NASAL DELIVERY CONCEPTS FOR PROTEASE INHIBITORS, VACCINES, AND TESTING FOR VIRAL INFECTION TREATMENT AND PROPHYLAXIS OF COVID-19

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Nasal Vaccines and Efficacy

- First FDA approved nasal vaccine was Flu Mist in 2003 [6].
- Intramuscular vaccine injections might reduce the severity, but not the transmission of the virus [7].
- COVID-19 intranasal vaccine was found to prevent infection in the nose and lungs [7] .
- The immune response is triggered by the B-cells producing IgA at the mucosal sites of pathogen access [7]
- Inducing serum IgG and mucosal IgA are necessary for enhanced efficacy of a vaccine [7].
- Hassan, et al. (2020) found intranasal immunizations produce cross protection from induced cross-reactive antibodies; hence a single dose immunization for COVID-19 prevents upper and lower airway infection and transmission [7].

6. U. S. Food and Drug Administration. (2003). Approval letter -iInfluenza virus vaccine live, intranasal. https://wayback.archive-it.org/ Hassan, A.O., Kafai, N.M., Dmitriev, I.P.7993/20170723030839/https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm123753.htm
7. Hassan, A.O., Kafai, N.M., Dmitriev, I.P. &……. Diamond, M.S., (2020). A single-dose intranasal ChAd vaccine protects upper and lower respiratory tractsagainst SARS-Cov 2, *Cell.* , doi: https://doi.org/10.1016/j.cell.2020.08.026

Nasal Function and Physiology

- The nose accounts for over 50% of the total upper airway resistance [9].
- Nasal breathing normally delivers 80% of airflow to the lungs [8].
- The nasal cavity is covered by a thin mucosa which is well vascularized.
  - A drug molecule can be transferred quickly across the single epithelial cell layer directly to the systemic blood circulation without first-pass hepatic and intestinal metabolism [4].
- The architecture, structure and physico-chemical characteristics of the mucosa are important criteria for drug delivery [9].
- Nasal obstruction can be a drug side effect [10].
  - 20 million Americans suffer from nasal airway obstruction (NAO); AAAAI Practice Statement for Provocation Testing and NAO.
- Nasal function studies identify and calculate the vertex, effective, and mean cross sectional area.

8. Hanif, J., Jawad, S. S., & Eccles, R. (2000). The nasal cycle in health and disease. *Clinical otolaryngology and allied sciences*, *25*(6), 461–467. https://doi.org/10.1046/j.13652273.2000.00432.x
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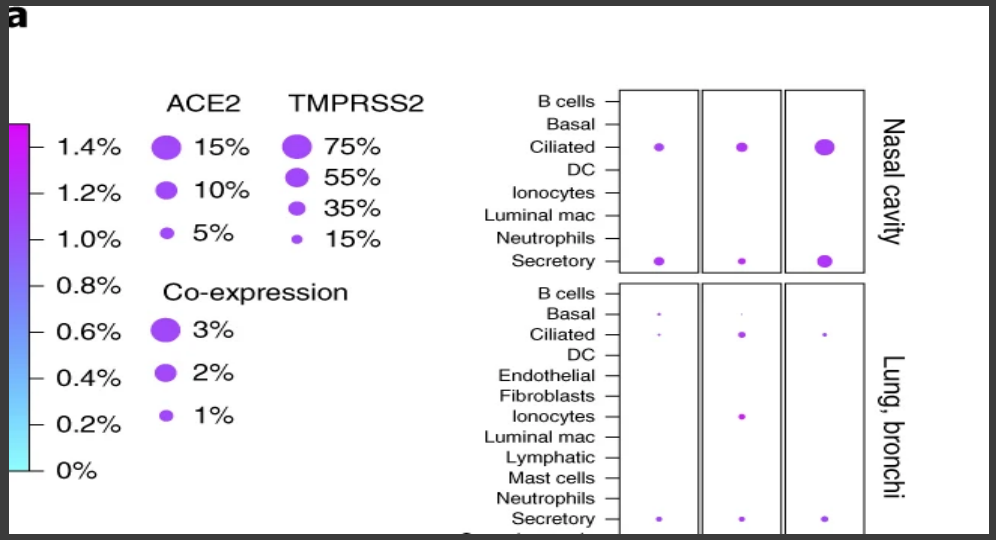
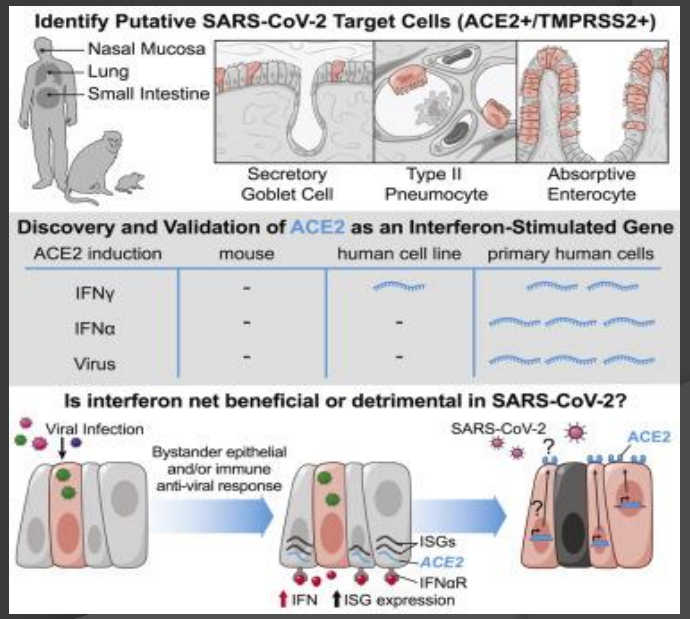
10. Cingi, C., Ozdoganoglu, T., & Songu, M. (2011). Nasal obstruction as a drug side effect. *Therapeutic Advances in Respiratory Disease*, 175–182. https://doi.org/10.1177/1753465811403348

Nasal Function and Physiology

- High concentration of ACE 2 and TMPRSS2 receptors in nasal goblet cells and ciliated epithelial cells are present in the nose than in the lungs and bronchi; ACE2 and TMPRSS2 co-expression in respiratory tissues is consistently found only among a rare subset of epithelial cell [12].
- The nasal epithelium encloses lymphoid tissues necessary for an immune response.





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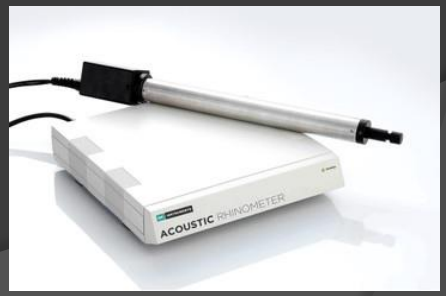
Nasal Function Testing

- The NR-6 filter is compliant and reported to be 99.999% efficient and validated against the passage of a variety of bacterial and viral species of varying particle sizes to include COVID-19.
- A major concern for some of nasal therapeutic agents is poor absorption [12].
- Objective, non-invasive nasal measurements confirm, qualify, and quantify the appropriateness of patient candidacy for nasal delivery through real-time data at the time of visit.
- Nasal function through 4-phase rhinomanometry should be tested first as intranasal pressure determined by flow and resistance, ciliary beat frequency, not geometrical measurements, assist in drug bioavailability.
- The only FDA approved measurement devices indicated for use in the pediatric, less than the age of 5, and adult populations.
  - 4-phase Rhinomanometry
  - Rhinometry



12. Ghadiri, M., Young, P. M., & Traini, D. (2019). Strategies to Enhance Drug Absorption via Nasal and Pulmonary Routes. *Pharmaceutics*, *11*(3), 113. https://doi.org/10.3390/pharmaceutics11030113

Intranasal Delivery

- Direct “nose-to-brain delivery” by-passing the blood-brain barrier
  - Nasal Compartmentalization treatment allows higher dosing and avoids tracheal and bronchial irritation / hemoptysis
- A growing body of evidence shows intra-nasal administration as a painless, controlled alternative for achieving systemic therapeutic effects of drugs that are
  - Comparable to the parenteral route, which can be inconvenient at times, and
  - Oral administration can result in unacceptably low drug bioavailability [13,14].
  - Nasal sprays are one of the simplest and most convenient delivery systems among all formulations with minimal side effects.
- Intranasal delivery demonstrates attacking virus at its point of invasion
- Easily accessibility of frequent sample feedback as to viral load and drug response
- Allows prophylaxis in a localized tissue area without total body saturation by drug.

13. Hanif, J., Jawad, S. S., & Eccles, R. (2000). The nasal cycle in health and disease. *Clinical otolaryngology and allied sciences*, *25*(6), 461–467. https://doi.org/10.1046/j.13652273.2000.00432.x
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Intranasal Drug Safety

- Intranasal antibody prophylaxis has shown great promise against viral respiratory tract infections in animals and is just beginning to show clear efficacy in human clinical studies [15].
- Once symptoms of respiratory tract disease manifest themselves Weltzin and Monath (1999) found it may be too late for antibody treatment to alter the course of illness [15].
- In a study to determine whether a topical formulation of camostat represents an efficacious and tolerable approach to reducing Na+ transport in the CF airway, Rowe et al. (2013) found there was no significant change in chloride transport or total nasal symptom score, nasal examination rating, and laboratory parameters [16].
- Yamaya et al. (2020) pretreated primary cultures of human nasal epithelial (HNE) cells with camostat and found that protease inhibitors may inhibit coronavirus 229E replication in human airway epithelial cells at clinical concentrations [17].

15. Weltzin, R., & Monath, T. P. (1999). Intranasal antibody prophylaxis for protection against viral disease. *Clinical microbiology reviews*, *12*(3), 383–39
16. Rowe, S. M., Reeves, G., Hathorne, H., Solomon, G. M., Abbi, S., Renard, D., Lock, R., Zhou, P., Danahay, H., Clancy, J. P., & Waltz, D. A. (2013). Reduced sodium transport with asal administration of the prostasin inhibitor camostat in subjects with cystic fibrosis. *Chest*, *144*(1), 200–207. https://doi.org/10.1378/chest.12-2431
17. Yamaya, M., Nishimura, H., Deng, X., Kikuchi, A., & Nagatomi, R. (2020). Protease Inhibitors: Candidate Drugs to Inhibit Severe Acute Respiratory Syndrome Coronavirus Replication. *The Tohoku journal of experimental medicine*, *251*(1), 27–30. https://doi.org/10.1620/tjem.251.27

Intranasal Drug Delivery

Figure 1 is the proprietary, patent protected Saved Nasal Inflammatory Fluid Flow Lavage Exhalation System (SNIFFLES) device for drug delivery and Figure 2 is the mechanism of action:

Drug powder or liquid picks up virus and launches airborne by exhalation from the contralateral nostril with droplet/powder transmission of the virus with an unknown duration of infectiousness dependent on the powder and liquid , its duration of being airborne and the surface that it is deposited on.

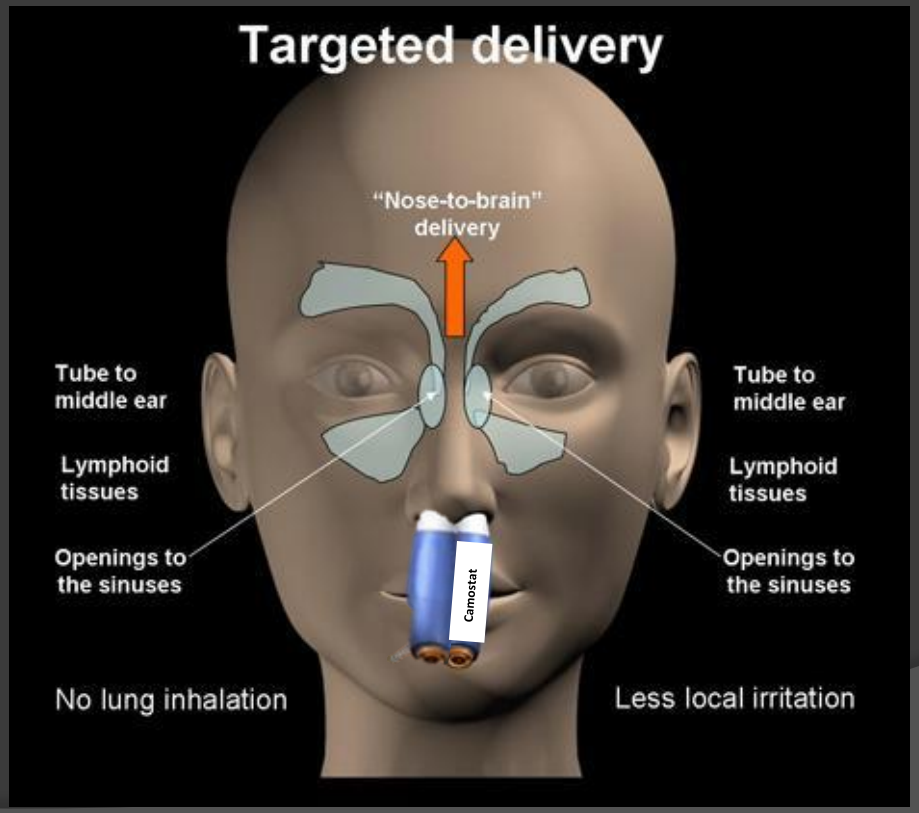


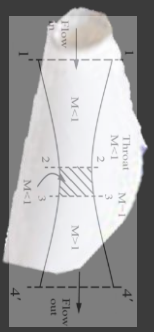
Figure 1. SNIFFLES

Receiving Nasal nozzle tapers with a movable flange . Nozzle contains a convergent-divergent double cone.

Trap door release

Receiving nozzle does not have a fluid well like delivery nozzle.

Reusable cartridge carrier with nasal nozzle



Port for aspirating contents with a pipette through one way valve .

One way valve .

Spring drop door
For unloading and loading nasal fluid retention cartridge.

Figure 2. Mechanism of action for the Saved Nasal Inflammatory Fluid Flow Lavage Exhalation System (SNIFFLES) Device

ADDITIONAL THERAPEUTICS

PROTEASE INHIBITORS

Camostat

- SARS-CoV-2 exploits the cell entry receptor protein angiotensin converting enzyme II (ACE-2) in the nasal cavity binding to 2-3% of the nasal mucosa [1].
- This process requires the serine protease TMPRSS2 [1].
- Camostat Mesilate is a potent serine protease inhibitor.
- Hoffman et al. (2020) demonstrated that SARS-CoV-2 cellular entry can be blocked by Camostat Mesilate [1].
- Zhou et al. (2015) found by employing a pathogenic animal model of SARS-CoV infection, they demonstrated that viral spread and pathogenesis of SARS-CoV is driven by serine rather than cysteine proteases and can be effectively prevented by Camostat [2].
- In mice, Zhou et al. (2015) found Camostat Mesilate dosed at concentrations similar to the clinically achievable concentration in humans reduced mortality following SARS-CoV infection from 100% to 30-35% [2].

1. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052
2. Zhou, Y., Vedantham, P., Lu, K., Agudelo, J., Carrion, R., Jr, Nunneley, J. W., Barnard, D., Pöhlmann, S., McKerrow, J. H., Renslo, A. R., & Simmons, G. (2015). Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral research*, *116*, 76–84. https://doi.org/10.1016/j.antiviral.2015.01.011

Bromhexine/Bromhexine Cocktail

- Brought to the market in 1963 as an over- the-counter drug.
- A derivative of a the *Adhatado plant,* used for respiratory conditions [3].
- Bromhexine has been found to improve respiratory symptoms [3].
- Bromhexine is safe, efficacious, global, and affordable.
- Endonasal Bromhexine is a good alternative to elude the first pass effect.
- Depfenhart et al. (2020) suggest that SARS-CoV-2 cellular entry can be inhibited by using Bromhexine to block TMPRSS2-specific viral activity [4].
- Bromhexine is able to inhibit serine protease and has a role in preventing influenze [4].
- In a clinical trial of 78 ICU patients, Tolouian and Mulla (2020) found a tremendous advantage after 2 weeks of use Bromhexine, 8mg tid, as an add-on for lowering morbidity and mortality rates [5].
- Qualitative C-reactive protein in all Bromhexine patients turned negative while 83% of the control group still had a positive [5].

3. Zanasi, A., Mazziloni, M., & Kantar, A. (2017). A reappraisal of the mucoactive activity and clinicall efficacy of bromhexine. *Multidisciplianry Respiratory Medicine, 12,* (7). https://doi.org/10.1186/s40248-017-0088-1
4. Depfenhart, M., de Villiers, D., Lemperie, G., Meyer, M., & Di Somma, S. (2020). Potential new treatment strategies for COVID-19: is there a role for bromhexine an add-on therapy? *Internal and Emergency Medicine, 15*(20). doi: 10.1007/s11739-020-02383-3
5. Tolouian, R., & Mulla, Z. D. (2020). Controversy with bromhexine in COVID-19; where we stand. *Immunopathologia Persa, 7*920. doi 10.34172/ipp.2021.12.

Clinical Protocol

- Phase I-III: In vivo, animal, and human studies
- Phase IV: Use of Nasal Camostat and/or Bromhexine for COVID 19 with nasal delivery device and testing system for early diagnosis and treatment with Nasal Function Studies
  - N = 100-300
  - Proposed dosing at 1.6mg ISIN, bid x 3-5 days
  - Placebo NS
  - Multi-center, double blinded, randomized study method
  - 5 weeks with primary and secondary outcome endpoints

- Phase V: Camostat and/or Bromhexine Cocktail consisting of Furin Cleavage Protease Inhibitor (PRRARSV) and Lisosome, Cathepsin-L Treatment.
  - Finalizing study protocol with Dr. Peter Libby, Harvard Medical School and Brigham and Women's Hospital, and Dr. Guo-Ping Shi, Harvard Medical School [11].
  - Patient Registry

18. Liu, T., Luo S, Libby, P., & Shi, G.P. (2020, May 25). Cathepsin L-selective inhibitors: A potentially promising treatment for COVID-19 patients. *harmacology & Therapeutics*. doi: https://doi.org/10.1016/j.pharmthera.2020.107587

Healthcare Financial Aspects of Nasal Therapeutics

Current COVID-19 Cases Valuation to the U.S. Healthcare System	
# number of confirmed positive tests*	6.6M
Cost of care reimbursed to the provider $51-100 per test	$336M-666M
Cost of Care to the U.S. Hospital System**	$179B
Cost of one treatment cycle [3-5 days] of Nasal Therapy/System (device + drug) per patient	$24.92 - 32
Cost of Nasal Function Studies (A1 & NR6) per patient	$15
National Avg Reimbursement Rate of Nasal Function Study (CPT 92512) and Revenue Flow to HCP per patient ***	$60.37
Total Cost of Treatment Using Nasal Therapy (HCP, Test, Tx, RI)	$997.9 – 1.15B
Cost Savings to the U.S. Hospital System Using Intranasal Tx and Prophylaxis to Avoid Hospital LOS	$177.5B

- CDC as of 9/13//2020

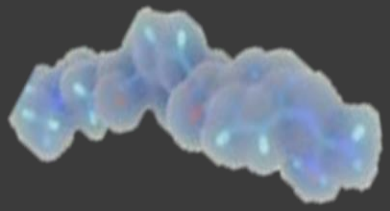
** American Hospital Association (AHA) (2020)

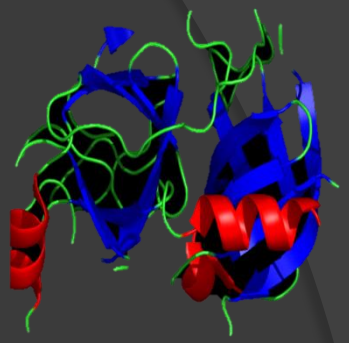
*** Centers for Medicare and Medicaid (2019)

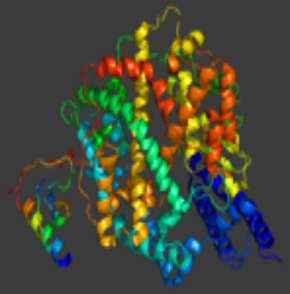
Conclusions

- We have to opportunity to cost effectively address a global pandemic among populations of various ages at a global scale.
- The research and therapeutic concept of nasal delivery therapeutics is
  - To prevent aspiration into the lungs, entry into the endovascular system, and prevent the cytokine storm from occurring.
  - To treat the nasal port of entry during the resting and proliferation phase of the virus
- Repurposing oral medications with a delivery device and nasal function testing Provides:
  - Early Diagnosis/Prophylaxis
  - Treatment
  - Evaluation of Treatment for COVID 19 Respiratory-EndoVascular Disease (SAREVs CoV-2)
- Global nasal delivery approach beyond Camostat and Bromhexine using SNIFFLES
  - Vaccines
  - Additional therapeutics (GERD)
  - Immunizations

COVID 19 Public Health Treatment Research Team







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