Pa

INTERFERON TO PREVENT RESPIRATORY ILLNESS EPIDEMICS

UNIVERSITY OF WASHINGTON STRATEGIC ANALYSIS, RESEARCH & TRAINING (START) CENTER

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Background

The COVID-19 pandemic has significantly impacted nearly all aspects of people's lives globally, and more than one year after its first recognition, highly effective agents for prevention and treatment of COVID-19 are still limited. One of the major lines of defense humans possess against viral infections is the interferon (IFN) system. IFNs are part of the innate immune system, and they help control most viral attacks, even in the absence of adaptive immunity (Randall and Goodbourn 2008). With most human cells having some type of IFN receptors on their surface, IFNs are capable of inducing expression of several hundred IFN-stimulated genes, encoding proteins that either block viral replication or trigger apoptosis of infected cells (Abernathy 1987; Katze, 2002).

Based on their genetic, structural, and functional differences and receptors on host cell surfaces, IFNs are grouped into three distinct types, namely type I IFNs (IFN-α, IFN-β, IFN-κ, IFN-ω, IFN-ν); type II IFNs (IFN-γ); and type III IFNs (IFN-λ) (Li, 2018). Although type II IFNs are known to possess antiviral activity, they are only secreted by a specific group of immune cells, whereas type I and type III IFNs can be produced by both immune and parenchymal cells, conferring upon them a robust antiviral activity (Stanifer, 2019; Kang, 2018). In addition, type III IFNs are produced by epithelial cells even in the absence of a strong inflammatory response (Stanifer, 2019; Lazear, 2019). These features make type I and III IFNs a powerful defense system against viral infections. If potent IFN-based agents, whether administered for prevention or early treatment of a viral infection, can be developed, and stored in sufficient quantities, the negative impact of future viral respiratory illness epidemics may be mitigated earlier in the epidemic response while pathogen-specific agents are being developed.

Similarly, given the critical role that IFN-stimulating agents, including toll-like receptors (TLRs), play in viral infections, they can also be potentially useful as a first line of defense against an emerging pandemic (Carty, 2010; Mifsud, 2014; To, 2019). Before IFNs can be released, the innate immune system detects the presence of chemical substances found on the pathogens' structures or released by pathogens through an array of protein molecules called pattern-recognition receptors (Katze, 2002). Pattern-recognition receptors recognize "danger signals", which include pathogen-associated molecular patterns, characteristic of the pathogens, or damage-associated molecular patterns. The most studied pattern-recognition receptors are TLRs, and, currently, 10 human TLRs have been well characterized (Katze, 2002, Amarante-Mendes, 2018).

IFN is related to viral respiratory disease susceptibility and progression. Recent studies have shown that IFN induces the so-called cytokine storm consisting of consisting of interleukin-1β, IL-6 and TNF-α—often markers of progression from mild or moderate to severe COVID-19 infection (García-Sastre, 2017; Acharya, 2020; Devasthanam, 2014). Similarly, individuals with IFN deficiency

or autoantibodies appear more susceptible to COVID-19 infection or greater severity of COVID-19 (Bastard, 2020). This indicates that progression of viral respiratory illnesses from mild to severe forms may be associated with impaired host IFN response. Relatedly, many viruses have developed evolutionary ways to suppress IFN induction or evade IFN responses (García-Sastre, 2017; Hoffmann, 2015). For instance, SARS-CoV-2, the causative agent of the ongoing COVID-19 pandemic, has developed the ability to suppress IFN induction, particularly type I IFNs (Acharya, 2020). Despite this finding, SARS-CoV-2 may be still be susceptible to the antiviral activities of type I and type III IFN, both in vitro and in vivo (Vanderheiden, 2020). For example, one recent study suggests that inhaled, nebulized IFN-β-1a may be safe and efficacious in the treatment of early and mild COVID-19 (Monk, 2020). Furthermore, there are at least thirty clinical trials underway that are testing the efficacy of IFN-based agents to prevent SARS-CoV-2 infection, reduce severe progression, and treat COVID-19.

Given the potential role IFN-based agents may play in the mitigation of future viral respiratory epidemics, our overall goal was to conduct a scoping review to summarize the literature on IFN or IFN-stimulating agents, such as TLR-agonists, on primarily viral respiratory infections to understand their role in preventing future human epidemics. This review was conducted to systematically map the existing literature on comparative efficacy and tolerability of IFN-based agents in humans in which IFN or TLR-agonist-based agents may play a critical role in enhancing the host antiviral response, reducing viral shedding, and preventing severe disease—and thus the need for hospitalization—in patients with an early viral respiratory illness.

Methods

This scoping review aims to identify and synthesize existing knowledge on comparative efficacy and tolerability of topical (intranasal or inhaled) versus injectable IFNs, type I versus type III IFNs, and IFN versus IFN-stimulating agents, such as TLR-agonists that trigger IFN or IFN-induced gene responses. This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) checklist and the Arksey and O'Malley framework, a widely used framework for scoping studies.

Research Question Identification

Based on the Arksey and O'Malley framework, we developed specific research questions to guide the scoping review. This is notably different from conducting a systematic review, which would have included one narrowly focused research question and an evaluation of the rigor of the studies. Our specific research questions are:

- What is known about the mode (topical vs. systemic) and timing (pre- or post-exposure prophylaxis vs. treatment) of administration of IFN-based agents, and if any differences emerge for direct IFN vs. TLR-based agents?
- What is known about the efficacy of these agents, including the location of response (local vs. systemic) and pharmacodynamic biomarkers offering insights into how these agents may succeed?
- What are the roles between Type I and Type III IFN in response to viral infections in the respiratory tract, and what IFN signatures may exist to predict severe disease or response to IFN-targeted therapies?

Literature Search

We conducted a complete systematic search of all relevant index peer-reviewed publications in October 2020. Academic databases included: PubMed/MEDLINE (National Library of Medicine); EMBASE (Excerpta Medica dataBASE); Cochrane Library; medRxiv. Search terms focused on clinical trials with humans with any IFN or TLR across several viral infections and disease areas. A summary of the search terms is included in ***Table 1*** below, and the full search string and detailed Medical Subject Headings (MeSH) are included in ***Supplementary Materials.*** A health sciences librarian systematically searched all four databases, and a team member (EJ) imported all articles into Zotero (Roy Rosenzweig Center for History and New Media, version 5.0.94), for de-duplication. All articles were then imported into REDCap for abstract screening.

Table 1: Search Term Concepts

Population	Humans: human; patient
Concept	Pathogen/Disease: Human respiratory syncytial virus; SARS coronavirus; coronavirus disease 2019; Coronavirus infection; Severe acute respiratory syndrome coronavirus 2; Middle East respiratory syndrome coronavirus; influenza; Human rhinovirus; Rhinovirus infection; asthma; pneumonia; chronic obstructive lung disease
	Treatment: IFN; TLR; IFN stimulated gene
	Drug Administration: inhalation; intranasal; drug; treatment; medication; therapy
	Safety & Efficacy: adverse drug reaction; complication; drug interaction; drug toxicity; side effect; unexpected outcome of drug; lack of drug effect; pharmacodynamics; safety; toxicity; tolerability; efficacy; drug interaction
Context	Clinical human studies; clinical trial; phase 1/2/3/4 clinical trial (i.e., exclusion of in vivo animal studies, and in vitro human cell line studies)

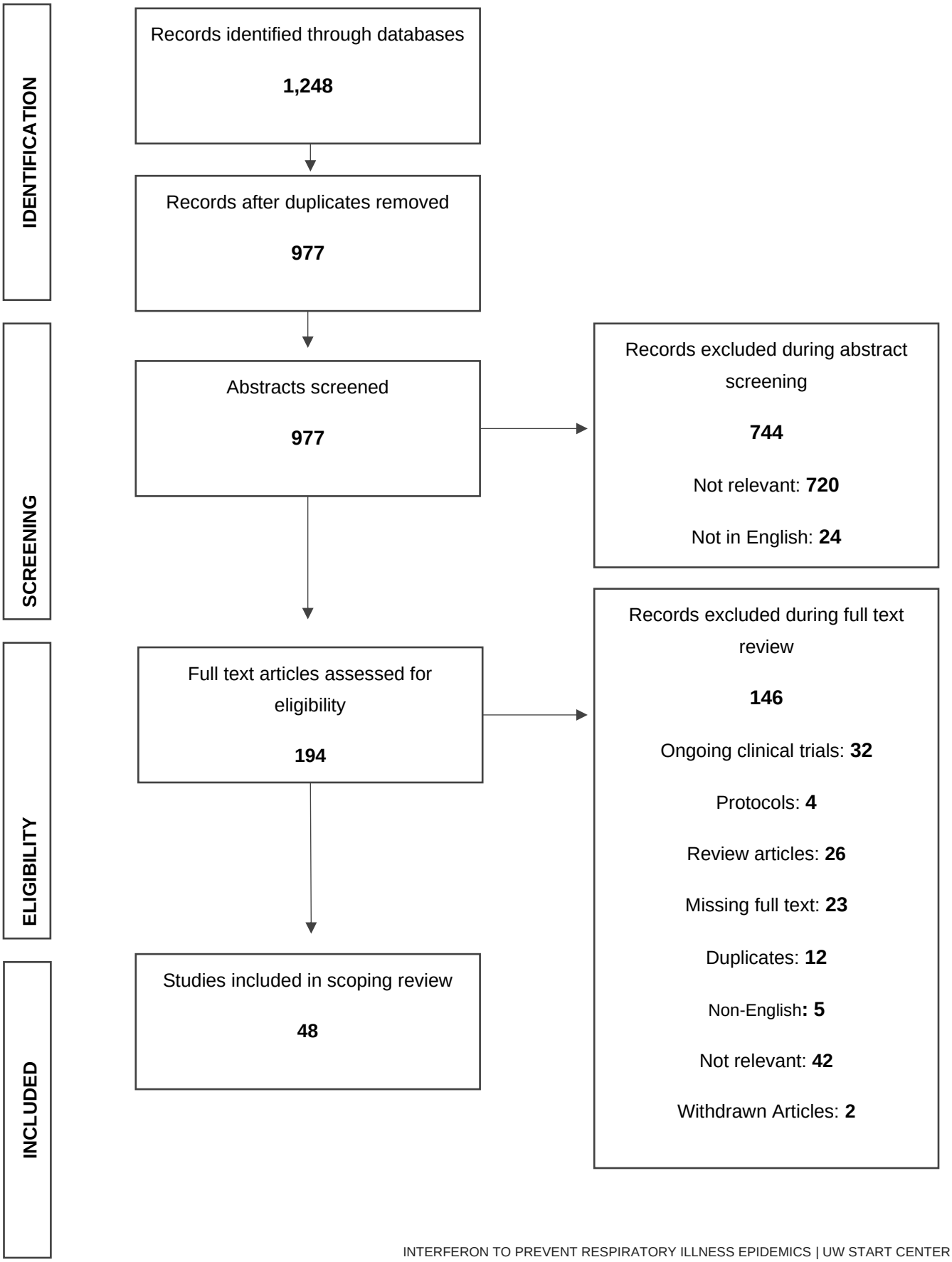
Study Selection

Team members (AM, EJ, ML) screened all abstracts for inclusion/exclusion criteria (***Table 2***), aiming to ascertain relevance to the research questions. Included studies were those that were English-language, peer-reviewed, able to be accessed using a library service, and reporting primary data. Commentaries, clinical trials, and any articles that did not report primary data, such as modeling studies or systematic reviews, were excluded. Lastly, one team member (ML) conducted a quality check of a random 5% of the articles to confirm inclusion/exclusion; this quality check yielded two discrepancies, which were resolved by discussion. Study selection results are reported below in Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) flow diagram in ***Figure 1.***

Table 2: Scoping Inclusion and Exclusion Criteria

Criteria	Inclusion	Exclusion
Publication Type	Peer-reviewed	Not peer-reviewed (i.e., grey literature)
Publication access	Full text available through a library service	Full-text not accessible
Language	English	Non-English
Study Design/Type	Randomized and observational studies	Animal studies, non-clinical trials, reviews, opinions/editorials, ongoing clinical trials
Time Limit	Any time	None
Treatment	IFN, TLR, IFN-stimulated gene	Not IFN, TLR, IFN-stimulated gene, TLR administered as an adjuvant for vaccines
Disease Area	Any disease area with potential relevance for the research questions	Diseases in which IFN was administered for a non-respiratory chronic condition (i.e., Hepatitis C, Multiple sclerosis)
Population	Healthy or infected individuals	None

Figure 1: PRISMA Study Selection Procedure Flow Chart



Data Extraction

Once articles were selected for inclusion, the reviewers (AM, EJ, ML) were randomly assigned articles for data extraction. Variables that were extracted included: article information, methods, and outcomes. We extracted detailed information related to administration including agent administered, mode of administration, frequency, and duration. We assessed the timing of administration as: pre-exposure prophylaxis (PrEP), disease prevention before an individual is exposed to a disease-causing agent; Post post-exposure prophylaxis (PEP), disease prevention after an individual has a possible exposure to the disease-causing agent but before disease; and lastly, as treatment to individuals with a disease. We assessed efficacy as it was reported by the authors, which included clinical, virological, and patient-reported outcomes. Detailed information on all variables extracted can be found in the ***Supplementary Materials***. This scoping review did not focus on reviewing the rigor or validity of the studies that were included.

Results

Overview

We identified 1,248 articles meeting our inclusion criteria based on title and abstract content. After deduplication, there were 977 articles remaining. After in-depth abstract review, we included 194 articles for full-text review. Articles that were not written in English or were deemed irrelevant (i.e., not related to administration of IFN) were excluded (n=24 and 720, respectively). 146 additional articles were excluded for various reasons: 32 were ongoing clinical trials, four were protocols, 26 were review articles, 23 were missing full text, 12 were duplicates, five were non-English, 42 were not relevant (i.e., not focused on respiratory illness), and two were withdrawn articles. All studies, including those excluded, are included in the ***Supplementary Materials.***

During the full-text review, we included 48 peer-reviewed articles, most of which were published between 1981-1990 (n=16), followed by 2020 (n=12). PrEP and treatment were the most common timing indication of administration (n=20 and n=22, respectively). Most studies administered IFN-based agents intranasally (n=26), followed by subcutaneously (n=11) and inhalation (n=9). The most common disease indication was rhinovirus (n=16), but many studies assessed both rhinovirus and other respiratory illnesses, including respiratory syncytial virus (n=5) and influenza (n=9). IFN-α was administered most commonly across these studies (n=22). Additional details related to the articles are included in ***Table 3***.

Table 3: Studies included in scoping review (N=48)

Variable	N (%)
Year of publication	
1970 – 1980	2 (4%)
1981 – 1990	16 (33%)
1991 – 2000	3 (6%)
2001 – 2010	4 (8%)
2011 – 2019	9 (19%)
2020	12 (27%)
Pre-print	2 (4%)
Timing of administration	
Pre-exposure prophylaxis	20 (41%)
Post-exposure prophylaxis[A]	2 (4%)
Treatment[A]	22 (46%)
Other	5 (10%)
Mode of administration[B]	
Intranasal	26 (54%)
Inhaled	9 (19%)
Intramuscular	3 (6%)
Intravenous	2 (4%)
Subcutaneous	11 (23%)
Pathogen or clinical condition indication[B]	
Adenovirus	5 (10%)
Allergic rhinitis	2 (4%)
Allergic asthma	3 (6%)
Asthma	2 (4%)
Bronchiolitis	1 (2%)
Cystic fibrosis	1 (2%)
Idiopathic pulmonary fibrosis	1 (2%)
Influenza	9 (19%)
Middle east respiratory syndrome	1 (2%)
Respiratory syncytial virus	5 (10%)
Rhinovirus	16 (33%)
Seasonal coronavirus colds	1 (2%)
Severe acute respiratory syndrome coronavirus 2	8 (17%)

Upper respiratory tract illness	1 (2%)
No indication	5 (10%)
Agent type	
IFN-α	23 (48%)
IFN-β	12 (25%)
IFN-γ	3 (6%)
IFN-κ	1 (2%)
IFN-λ	1 (2%)
IFN-stimulating agents	1 (2%)
Toll-like receptor-agonists	8 (17%)
Biomarkers assessed	
Gene expression	3 (6%)
Inflammatory cascade	9 (19%)
None or not indicated	36 (75%)

A. One study gave IFN-α as treatment to the index case of cold, and as PEP to the household members without illness.

B. Multiple categories possible for each article.

Pre-Exposure Prophylaxis

We identified a total of 20 studies that offered IFN-based agents as PrEP, as disease prevention before an individual is exposed to a disease-causing agent. Of these, 15 studies (75%) administered IFN-α, two studies (10%) administered IFN-β, two (10%) administered a TLR-agonist, and one study (5%) administered other IFN-stimulating agents.

IFN-α as PrEP (n=15)

A total of 15 studies administered IFN-α as PrEP, most often as human challenge studies. Nearly all the IFN-α was given intranasally (n=15), with one study administering IFN orally (Bennett, 2013). The frequency varied from once daily to four times daily, for a duration of four days to 16 weeks. Most of the PrEP studies aimed at preventing common colds (defined as rhinovirus, coronavirus, and respiratory syncytial virus, and adenovirus) and one study focused on SARS-CoV-2.

Table 4: IFN-α as PrEP studies (n=15)

Study	Indication	Agent	Mode	Frequency	Duration
Bennett, 2013	Influenza B, adenovirus, respiratory syncytial, virus and parainfluenza	3:1 ratio of IFN-α-2b and IFN-α-8	Oral	Once daily	16 weeks
Farr, 1984	Rhinovirus	IFN-α-2	Intranasal	Once daily	22 days
Gao, 2010	Influenza A virus, influenza B virus parainfluenza viruses 1-3 and adenovirus species B	IFN-α-2b	Intranasal	Twice daily	Five days
Hayden, 1983	Rhinovirus	IFN-α-2a	Intranasal	Four times daily	4 days
Higgins, 1990	respiratory syncytial viruses	IFN-α-2a	Intranasal	Three times daily	4.3 days
Meng, preprint	SARS-CoV-2	IFN-α-2a	Intranasal	Four times daily	28 days
Monto, 1986	Rhinovirus	IFN-α-2b	Intranasal	Twice daily	4 weeks
Monto, 1988	Influenza, rhinovirus	IFN-α-2b	Intranasal	Twice daily or once daily	4 weeks
Phillpotts, 1984	Influenza, Rhinovirus	IFN-α-n1	Intranasal	Three times daily	4.3 days
Samo, 1984	Rhinovirus	IFN-α-A	Intranasal	Two doses daily	Four days
Sarno, 1986	Rhinovirus	IFN-α-A	Intranasal	Daily	Four days
Scott,1983	Common cold (defined as upper respiratory symptoms)	IFN	Intranasal	Twice daily	28 days
Higgins, 1988	Rhinovirus 9 and 14	IFN- α-2a	Intranasal	Four times daily	4.25 days
Tannock, 1988	Influenza, Human rhinovirus, respiratory syncytial virus, adenovirus	IFN- α-2a	Intranasal	Once daily	28 days
Turner, 1986	Coronavirus	IFN-α-2b	Intranasal	Twice daily	15 days

Tolerability and safety

Amongst these 15 studies, several assessed tolerability (n=10; Meng, 2020, Samo, 1984, Herzog, 1986, Phillpotts, 1984, Scott, 1983, Sarno, 1983, Gao, 2010, Monto, 1986, Hayden, 1983, Tannock, 1988) and found that IFN-α was well tolerated (Herzog, 1986, Ruthman, 2015, Phillpotts, 1984, Gao, 2010, Hayden, 1983), with only three studies having any participants withdraw due to drug-related symptoms. In one study, three of 20 volunteers withdrew due to the side effects of bloody mucus and superficial mucosal erosions (Samo, 1984), and another study where three participants (two in the treatment arm, one in control) of 412 withdrew due to nasal erosion requiring cauterization (Tannock, 1988). Scott et al. administered three doses of IFN (0.44 million units, 1.52 million units, or 4.4 million units), and nearly all 68 participants completed all 28 days of treatment, with only four participants inadvertently stopping the treatment or missing doses.

Most of the 15 PrEP IFN-α studies also reported safety outcomes (n=12; Meng, 2020, Samo, 1984, Phillpotts, 1984, Farr, 1984, Sarno, 1983, Gao, 2010, Scott, 1985, Monto, 1986, Monto, 1988, Hayden, 1983, Tannock, 1988, Higgins, 1988), which included mild side effects such as nasal symptoms (e.g., dry nose, nasal stuffiness, burning, irritation, stinging, erosion), upper respiratory infection symptoms, flu-like symptoms (cough, sneeze, nasal congestion), blood-tinged mucus, fever, headache, and diarrhea. In one study, dry pharynx was significantly higher in the treatment group compared to the control group during the treatment and follow-up period (Gao, 2010).

Efficacy

Of the total 15 PrEP IFN-α studies, 13 assessed efficacy and found mixed results. Positive results included one study found that rhIFN-α nasal drops may potentially protect susceptible healthy people during the COVID-19 pandemic (Meng, 2020), a lower frequency of illness among the group given rIFN-α A compared to the corresponding placebo group (Samo, 1984), a reduction on the duration and severity of common colds (Herzog, 1986, Monto, 1986), significantly lower clinical scores and nasal secretion weights (Phillpotts, 1984), a significant reduction in the frequency of illness (Sarno, 1983), a lowered risk of developing viral respiratory infections caused by adenovirus, influenza-A, influenza-B and parainfluenza 1-3 (Gao, 2010), and by rhinovirus (Hayden, 1983). However, these results were inconsistent. IFN-α did not prevent influenza infections (Phillpotts, 1984, Monto, 1988), did not lead to a difference in infection rates (Sarno, 1983), did not decrease the respiratory syncytial virus infection rate (Gao, 2010), parainfluenza infections (Monto, 1986), or common cold rates (defined as self-reported symptoms; Scott, 1983).

Pharmacodynamics and kinetics

In terms of pharmacodynamic and pharmacokinetic outcomes, one of the studies found that with a daily dose, IFN-α was detected in nasal washes of all the participants on days 2, 3, and 4, with a rapid decrease on days 5, 6, 7, and 8 after daily administration (Farr 1984). However, no serum anti-IFN-α was detected during follow up in any of the volunteers. The other study showed a similar pattern in the decline of recombinant IFN-α 2b, where IFN-α was detected in nasal washes was 75 U/ml or below on day seven, even in a dose as high as 20 MU/day after four days of treatment (Sarno, 1984; Hayden 1983).

Biomarkers assessed

Three studies, which administered intranasal IFN-α-2 and IFN-α-A, detected no antibodies against the agents (Farr 1984; Sarno, 1984; Hayden 1983).

IFN-β as PrEP (n=2)

In the PrEP IFN-β studies (n=2), the IFN agent was given intranasally (Sperber, 1988, Sperber, 1989). To assess tolerance, IFN-β was administered once daily for 21 to 26 days in both studies. The IFN agent was administered daily beginning 36 hours before the challenge, for three days. In both studies, the IFN agent was administered against human rhinovirus. Participants included in these PrEP studies were healthy individuals.

Table 5: IFN-β as PrEP studies (n=2)

Study	Indication	Agent	Mode	Frequency	Duration
Sperber, 1988	Human rhinovirus	IFN-β-serine	Intranasal	Once daily	25 days
Sperber, 1989	Human rhinovirus	IFN-β-serine	Intranasal	Once daily (except Sundays)	4 weeks

Tolerability and safety

Both studies assessed tolerability and found that IFN-β was well-tolerated. In one of the two studies (Sperber, 1988), one volunteer withdrew due to persistent microscopic hematuria of unclear

etiology. Another study had seven withdrawals (four from the IFN group and three from the control) due to abnormalities (i.e., ulcers and erosions) found on the nasal exam after two weeks (Sperber, 1989). All studies reported safety outcomes, including mild side effects such as nasal stuffiness, mucosal irritation, nasal dryness, and blood-tinged mucus. Studies reported no significant change in the peripheral white blood cell counts.

Efficacy

Both PrEP IFN-β studies assessed the efficacy and could not establish that the IFN agent could prevent rhinovirus-driven coryza. However, one of the studies (Sperber, 1988) showed that a high dose of recombinant IFN-β_{ser} (24×10^6 units) significantly reduced the frequency and the severity of the illness. Two studies, conducted in 1986 and 1987, evaluated the safety and efficacy of nasal drops of IFN-β and found that though the agents were safe and tolerable, the illness frequency and the number of days with subjective coryza did not differ between the groups (Sperber, 1989).

TLR-Agonists as PrEP (n=2)

One study from 1972 explored the effects of PrEP intranasal administration of the TLR-3 immunostimulant polyinosinic:polycytidylic acid on the incidence and the severity of rhinovirus 13 or type A2 influenza virus/Hong Kong/68 (Hill, 1972). Over the course of three separate trials within the study, the incidence of infection, as well as viral shedding, were not reduced but there was a measurable reduction in the symptomatic severity of both viral infections. No toxicity was detected in any of the trials. The authors did not report on safety, tolerability, or biomarker signatures of IFN.

Another study assessed the safety of an intranasally-administered TLR4-agonist, CRX-675, among patients with allergic rhinitis and challenged patients with intranasal ragweed challenges (Casale, 2006). Though the agent was shown to be safe (i.e., with only mild adverse events), not toxic, and tolerable, there was no evident trend that the agonist was able to inhibit allergen responses. However, at 100 microgram dose, there was an improvement in nasal symptom score.

Table 6: TLR-agonists as PrEP studies (n=2)

Study	Indication	Agent	Mode	Frequency	Duration
Casale, 2006	Allergic rhinitis	CRX-675, 2, 20, 100, or 200 microgram doses	Intranasal	Once, 24 hours before	

				ragweed challenge	
Hill, 1972	human rhinovirus 13, type A2 influenza virus/Hong Kong/68	polyinosinio-polycytidylic acid, 0.7 mg (0.01 mg/kg) for one day followed by 0.35 mg (0.005 mg/kg) or 7 mg (0.1 mg/kg) for one day followed by 3.5 mg (0.05 mg/kg).	Intranasal	Daily	Six days

Other IFN-Stimulating Agents as PrEP (n=1)

In a 1973 randomized controlled trial of 25 healthy subjects challenged with rhinovirus 21, an IFN-stimulating agent, N,N-dioctadecyl-N′-bis-(hydroxyethyl)-propanediamine, was administered via nebulizer inhalation to 14 subjects for 24 hours prior to the challenge (Gatmaitan, 1973). Compared to the 11 control subjects challenged with rhinovirus alone (i.e., without administration of the IFN-stimulating agent), there was no sufficient evidence that prophylactic immunostimulation of IFN reduced viral infection nor illness incidence. The authors did not report on safety, toxicity, or tolerability.

Table 7: IFN-Stimulating Agents as PrEP studies (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Gatmaitan, 1973	Human rhinovirus	N,N-dioctadecyl-N'-bis-(hydroxyethyl)-propanediamine	Intranasal	Three times daily on day the before the challenge, two more doses given 4 and 8 hours after the challenge	Two days

Post-Exposure Prophylaxis

The following studies administered IFN-based agents as post-exposure prophylaxis (PEP), a disease prevention strategy after an individual has a possible exposure to the disease-causing agent but before disease development. One study administered IFN-α and another IFN-β.

IFN-α as PEP (n=1)

One study administered IFN-α after exposure to a pathogen, by enrolling 191 families in which one household member had a self-reported cold (i.e., without pathogen identification) for one to two days (Herzog, 1986). IFN-α was administered as PEP among 337 initially symptom-free contacts. IFN-α was applied intranasally twice daily (morning and evening) in each nostril for five days and found to be well-tolerated and safe. However, it did not influence common cold infection rates, but did reduce the duration and severity of ensuing common colds.

Table 8: IFN-α as PEP studies (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Herzog, 1986	Rhinovirus, Adenovirus, Influenza Virus A/B, Parainfuenza Virus 1/2/3, Respiratory Syncytial Virus	IFN-α-A	Intranasal	Twice daily	Five days, starting within two days after appearance of self-reported common cold in household

IFN-β as PEP (n=1)

One study administered IFN-β after exposure to a pathogen which enrolled 40 adults, 20 of whom received IFN-β 36 hours after infection with rhinovirus (Sperber, 1992). No safety, toxicity, or tolerability results were reported. The rates of illness and severity did not differ between groups, but the frequency of virus shedding was reduced in IFN receipts on the fourth (37% vs. 74%) and sixth day (11% vs. 42%). The authors also explored the effect of prophylaxis on parameters of eustachian tube function, given that colds can often cause acute otitis media. Middle ear pressure abnormalities were reduced in the IFN group compared to placebo (18% vs. 38%), indicating that PEP treatment with IFN- β may reduce rhinovirus-associated middle-ear dysfunction.

Table 9: IFN-β as PEP studies (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Sperber, 1992	Rhinovirus	IFN-β-serine	Intranasal	Three times daily	4.3 days, beginning 36 hours after infection

Treatment

We identified several studies (n=22) that administered IFN-based agents as treatment to individuals with a disease. Seven studies (32%) administered IFN-α, six (27%) administered IFN- β, two (9%) administered IFN- γ, one (5%) administered IFN- κ, one (5%) administered IFN-λ, and five (23%) administered TLR-agonists.

IFN-α as Treatment (n=7)

Seven studies administered IFN-α for treatment after the onset of infant bronchiolitis symptoms, upper respiratory tract illness, common cold (caused by rhinovirus, adenovirus, parainfluenza, and respiratory syncytial virus), and SARS-CoV-2. The modes of administration included intramuscular, inhalation, and intranasal, with a frequency of three times weekly to four times daily for a minimum duration of five days to a maximum duration of 17 days.

Table 10: IFN-α as treatment studies (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Chen, 2020	Infant bronchiolitis	IFN-α-1b; inhaled dose includes 1 microgram per kilogram or 2 microgram per kilogram	Intramuscular; inhalation	Daily	Seven days
Hayden, 1988	Upper respiratory tract illness (defined as self-reported sneezing, rhinorrhea, nasal congestion, sore	IFN-α-1b, 10 or 20 micrograms	Intranasal	Four times daily	Five days

	throat, hoarseness, cough)				
Herzog, 1986	Rhinovirus, Adenovirus, Influenza Virus A/B, Parainfuenza Virus 1/2/3, Respiratory Syncytial Virus	IFN-α-A	Intranasal	Twice daily	Five days
Huang, 2020	SARS-CoV-2	IFN-α	Inhalation	Twice daily	14 days
Jiang, 2020	Non-influenza viral pneumonia	IFN-α-1b	Inhalation	Once daily	Seven days
Pereda, 2020	SARS-CoV-2	IFN-α-2b	Intramuscular	Three times weekly	Two weeks
Wang, 2020	SARS-CoV-2	IFN-α-2b	Not reported	Not reported	5-17 days

Tolerability and safety

Across studies, treatment was generally well-tolerated without any study withdrawals due to treatment. Most side effects were mild (Turner, 1986, Huang, 2020, Jiang, 2020, Chen, 2020), and one study found no significant differences in adverse events (Huang, 2020). One study noted that nasal bleeding was seen in eight of the IFN-receiving participants compared to one placebo participant (p=0.05; Turner, 1986). Similar outcomes were observed in another study, which found blood in nasal mucus and nasal bleeding in 19% of the 10-MU, and 25% of 20-MU IFN recipients, compared with 6% of placebo (p<0.02; Jiang, 2020). Other notable symptoms included sore/dry throat, low white blood cell count, rash, diarrhea, and poor appetite (Jiang, 2020, Huang, 2020, Chen, 2020).

Efficacy

Efficacy results were highly variable and appeared dependent on administration timing and disease progression. In one study of inhaled IFN-α for non-viral pneumonia, the overall response rate was significantly higher in the IFNα1b group compared with that in the control group (p<0.05; Jiang, 2020). Scores of expectoration, respiratory rate, and pulmonary rates decreased rapidly during treatment, particularly among the IFNα1b group. Though some of these findings are promising, including that the response rates of expectoration and pulmonary rales were significantly higher in the

IFNα1b group than that in the control group (p<0.05), there were no significant differences in coughing, respiratory rate, or chest pain between treatment and control. In a study of 600 infants admitted with bronchiolitis, administration of IFN effectively alleviated wheezing and coughing in bronchiolitis, and the inhalation mode of administration showed significant advantages compared to intramuscular injections to infants (Chen, 2020).

In another study in which 446 COVID-19 patients were given inhaled IFN-α, early administration was associated with reduced in-hospital mortality, whereas later IFN increased mortality and delayed the recovery, indicating that early timing is crucial. Though IFN was not associated with COVID-19 recovery time, it did have better outcomes compared to lopinavir/ritonavir (Wang, 2020). Another similar study of 812 COVID-19 patients saw positive findings related to IFN, including significantly better hospital discharge rates between IFN and placebo (95% vs. 26%, p<0.01), lower case fatality (1% vs. 32%, p <0.01), and lower case fatality among severe/critical cases (22% vs. 49%, p<0.05). Another study treated 761 hospitalized COVID-19 patients with lopinavir/ritonavir and chloroquine with intramuscular administration of IFN-a2b three times per week, for two weeks, and found that the proportion of patients discharged was higher in the IFN group compared to the control group (95.4% vs. 26.1%, p<0.01). Further, the study indicated that the case fatality rate was lower for the IFN group compared to the control group (0.92% vs. 2.95% p<0.01; Pereda, 2020).

However, some studies did not find a positive effect of IFN. IFN-α did not affect the duration or severity of the common cold illness (Hayden, 1988). Another study found no statistically significant differences between the combination therapies Ribavirin Plus IFN-A, Lopinavir/Ritonavir Plus IFN-A, and Ribavirin Plus Lopinavir/Ritonavir Plus IFN-A in patients with mild/moderate COVID-19 (Huang, 2020). However, it is impossible to isolate the impact of IFN-α within this study. One study showed worse outcomes when patients were treated with IFN-α. In one study (Hayden, 1988), the median duration of colds was shown to be longer in the 20 MU group, compared to 10 MU IFN, or placebo (p=0.06), and this was found to be statistically significant among patients with proven rhinovirus colds that were treated within 24 hours. Relatedly, no differences were found in respiratory symptom scores or resolution of symptoms (Hayden, 1988).

IFN-β as Treatment (n=6)

In studies where IFN-β was administered as a treatment, the agent was given via subcutaneous route (n=4), inhaled (n=2), or intravenously (n=1). IFN- β was given once daily or every alternate day for 6-14 days to treat SARS-CoV-2 (n=4), Middle East respiratory syndrome (n=1), and asthma exacerbation (n=1).

Table 11: IFN-β as treatment studies (n=6)

Study	Indication	Agent	Mode	Frequency	Duration
Arabi, 2020	Middle East respiratory syndrome	IFN-β-1b	Subcutaneous	Every alternate day	14 days
Djukanovic, 2014	Asthma and cold symptoms	IFN-β-1a	Inhalation	Once daily	14 days within 24 hours of developing a cold
Hung, 2020	SARS-CoV-2	IFN-β-1b; group treated on day 7-14 of symptoms did not receive IFN-β-1b due to proinflammatory effects	Subcutaneous	Every alternate day	14 days
Monk, 2020	SARS-CoV-2	IFN-β-1a	Inhalation	Once daily	14 days
Rahmani, 2020	SARS-CoV-2	IFN-β-1b	Subcutaneous	Every alternate day	14 days
World Health Organization Solidarity Trial Consortium, 2020	SARS-CoV-2	IFN-β-1a	Subcutaneous; intravenous	Subcutaneous: 3 doses; intravenous: daily	6 days

Safety and tolerability

Administered via inhalation, IFN-β was shown to be well tolerated. The IFN agent was not associated with severe adverse events. Observed side effects included injection site reactions and flu-like syndrome, blood dyscrasias (leukocytosis, leukopenia, lymphopenia, thrombocytopenia, and anemia), and electrolyte imbalance (e.g., hyperkalemia, hypokalemia, and hyponatremia) with the subcutaneous IFN-β in one study (Rahmani, 2020).

Efficacy

Three studies (Rahmani, 2020, Djukanovic, Hung, 2020) showed that IFN-β has varying degrees of efficacy. One study found that 14-day triple combination of lopinavir 400 mg and ritonavir 100 mg every 12 hours, ribavirin 400 mg every 12 hours, and three doses of 8 million international units of IFN-β-1b on alternate days (combination group) led to a significantly shorter median time from the beginning of treatment to negative nasopharyngeal swab (seven days [IQR 5-11]), compared to the control group (14 days of lopinavir 400 mg and ritonavir 100 mg every 12 hours, 12 days [IQR: 8-15]; Hung, 2020).

Relatedly, a similar study comparing the triple combination as subcutaneous injection on alternate days for 14 days to placebo for the treatment of middle east respiratory syndrome found that the triple combination yielded a risk difference of -19% percentage points for all-cause mortality (p<0.024; Arabi, 2020). Efficacy was related to the timing of administration; treatment within seven days after symptom onset led to lower 90-day mortality than the use of placebo (relative risk, 0.19; 95% CI, 0.05-0.75), and later treatment did not. One study (Rahmani, 2020) reported that, as an add-on, IFN-β-1b significantly shortened the time to clinical response, increased the discharge rate at day 14, and decreased the need for ICU admission in patients with severe COVID-19. Another study concluded that this IFN agent improves morning positive lung function. Lastly, another study (Monk, 2020) reported that inhaled nebulized IFN-β-1a is associated with improving the WHO Ordinal Scale for Clinical Improvement in COVID-19 patients. In contrast, the WHO Solidarity Trial Consortium reported that IFN-β did not improve hospitalization duration, illness rates, or severity in patients with COVID-19 (2020).

IFN-γ as Treatment (n=2)

Our search suggested that IFN-γ has been administered more frequently as a treatment in chronic respiratory disease, compared to acute illness. Two randomized controlled trials (Moss, 2005, Antoniou, 2006) explored longer-term administration of 12-week by inhalation (Moss, 2005) or two-year subcutaneous administration (Antoniou, 2006) IFN-γ for cystic fibrosis and idiopathic pulmonary fibrosis, respectively.

Table 12: IFN-γ as treatment studies (n=2)

Study	Indication	Agent	Mode	Frequency	Duration

Antoniou, 2006	Idiopathic pulmonary fibrosis	IFN-γ-1b	Subcutaneous	Three times weekly	2 years
Moss, 2005	Cystic fibrosis lung disease	IFN-γ-1b, 500 micrograms or 1,000 micrograms	Inhalation	Three times weekly	12 weeks

Safety and tolerability

One study had 15% drop out (five in placebo, two in the lower dose, and three in the higher dose). The 500-mg dose was well-tolerated. However, the 1,000-dose cohort, with a higher baseline bacterial density than placebo patients, had 24% more hospitalizations for exacerbation than placebo patients (P<0.05; Moss, 2005). In another study, no patients withdrew for treatment-related reasons (Antoniou, 2006). Symptoms such as myalgia, headache, fever, and influenza-like symptoms were significantly more common in the IFN- γ group (P<0.01). However, most of these symptoms subsided within the first 9-12 weeks.

Efficacy

Efficacy findings were mixed. There was no statistically significant effect of either dose of IFN-γ on 12-week change in FEV, or reduction of sputum bacterial density, or in the secondary endpoints of reducing systemic antibiotic usage or hospitalization (Moss, 2005). for idiopathic pulmonary fibrosis, patients treated showed a better outcome after two years of therapy, and fewer symptoms, as assessed using the St George's Respiratory Questionnaire, after 12 months of therapy. The group demonstrated a higher forced vital capacity at two years of treatment. However, there were significant differences in resting arterial oxygen tension, total lung
capacity, transfer factor of the lung for carbon monoxide, and high-resolution computed tomographic scoring between the two groups (Antoniou 2006).

Biomarkers

In terms of biomarkers, there was no impact of IFN-γ on inflammatory biomarkers in the sputum (Moss, 2005).

IFN-κ as Treatment (n=1)

Fu et al. assessed the use of inhaled IFN-κ in combination with Trefoil factor 2 (TFF2) as a treatment for COVID-19 (2020). The aerosol agents were delivered by a nasal mask for 20-30 minutes, three times, every 48 hours from the first day of hospitalization. The group receiving IFN-κ and TFF2 had significantly shortened median time in cough relief, viral RNA reversion, and median hospital stay duration (Fu, 2020).

Table 13: IFN-κ as treatment study (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Fu, 2020	SARS-CoV-2	IFN-κ	Inhalation	Three times every 48 hours	10 days

IFN-λ as Treatment (n=1)

IFN-λ was given as a single subcutaneous injection to patients seeking care for COVID-19 in an outpatient setting. The agent was found to be well-tolerated with similar side effects to placebo. Further, there were no differences in severe or any adverse events, including laboratory adverse events (e.g., hemoglobin, white blood cells; Feld, pre-print).

Table 14: IFN-λ as treatment study (n=1)

Study	Indication	Agent	Mode	Frequency	Duration
Feld, pre-print	SARS-CoV-2	IFN-λ	Subcutaneous	Once	N/A

TLR-Agonists as Treatment (n=5)

TLR-agonists were administered in the treatment of chronic disease symptoms in five studies for allergic rhinitis (n=2), allergic asthma (n=2), and asthma (n=1). Three studies administered TLR-agonists intranasally, whereas two administered it subcutaneously. Most treatments were given weekly or bi-weekly for a duration of five to 13 weeks.

Table 15: TLR-agonists as treatment studies (n=5)

Study	Indication	Agent	Mode	Frequency	Duration
Beeh, 2013	Allergic asthma	QbG10 (bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside), a TLR9-agonist	Subcutaneous	First three doses were given weekly, followed by every second week, 7 total doses	10 weeks
Casale, 2015	Allergic asthma	CYT003, a TLR-9-agonist immunomodulator	Subcutaneous	Weeks 1, 2, 4, 6, 8, and 10	10 weeks
Greiff, 2012	Allergic rhinitis	AZD8848, A TLR-7-agonist	Intranasally	Once weekly	Five weeks
Leaker, 2012	Allergic asthma	AZD8848, A TLR-7-agonist	Intranasally	Once weekly	Eight weeks
Psallidas, 2020	Asthma	AZD1419, a TLR-9-agonist	Intranasally	Once weekly	13 weeks

In one study (Greiff, 2012), a TLR-7-agonist (known as *AZD8848*) was administered intranasally in two arms: 1) 12 subjects with chronic, active allergic rhinitis and 48 patients without known allergies, and 2) 74 subjects with chronic, out-of-season allergic rhinitis. Multiple measures were monitored, including safety, tolerability, pharmacokinetics, and biomarkers (blood lymphocyte levels, plasma interleukin-1 receptor-agonist levels). Greiff concluded that administration of *AZD8848* was a safe and effective treatment for reducing active allergic rhinitis symptoms, but there was no evidence of reduced symptom severity when administered as an out-of-season treatment. Another

related study (Leaker, 2019) assessed the efficacy and safety of once-weekly intranasal *AZD8848* (given eight weeks) among participants with mild-to-moderate allergic asthma and found that the agent was safe and tolerable, but with limited efficacy; *AZD8848* reduced late asthmatic response (LAR) fall in forced expiratory volume in one Forced Expiratory Volume (FEV) 27% vs. placebo after a week of treatment (p<0.035), which was sustained four weeks after treatment, however not clinically significant.

Two studies assessed TLRs on allergen-induced responses in patients with allergic asthma. One study administered seven doses of *CYT003*, a TLR-9-agonist immunomodulator, subcutaneously and assessed change from baseline in the Asthma Control Questionnaire, change in FEV, Mini Asthma Quality of Care, and safety. Though the agent was safe and tolerable, there was no significant difference between the *CYT003* and placebo groups at week 12 in any of the outcomes (Casale, 2015). Another study administered the TLR-9-agonists, *QbG10,* for allergic asthma (Beeh, 2013). QbG10 in seven subcutaneous injections over 12 weeks while incorporating a controlled steroid withdrawal. QbG10 was tolerable, with only two of 63 participants withdrawing due to adverse events. Most side effects were mild and included injection side pain and edema, rhinitis, and dyspnea. About 68% of all the adverse events were reported in the QbG10 group (Beeh, 2013). All patient-reported outcomes improved during the study period, including the Asthma Control Test score ($p<0.05$). FEV 1 had worsened among the placebo patients (likely due to the steroid withdrawal) but remained stable in the QbG10 group.

Another study administered intranasal AZD1419 to adult asthmatic patients for 13 weeks (Psallidas, 2020). The patients were observed for 40 weeks for asthma symptom reduction and incidence of adverse events or toxicity. Tolerability and safety were also reported. While type 2 inflammatory markers were reduced in most patients receiving *AZD1419*, asthma control was not improved significantly compared to placebo

Pharmacodynamics and kinetics

One study that administered inhaled AZD1419 found that target engagement biomarker CXCL10 levels were significantly higher compared to placebo at 24 hours post-dose, suggesting that AZD1419 dose (4 mg to 8 mg, for 13 days) was sufficient to trigger an innate type 1 immune response in the study population (Leaker, 2012; Psallidas, 2020).

Biomarkers

One study found that AZD8848 did not significantly change plasma cytokine, sputum Th2 cytokine, or sputum eosinophil levels at 1 and 4 weeks after the last dose.

Other Studies

Five studies focused on safety, tolerability, pharmacokinetics, and pharmacodynamics among healthy participants, and did not assess the efficacy of IFN or TLR-agonists on specific diseases or intended uses (e.g., for PrEP, PEP, or treatment). Three of these studies administered IFN-β (60%), one administered IFN- γ (20%), and another administered a TLR-agonist (20%).

IFN-β (n=3)

Three studies assessed the safety, pharmacodynamics, and pharmacokinetics of IFN-β. Modes of administration included subcutaneous, inhalation, intramuscular, and intravenous. The IFN agent was given once or once a week.

Table 16: Other IFN-β studies (n=3)

Study	Agent	Mode	Frequency	Duration
Buraglio, 1999	IFN-β-1a or IFN-β-1b	Subcutaneous	Once	N/A
Norris, 2016	IFN-β-1a	Inhalation	Once a week	Four weeks
Salmon, 1996	IFN-β-1a	Intravenous; intramuscular; subcutaneous	Once a week	Four doses, separated by one week washout period

Norris found that IFNb-1a 300 micrograms given by inhalation once a week for four weeks, in comparison to room air and placebo, significantly reduced carbon monoxide transfer factor corrected for hemoglobin after two, three, and four doses, compared with room air, indicating that this dose and administration may not be safe for general use (2016). The authors also found that circulating IFN-β-1a concentrations were about one-third those of the intramuscular dose regimen.

Salmon compared IFN-β modes of administration (intravenous, intramuscular, and subcutaneous) to matching placebo four times with a weeklong washout period (1996). Each type of administration was well tolerated with mild flu-like symptoms. Intramuscular and subcutaneous injection led to one-sixth of the administered dose being available systemically. The authors concluded that the clinical and biological effects (including the IFN-β serum levels) were independent of the route of administration, and that the subcutaneous route may be preferred for immunomodulatory activity.

In contrast the intravenous route may be better for enhancing antiviral or antiproliferative activities (Salmon, 1996).

Another study compared single injection doses of subcutaneous IFN-β-1a (6 MU) versus IFN-β-1b (8 MU) and found that both treatments had similar pharmacodynamic effects (i.e., produced similar increases in β2-microglobulin and neopterin) but that IFN-β-1a may be better tolerated. Notably, fewer individuals in the IFN-β-1a group experienced moderate fever and a smaller increase in body temperature, whereas headache was similar across groups (15 episodes in the IFN- β-1a group vs. 11 episodes in the IFN-β-1b group; Buraglio, 1999).

IFN-γ (n=1)

Devane studied the effect of a two-week titration regime in reducing the severity of treatment-associated flu-like symptoms with initial IFN-γ-1b treatment (2014). Forty participants were randomized to receive one of two treatment regimens: 1) No titration dosing (full 50 mcg/m(2) subcutaneous three times weekly for three weeks) and titration (15 mcg/m(2) subcutaneous three times weekly during week one, 30 mcg/m(2) subcutaneous three times weekly during week two followed by the full dose of 50 mcg/m(2) subcutaneous three times weekly during week three). Each regimen included a 15-day minimum washout period. Though there was a high discontinuation rate (37.5%), it was found that titration results in a significant reduction in flu-like symptoms at eight hours (p =0.023) over the treatment period. There was a particularly stark difference in fever severity (Devane, 2014), indicating that titration may increase the tolerability of IFN-γ.

Table 17: other IFN-γ study (n=1)

Study	Agent	Mode	Frequency	Duration
Devane, 2014	IFN-γ-1b	Subcutaneous, titration vs. no titration[A]	Three times weekly	Three-week study period with 15 day washout between administrations (total=51 days)

A. No Titration dosing (full 50 mcg/m2 subcutaneously three times weekly for 3 weeks) and Titration (15 mcg/m2 subcutaneously three times weekly during week 1, 30 mcg/m2 subcutaneously three times weekly during week 2 followed by the full dose of 50 mcg/m2 subcutaneously three times weekly during week 3)

TLR-Agonists (n=1)

The first-in-human study of *AZD1419,* a TLR9 oligonucleotide-agonist, (Jackson, 2018) with healthy subjects, involved an ascending inhalation dose given as four once-weekly doses (ranging from 0.8-23.1 mg). The trial demonstrated that *AZD1419* was safe, well tolerated, and that target engagement in the lung was demonstrated at all dose levels tested. There were indications that a higher dose level may have affected the frequency and severity of flu-like adverse events (i.e., chills, fatigue, myalgia, and pyrexia). Pharmacodynamic and pharmacokinetic markers indicated that there was a dose-dependent relationship between the dose of *AZD1419* and the protein levels of *CXCL10*, an IFN-inducible chemokine in the plasma and sputum, and that deposition of inhaled *AZD1419* was primarily restricted to the lung, as expected.

Table 18: TLR-agonist study (n=1)

Study	Agent	Mode	Frequency	Duration
Jackson, 2018	AZD1419	Inhalation; ascending doses included: 0.8, 2.3, 7.7, 15.4, and 23.1 mg per administration	Single ascending inhalation dose; once weekly doses	Four weeks

Discussion

In this scoping review, we described what is already known about efficacy and safety/tolerability by mode (topical vs. systemic) or timing of administration (PrEP vs. PEP vs. treatment) of IFN-based agents, and if any differences emerge for direct IFN vs. TLR-based agents. Studies included in this review demonstrated mixed efficacy results, with some indicating strong efficacy to others with no effects to some with even worsening of respiratory symptoms.

First, as for efficacy of IFN-based agents included in this review, we found that efficacy was highly dependent on IFN-agent type, frequency/duration of administration, and timing of administration during disease progression or stage. As PrEP, IFN-α generally reduced the incidence of viral infections, symptoms count, and symptom severity (Meng, 2020; Sarno, 1984; Herzog, 1986; Monto, 1986). IFN-β, though ineffective in infection prevention, did reduce the severity of disease (Sperber, 1988; Sperber 1989). TLR-agonists were shown to improve nasal symptom scores and reduced viral infection symptoms (Casale, 2006; Hill 1972). However, IFN-stimulating agents, did not reduce incidence of viral infection (Gatmaitan, 1973). For PEP, though IFN-α and IFN-β did not affect illness rates, both reduced duration and severity of illness and viral shedding, which is an important finding for epidemic prevention efforts (Herzog, 1986; Sperber, 1992). As treatment, IFN-α reduced in-hospital mortality with COVID-19 and increased discharge rates, though appeared to have no effect on or lengthen the duration or severity of colds (Pereda, 2020; Wang 2020; Hayden, 1988). IFN-β for treatment increased discharge rates for COVID-19, shortened time to clinical response, improved morning positive lung function, and lastly, reduced all-cause mortality among patients with Middle East Respiratory Syndrome (Rahmani, 2020; Monk 2020; Arabi, 2020). For COVID-19, as a combination therapy with TFF2, IFN-κ provided cough relief and reduced median hospital stay duration, and IFN-λ was significantly associated with virus clearance (Fu, 2020; Feld, pre-print). Overall, though these findings are mixed, the positive signals of efficacy underscore the potential role that IFN-based agents, IFN-β, may play for COVID-19 and other future pandemics.

Second, the various IFN-based agents were well-tolerated with favorable safety/tolerability profiles. Most agents were administered intranasally, subcutaneously, or via inhalation and were consistently well-tolerated, with few study withdrawals reported due to mild side effects, adverse events, or serious adverse events. The consistent safety and tolerability findings across studies and disease areas (e.g., rhinovirus, COVID-19, bronchiolitis, or asthma) may imply IFN-based agents administered intranasally may be the safest option for future unknown respiratory illnesses.

Third, as for pharmacodynamic and pharmacokinetic outcomes that offer insight into how IFN-based agents may succeed, we found limited data to this end in the human clinical trials. Only a few studies commented on these parameters and found that IFN-based agents appear to have a reasonable half-life (i.e., in terms of the durability of the administered agents and the duration of downstream biological effects). However, the effect of these antibodies on efficacy and impact of endogenous IFN were notably understudied areas.

While our scoping review identified several human clinical trials with IFN-based agents for respiratory illnesses, there are still apparent gaps in the literature that require further study to more fully determine the potential role of IFN-based agents in mitigating future viral respiratory epidemics. We highlight these gaps as: 1) lack of studies that concurrently assess various elements of IFN-based agents; 2) limited research on biomarkers of IFN induction. First, few studies concurrently assessed different modes, dosages, frequencies, or duration of treatment, making comparisons difficult given marked variations in study designs. Only one study assessed differences in safety, tolerability, and pharmacodynamics or kinetics between intravenous, intramuscular, and subcutaneous administration (Salmon, 1996). Few studies assessed different dosages and one assessed dose titration (Moss 2005; Hayden 1988; Chen 2020; Devane, 2014). Additionally, only one study assessed different types of IFNs (i.e., IFN-β-1a vs. IFN-β-1b), which concluded there were differences in tolerability based on the type of agent (Buraglio, 1999). Thus, there is a need to understand optimal IFN type, mode of administration, dosage, frequency, and duration to ensure the highest safety, tolerability, and efficacy of these treatments. In terms of timing, there was limited research (i.e., only two studies) on the impact of IFNs or TLR-agonists as PEP (Herzog, 1986; Sperber, 1992). Understanding the role that IFN-based agents can have for those exposed, but not necessarily with disease symptoms, is critical for future potential epidemics.

Second, we also noted limited research on biomarkers of IFN induction, or downstream effects, or virologic endpoints. Our review aimed to understand the roles between Type I and Type III IFN in response to viral infections in the respiratory tract, and what IFN signatures may exist to predict severe disease or response to IFN-targeted therapies. There were no studies which corresponded to this research question, which we suspect is due to our exclusion of non-clinical studies and our identifying few studies assessing IFN signatures and biomarkers in human clinical trials. The lack of understanding of how IFN or IFN-inducers influence local or systemic interferon stimulated gene responses is critical in more holistically understanding the signals for the impact of IFN-based agents in humans. RNA sequencing, multiplexed cytokine measures, and other highly dimensional modern assays can readily address this outstanding question of gene responses. For example, there were no studies on IFN-induced gene signatures to determine optimal dosing of IFN-based agents as treatment. This would be important to address in future research given the need to understand target

coverage across tissue types. Also, notably missing, most human challenge studies used clinical, rather than quantitative/viral shedding endpoints. This is an important limitation of these studies, as understanding viral shedding and community spread is of utmost importance during a pandemic. Therefore, studies of innate immunity agents should employ frequent upper respiratory sampling for quantitative PCR/RT-PCR to determine if these agents are truly reducing viral replication. Lastly, previous work has not included study of pre-existing autoantibodies to IFN, now shown to be associated with poor outcome after COVID-19, or the effect of genetic variation in innate immunity genes, which could be associated with differential responses to IFN-pathway drugs (Bastard, 2020). Thus, future clinical studies need to be attuned to this phenomenon of antibody generation, as efficacy of the agent may be reduced in light of potential antibodies.

While this is one of the first scoping reviews to examine IFN-based agents on viral respiratory illnesses, it has several limitations. First, many of the studies included in our review were older (from before the 1990s) when IFN-based agents were first discovered, and knowledge of IFN-inducing agents was emerging. These older studies rarely included data on mechanisms of action or biomarkers, and measurements of efficacy and even identifying the IFN-agent used were difficult to ascertain. This lack of more recent data may have resulted from early studies demonstrating low efficacy as well a general decline in the use of human challenge models. Additionally, a general shift in interest in the research community in small molecules and pathogen-specific agents may have driven disinterest in the broadly acting IFN-based agents; however, the number of IFN-related studies published and ongoing during the COVID-19 pandemic signal a renewed interest in these agents. Second, our review included only English-language literature and studies with full text accessibility through a library service, which may have excluded some relevant studies. However, we sought out several library services to include as many articles as possible. Third, we limited our search to human clinical trials and excluded human cell lines and animal studies, which may have provided important insights, particularly on biomarkers. Fourth, we may not have captured all agents that may induce or stimulate IFN, particularly if any newly discovered agents evaded our search terms. Fifth, we excluded studies that used IFN-based agents for non-respiratory illness, which excluded dozens of studies on multiple sclerosis and hepatitis or on the use of TLRs as adjuvants for vaccines. However, we did not believe these studies on chronic administration would provide relevant insight on the role of IFN-based agents in the context of an epidemic. Lastly, though we re-ran the searches several times throughout the study, articles published after January 2021 may not have been included in this analysis given how quickly and continuously the literature is evolving for COVID-19.

Conclusion

In conclusion, through a systematic mapping of the literature of IFN-based agents, including TLR-agonists, for viral respiratory infections, this scoping review has provided insight on the existing literature and gaps with an eye for epidemic preparedness for the future. IFN-based agents warrant further research, particularly on the administration routes, efficacy, and biomarkers, including IFN signatures, autoantibodies, and gene alterations that may impact patient response. Several studies investigating the use of IFN-based agents in viral respiratory infections—including three large trials led by WHO, the National Institutes of Health, and Oxford University—are ongoing, signaling the potential of developing further knowledge to inform the role of IFN-based agents in mitigating future viral respiratory epidemics and pandemics.

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