated dose, even though animals were dosed with a 30 mg/mL gel formulation that delivered a tissue concentration measured in PK assays at more than 1,000 fold the EC_{90} of HIV-1.[39] Both mice and Syrian Golden hamsters have been treated with GRFT intranasally in efficacy studies against SARS-CoV [22] and NiV [18], respectively. Rhesus macaques have been exposed to nebulized GRFT. No toxicity has been reported in these studies, and this anticipation extends to the studies described in this proposal. Evidence of Q-GRFT distribution to the brain is perhaps the most significant toxicity risk that we may encounter, which will result in the need for extensive neurotoxicity assessments in the toxicology studies planned to support Phase 2 clinical studies.

Based on efficacy observed in the SARS-CoV, NiV, and MERS-CoV models, 7.5 mg/mL of Q-GRFT (total dose of 3.0 mg) will be administered to participants in Phase 1a. This dose is 1,000-fold higher than the effective dose (EC_{50}) that was protective in tissue exposure experiments performed by our group (unpublished).

Outcome measures

Phase 1a will determine the safety, acceptability, and PK of a single dose exposure of Q-GRFT administered intranasally. Phase 1b will evaluate the safety, tolerability, and pharmacokinetics of multiple doses of Q-GRFT administered intranasally. Safety will be assessed by incidence of adverse events, acceptability by a rating score from participants, and PK by detection of drug levels in nasal fluids collected.

Safety and tolerability

Clinical laboratory tests: Hematology, blood chemistry, and urinalysis will be performed for participants, as described in **Figures 3** and **4**. A complete blood count with differential, blood chemistry, urinalysis, and pregnancy test (βhCG) for female volunteers will be performed at the screening visit, with repeat hematology and a comprehensive metabolic panel performed at Visit 3, for Phase 1a participants. Evaluation for hematology, blood chemistry, and urinalysis will be performed for Phase 1b participants at screening, with repeat tests on days 2, 5, and 8. The screening tests will establish baseline values, while repeat tests will allow the assessment of any impact of intranasal Q-GRFT on body physiologic parameters.

Physical examination and nasal endoscopy: A physical examination will be performed per **Figures 3** and **4**. An abbreviated physical examination—including a nose and throat exam and routine examination of the lungs, heart, abdomen, skin, and central nervous system—will be performed. Subsequent nasal endoscopy will be performed by the study rhinologist during PK sample collection to ensure accuracy and consistency at all time points. The direct visualization afforded by endoscopy will allow investigators to swab the same anatomic location at each sampling time point, reducing sampling variability.

Vital signs: Vital signs (heart rate, blood pressure, temperature, and respiratory rate) will be measured as specified in **Figures 3** and **4** after the subject sits quietly for five minutes on all visits and as clinically indicated. This will allow the determination of any changes that may be attributed to product use.

b) Group 2

	Study period								
	Screening	Enrollment	Follow-up						Study completion
Timepoint	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Enrollment									
Eligibility screen	×	×							
Informed consent	×								
Medical history	×	×	×	×	×	×	×	×	×
COVID-19 testing	×								
Interventions									
Study product dosing		×	×	×	×	×			
Assessments									
Physical exam and vitals	×	×	×	×	×	×	×	×	
Nose and throat exam	×	×	×	×	×	×	×	×	
Nasal, nasopharyngeal swab	×	×	×	×	×	×	×	×	×
Complete blood count	×				×	×			×
Comprehensive metabolic panel	×				×	×			×
Urinalysis	×				×		×		×
Plasma (SARS-CoV-2 antibodies)	×								
Olfactory function	×							×	
Quality of life	×							×	
Product acceptability questionnaire					×	×		×	×
Adverse events		×	×	×	×	×	×	×	×
Optional rectal sampling							×		

Figure 4 (cont'd).

Bodyweight and height: Bodyweight and height will be recorded at the initial health assessment.

Olfactory assessment: Evaluation of olfactory function will be performed at the time points specified in **Figures 3** and **4**. Sense of smell will be determined using the University of Pennsylvania BSIT, a validated assessment of olfactory function.[40] The BSIT assesses an individual's ability to detect odors at a suprathreshold level. The test consists of 12 items provided in a 12-page booklet. On each page, there is a "scratch and sniff" strip embedded with a microencapsulated odorant along with four choice options. The participant smells the strip and chooses the odor from the four choices listed.[41] Scores range from 0 to 12. The BSIT has demonstrated good reliability (r=0.71) and takes less than five minutes to complete.

Any clinically significant change from baseline on follow-up assessments will be recorded as an adverse event (AE). If the changes are persistent, a full neurological evaluation will be performed.

Quality of life: Quality of life will be determined by the SF-12, a truncated, reliable version of the SF-36. The SF-12 uses 12 questions to evaluate eight domains that provide a general assessment of health-related quality of life from the participant's perspective.[42] The SF-12 includes subscales to assess mental and physical functioning. Subscales are scored according to publisher specifications and standardized to allow comparison with the general US adult population.

Any clinically significant change from baseline on follow-up assessments will be recorded as an adverse event (AE). If the changes are persistent, a full neurological evaluation will be performed.

Product acceptability assessment: Product acceptability, feasibility, and tolerability will be evaluated by a questionnaire as specified in **Figures 3** and **4**. Questionnaire items are derived from existing, validated questionnaires, adapted for the current study.[43] Participant experience and opinion of efficacy, sensory perceptions, spray characteristics, administration process, applicator design, and use regimen will be assessed. Responses help determine product characteristics disliked or considered likely to challenge future sustained use by participants. Items are rated on 5-point Likert scales. The proportion of participants reporting product characteristics considered a barrier to use will be calculated. A Likert scale rating of lower than 3/5 on a given item will be considered a 'dislike' or potential barrier to future

product use.

Pharmacokinetics assessment and *ex vivo* efficacy: Per **Figure 3**, in Phase 1a, nasal, nasopharyngeal, and oropharyngeal swabs will be collected at visits 0 (baseline), 1 (enrollment), 2 (24 hours post dose), and 3 (3 days post dose), to determine the drug concentration. In Phase 1b, Group 1 (**Figure 4a**), nasal and nasopharyngeal samples will be collected at visits 0 (baseline), 1 (enrollment), 2 (day 2 of dosing), 3 (day 4 of dosing), 4 (day 7 of dosing), 5 (24 hours after final dose), and 6 (48 hours post final dose). In Phase 1b Group 2 (**Figure 4b**), nasal and nasopharyngeal samples will be collected at visits 0 (baseline), 1 (enrollment), 2 (day 2 of dosing), 3 (day 3 of dosing), 4 (day 5 of dosing), 5 (day 8 of dosing), 6 (24 hours post final dose) and 7 (48 hours post final dose). Inhibition of SARS-CoV-2 infectivity will be assessed *ex vivo* by plaque reduction neutralization assay (PRNT), and correlations with Q-GRFT drug levels will be established.

Tolerability assessment and management—adverse events: Throughout the study, each participant's condition will be closely monitored. Signs and symptoms of possible adverse events (AEs) will be reported by the study participants or observed by staff. They will be elicited from the participants by using direct or indirect questions, such as "How have you felt since your last visit to the clinic?" and "Have you experienced any changes in your wellbeing since receiving the study product?" All AEs, whether reported by the subject, elicited by staff, or observed by the investigator, will be recorded. The start and end dates, AE-specific severity, relationship to study drug, and any actions taken to address the AE will also be documented. Outcomes, such as whether an AE results in death, requires or necessitates hospitalization, or causes any persistent or significant disability/incapacity; any intervention to prevent these outcomes; and whether the events and actions taken were reported to the Data and Medical Monitor, Institutional Review Board (IRB), and study sponsor, will be recorded.

Serious adverse events (SAEs) occurring in a study participant or any worsening of SAEs at any time during the study will be elicited and reported within 24 hours to the investigator, who will then immediately inform the Medical Monitor and IRB. If any pregnancy occurs during the study, the investigator will immediately notify the Medical Monitor and IRB upon learning of the occurrence.

Tolerability of Q-GRFT in study volunteers will be derived from the frequency of AEs and study withdrawal due to any reported discomfort during the dosing period. Per **Figures 3** and **4**, blood samples will be collected as previously described. Although not expected, if systemic toxicity is identified following drug administration, the subject will be re-evaluated with a full physical examination and repeat blood tests to confirm toxicity. If the repeat tests are normal, this will be documented. Additionally, if still abnormal, appropriate action will be taken and/or subsequent blood draws performed serially until resolution is documented.

Sample and data storage: Samples and data collected will be stored at the University of Louisville Clinical Trials Unit (CTU) and the Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases (CPM) laboratory, Louisville, KY. IRB approval will be sought for all the study procedures. Consent for sample collection and data storage will be provided by participants. All biospecimens collected will be de-identified, processed, and analyzed blindly until study completion. Similarly, electronic data will be de-identified, stored on secured servers with encryption, and accessed only by authorized users. A Research Electronic Data Capture (REDCap®) online database system hosted at the University of Louisville will be utilized to store, secure, organize, and analyze data.[44] Responses on paper questionnaires will be entered into REDCap® by study personnel. Upon conclusion of the study, the de-identified stored specimens and data will be made available for use by other researchers and investigators upon request to the Principal Investigator.

Planned analyses

Data analyses: All participants who receive the study product (placebo and active drug in Phase 1a, and active drug in Phase 1b) will be included in the safety and efficacy analyses. All intra-and inter-participant outcome measures collected during the study period will be analyzed by an experienced biostatistician.

Data will be summarized following recommended standard guidelines. Data from sets of patients that complete each study visit and any reasons for early study termination will be noted and compared across study arms. Descriptive statistics (median, mean, standard error, standard deviation, minimum, and maximum) will be used to summarize data for doses received and study visits completed. Similarly, demographic and baseline characteristics for participants in all groups will be summarized using descriptive statistics. Drug concentrations over the study period will be compared in both nasal cavities/epithelia and plasma to determine changes over time. Biomarker changes between pre- and post-dosing will be analyzed using paired tests. Descriptive summary statistics will be reported according to observed data, and missing data will not be imputed. Bayesian and likelihood estimates will be used for limited analyses where formal inferential statistics will be required for participants with missing follow-up assessments. A P-value ≤ 0.05 will be considered statistically significant.

Table 3. Analysis of safety event frequency for arms of size 12 (Q-GRFT nasal spray).

Event rate (%)	P (0 events)	P ($\geq$1 events)	P ($\geq$2 events)	P ($\geq$3 events)
1	0.886	0.114	0.006	0.000
5	0.540	0.460	0.118	0.020
10	0.282	0.718	0.341	0.111
15	0.142	0.858	0.557	0.264
25	0.032	0.968	0.842	0.609
35	0.006	0.994	0.958	0.849
45	0.001	0.999	0.992	0.958

Table 4. Analysis of safety event frequency for arms of size 6 (placebo spray).

Event rate (%)	P (0 events)	P ($\geq$1 events)	P ($\geq$2 events)	P ($\geq$3 events)
1	0.941	0.059	0.001	0.000
5	0.735	0.265	0.033	0.002
10	0.531	0.469	0.114	0.016
15	0.377	0.623	0.224	0.047
25	0.178	0.822	0.466	0.169
35	0.075	0.925	0.681	0.353
45	0.028	0.972	0.836	0.558

Safety analyses:

Concomitant/ongoing medications: Any concomitant medications, including those not related to the study product, will be coded according to the World Health Organization (WHO) drug dictionary and tabulated by dosing group.

Adverse events: Incidence of all treatment-related AEs and treatment-emergent AEs (TEAEs) will be summarized. A TEAE is an event that first occurs or worsens in intensity following the administration of a study drug. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to classify AEs by system organ class and preferred term. For incidence reporting, when participants report more than one AE coded to the same system organ class or preferred term, the participant will be counted only once for that system organ class or preferred term. Events that occur between the signing of informed consent and the first study drug administration will be recorded. A list of possible AEs, including the participant incidence of TEAEs, treatment-related AEs, SAEs, deaths, and AEs resulting in study termination, will be constructed. For the stratification of AEs by severity, the worst severity for each participant during the study will be presented. Appropriate generalized linear models will be used for comparisons of significant differences between groups.

In addition, for the safety analysis, the number and frequency of $\geq$Grade 2 AEs and $\geq$Grade 1 nasal cavity-related AEs will be tabulated for the intranasal administration method. To determine whether AEs are occurring excessively, the proportion of subjects that experience an AE will be calculated for each method of administration. A single summary outcome of this type (yes/no) can be reasonably assumed to follow a Bernoulli distribution. **Tables 3** and **4** show, for selected true underlying rates between 0.01 and 0.45, the probability of zero, one or more, and two or more subjects experiencing AEs in a sample group of participants, for the Phase 1a study.

With regard to safety, the Phase 1a study will be paused for clinical data safety review in the event that four or more study participants in the treatment arm or two or more participants in the placebo arm experience an AE $\geq$Grade 3. With 18 total subjects, the probability of three or more AE $\geq$Grade 3 events occurring given a true $\geq$Grade 3 AE rate is shown in **Table 5**.

In Phase 1b, the study will pause for clinical data safety review in the event that three or more study participants experience an AE $\geq$Grade 3. With up to 24 total participants, the probability of three or more AE $\geq$Grade 3 events occurring given a true $\geq$Grade 3 AE rate is shown in **Table 6**. **Table 7** shows the analysis of safety event frequency with a group size of 12 for participants who receive the Q-GRFT nasal spray.

Serious adverse events: In a similar manner to AEs above, SAEs will be listed and summarized. The sponsor will be notified of any SAEs within 24 hours of their discovery.

Clinical laboratory results: Blood samples will be evaluated in a qualified clinical laboratory. Descriptive statistics will be

Table 5. Analysis of safety event frequency for both arms of size 18.

Event rate (%)	P (0 events)	P ($\geq$1 events)	P ($\geq$2 events)	P ($\geq$3 events)
1	0.835	0.165	0.014	0.001
5	0.397	0.603	0.226	0.058
10	0.150	0.850	0.550	0.266
15	0.054	0.946	0.776	0.520
25	0.006	0.994	0.961	0.865
35	0.000	1.000	0.995	0.976
45	0.000	1.000	1.000	0.997

Table 6. Analysis of safety event frequency for both groups of size 24.

Event rate (%)	P (0 events)	P ($\geq$1 events)	P ($\geq$2 events)	P ($\geq$3 events)
1	0.786	0.214	0.024	0.002
5	0.292	0.708	0.339	0.116
10	0.080	0.920	0.708	0.436
15	0.020	0.980	0.894	0.720
25	0.001	0.999	0.991	0.960
35	0.000	1.000	1.000	0.997
45	0.000	1.000	1.000	1.000

used for actual values, and any changes from baseline will be tabulated by study visit. Clinical laboratory values outside of normal reference ranges post baseline will be recorded and analyzed using appropriate binary measures. Any changes from baseline to worsening severity in laboratory values will be recorded and analyzed with appropriate test statistics.

Vital signs: Data observed at baseline and any change from baseline will be determined for all measurements at clinic visits. These data will be summarized with descriptive statistics and analyzed using an appropriate generalized linear mixed model.

Statistical analysis for study objectives: As mentioned above, patients will be closely monitored for any AEs or toxicity with repeat blood draws until AE resolution. To account for inherent correlation, repeated measures statistics will be included in a repeated measures factor analysis with random effects. For the exploratory objectives, correlations will be determined between Q-GRFT drug levels obtained at PK sampling and SARS-CoV-2 infectivity *in vitro* and *ex vivo*. For olfactory sensation and quality of life, Spearman's statistics and Student's t-tests will be used for correlation analysis and comparisons between groups, respectively.

Discussion

The initial SARS-CoV pandemic was reported in 2002–2003 [13] while MERS-CoV outbreaks have continued to date since the first human case was identified in 2012.[14, 15] Coronaviruses have also been responsible for seasonal noncomplicated upper and lower respiratory tract infections.[45, 46] The ongoing COVID-19 pandemic caused by SARS-CoV-2 has resulted in unprecedented disruption in peoples' lives and had a serious negative impact on healthcare operations and the economy. Unlike the responses to the multiple epidemics and pandemics caused by coronaviruses in the past, the current COVID-19 pandemic has resulted in rapid and fast-paced development of effective vaccines to curb infection.[47]

Treatments that have received either Emergency Use Authorization or full approval by the U.S. Food & Drug Administration (FDA) for utility in treatment and/or prevention include Remdesivir [48]; monoclonal antibodies, such as sotrovimab and casirivimab plus imdevimab; and vaccines, including products marketed by Pfizer-BioNTech, Moderna, and Johnson & Johnson.[49] The search for more effective approaches and treatments is on-going.[50, 51] To supplement these strategies, a non-vaccine broad-spectrum prophylactic nasal spray is an ideal adjunctive approach to prevent virus infection and transmission. This is warranted since the long-term durability of antibodies in the elderly and immunocompromised individuals following vaccination is still under investigation.[52] Furthermore, vaccinated and previously infected individuals are still susceptible to re-infection with SARS-CoV-2.[29, 53] In addition, viral loads in asymptomatic individuals are high, with the virus easily transmissible.[54] The current spread and transmission of new viral variants complicate the current authorized/approved therapeutic and

Table 7. Analysis of safety event frequency for group size of 12 (Q-GRFT nasal spray).

Event rate (%)	P (0 events)	P ($\geq$1 events)	P ($\geq$2 events)	P ($\geq$3 events)
1	0.886	0.114	0.006	0.000
5	0.540	0.460	0.118	0.020
10	0.282	0.718	0.341	0.111
15	0.142	0.858	0.557	0.264
25	0.032	0.968	0.842	0.609
35	0.006	0.994	0.958	0.849
45	0.001	0.999	0.992	0.958

prevention modalities.[55]

Studies have shown that intranasal sprays can decrease the risk of acquisition and establishment of viral respiratory infections. In rodent models, an intranasal spray with low pH gel and antibody prophylaxis prevented infection with influenza virus.[56, 57] Pilot studies in humans with hypertonic nasal saline irrigation [58] and rupintrivir nasal spray [59] have demonstrated efficacy against viral upper respiratory infections, including rhinovirus colds. Our group and other researchers have shown that GRFT inhibits viral entry of all coronaviruses tested, including SARS-CoV, MERS-CoV, and SARS-CoV-2.[16, 21, 22] Moreover, delivery of GRFT to the upper respiratory tract provides significant protection from SARS-CoV [60], MERS-CoV (unpublished), and paramyxovirus Nipah virus [18] in animal models.

Given the existential need for prophylactic interventions to prevent the spread of the SARS-CoV-2 and other coronaviruses, Q-GRFT API in an intranasal spray will likely provide an additional opportunity to protect against SARS-CoV-2 infection and transmission.

Our PREVENT-CoV Phase 1a/1b study will assess the safety, acceptability, and pharmacokinetics of Q-GRFT intranasal spray as broad-spectrum prophylactic for coronavirus infections. This study will be the first proof of concept trial to determine whether a Q-GRFT intranasal spray formulation is safe and acceptable for human use. Clinically relevant endpoints have been carefully selected, including olfactory function and determination of PK parameters. Q-GRFT intranasal spray is a topically administered product with a low likelihood of systemic side effects.[39, 61] Although the expectation is the demonstration of a low risk of toxicity, careful assessments of safety, including systemic symptomatology, will be undertaken in this trial with special consideration for issues related to central nervous system toxicity. This is due to the potential for Q-GRFT exposure via the olfactory epithelium when administered through the nasal route. The design of this study will allow for the exploration of these questions while protecting the safety of participants.

For the drug to prevent coronavirus infection, it is critical that the product must be present in the appropriate anatomical site and at the ideal concentration throughout the period of exposure to infection. Consequently, the multicompartmental PK included in this study will generate important data that, in combination with the SARS-CoV-2 neutralization findings, will help determine whether the test product has the appropriate profile to support further development in future clinical trials.

Trial status

Approval to conduct this study was granted by the University of Louisville Institutional Review Board (IRB), (Phase 1a IRB# 21.0704 and Phase 1b IRB# 22.0224). FDA approval for use of intranasal Q-GRFT has been granted under IND 151381. The ClinicalTrials.gov identifier for the Phase 1a study is NCT05122260 and for Phase 1b is NCT05437029. Participant recruitment for the Phase 1a trial commenced on November 11, 2021 and was completed on February 14, 2022. Recruitment for the Phase 1b study started on June 15, 2022, and the last study visit occurred on September 8, 2022. The anticipated study completion date is November 1, 2022.

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