New therapeutic targets for the prevention of infectious acute exacerbations of COPD: role of epithelial adhesion molecules and inflammatory pathways

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Abstract

Chronic respiratory diseases are among the leading causes of mortality worldwide, with the major contributor, chronic obstructive pulmonary disease (COPD) accounting for approximately three million deaths annually. Frequent acute exacerbations of COPD (AECOPD) drive clinical and functional decline in COPD and are associated with accelerated loss of lung function, increased mortality, decreased health-related quality of life and significant economic costs. Infections with a small subgroup of pathogens precipitate majority of acute exacerbations (AE) and consequently constitute a significant comorbidity in COPD. However, current pharmacological interventions are ineffective in preventing infectious exacerbations and antibiotic therapy is compromised by the rapid development of antibiotic resistance. Thus, we need to consider alternative preventative therapies. Pathogen adherence to the pulmonary epithelium through host receptors is the prerequisite step for invasion and subsequent infection of surrounding structures. Thus, disruption of bacterial-host cell interactions with receptor antagonists or modulation of the ensuing inflammatory profile present attractive avenues for therapeutic development. This review explores key mediators of pathogen-host interactions that may offer new therapeutic targets with the potential to prevent viral/bacterial mediated AECOPD. There are several conceptual and methodological hurdles hampering the development of new therapies that require further research and resolution.

Key words: chronic obstructive pulmonary disease, exacerbation, infection, inflammation, novel therapies.

Abbreviations:

COPD	Chronic obstructive pulmonary disease
AE	Acute exacerbation
NTHi	Nontypeable *Haemophilus influenzae*
HRV	Human rhinovirus
FEV_1	Forced expiatory volume in 1 second
GOLD	Global Initiative of Chronic Obstructive Lung Disease
LABA	Long acting β2-adrenergic receptor agonists
LAMA	Long-acting muscarinic antagonists
ICS	Inhaled corticosteroids
EC	Electronic cigarettes
PAFr	Platelet-activating receptor
pCHo	Phosphorylcholine
CSE	Cigarette smoke extract
ICAM-1	Intracellular adhesion molecule-1
NF-κβ	Nuclear factor-kappaB
IL	Interleukin
IFNγ	Interferon gamma
TNF	Tumor necrosis factor
hpBECs	Primary human bronchial epithelial cells
RSV	Respiratory syncytial virus
NHBE	Normal human bronchial epithelial cells
CEACAM-1	Carcinoembryonic antigen-related cell adhesion molecule-1
TLR	Toll-like receptor
UspA1	Ubiquitous surface protein A1
LPS	Lipopolysaccharide
LOS	Lipooligosaccharide
OMP	Outer membrane protein
PDE	Phosphodiesterase
IPF	Interstitial pulmonary fibrosis
CF	Cystic fibrosis
ROS	Reactive oxygen species
NOX	NADPH oxidase
DUOX	Dual oxidase
Gpx	Glutathione peroxidase
SOD	Superoxide dismutase

RESPIRATORY INFECTIONS ARE A MAJOR DETERMINANT OF PULMONARY MORBIDITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Chronic respiratory diseases are among the leading causes of mortality worldwide, with the major contributor, COPD, accounting for approximately three million deaths annually [1-4]. Acute exacerbations of COPD (AECOPD) are most often precipitated by respiratory tract infections, and are associated with accelerated loss of lung function, increased mortality, decreased health-related quality of life and significant economic costs [1, 3, 5-11]. However, as is the case for many chronic respiratory diseases, the mechanisms that promote increased susceptibility of COPD patients to infections are poorly understood [12-14].

Recurrent acute infections with bacterial and/or viral pathogens are strongly linked with the occurrence of exacerbations and contribute considerably to the clinical course of COPD. As such, these infections constitute a significant comorbidity in COPD [15]. Approximately 50% of AECOPD are caused by bacterial pathogens and in severe exacerbations requiring ventilatory support, the frequency exceeds 70% [12, 13]. In AECOPD, nontypeable *Haemophilus influenzae* (NTHi) is the most frequent bacterium isolated, followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*. In healthy individuals and stable COPD, these bacteria are common colonisers of the nasopharynx and migration to privileged anatomical sites is typically facilitated by a combination of bacterial pathogenic mechanisms and defects in host innate immune defences [16-18].

Despite enhanced expression of an antiviral immune response, COPD epithelial cells exhibit increased vulnerability to viral infection [19]. PCR-based studies have detected viral contribution in 34-60% of cases, most frequently with human rhinoviruses (HRV) accounting for 36-50% of viruses [20-23]. Influenza virus and respiratory syncytial virus (RSV) are also detected in approximately 16% and 29% of exacerbations, respectively [22]. Viral and bacterial co-infection is a frequent occurrence and is strongly associated with worse exacerbations of respiratory disease and enhanced inflammation [3, 24, 25].

The healthy human bronchial tree and lung parenchyma have a remarkable ability to maintain a healthy status in spite of repeated exposure to microbial aspiration [15]. However, in COPD this innate lung defence appears to be disrupted, enabling proliferation of a dysbiotic lung microbiome [26-29]. Pathogen adherence to the pulmonary epithelium through host receptors

is the pre-requisite step for migration and subsequent infection of surrounding structures [30]. After attachment to the mucosal surface, pathogens use a variety of mechanisms to enhance their persistence such as antigenic variation, immune evasion and invasion of host respiratory cells [3, 31-33]. Persistence of pathogens in the lower respiratory tract has significant implications for patients with COPD and has been suggested to be a major determinant of pulmonary morbidity or a source of infections that induce AECOPD [7, 18, 28]. Interestingly, even in the absence of clinical exacerbations, airway colonisation by bacterial pathogens in COPD has been associated with a clinically significant increase in daily symptoms [3, 34, 35]. Thus, elucidating key mediators of pathogen-host interactions may reveal new therapeutic targets with the potential to prevent viral/bacterial mediated exacerbations in COPD.

CURRENT COPD MANAGEMENT NECESSITATES ALTERNATIVE PREVENTATIVE STRATEGIES

Management of COPD typically involves avoidance of risk factors to prevent disease progression and pharmacotherapy to control symptoms, exacerbation rates and severity [2]. Existing therapies are of limited effectiveness and do not affect the overall course of COPD. Despite prescription of therapies shown to reduce exacerbation frequency, approximately one-third of COPD patients still experience one or more exacerbations every year [36, 37].

Loss of lung function and disease progression is exemplified by declines in forced expiatory volume in 1 second (FEV_1) over time, and frequency of exacerbations [38]. However, it is important to consider the inherent limitations of measuring these disease features. FEV_1 measurement requires an artificial manoeuvre that does not always correlate with clinically relevant outcomes such as dyspnoea, exacerbations, health status or exercise capacity [38]. There is also no standardised definition of an exacerbation; differential diagnoses such as pneumonia, heart failure, ischaemic heart disease and pulmonary embolism have to be taken into account [39]. These limitations make comparative evaluations of clinical studies difficult. This is further complicated by the lack of established biomarkers that reflect the inflammatory and destructive process in the lung or that indicate responsiveness to treatment [40, 41].

Pharmacotherapy

The most widely adopted therapeutic approach for managing COPD are the recommendations of the Global Initiative of Chronic Obstructive Lung Disease (GOLD). The GOLD approach recommends combination therapy with long acting β2-adrenergic receptor agonists (LABAs), long-acting muscarinic antagonists (LAMAs) and inhaled corticosteroids (ICSs) for patients with severe disease or frequent exacerbations [42]. LABAs and LAMAs achieve bronchodilation to increase lung function parameters such as FEV_1 while ICSs are administered for their anti-inflammatory effects [43]. Although these treatments may be effective in improving quality of life, FEV_1 and reduce exacerbation rates, there is currently no conclusive evidence that any existing medications for COPD modify mortality rates or the long-term decline in lung function [44-48]. The highly variable nature of responses to pharmacological treatment has further complicated treatment options. Irrespective of symptom severity and/or the level of risk, patients do not respond equally to all drugs [49]. The GOLD approach attempts to address this by stratifying patients into phenotypes based on disease severity, assessed by spirometry, symptoms and exacerbation history, to determine the most appropriate therapy. However, these clinical phenotypes require prospective validation regarding their prognostic value and treatment responses [50].

The anti-inflammatory effects of ICS therapy is important in the management of mild-moderate persistent asthma [14, 51], however; the role of ICS in the treatment of COPD remains controversial [52]. The inflammatory process in COPD has been shown to be fundamentally different to that of asthma [53], and there is conflicting evidence surrounding the efficacy of ICSs in preventing exacerbations [42, 54]. Double-blind placebo-controlled studies found that even high doses of ICS do not reduce inflammatory cell numbers, concentrations of cytokines, or proteases [55-57]. Additionally, early trials found no clinical benefit of long-term ICS in the treatment of mild-advanced COPD [58, 59]. Despite this, several trials revealed some benefit of ICS treatment showing a reduction in the overall frequency of AECOPD [47, 60-62]. However, it has been suggested these conclusions were based upon questionable statistical analyses and that other studies that used potentially more appropriate statistical approaches found no significant effects of ICS treatment [63-65]. Those that did find a reduction in exacerbations, had no significant effect on the survival of COPD patients [66]. These inconsistencies may be confounded by large patient-to-patient variability in responsiveness to ICS treatment [67], or difficulties in measuring ICS efficacy, particularly as commonly used variables such as FEV^1 may not adequately reflect the overall health status of patients [48, 50,

68]. Additionally, the use of ICS is associated with adverse effects such as bruising, accelerated bone loss, and increased risk of subcapsular cataracts [69]. Recent GOLD recommendations propose an individualised approach to ICS administration to mitigate the associated risks in patients unlikely to benefit from this treatment. Data modelling shows that blood eosinophilia may be predictive of a higher risk of exacerbations [70] and a favourable response to ICS therapies in patients with stable and moderate-severe COPD [71-75]. This evidence has formed the basis for a predictive (>300 cells/uL) threshold which can be used to identify patients with the greatest likelihood of treatment benefit with ICSs (in combination with LABAs). ICS-containing regimes have little or no effect at a blood eosinophil count <100cells/uL [76]. Although studies assessing the benefit of ICS treatment in eosinophilic COPD consistently report a reduction in exacerbation frequency, they are all based on retrospective evidence [72, 75]. Thus, clinical trials are required to validate these benefits and elucidate underlying mechanisms.

The clinical use of ICS is based on the supposition that airway wall inflammation is a primary driver of disease progression, a paradigm that now being challenged [77-81]. Recent evidence suggests the contrary, whereby COPD lungs exhibit dysfunctional immune responses due to decreases in key immune cells and/or their function [79]. We have previously reported that "total" airway wall cellularity decreases in COPD patients compared to never-smokers, with a strong association with smoking history [26, 82]. A similar trend was observed for airway wall vascularity [83]. Inhaled fluticasone propionate did not change total airway wall cellularity but improved *lamina propria* vascularity in COPD ex-smokers [82, 83]. Our data from a confirmatory cross-sectional study demonstrated hypocellularity of both large and small airway walls in COPD compared to never-smokers. However, there was no change in the proportions of key immune cell populations such as neutrophils, macrophages (CD68+), CD8+ and CD4+ cells [26]. The only increase was observed for CD8+ cells in small airways but not in the large airways of COPD [26]. In addition, we noted that CD68+ expression was non-specific and localised to macrophages and a mesenchymal cell population [26]. We further identified differential macrophage switching in small airway wall, lumen and alveolar macrophages (AMs) [84]. The airway wall in never-smokers was predominantly M2 (CD163+), which switched to a more M1 phenotype (CD68+ iNOS+ dual positive) in COPDs, while, the AMs in contrast switched towards a more M2 phenotype. Furthermore, AMs from COPD patients had comparatively reduced iNOS expression than never-smokers, again confirming immune

dysfunction and possibly explaining their inability to mount an effective response to infection and efferocytosis [85].

Thus, the immune-inhibitory function of ICSs may exacerbate the inherent immune dysfunction and increase susceptibility to persistent infections [77]. This may explain the increased incidence of pneumonia in ICS users compared to non-users [42, 86, 87]. Several studies have associated ICS use with a 70% increase in the risk of being hospitalised for pneumonia, the effect of which is ICS dose-dependent [42, 69, 86, 87]. This association is strengthened by a recent study of over 10,000 COPD patients that compared a fifty-two-week, once-daily combination of fluticasone furoate (ICS), umeclidinium (LAMA) and vilanterol (LABA) either in triple or dual therapy. There was a higher incidence of pneumonia in the triple therapy (ICS-LABA-LAMA) and fluticasone furoate-vilanterol (ICS-LABA) groups than in the umeclidium-vilantrol (LAMA-LABA) group [88]. A recent study also found a 37% decrease in the risk of pneumonia in patients withdrawn from ICS therapy over three-six months [89]. Experimental mouse models have also described ICS-mediated impairment of macrophage bactericidal properties and the IFN-mediated antiviral immune response, resulting in impaired clearance of pneumococci, *Klebsiella pneumoniae* [90, 91] and HRV [92]. Despite the predictiveness of eosinophilia in ICS treatment response, a meta-analysis of five studies comprising of 12, 496 patients with moderate-to-severe COPD showed an increase in the risk of pneumonia in the high eosinophil group with ICS-including treatments, compared to the low eosinophil group [72]. This indicates that the risk of pneumonia is a result of ICS treatment, irrespective of eosinophil count [74, 75, 93]. Proposed drugs targeted against eosinophil recruitment by antibody inhibition of IL-5, such as Mepolizumab may therefore be ineffective in reducing pneumonia and infectious exacerbations. Indeed, trials have found no beneficial effects of IL-5 inhibitors on pneumonia or infectious exacerbations [94-96] and may even be detrimental. Eosinophils have been shown to contribute to the immune response against infection and counts ≥2% predicted an overall lower risk of bacterial presence at exacerbation [70, 97-99]. Therefore, their inhibition may increase susceptibility to infectious exacerbations.

Clinical and experimental studies suggest that there are ICS intra-class differences regarding pneumonia risk with some evidence of increased risk with fluticasone propionate (43-78%) compared to budesonide therapy [100]. Such disparity may be the consequence of physiochemical and pharmacokinetic differences resulting in the prolonged presence of slowly dissolving fluticasone propionate particles in the airway epithelial lining fluid compared with

budesonide. This may cause protracted local immunosuppression that could impair the clearance of airway pathogens, leading to their airway/lung colonisation, which may develop into pneumonia. The correlation between higher bacterial load and higher ICS dose in stable COPD patients exemplified this theory [100]. Current therapies and clinical trials in COPD are summarised in Table 1.

The role of antibiotics

Although first-line pharmacological interventions reduce AECOPD, they are not an effective means of preventing infectious exacerbations [101]. Thus, current guidelines advocate the use of intermittent antibiotic therapy for moderate and severe exacerbations that exhibit symptoms consistent with respiratory infection, i.e. increase in dyspnoea, sputum volume and sputum purulence [42]. Usual initial empirical treatment is aminopenicillin with clavulanic acid, doxycycline or macrolide treatment due to their additional anti-inflammatory properties [36, 102]. Studies suggest that long-term or intermittent antibiotic therapy may have a beneficial effect on the outcome of COPD patients and may improve quality of life by reducing exacerbation frequency or by prolonging time to next exacerbation through local and systemic effects [103-107]. Antibiotic therapy may reduce exacerbation frequency by eradicating colonising bacteria with the potential to cause infection, however there is limited information to support this hypothesis [35]. This hypothesis is also not correlated with microbiome data that show an overrepresentation of potential pathogens and associated inflammatory response in COPD airways despite antibiotic therapy [18, 28, 29, 108].

Antibiotic therapy is complicated by the lack of guidelines pertaining to the most appropriate drug and administration regime, as well as the optimal duration of therapy [101]. Furthermore, antibiotics do not offer long-term protection against re-infection and relapse is common [109]. Inadequate antibiotic efficacy, which through incomplete resolution of the initial exacerbation and persistent bacterial colonisation is likely to influence risk of relapse [35, 110, 111]. Prolonged and sub-inhibitory antibiotic concentrations in COPD airways may also promote antibacterial resistance by providing a conducive environment for the amplification of antibiotic-resistant subpopulations [101, 111]. Management of pneumococcal and NTHi infections, have been compromised (in particular NTHi) by the rapid development of antibiotic resistance and infections are frequently refractory to first and second line antibiotics [109]. Intermittent or long-term use of macrolides and penicillins have been associated with the failure to eradicate resistant strains of *S. pneumoniae* and *H. influenzae* from the sputum of COPD

patients [35, 102, 111, 112]. Fluoroquinolones, in particular moxifloxacin, have proved to be effective at eradicating common COPD pathogens and resistance to this class of antibiotic is currently low [102, 113]. However, fluoroquinolone-resistant NTHi isolates have been detected worldwide and treatment failure with other fluoroquinolones such as ofloxacin or levofloxacin has also been described [114-116]. A randomised control trial reported a direct comparison of 13-week antibiotic therapy using tetracyclines, macrolides and quinolones. Total bacterial load did not decrease significantly after three months and there was a large increase in antibiotic resistance in all treatment groups where mean inhibitory concentrations of culture isolates increased by at least three-fold over the placebo group [117].

Long-term use of particular antibiotics has also been associated with adverse side effects, further reducing antibiotic treatment options. For example, azithromycin use has been associated with hearing impairment and increases in cardiovascular events [36].

Vaccination

Prevention of infectious exacerbations by vaccination has the potential to increase quality of life and patient survival. However, the availability of vaccines that protect COPD patients from key pathogens is restricted and the efficacy of currently used formulations are variable [118]. International guidelines recommend that patients with COPD should be immunized with influenza and pneumococcal vaccines. A systematic review of injectable polyvalent pneumococcal vaccines in COPD patients identified seven studies for inclusion (2 for 14-valent vaccine and 5 for 23-valent) and observed reductions in the incidence of pneumococcal pneumonia and AECOPD that did not reach statistical significance [119]. However, the pneumococcal conjugate vaccines, PPSV13 and PPSV23, have been shown to reduce the incidence of community-acquired pneumonia in COPD patients [120, 121].

The majority of clinical trials evaluating the efficacy of influenza vaccines in COPD patients indicate long-term benefits of annual seasonal influenza vaccination, such as reduced frequency of exacerbations, reduced hospitalisations and outpatient visits, and decreased all-cause and respiratory mortality [122-124]. Despite this, patients with COPD still remain highly vulnerable to adverse outcomes from influenzae infections. [123, 125] There is also evidence that suggests COPD patients exhibit a significantly lower humoral immune response to the influenza vaccination compared to healthy participants. A study assessing the immunogenicity of the 2010 trivalent influenza vaccine found a lower rate of seroconversion in COPD

participants (43%) compared to healthy participants (90%) [126]. This altered immune response was associated with reductions in both T-cell and B-cell function. [127, 128] Further investigations are warranted to examine the mechanisms behind this altered immune response with the potential for new vaccination approaches in COPD that maximise protection against influenza infections.

Development of new vaccination strategies is further complicated by the intrinsic heterogeneity of pathogen target proteins, as is the case with an oral whole-cell killed NTHi vaccine developed to reduce acute exacerbations in COPD. Trials with this vaccine showed no significant beneficial effects to warrant widespread vaccination [7, 129]. Similarly, vaccination is not feasible against viruses such as HRV due to the large number of serotypes with low antibody cross-reactivity [130]. Despite the feasibility of vaccination, no vaccine is currently licensed to prevent RSV infection. [131, 132].

Antiviral Agents

Although not currently utilised in COPD, antiviral agents have been suggested as an approach to mitigate viral-induced exacerbations and associated complications particularly as there are currently no therapies recommended for treatment of viral infections in COPD. [133, 134] Although vaccination may be a useful prophylactic treatment it has no benefit during an active infection.

Only two classes of drugs are currently approved for the treatment of influenza: M2 ion channel blockers (adamantanes) and neuraminidase (NA) inhibitors. Adamantanes inhibit influenza A replication by blocking virus entry while NA inhibitors (e.g., Peramivir, Zanamivir and Oseltamivir) block the release of virions after budding from the host cell in both influenza A and B viruses [135-138]. Zanamivir has been shown to prevent influenza infection or reduce symptoms if begun early in the infection process. The clinical efficacy and safety of Zanamivir has been demonstrated in all age groups and in healthy and immunocompromised individuals [137, 139-141]. Albeit limited, evidence suggests similar benefits and safety profile of Zanamivir for the treatment of influenza in COPD [125]. However, the evidence produced by this study was unable to rule out adverse events such as bronchospasm. Zanamivir was shown to promote resolution of influenza, without adversely affecting pulmonary function [142]. Oseltamivir has shown a similar efficacy to Zanamivir in healthy adults and children with reductions in illness severity and duration, viral shedding, and lower respiratory tract

complications [143]. Oseltamivir has not been associated with any significant respiratory adverse effects in healthy individuals, but no data exists on the safety and efficacy in patients with underlying respiratory disease [144, 145]. Likewise, there are no trials of M2 inhibitors in COPD patients, however there are serious safety concerns in otherwise healthy patients with this class of antiviral. Side effects involving the central nervous system occur in 5%–29% of patients treated with amantadine, and include headache, light-headedness, dizziness, difficulty in concentrating, and insomnia [142].

Of growing concern is the reduced susceptibility to currently used antivirals, which has been demonstrated in influenza A and B strains. [146, 147]. Clinical cases of influenza viruses harbouring single or multiple NA or haemagglutinin (HA) substitutions or deletions have been reported [148]. H1N1 influenza viruses containing a mutation conferring resistance to oseltamivir, one of the most common resistance mutations seen in treated patients since 2004, have now circled the globe [149]. Adamantane resistance increased in frequency from 0.4% of influenza A viruses isolated in 1994–1995 to 12.3% in 2003–2004. Of concern is the fact that the currently circulating strains of avian H5N1 influenza are highly resistant, thus if human infections do become more frequent, the M2 inhibitors will have no useful therapeutic role [142]. Combination therapy of antivirals with different mechanisms of action has demonstrated greater clinical efficacy than monotherapy [150, 151] and has been promoted for the treatment of strains resistant to one of the NA inhibitors [152]. However, mutations resulting in a cross-resistant phenotype to Zanamivir and Oseltamivir have been reported and are more likely to occur in immunocompromised individuals. [147, 153]. While these resistant phenotypes tend to arise from prolonged treatment with NA inhibitors, it is generally immunocompromised patients that required prolonged therapy in the first place. Given that the only approved antivirals against influenza are already inducing emergence of resistant mutants, there is an urgent need for the development of new antiviral approaches.

A number of other HRV-targeted antiviral agents have been developed but have so far not been approved for human use. These agents typically target the HRV capsid to prevent binding to host cells, or inhibit viral 3C protease, the activity of which is essential to viral replication [154, 155]. However, considering these are viral targets and the high mutation rates of RNA viruses, the efficacy of these antiviral classes are likely to be undermined by resistant mutants. During phase II clinical trials with the capsid binder pleconaril, virus variants with reduced susceptibility or even full resistance were detected in, respectively, 11% and 3% of the drug-

treated subjects. All capsid binders, including vapendavir and pleconaril, share the same target so drug-resistant mutants exhibit cross-resistance [133]. Development is further complicated by formulation bioavailability and toxicity [133].

Thus, whilst the treatment of viral-induced AECOPD may confer long-term benefits in COPD patients, the need for new antiviral targets that will not instigate resistance is needed, particularly given the likelihood of prolonged treatment regimens in these patients.

Non-pharmacological therapy

Non-pharmacological therapy, such as patient education, pulmonary rehabilitation, long term oxygen and minimising exposure to particulate matter are also important for improving health-related quality of life in COPD patients [156, 157]. However, cessation of smoking is one of few interventions that slows the rate of progression of COPD and prolongs life expectancy [158, 159]. The Lung Health Study, a five-year early intervention study involving 3,926 smokers with mild to moderate airflow limitation due to COPD demonstrated the benefit of sustained smoking cessation. Participants who ceased smoking experienced half the rate of decline in FEV_1 than continuing smokers, which was comparable to those that had never smoked [160]. This benefit persisted for at least an additional six years in those that remained abstinent [161]. In addition, smoking cessation, regardless of the severity of lung disease, has been shown to have a mortality benefit, secondary to the reduction in cardiovascular and lung cancer mortality [162]. The addiction to the nicotine component of tobacco and behavioural dependence has resulted in a large proportion (30.4% to 43.0%) of moderate to severe COPD patients continuing to smoke [156]. This is in spite of the two-fold increase in smoking cessation in patients undergoing nicotine replacement therapy as relapse rates are high in COPD compared to the general population [163, 164]. The recommendation of oxygen therapy came from early experiments that showed long-term treatment with supplemental oxygen reduced mortality among patient with COPD [165]. However, recent studies have refuted the benefits of oxygen therapy, finding no effect on survival of COPD patients with moderate hypoxemia [165-167]. The Long-Term Oxygen Treatment trial of 738 COPD patients followed for 1-6 years, found no significant benefits of long-term oxygen therapy on survival, hospitalization rates, exacerbations or other outcomes such as quality of life, lung function and exercise capacity [165, 168].

Nicotine replacement therapy can be administered in various formulations such as gums, lozenges, transdermal patches, inhalers and nasal sprays. However, each method comes with issues regarding nicotine delivery and/or side effects [156]. Electronic cigarettes (EC), have gained rapid popularity as a mode of nicotine replacement therapy, with the concept that they are a safer alternative to cigarette smoking. Despite their increasing use, there is limited research regarding the impact of EC on human health and effects on the lungs [164, 169]. EC vapour typically contains nicotine, together with a variety of flavourings, additives, and other contaminants that have the potential to affect normal lung biology. They also may be gateway formulations to conventional smoking and be particularly deleterious in COPD patients [170].

Evidence shows that EC aerosols have immune modulatory activity on human and mouse airway epithelial cells *in vitro* and mice *in vivo*, resulting in oxidative stress [171], low levels of inflammatory cell recruitment [172-174], and DNA damage that reduced repair activity [175]. Two-week EC aerosol exposure in mice resulted in reduced bacterial clearance and increased susceptibility to influenza virus infection compared to air-exposed controls, despite appropriate recruitment of neutrophils and alveolar macrophages. The impaired immune defence was attributed to reduced levels of cytokines (IL-17A, IFNγ, and TNF) involved in protective immunity against infection. Reduced IL-17 responses and defective bacterial phagocytic function of alveolar macrophages has also been observed. Increases in phosphatidylinositol 3-kinase, increases in miR-125a/b and impaired stress granule formation have all been implicated in increased susceptibility to influenza virus infection in COPD [31, 176] . Miyashita et al reported that nicotine-containing EC vapour increases primary cell and mouse nasal PAFR expression, hence increasing susceptibility to pneumococcal infection [177].

It is unclear which component(s) of EC vapour elicits these immunomodulatory effects. Nicotine has many immunosuppressive effects, including impairing antibacterial defences against *S. pneumoniae* and *Legionella pneumophilia* [178, 179], and alteration of macrophage activation that suppresses adherence, chemotaxis, phagocytosis and killing of bacteria [180-182]. The effects of other components on pulmonary function are less clear but constituents such as propylene glycol are known to elicit detrimental effects on lung function of workers exposed to theatrical smoke machines [183]. In a small study, acute exposure to nicotine-rich aerosols in healthy naïve subjects affected the biology of small airway epithelium, alveolar macrophages and the alveolar capillary endothelium. Gene expression changes in small airway

epithelium and alveolar macrophages were also observed in the nicotine-free group, suggesting that EC vapour may have harmful long-term effects [175]. We recently showed in a ground-breaking study, that in a manner very similar to cigarette smoke, EC and IQOS (heat-not-burn smoking device) has the potential to increase oxidative stress and inflammation, infections, airway remodelling and initiate epithelial mesenchymal transition (EMT) related changes in the airways of users of these devices. However, prospective clinical studies must be conducted to verify our *in vitro,* cell-based but highly important and novel findings on EC and IQOS [184].

NEW THERAPEUTIC TARGETS FOR THE PREVENTION OF INFECTIOUS AECOPD

Microbial pathogenesis involves a number of vital steps in order to infect and survive in the host environment. Upon access to the respiratory tract, pathogens must find a way to attach to host tissue, avoid destruction by host immune defences and replicate despite harsh environmental pressures and antimicrobial therapies **(Figure 1).** Development of new therapeutic agents has focussed on subverting these pertinent steps; inhibition of attachment/invasion, disruption of cell membrane, inhibition of microbial replication or enhancing clearance by the immune response **(Figure1)** [185]. The rising concern of antiviral and antibiotic resistance has also lead to the focus on specific host cellular processes essential for viral replication and modulation of the inflammatory profile in COPD, which is currently unresponsive to current pharmacotherapies [186]. These strategies are less likely to induce antimicrobial resistance as a missing cellular function is more difficult for a pathogen to adapt to, and should affect replication independent of the type, strain and antigenic properties of the invading pathogen [138]. New therapeutic targets that mitigate host-pathogen attachment and immune subversion have been summarised in Table 2 and Table 3, respectively.

Mediators of pathogen-host cell attachment

Platelet-activating receptor (PAFr)

The PAFr is a widespread G protein-coupled receptor that is expressed on the surface of the majority of innate immune cells and is a potent mediator of phospholipid-induced inflammation [187]. The PAFr recognises the phosphorylcholine (pCHo) determinant on the eukaryotic pro-inflammatory chemokine PAF, which is synthesised by various cells, such as leukocytes, macrophages and platelets [188]. PAFr-PAF binding mobilises Ca^{2+} and activates numerous

signalling pathways, such as those involving phospholipase C and A_2, and protein kinase C with downstream immune modulatory effects [189, 190]. Like PAF, the cell wall of NTHi and *S. pneumoniae* express pCho residues which engage the PAFr through molecular mimicry and induce cytoskeleton remodelling and subsequent host cell internalisation [187, 191]. This exploitation confers several persistence-related effects, including adherence, invasion and resistance to some host-derived antimicrobials [129, 192-194].

PAFr is of particular interest as cigarette smoke exposure, the major risk factor for the development of COPD [5, 187], has been shown to significantly upregulate its expression in the airway epithelium [195-198]. Studies have found significant increases in PAFr expression in both large and small airway epithelium and alveoli of active smokers and COPD patients, suggesting a ubiquitous expression of the receptor throughout the airways. The effect on adhesion is not limited to the most distal epithelial cells, since cigarette smoke extract (CSE)-stimulated adhesion has been shown in the bronchial epithelial cell line (BEAS2-B) and human primary bronchoepithelial cells (hpBECs) [195, 197]. Increased PAFr mRNA and protein levels in CSE-exposed A549 cells suggests a major role in CSE-induced adhesion [195]. Similar mechanisms could also be active in idiopathic interstitial lung disease [199].

Significant increases in *S. pneumoniae* and NTHi adherence to cultured primary human bronchial epithelial cells (hpBECs) exposed to CSE has also been demonstrated [195, 196]. Adhesion by these pathogens was attenuated by the PAFr antagonist, WEB-2086, to levels in non-CSE-exposed cells [196]. We also found that adhesion was attenuated by a specific PAFr antagonist (CV-3988). Compatible with previous studies in cytokine-stimulated A549 cells, blocking of PAFr attenuated stimulated, but not unstimulated adhesion of pneumococci to these cells [195]. The exact constituents of CSE responsible for PAFr upregulation remains unknown. However, a recent study showed upregulation of PAFr expression in nasal epithelium in response to nicotine-rich E-cigarette vapour *in vitro*, suggesting that nicotine may be important [200].

These investigations suggest that the upregulation of PAFr levels on the respiratory epithelial cell surface may increase susceptibility to infections in COPD patients [197]. PAFr antagonists may therefore be a new drug target for inhibiting PAFr-dependent respiratory infections in smokers and COPD patients [196]. The PAFr bacterial ligand, PCho would be a poor vaccine target for NTHi given the constant phase variation and variable nature in which it stimulates

protective immunity [192, 201]. However, it may be more promising if combined with immunogens (e.g. proteins) that are conserved across NTHi strains.

Intracellular adhesion molecule-1 (ICAM-1; CD54)

ICAM-1 is expressed constitutively on a wide variety of cells and is a key molecule in processes critical to normal leukocyte recruitment and T-cell development such as reversible cell-cell adhesion that enables intercellular communication and signal transduction [202]. Additionally, aberrant ICAM-1 levels have been implicated in pathological complications such as acquired immunodeficiency syndrome, malignancies and allergic asthma [203]. NTHi and 60-90% of HRV serotypes utilise ICAM-1 in attaching to host bronchial and alveolar epithelial cells [204-206]. More data are required to determine the frequency at which NTHi uses this mechanism of adhesion. However, blockade of cell surface ICAM-1 with a specific antibody reduced the adhesion of NTHi to A549 respiratory epithelial cells by 37% and to CHO-ICAM-1 cells by 69%; suggesting a cell-dependent role of ICAM-1 as a binding target [202].

Evidence suggests that expression of ICAM-1 is stimulated *via* the activation of nuclear factor-kappa-B (NF-κβ) pathways by NTHi and HRV [207]. We previously reported increased ICAM-1 levels on the airway epithelium of patients with chronic bronchitis and smoking-related chronic airflow limitation [208]. *In vitro,* incubation of respiratory epithelial cells with NTHi increased ICAM-1 expression four-fold and the binding of HRV39 in a TNF-dependant manner [129, 202, 205, 209]. Additionally, all HRV serotypes, regardless of the receptor used for cell attachment, have been shown to enhance ICAM-1 expression [207]. This suggests a mechanism for additive or synergistic effects between bacterial and viral infection that may explain why COPD patients are more susceptible to viral infections and NTHi-HRV co-infections [209].

Vaccine development against NTHi and HRV infections is difficult given the high degree of antigenic heterogeneity. Antibodies produced in response to vaccines provide little or no cross-protection against newly-acquired strains [201, 210]. This makes host ICAM-1 an enticing therapeutic target for the prevention of both NTHi and HRV infection in COPD patients. However, inhibiting ICAM-1 may induce deficient normal host defences and cell trafficking as a side-effect. One trial assessed this using a mouse anti-human ICAM-1 antibody (14C11) that specifically binds domain 1 of human ICAM-1. When administered topically or systemically, the antibody prevented the entry of two major groups of HRVs and reduced

cellular inflammation, pro-inflammatory cytokine production and virus load *in vivo* [211]. Inhibiting ICAM-1, therefore, offers a promising means of supressing HRV entry and may also have application in preventing the common cold and exacerbations of bronchiolitis, asthma, COPD and pneumonia in patients [210, 212].

NTHi ligands for ICAM-1, namely the outer membrane protein P5 fimbria and the type IV pilin, PilA, have also been investigated as potential therapeutic targets [129, 205]. Expression of PilA contributes significantly to ICAM-1-mediated adherence and mucosal colonisation and also has important roles in biofilm formation and the ability of NTHi to exhibit competence [213]. Each of these phenotypes has important implications for the initiation and establishment of long-term colonization of humans, as well as ability to induce disease, cause recurrence and promote the chronicity of NTHi-induced diseases [205]. It has also been shown that respiratory syncytial virus (RSV) enhances the attachment of NTHi to epithelial cells which is largely mediated by the bacterium's P5-fimbria-binding receptors [214].

NTHi PilA may also be a therapeutic target. Disruption of the *pil* locus diminished the ability of NTHi to adhere to normal human bronchial epithelial cells (NHBEs) *in vitro* or to form stable, robust biofilms in a continuous flow chamber. Additionally, a *pilA* mutant was not as capable of maintaining colonization of the mucosal epithelial surface in the chincilla nasopharynx [213]. Antibodies against PilA induces the formation of antibodies that eradicate NTHi from middle ear fluids and mucosal biofilms of experimental otitis media from the chinchilla middle ear [215]. Antibodies directed against P5 have also shown to be protective in rat and chinchilla models of middle-ear infection [205]. During chronic infections with NTHi, P5 is antigenically variable, potentially permitting the bacteria to evade immune recognition and killing [202]. However, this may be overcome by delivering P5- and PilA-targeted immunogens together with an adjuvant. This immunisation strategy has been shown to induce significantly earlier clearance of NTHi from the nasopharynges and middle ears of chinchillas compared to immunogen or adjuvant alone [215].

Carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1)

NTHi and *M. catarrhalis* are able to utilize CEACAM-1 as a receptor for efficient adhesion, and internalisation, and to induce apoptosis and suppression of Toll-like receptor-2 (TLR2) and TLR4 as an immune-evasion strategy [216]. The CEACAM family is a group of 12 highly glycosylated intercellular adhesion molecules involved in signalling events that mediate key

processes including cell adhesion, proliferation and differentiation, as well as tumour suppression and apoptosis [217]. CEACAM-1 binding is mediated by P1, an outer membrane protein in NTHi, and ubiquitous surface protein A1 (UspA1), an outer membrane protein in *M. catarrhalis* [218]. Both pathogens can increase the expression CEACAM-1 on host cells, thereby increasing host susceptibility to bacterial infection [218]. An *in vivo* study with chinchillas reported that anti-CEACAM-1 antibody (YTH71.3) effectively blocked NTHi attachment to the nasopharynx [218]. However, a direct correlation between CEACAM-1 levels and COPD has not yet been found [217]. Despite a lack of correlation, inhibition of binding to CEACAM-1 may be effective in preventing infection with NTHi and *M. catarrhalis*. However, studies have also shown that the intrinsic signalling of CEACAM-1 is also essential for generating efficient B-cell survival and protective anti-viral antibody response. CEACAM-1 may function as broad-spectrum antiviral suppressor including influenza virus [219] and the absence of CEACAM-1 on B-cells leads to insufficient anti-viral B-cell response in mice [220]. Similarly, CEACAM1 is found to be an important regulator of virus-specific CD8+ T cell functions in mice and humans [221]. Thus, blocking this pathway may increase susceptibility to viral infection. However, a study of mice with altered expression *Ceacam1a* failed to develop clinical signs of viral infection with mouse hepatitis virus, produced smaller viral load in the liver and survived a dose 100-fold higher than wild-type mice. This was a result of the reduced expression of CEACAM-1a/D1-4 proteins and altering the ratios between two- and four- domain CEACAM-1a isoforms *in vivo.* These results suggest an isoform-dependent activity of CEACAM-1, which may be relevant in COPD [222].

Sialic acid (SA) and Haemagglutinin A (HA-A)

SAs are a group of nine-carbon monosaccharides that decorate the surface of most mammalian cells, mostly in cell membrane glycoproteins. These residues contribute to the functioning of several biological systems, including the stabilisation of glycoproteins and cellular membranes, and assisting in cell-cell recognition and interactions [223]. There have been reports of increased levels of SA in the serum and saliva if COPD patients [224, 225]. Influenza viruses primarily infect bronchoepithelial cells (BECs) through the binding of viral HA-A to specific host SA–terminated glycoproteins. SAa2,6Gal and SAa2,3Gal residues are mainly found on upper and lower respiratory epithelial cells and are preferentially bound by human and avian influenza viruses, respectively [31, 226]. Following anchorage to host cells, the influenza virus is internalised by endocytosis into the endosome. The low pH environment of the endosome

allows the viral HA-A to undergo a conformational change and fuse with the endosomal membrane, thereby allowing the release of viral genes into the host cytoplasm [125, 227, 228].

Disruption of viral attachment has been proposed by two main mechanisms. The first is direct competition with SA binding by blocking receptor sites of HA-A, either directly with small peptide molecules [229, 230] or by neutralising antibodies targeted against HA-A subunits [231]. The second strategy involves interference of the HA-A conformational change necessary for viral fusion [232-234], thus preventing the subsequent release of viral RNA into host cells [235]. Preventing viral replication early in the infection cycle has proved to be a promising tactic in preventing and treating viral infections and may provide cross-protection of different viral strains [236].

A plethora of entry-blocking peptides are in development that display broad anti-influenza virus activity [236, 237]. Of these peptide-based inhibitors, only Arbidol has been approved for administration in humans, albeit only in Russia and China [236]. Arbidol has the ability to elicit protective broad-spectrum antiviral activity against a number of respiratory viruses such as influenza, RSV, HRV, coxsackie virus and adenovirus *in vitro* and *in vivo [152, 238]*. By functioning as molecular glue, Arbidol stabilizes the prefusion conformation of HA that inhibits the large conformational rearrangements associated with membrane fusion in the low pH of the endosome [239]. Arbidol has been found to reverse the main symptoms, reduce incidence of complications and contribute to the stabilisation of adaptive reactions in patients with acute respiratory viral infections [240]. A study of 100 patients with COPD or asthma found that seasonal and post-exposure prophylaxis using Arbidol (Umifenovir) was associated with 2.6-times reduction in influenza and acute respiratory viral infection related morbidity during an epidemic period. This study also showed that prophylactic administration of Arbidol before and during flu season reduced the frequency of infectious exacerbations of COPD [241]. Although clinical effectiveness has been shown, anticipating adverse events are difficult as Arbidol is the only available drug that targets HA-A and the structural information on drug-HA complexes is limited [239]. Although there are no reports of Arbidol resistance so far, [242] drug resistance is still a concern as influenza viruses mutate frequently and unpredictably, especially in the HA-A gene [243].

As an alternative to vaccines, neutralizing monoclonal antibodies (MAbs) represent a passive therapeutic strategy to provide immediate protected against influenza virus infection [244]. The

MAb, HA-7 was found to potently neutralise and completely protected mice against lethal challenges of H5N1 [231]. This MAb specifically targets the highly conserved epitope HA-A "head" subunit (HA1), which is mainly responsible for receptor binding [231]. MAb CR6261 was found to neutralize multiple influenza subtypes by blocking the conformational rearrangements associated with membrane fusion. It was also protective from lethal challenge of mice from H5N1, and also with H1N1 when administered up to 5 days post-infection [245]. MAb CR8020 exhibited neutralizing activity against most group 2 viruses, including H3N2 and H7N7 [246] and MAbs 5A7 and 46B8 have shown anti-influenza B activity conferring protection in mice. [247] Finally, a MAb that recognizes the HA glycoprotein of all 16 subtypes and neutralizes both group 1 and group 2 influenza A viruses confers protection to mice and ferrets when passively transferred [248]. It is evident that there are a range of MAbs with varying subtype binding and/or neutralization spectra which raises the possibility of combining different MAbs that could provide broad protection against all seasonal and pandemic influenza A viruses [249].

Entry-blocking with small molecule peptides and MAbs both manipulate the HA-A-mediated attachment to host SA. However, two HA-A subtypes, H17 and H18 from influenza viruses isolated from bats, do not bind to SA and the receptor for these viruses is not yet known [250]. This suggests a novel mechanism of influenza A virus attachment and activation of membrane fusion for entry into host cells. Thus, bats may constitute a potentially important reservoir for influenza viruses [251]. Although it is unlikely, there is the possibility that bat-derived genomes could exchange genetic information with human influenza viruses through reassortment, resulting in an immunologically unrecognisable influenza strain. The prospect of natural reassortment has been realised by the *in vitro* reassortment of a H1N1 virus with NS1 from the bat strain H17N10 that was able to propagate [252]. Furthermore, an infectious bat-derived chimeric influenza virus containing HA-A and NA from a human influenza virus was shown to replicate well in a broad range of mammalian cell cultures including, human primary airway epithelial cells and in mice *in-vivo* [253].

Host immune defence

Macrophage function and phenotype

Macrophages have important roles in host immune defence against bacterial and viral pathogens in the lung, and their involvement includes recognition of pathogen components *via* TLRs. Lung macrophages exhibit phenotypic and functional plasticity in order to adapt and

respond to a variety of insults [254]. Although oversimplified, macrophages can be crudely divided into M1 classically-activated and M2 alternatively-activated subtypes. [255] M1 macrophages promote Th1-type immunity by producing high levels of pro-inflammatory cytokines, such as IL-1β, TNF, and IL-12 induced by exposure to bacterial proteins and IFN-γ. Lipopeptides from NTHi and *S. pneumoniae* bind to and activate TLR2 [256], while the lipopolysaccharide (LPS) and lipooligosaccharide (LOS) from Gram-negative bacteria bind to TLR4; viruses activate TLR3,4,7 and 8 [254]. M1 macrophages have potent bactericidal properties but are cytotoxic to host cells due to their production of high levels of reactive nitrogen and oxygen species. Thus, their tissue damaging effects must be balanced by M2 macrophages that are involved in immunomodulation, tissue remodelling and fibrosis, and promote macrophage efferocytic function. M2 macrophages secrete anti-inflammatory cytokines, including IL-10, TGF-β, CCL18, and CCL22 upon exposure to fungi, immunocomplexes, helminths, complement components, apoptotic cells, macrophage colony-stimulating factors, IL-4, and IL-13 [257].

Despite significant increases in the total number of airway macrophages in COPD, their phagocytosis and elimination of microorganisms and apoptotic cells are impaired, suggesting defective functional properties [258]. We have found evidence for a macrophage phenotypic switch in COPD airways promoting a shift towards the M1 phenotype in the airway wall but towards M2 in the airway lumen compared to normal airways [258]. The divergent biological functions of M1 and M2 macrophages is dictated by the dichotomous regulation of arginine metabolism [259]. Arginase-1 (upregulated in an M2 environment) was found to suppress iNOS activity (M1 inflammatory marker) in the airway wall by competing with the common substrate arginine. The suppression of anti-bacterial iNOS activity by increased arginase-1 activity, along with its own contribution to collagen-1 formation promotes a pro-infectious environment along with increased wall thickening [258]. The cause of this phenotypic switch is unknown but may be an adaptive response to ongoing insult to the COPD airways by cigarette smoke and chronic colonisation/infection with bacteria [254].

In addition to immune-related aberrations, COPD macrophages also exhibit dysfunctional bacterial phagocytosis and uptake of dead and apoptotic cells (efferocytosis) which are essential functions in maintaining a healthy lung environment [257, 260, 261]. Impaired phagocytic ability of alveolar macrophages has been observed in both COPD patients and healthy smokers, compared to control subjects. Phagocytic capacity was greater in COPD

patients who had ceased, compared with those continuing to smoke, implicating continuous smoking as a key mediator of macrophage dysfunction [262]. Attenuated phagocytosis and efferocytosis in combination with a pro-inflammatory environment may promote bacterial colonisation and lung tissue damage, which collectively contribute to the progression of COPD.

Specific treatments that manipulate macrophage phenotype, efferocytosis and/or bactericidal properties may provide an effective means of clearing the respiratory tract and hasten the resolution of inflammation. The efferocytic ability of lung macrophages can be restored with phosphodiesterase (PDE) inhibitors, such as aminophylline/theophylline, and by levostatin, low-dose oxygen and acute phase reactant α-1 antitrypsin in mouse models [263-265]. Recent studies using Roflumilast, a newer PDE-4 inhibitor, revealed alterations in the lung macrophage phenotype to a more reparative M2 type [266]. Macrolides and statins have been shown to improve AM phagocytosis *in vitro* but further research is required to determine if they can correct defective bacterial phagocytosis in COPD [263, 267].

Manipulation of endogenous pro-resolving mediators including lipoxins, E-series resolvins, D-series protectins and maresins may also promote resolution of inflammation in COPD [268]. These mediators are derived from polyunsaturated fatty acids and act on distinct receptors to promote resolution of neutrophilic inflammation by preventing neutrophil recruitment and enhancing the removal of these cells by efferocytosis [269]. The pro-resolving molecule annexin A1 is gaining attention as a potential therapeutic target for both its roles in inflammation and influenza infection [270, 271]. Roles in monocyte recruitment, enhanced clearance of apoptotic cells by macrophages, and macrophage reprogramming toward a resolving phenotype, have been described [271]. Annexin A1 is also upregulated during influenza infection and enhances viral binding, replication, apoptosis and endosomal trafficking of the virus to the nucleus [270]. Annexin A1 deficient mice exhibit a survival advantage, and lower viral titres after infection with influenza A virus, accompanied by enhanced inflammatory cell infiltration [270]. Recently, it has been observed that serum Annexin A1 expression is upregulated in COPD patients and is positively correlated with the severity of the disease. The inability of Annexin A1 to resolve the chronic inflammation profile of COPD suggests that this resolution pathway is dysfunctional. Indeed, systemic levels of pro-resolving mediators are increased and dysfunctional in other chronic inflammatory diseases such as inflammatory bowel disease, preeclampsia, and Alzheimer's disease [272-274]. Interestingly, in a separate study with a slightly larger sample size, serum Annexin A1

was found to be decreased in COPD compared with healthy non-smokers [275]. However, results of both studies agreed that HBE Annexin A1 levels increased in response to CSE exposure and that annexin A1 correlated positively with the severity of COPD [275, 276]. The discrepancies between these two studies are inexplicable, and the similar methodologies suggest a sample-related factor. However, both can agree that Annexin A1 potentially plays a role in COPD pathogenesis and more studies are needed to solve this problem and determine the true role if there is to be any therapeutic development. As well as inhibiting infectious exacerbations, modulation of Annexin A1 or other pro-resolution molecules may be able to reduce thickening of the airway wall and arrest disease progression in COPD [277]. Such treatments may also be beneficial in other lung diseases such as interstitial pulmonary fibrosis (IPF) and cystic fibrosis (CF) where macrophages also exhibit impaired efferocytosis [278].

Macrophage TLR-mediated response

Dysregulation of TLR-mediated macrophage responses in COPD airways is also implicated in the pathophysiology of the disease. It has been suggested that overactive TLR responses, stimulated by chronic bacterial colonisation/infection and cigarette smoke, contributes to excessive inflammation and subsequent airway remodelling and lung tissue damage induces long-term declines in lung function [279]. Whole tissue explants from patients with COPD released higher levels of pro-inflammatory cytokines and chemokines TNF and CCL5 following activation of viral-associated TLR3, 7 and 8, compared with those from smokers [280]. Another study showed that TLR4 followed by TLR2 stimulation of alveolar macrophages enhanced cytokine production from COPD patients compared to those from smokers [281]. While these studies describe mechanisms of how COPD airways may undergo prolonged and excessive inflammatory responses during exacerbations, they fail to explain the inability of these responses to clear pathogens. They also compare tissue and macrophages from smokers and COPD patients without employing a normal non-smoker control for baseline comparison. Contrary to these findings, there is evidence to suggest that alveolar macrophages from the airways of chronic smokers and COPD patients exhibit dysfunctional responses to TLR stimulation [254]. Studies have shown an impaired ability of COPD alveolar macrophages to sense bacterial components via TLR2 and TLR4, a response that was not replicated by TLR3 stimuli [282]. Smoking-mediated oxidative stress reduces the production of a range of cytokines from stimulated and non-stimulated alveolar macrophages compared to those from non-smokers, which has been associated with reduced MAPK and NF-κB signalling [282-287]. Comparative induction of COPD and non-COPD alveolar macrophages by NTHi antigens,

LOS and outer membrane protein (OMP) P6 revealed diminished IL-8, TNF, and IL-1β responses of COPD alveolar macrophages [288]. These cytokines are important mediators of acute inflammatory responses, with downstream roles in neutrophil recruitment, cell growth and tissue remodelling [289, 290]. These findings support a paradigm of defective alveolar macrophage responsiveness, the inability of the immune response to clear infections and dysfunctional tissue repair in COPD.

Synthetic TLR activators are currently in clinical development for treating asthma, allergies and infections but may also be useful in COPD during infectious exacerbations [291]. TLR agonists or adjuvant therapy with the ability to correct the dysfunctional TLR-mediated responses of COPD macrophages could boost protective inflammatory responses to quickly clear pathogens and reduce the oxidative stress in the airway epithelium. Specific TLR agonists could be used to induce desirable response, such as a Th1 responses that could lead to neutrophilic inflammation, in addition to the suppressive effects on Th2 or regulatory T cells [292]. Intranasal delivery of the TLR7 agonist, imiquimod prevented peak viral replication, bodyweight loss, airway and pulmonary inflammation, and lung neutrophils in mice following influenza A infection. Imiquimod treatment also resulted in a significant reduction in pro-inflammatory neutrophil chemotactic cytokines and prevented the increase in viral-induced lung dysfunction [293]. However, this approach may be difficult to manage as to not amplify unwanted inflammatory tissue destruction. Before utilizing such therapies, the exact mechanisms surrounding inflammatory signalling pathways in COPD need to be fully elucidated.

Oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by immune cells exert antimicrobial activity against a broad range of invading pathogens, performing an important role in immune defence. However, their excessive accumulation or impaired clearance by antioxidant mechanisms results in oxidative tissue damage, including DNA damage, lipid peroxidation, and protein denaturation [294-296]. An increase burden of oxidative stress is recognised as an important feature in airway tissue damage and pathogenesis of COPD as a result of continuous ROS production [296-299]. Despite involvement in driving the onset of COPD, cigarette smoke-induced oxidative stress is not resolved after smoking cessation [300], implicating other endogenous factors, particularly persistent infection or pathogen presence as primary drivers of excessive ROS accumulation [297]. Key COPD

pathogens such as Influenza A, RSV and *S. pneumoniae* have been shown to elicit excessive production of oxidative stress markers in mouse models of COPD [298, 301, 302]. Despite the defensive role of ROS, their excessive accumulation may have deleterious functional effects on AM phagocytosis and efferocytosis, impairing clearance of pathogens, leading to an increased ROS response with the inability to clear an infection [303]. Thus, interrupting this cumulative cycle of excessive ROS production and impaired bacterial clearance and resolution of inflammation may be the key to managing COPD disease progression as well as reducing infectious exacerbation risk. Decreasing the excessive oxidative burden in COPD may be achieved by either, increasing endogenous antioxidant enzyme activity via enzyme modulators, or by inhibiting enzymatic sources of ROS production [296].

The family of NADPH oxidases (NOX) are important enzymatic sources of ROS and while they share the capacity to transport electrons across the plasma membrane, family members exhibit markedly different activation mechanisms and tissue distribution [304]. Of this family the primary source of inflammatory cell ROS is the NOX2 isoform which targets killing of bacteria and fungi via phagosomal ROS production [301]. However, there is evidence suggesting this elicited ROS response is not protective against and may even promote viral infection. In the absence NOX2, influenza A virus causes substantially less lung inflammation, injury and dysfunction, and leads to lower viral burden in mice [301, 305]. Conversely, prophylactic inhibition of NOX2 activity, ameliorated influenza A virus-induced lung inflammation, injury [295] and viral replication in mice [302]. The pivotal role of NOX2 as a primary generator of ROS and its potential role in viral-induced exacerbations makes it an enticing therapeutic target. However, there is also evidence that potentially implicates NOX2 in the pathogenesis of COPD. Gene expression of NOX2 is significantly upregulated, and associated with airway inflammation in COPD [306]. Similarly, macrophage-specific NOX2 has been implicated in the pathogenesis of elastase-induced emphysema in mice [307]. Of the studied NOX2 inhibitors, the most common is apocynin, which preferentially blocks NOX2 by preventing assembly of enzyme subunits [308]. Studies in mice models of COPD have shown that *in vivo* administration of apocynin significantly suppresses viral titre, airway inflammation and cell superoxide production following viral infection [298, 301] and exposure to cigarette smoke [296]. Clinically, COPD patients treated with apocynin exhibited reduced H_2O_2 and NO_2^- in their exhaled breath concentrate compared to placebo control [296]. However, there are also concerns about the possible immunosuppressive side effect of NOX2 inhibitors, that may contribute to increased infection and/or autoimmune disorders [309]. Although infection-

related symptoms are only seen when the NOX activity is <15–20% of normal in otherwise healthy individuals, [310] it may be difficult to predict a therapeutic threshold in COPD which displays a multifaceted susceptibility to infections. Although NOX2 has taken the spotlight, a study examining the NOX expression in COPD bronchial brushings has highlighted the importance of the different NOX family isoforms [306]. Of note, gene expression of NOX4 and dual oxidase 2 (DUOX2) were increased and NOX1 was decreased compared to normal controls. Multiple regression analyses also identified independent associations between: NOX1 and DUOX2 with airflow obstruction; NOX1 with airway inflammation (neutrophils and eosinophils) [306]. NOX4 may also be involved in promoting airway smooth muscle remodelling in the small airways of COPD [311, 312] and is readily induced by cigarette smoke [307]. Thus, isoform differences may impact effectiveness of new therapies targeting NOX-generated ROS, particularly as drugs like apocynin are isoform-specific [308].

Alternatively, increasing antioxidant capacity in COPD has been proposed via manipulation of the glutathione peroxidase (Gpx) family of enzymes or superoxide dismutase (SOD). These enzymes are work in tandem to inactivate superoxide produced by cellular respiration and enzymatic ROS generators such as NOX [313, 314]. SOD3 is a major extracellular antioxidant enzyme that is highly expressed in lungs and accounts for the majority of SOD activity in airways and vessels [315]. SOD3 plays a major role in the formation of H_2O_2 by dismutation of superoxide, while the he Gpx family of enzymes, along with catalase, are responsible for the termination of the ROS cascade primarily by reduction of this H_2O_2 to H_2O and O_2 [314]. Of the known eight isoforms, Gpx1 is the predominant isoform of cellular Gpx and is ubiquitously expressed throughout the body, including lung epithelium, alveolar epithelial lining fluid and alveolar macrophages [294]. Gpx and SOD3 levels are overexpressed in erythrocytes and sputum, respectively in both COPD and smokers [316, 317]. However, the inability of these antioxidants to resolve oxidative burden suggests an overwhelmed or dysfunctional antioxidant capacity. Multiple classes of SOD or Gpx mimetics have been developed and have generally demonstrated effectiveness in animal models of COPD but are yet to be translated into humans. Mice lacking the *gpx-1* gene were highly susceptible to oxidative stress [294, 299] and administration of the Gpx mimetic ebselen, or a SOD mimetic, significantly inhibited cigarette smoke-induced lung inflammation when given prophylactically and during established inflammation [294, 296, 299]. Ebselen caused a reduction in influenza A virus-induced lung inflammation [318] and mice with selective overexpression of extracellular SOD displayed significantly less lung injury from influenza virus [313]. This suggests that Gpx-1 or SOD

mimetics may have therapeutic utility in preventing viral-induced AECOPD. These antioxidant enzymes have also shown *in vivo* efficacy in protecting against diseases hallmarked by oxidative stress such as emphysema [299, 319], whereas Gpx-1 knockout mice exhibited an exaggerated emphysema phenotype [299]. Several genetic studies have identified an association between SOD3 polymorphisms and altered risk of COPD. A mutation associated with hyper-production of SOD3 was protective against development of COPD among smokers, but not in non-smokers [320]. A different pair of polymorphisms was also associated with reduced lung function in COPD patients compared to normal population [315].

While inhibiting ROS-production or enhancing ROS-resolution shows potential clinical utility in preventing viral-induced exacerbations and disease progression of COPD, there is limited research regarding their efficacy and safety in humans. Elucidating mechanism by which ROS production paradoxically promotes virus pathogenicity and COPD pathogenesis will allow development of a multifaceted therapy.

Respiratory Microbiome

The role of a beneficial, healthy bacterial microbiota is well established in the gastrointestinal tract, where resident bacteria aid in establishing a balanced immunological phenotype, compete with potentially harmful micro-organisms and synthesize a variety of beneficial biomolecules [321]. The respiratory system was initially thought to be a sterile environment but novel culture-independent techniques of microbial identification have since revealed the previously unappreciated complexity to the respiratory microbiome [322]. Recent research has made it evident that a variety of chronic lung disorders, including asthma, COPD, and cystic fibrosis, are strongly linked to airway dysbiosis, generally accompanied by a loss in bacterial diversity due to the outgrowth of certain pathogenic bacteria [323, 324]. Bacterial composition of the bronchial microbiome remains relatively stable in clinically stable COPD but exhibits overrepresentation of COPD-associated bacteria such as *Haemophilus*, *Moraxella*, and *Neisseria* during an exacerbation [325-329]. Potentially pathogenic members of the Pseudomonadaceae, Enterobacteriaceae, and Helicobacteraceae families, not typically associated with COPD, were also isolated from COPD patients experiencing severe exacerbations [330] and strongly correlated with presence of *Haemophilus influenzae* [325,

326]. These correlative relationships across Proteobacteria members suggests that pathogenesis of exacerbations could involve a polymicrobial process. COPD airways have also been found to be enriched with oral taxa associated with subclinical lung inflammation, such as *Prevotella* and *Veillonella* [331], as well as gut-associated species [330]. These findings suggest that the URT and gastrointestinal tract may act as a microbial reservoir for seeding the airways in COPD, and provide an association between a distinct human microbiome and inflammation in the lung [331, 332]. COPD airways also exhibit a decreased abundance of other bacteria whose predicted metagenomes suggest functional capacities to produce a variety of anti-inflammatory compounds [325].

Given that carriage is prerequisite for the development of disease, maintaining balance of the upper respiratory tract (URT) microbiome is vital in maintaining respiratory health. Introduction of bacteria to the lower respiratory tract (LRT) occurs through physiological episodes of micro-aspiration [333]. The importance of oral health and micro-aspiration was highlighted by a study in residents of nursing homes, showing that neglect of oral health measures leads to a dramatic increase in incidence of and mortality by pneumonia [334]. Likewise, absence of MRSA carriage in the nasopharynx negatively predicted a lower respiratory tract infection caused by the organism, with a negative predictive value of 98.5% [335]. Strong evidence suggests that a healthy URT microbiome infers colonisation resistance by preventing acquisition and establishment of a new pathogens, or by containment of potentially pathogenic bacteria residing amidst harmless commensals [336]. Although potential pathogens such as NTHi and *S. pneumoniae* comprise the normal URT flora, a state of bacterial symbiosis may be protective against short-term infection and inflammation [329]. However, natural microbiome and immune defences against overgrowth of a potential pathogen are susceptible to malfunction following acquisition of a new bacterium or viral co-infection [329]. This resultant state of temporary dysbiosis in the absence of adaptive immunity may be resolved by administration of a probiotic that can diminish pathogen colonisation either by direct competition or indirect stimulation of innate and adaptive immunity [336].

Immune modulation

Viral and bacterial pathogens have a complex and potentially synergistic relationship in the respiratory tract. Viral infection, particularly with HRV, RSV or influenza results in enhanced growth of pathogenic bacteria, mainly of the Proteobacteria phylum (e.g., *H. influenzae* and *Moraxella*), which could explain the predisposition to secondary bacterial infections in COPD

[323]. Several studies have also reported a persistent presence of potentially pathogenic microbes in the LRT of individuals with COPD coinciding with a reduction in microbe diversity [328, 332, 337]. Conversely, presence of potentially pathogenic bacteria is predictive of RSV bronchiolitis in children and in some cases could be linked to severity of the infection [338]. TLR3 expression, and consequently recognition of viral structures and antiviral type I/III IFN production, was shown to be impaired in bronchial epithelial cells exposed to *Moraxella catarrhalis* [338]. Thus, a healthy respiratory microbiome may also aid in limiting risk and severity of viral infections as well as the tissue damage following excessive inflammation induced by viral infection [321]. Colonization of the URT with commensal bacteria has been shown to drastically reduce influenza-induced acute lung injury and mortality in mice [339, 340]. Microbiome modulation was more effective in preventing virus induced hyperreactivity than pre-treatment with synthetic TLR agonists [341]. An intact microbiome has also been shown to be required for the formation of an adaptive response against influenza virus in mice [342].

These protective benefits have been implicated with probiotics regulating monocyte recruitment, production of anti and proinflammatory cytokines, and balance between types of T cell responses such as Th1/Th2, Treg, and Th17 responses [340, 343]. It has also been proposed that the protective effects of probiotics (both intranasal and oral) are also associated with stimulation of NK cell function including production of IFN-γ, as well as induction of M2 macrophages within the airway mucosa [343]. NK cell activity and cytokine release is critically important to the early control of viral infection but is impaired in smokers [343], and in COPD [344-346].

Direct competition

The battle for similar nutrients and the bacterial secretion of antimicrobials provide a direct means of competition between beneficial and harmful microbes [336]. For commensals and pathogens living in or invading human tissues, iron is often a limiting nutrient, particularly in the respiratory tract where iron concentrations are considered to be low [347]. In bacteria, iron is a co-factor for many enzymes and plays a crucial role in diverse physiological processes such as DNA replication, transcription and central metabolism [348]. The primary iron source for bacteria is haem, protoporphyrin IX (ppIX) that contains an iron ion at its centre [349]. The entire haem molecule is also essential to the function of haemoproteins, which are involved in energy generation by the electron transport chain, detoxification of host immune effectors and

other vital processes [349]. To restrict microbial growth, free haem and iron are tightly regulated by the host and is sequestered in high-affinity haemoproteins or macrophages, respectively [348, 350]. Iron-regulatory responses differ between macrophage phenotypes: M1 cells accumulate iron as part of a bacteriostatic stratagem linked to the anaemia of chronic inflammation, whereas the opposite is the case for M2 cells, where iron release is favoured [347].

Indirect evidence provides insight whereby increased iron levels in the lungs of smokers, COPD or CF patients may contribute to the increased susceptibility to airway infections [351]. A rat study showed that airway iron was elevated after exposure to cigarette smoke, leading to increased tissue-damaging oxidative stress and release of IL-8 [352]. An increase in IL-8 may contribute to the increased susceptibility to viral infection via activation of EGFR [353-355]. Increased iron levels in COPD lung occurs as a result of genetic dysregulation of iron regulatory protein (IRP2) and deposition of inhaled iron from cigarette smoke [356]. The iron status of the host is very important in determining the risk of pulmonary infection. For example, correction of iron deficiency led to activation of previously suppressed, pre-existing infections including malaria, brucellosis and tuberculosis, in a group of Somali nomads [356]. As iron repletion advanced, infectious activity reached a peak, showing that iron repletion can also allow infectious diseases to become more clinically overt [356].

Given the role of iron in the pathophysiology and increased susceptibility to infection in COPD lungs, inhaled iron chelators may be a potential therapeutic approach to AECOPD [347]. Intracellular iron chelators such as deferiprone, which specifically target intracellular iron deposits and relocate them outside the cell to prevent cellular iron loading [357, 358] may have more therapeutic efficacy than regular cell-impermeable iron chelators such as deferoxamine [359]. A surgical wound-gel loaded with the iron chelator deferiprone and the heme-analogue gallium-protoporphyrin showed significant antibiofilm activity against multi-drug resistant *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa and Acinetobacter johnsonii*. The gel also potentiated the antibacterial activity of ciprofloxacin against these organisms [358]. This study provides a "proof of principle" for the efficacy of therapies that take advantage of bacterial nutritional requirements and may be useful in the inhibition on diverse bacterial species. However, applying this concept to an inhaled preparation would require further studies that assess the effects of using inhaled iron chelators on systemic iron metabolism. [347]

NTHi is uniquely susceptible to haem restriction as, unlike most bacteria, it cannot make its own and relies solely on haem acquisition from the host [349, 360, 361]. As such, expression of haem-acquisition genes in NTHi is an important determinant of survival and modulation of virulence factors, which is highlighted by the increased prevalence of these genes in middle ear strains than colonising throat strains [362]. Deletion of multiple genes related to haem-iron scavenging, utilisation and regulation have resulted in the attenuation these virulence factors and disease severity and duration in animal models of otitis media [350, 361, 363]. Similarly, an isogenic mutant of two haem-acquisition pathways was unable to sustain bacteraemia or produce meningitis in a rat model of invasive disease [364]. Thus, haem-acquisition pathways represent potentially high value targets for the development of novel therapies for the eradication NTHi from the respiratory tract, particularly in COPD [365, 366]. Alternatively, a probiotic approach that takes advantage of NTHi's reliance on haem-acquisition has been proposed. *Haemophilus hameolyticus* (Hh) is considered a respiratory commensal and recent observations support the bacterium as a potential probiotic candidate against NTHi colonisation and infection [367]. In an *in vitro* cell culture model of human lung epithelium, pre-treatment with Hh significantly inhibited colonisation and transcytosis of NTHi to cell monolayers [6]. A bacteriocin-like substance produced by a minority of Hh strains was isolated with the ability to inhibit NTHi growth *in vitro*. The inhibitory mechanism of this substance has not been fully elucidated but ongoing research indicates the inhibitory effect of this NIS is due to its capacity to bind haem, thus limiting its access to NTHi [368]. Eradication of NTHi carriage from the nasopharynx and subsequent migration to the lower airways would be an effective means of preventing infection with the organism. However, further research is required to investigate the inhibitory effect of this substance in cell culture models and implications on co-colonisation of the shared nasopharyngeal niche.

Immune signalling pathways

Multiple kinases play a critical role in modulating inflammation and downstream antiviral immune responses [369, 370]. However, respiratory viruses possess numerous mechanisms of subverting these responses to facilitate viral entry into host cells and replication. Kinases are an enticing therapeutic target but must overcome developmental hurdles, such as specificity and off-target effects, before implementation as effective preventive therapy for viral-induced AECOPD [369, 370].

Protein kinase C (PKC)

The protein kinase C (PKC) superfamily is linked to several downstream signalling pathways and is therefore responsible for diverse regulatory roles in membrane structure events, immune responses, gene transcription and cell growth [371]. The influenza virus and the viral HA can rapidly activate PKCs upon binding to host-cell surface receptors [138]. *In vitro* experiments show that host cell entry and infectivity of several enveloped viruses, including influenza A and B, could be inhibited by PKC inhibitors, implicating the critical involvement of PKCs critical the enveloped viral entry process [372, 373]. Although the full mechanism of PKC-mediated viral entry has not been elucidated, the PKCbII isoform has been implicated as an important regulator of late endosomal sorting events needed for influenza virus entry and infection [138, 374]. *In vitro* kinase activity assays of infected A549 cells showed that RSV infection also resulted in the upregulation of multiple PKC isoforms with subsequent downstream activation of extracellular signal-regulated kinases. The RSV-mediated upregulation of PKC activation was implicated in the host cell fusion process, a pivotal step in inducing a successful infection [371]. Blocking of PKC was also found to significantly impair RSV infection of NHBE cells when the inhibitory agent was administered at the onset of infection [375].

These observations suggest pharmacological interference of PKC might help to prevent influenza and RSV infections during initial entry of virions into their target cells [138]. One approach to interference is the use of defensins. Defensins are antimicrobial peptides of polymorphonuclear neutrophils and other leukocytes that are important effectors of the innate immune system and have been shown to inhibit a variety of bacteria and fungi [376]. Recent evidence also suggests alpha defensins can modulate viral infection with both enveloped and nonenveloped viruses, including influenza [237, 377] via inhibition of PKC activation [377]. Beta-defensin has also been shown to act as an antiviral molecule against RSV [378]. Treatment of cell cultures with alpha-defensin soon after infection resulted in marked inhibition of influenza virus replication and viral protein synthesis. Additionally, treatment of cells with alpha-defensin followed by its removal before infection also inhibited viral replication, suggesting an effective prophylactic approach [377]. However defensin-mediated inhibition of PKC would have little effectiveness during an active infection as inhibitory activity is only effective when administered during the first 60 minutes of viral infection [373].

Regardless of the naturally occurring *in vitro* antiviral effects of defensins, humans still become infected, suggesting that baseline defensin levels are not sufficient to fully protect the host from viral infections. Prophylactic overexpression of defensins in mice were protective against a lethal influenza A infection and a reduction of the viral lung titre was observed [379]. However, more studies are needed to determine if this strategy can be translated to preventing viral infection in COPD. [376] Basal alpha-defensin was found to be elevated in COPD and further increased post infection with HRV but did not correlate to any changes in viral load [380-382]. Additionally, exposure of pBECs to CSE decreased alpha-defensin 1 production in healthy controls but remained unchanged in pBECs from COPD patients. [380] Basal beta-defensin 2 levels have been described in very low titres in COPD central airways ,but not in distal airways, and are not affected by viral infections [380, 383]. These results suggest a variable role of defensins which is dependent on type and may point toward dysregulation or dysfunction of defensins in COPD. Long-term increases in defensin levels carry safety concerns as high concentrations of human alpha defensin have been found to induce cytotoxicity *in vitro* [384]. Additionally, higher expression of beta-defensin genes can be associated with diseases such as psoriasis [385], and human defensins have also been shown to have effects on tumour microenvironment by either promoting or supressing tumour growth [386]. There is also a potential that defensins may enhance certain viral infections, while inhibiting others. For example, human alpha-defensin 5 and 6 were shown to increase HIV infectivity by enhancing attachment by concentrating virus particles on the target cells [387].

The plant polyphenol, Resveratrol (RV), is an antiviral compound with a broad-spectrum of antiviral activity against DNA and RNA viruses, including HRV and influenza A [130, 388]. The antiviral effects of RV are elicited by interfering with several intracellular signalling pathways, including PKC and its dependent pathways [136, 389]. In *in vivo* studies, RV also improved survival and decreased pulmonary viral titres in influenza virus–infected mice [136]. When administered in the form of a nasal spray, RV was found to be safe and effective in reducing the severity and recurrence of respiratory infections in children [130, 388].

Protein kinase R (PKR) and viral non-structural (NS1) protein

The presence and replication of viral nucleic acids in vertebrate cells triggers a potent antiviral innate immune response, in particular the induction of type-I IFN genes and the activation of viral enzymes [390]. The double-stranded RNA-dependent PKR is a key executor of this antiviral response, along with other IFN-stimulated gene products [390]. As a defence against

this antiviral response, influenza viruses express a multifunctional NS1 protein with the major function being to counteract or prevent the PKR-mediated antiviral response [391, 392]. NS1 protein can prevent PKR detection by binding to viral RNA [393, 394] or, by direct binding, can inhibit PKR-mediated viral mRNA suppression and induction of apoptosis. Additionally, some strains are able to directly inhibit the expression of IFN [395, 396]. NS1 not only mediates antiviral suppression but also modulates other important aspects of the virus replication cycle, which may additionally contribute towards efficient virus replication and virulence during infection [397]. Additional roles of NS1 include viral RNA replication, viral protein synthesis, and general host-cell physiology [125, 390, 392].

Given the numerous roles during virus replication, antiviral compounds that inhibit NS1-actived signalling cascades or augmentation of IFN antiviral pathways could be a promising preventative strategy against influenza infection in COPD. Influenza A virus mutants unable to express NS1 (delNS1), or that possess a truncated form of NS1, induce high levels of IFN in infected cells, and are consequently attenuated in IFN- α/β-competent systems [398, 399]. Inhibition of IFN pre-mRNA was found to be mediated by the binding of viral NS1 to cellular CPSF30, thus indicating this interaction as a potential therapeutic target [400]. Peptide-mediated inhibition of NS1-CPSF30 binding has been shown to limit virus replication in tissue culture [399, 401, 402]. However, this strategy is specific to influenza A and is yet to be tested *in vivo* [399]. Recombinant influenza viruses with truncated or mutated NS1 proteins have also been investigated for their potential as live-attenuated vaccine candidates [392]. Such viruses are partially debilitated in their ability to counteract the host IFN response, but retain immunogenicity and replicative ability, making them an ideal vaccine candidate [392, 403, 404]. NS1-truncated viruses are generally attenuated, except in cells lacking the ability to produce appropriate levels of IFN [398, 399]. There is evidence of deficient IFN-induction in COPD cells *ex vivo*, and COPD mice [403, 405]. Therefore, further studies are required to properly assess the safety of this approach in COPD, perhaps with the consideration of administering recombinant interferon in combination with vaccination. The type- and strain-specific IFN-antagonistic and binding properties of NS1 [392, 406, 407] may also complicate development of therapies that target NS1. Influenza H5N1 encodes an NS1 protein that is highly effective in inhibiting host antiviral responses that contributes to it high fatality in humans [408, 409]. Transfected NS1 of a human H3N2 strain was also found to be more effective in supressing IFN-mediated antiviral response than that of a low pathogenic avian influenza, H11N9, which resulted in a higher replication rate [410].

An alternative approach is augmentation of the IFN response. Mice lacking functional IFN-λ receptors are unable to restrict virus dissemination from the upper airways to the lungs and transmit the virus much more efficiently to naïve contacts compared with wild-type mice [411]. Prophylactic intranasal treatment with IFN-α inhibited initial virus replication in mice, ferrets, guinea pigs or rhesus macaques infected with influenza A [412-414]. However, IFN-α was only effective in protecting ferrets from seasonal influenza viruses, which replicate mainly in the upper respiratory tract, but not from highly pathogenic influenza viruses, which also disseminate to the lung [412]. However, only IFN-λ conferred long-lasting antiviral protection in the upper airways and blocked virus transmission in mice [411]. IFN pre-treatment reduced viral replication in COPD pBECs exposed to HRV, suggesting a broad-spectrum antiviral potential of IFN treatment [415]. Intranasal recombinant IFN-α2b (SNG001) was shown to be an effective prophylactic treatment against HRV and prevented exacerbations in asthmatics. However, stage 2 clinical trials are yet to reveal any efficacy in preventing viral-induced AECOPD [416]. Another promising avenue is the family of interferon-inducible transmembrane IFITM proteins that mediate IFN's broad-spectrum antiviral role against influenza A virus, West Nile virus and dengue virus. IFITM 1, 2 and 3 restricted early steps in influenza A infection, and there are no reports of viral escape from IFITM restriction [417-419]. Evidence also suggests that *Ifitm3*$^{-/-}$ mice are more susceptible to RSV infection than their wild-type littermates. However, RSV restriction has not been examined in cultured cells, and it is possible that IFITM proteins protect mice from RSV challenge via means other than inhibition of viral entry [419]. IFITM expression has also been shown to influence the quality of the adaptive immune response to influenza A virus in mice [419].

Augmentation of the antiviral IFN response appears to be an effective prophylactic strategy in animal studies but more trials are required to determine if this effect is translatable to humans and whether there is any benefit in preventing AECOPD. The inability of exogenous IFN-β to fully restore antiviral responses in COPD pBECs, indicates a partially dysfunctional IFN-β-mediated signalling in COPD [31]. Thus, simply artificially increasing IFN levels in COPD airways may not be sufficient to mount an appropriate antiviral response. It is also not clear how augmentation of such a vital host immune response would affect uninfected tissue [392]. Mice studies have shown that excessive IFNαβ signalling in response to acute influenza infection can result in uncontrolled inflammation leading to lung damage and increased morbidity and mortality [420]. Additionally, it causes increased expression of IFN-γ

suppressed innate protection against extracellular bacterial pathogens in the lung [421]. This may have serious implications for bacteria-induced exacerbations in COPD.

P13K-p110a signalling pathway

Influenza A NS1 protein is also responsible for the activation of the phosphoinositide 3-kinase (PI3K) and the downstream effector protein kinase Akt by direct interaction with the p85 subunit of PI3K [422, 423]. The normal physiological role of PI3K is in various host cell functions such as anti-apoptosis, cell proliferation, growth, metabolism and cytokine production/signalling [125, 392]. However, several studies have implicated PI3K in supporting efficient virus propagation. Blocking of the PI3K/Akt pathway results in the inability of influenza to endocytose into AECs [424], a reduction in virus yield, suppression of viral RNA and protein synthesis, and suppression of premature apoptosis at later stages of infection [422, 423, 425-427]. The role of influenza-induced PI3K activation appears to be dependent on virus type. PI3K is activated and regulates early stages of viral entry upon infection with influenza B viruses as opposed to the post-entry events at later stages of infection with influenza A [422]. Although initial viral replication is dependent on PI3K activation, influenza virus produces NS1, which further activates PI3K to further amplify replication [31, 428]. Influenza viruses containing a truncated NS1 failed to activate the PI3K/Akt pathway, grew slower and formed small plaques in tissue culture [429]. The truncated NS1 protein also conferred an attenuated phenotype due to its inability to inhibit host antiviral responses [430].

Hyperexpression of PI3K in lungs of COPD patients has been described, not only providing a mechanism of increased viral susceptibility but also may be linked to pathogenesis of the disease [431]. Increased expression of PI3K and decreased expression of a negative regulator of PI3K (PTEN) correlated with more severe airflow obstruction, regardless of disease stage [431]. In primary bronchial epithelial cells and BEAS-2B cells, CSE decreased PTEN protein, and PTEN knockdown potentiated Akt phosphorylation and enhanced production of proinflammatory cytokines [432]. Exaggerated PI3K response has also been described in COPD pBECs, which conferred increased susceptibility to influenza infection. Smoke exposed mice also supported increased influenza viral replication and produced deficient antiviral responses, with exacerbated inflammation and impaired lung function [31]. PI3K-p110α was identified as the predominant isoform hyper-expressed in COPD pBECs. Specific inhibition of this isoform prior to infection attenuated influenza infection and enhances IFN-β antiviral responses in COPD, as well as healthy pBECs [31]. Identification of offending isoforms is

beneficial to developing more specific inhibitors and limit off-target activity. The PI3Ky subunit has also been shown to have a critical role in the clearance of viral infection [433]. PI3Ky is a more specific target as it is only expressed on cells of haemopoietic origin as opposed to p100α which is expressed on all cells [434], however PI3Ky has not yet been characterised in COPD.

Epidermal Growth Factor Receptor (EGFR)

EGFR is a receptor kinase which is well known to play an important role in the regeneration of damaged tissue, mucin production and elastase-induced IL-8 production in lung epithelial cells [353, 435, 436] IL-8 production is particularly important in the context of COPD as concentrations are increased during an exacerbation or in patients that experience frequent exacerbations [437, 438]. EGFR has been shown to promote influenza A infection of host cells either directly or indirectly through activation of PI3K/Akt signalling [354]. Influenza virus and HRV induce upregulation of ligands that further activate EGFR and IL-8 production in bronchial epithelial cells *in vitro,* further facilitating viral attachment to airway epithelium [353, 355]. EGFR inhibition has been shown to completely block IL-8 and the EGFR ligand, ICAM-1, expression to basal levels during HRV infection [355]. Furthermore, aberrant EGFR activity has also been described in COPD which subsequently increases PI3K/Akt signalling and IL-8 secretion [439], the effect of which is exacerbated by long-term smoking [440, 441]. Persistent activation of EGFR by viral infection or through a defect in glutathione secretion in COPD may contribute to airway remodelling, an important driver of airway lumen obstruction [442-444]. EGFR has also been shown to have an indirect role in the IFN-mediated antiviral response. Activation by influenza A virus and HRV suppressed IFN-γ production and increased viral replication in BEAS-2B cells [445]. Inhibition of EGFR in mice during viral infection augmented IFN- γ production, which resulted in decreased viral titres *in vitro* and *in vivo* [445]. *M. cattarrhalis*, another common precipitant of COPD exacerbation, has also been shown to activate EGFR. [446] This evidence suggests that inhibition of EGFR may be a multifaceted therapeutic approach with the potential to not only prevent bacterial and viral-induced exacerbations, but also affect the overall pathophysiology of COPD.

Strategies developed for antagonising EGFR signalling include MAbs that prevent endogenous ligand engagement, [447, 448] or small molecule inhibitors which target specific downstream EGFR signalling pathway components [369]. However, research in MAbs has primarily focused on treatments for lung cancer and have not yet been contextualised for COPD [369].

Suppressor of cytokine signalling (SOCS) protein regulation of EGFR has shown a pivotal role in restricting influenza A virus in airway epithelium. Socs5-deficient mice exhibit heightened disease severity, with increased viral titres and weight loss [32, 449]. In COPD airway epithelial cells, Socs5 was found to be downregulated, which correlated with increased susceptibility to influenza infection. Importantly, restoration of SOCS5 levels restricted influenza virus infection, suggesting that manipulating SOCS5 expression and/or SOCS5 targets might be a novel therapeutic approach to influenza [32]. Fucoidan, an algae-derived sulphated polysaccharide has also exhibited broad anti-influenza activity in mice by enhancing the immune response [450] and blocking EGFR-mediated viral entry [354, 451]. Studies assessing the therapeutic potential of fucoidan in humans is limited but reveals an additional immune-modulatory role that may increase vaccine efficacy in immunocompromised individuals. Elderly participants treated with Mekabu fucoidan produced higher antibody titres and exhibited an increase in natural killer activity against influenza strains in a seasonal influenza vaccine [450]. Further studies are required to assess these effects in COPD.

CONCLUSIONS

Infectious exacerbations are a primary driver of clinical and functional decline in COPD, but existing therapies are of limited effectiveness and do not effect the overall course of the disease. The rapid development of antibiotic resistance and microbial evasion of vaccination strategies further complicates the treatment and prevention of these infections. In order to mount an infection, a pathogen must exploit host-cell surface receptors for attachment and evade clearance by host immune responses. Therefore, novel therapeutic strategies that disrupt bacterial-host cell interactions or that mediate the host inflammatory response present an attractive approach to reducing infectious exacerbations in COPD. The usefulness of pathogen proteins as vaccine candidates are limited by antigenic variation, thus targeting specific host receptors may be a more promising approach. Modulation of the aberrant inflammatory response in COPD by altering immune cell function, signalling pathways, balancing the microbiome or reducing oxidative burden may have added utility of affecting disease progression as well as preventing infectious exacerbations. However, due to integrated nature and complexity of the immune response, a combination of approaches may be required to elicit any benefits. These strategies are less likely to induce antimicrobial resistance as a missing cellular function is more difficult for a pathogen to adapt to, and should affect replication independent of the type, strain and antigenic properties of the invading pathogen. However, host-targeted approaches may be more complex to manage due to the increased concern of off-

target side effects, and their development may be hindered by the uncertainty surrounding the mechanisms of COPD pathogenesis. It is imperative that the mechanisms driving COPD pathogenesis be resolved, which will facilitate the development of effective treatment strategies. Further development of such preventative strategies has the potential to significantly reduce AECOPD, prevent disease progression and associated mortality and economic costs, as well as improving health-related quality of life for affected individuals.

Funding

SSS is supported by Clifford Craig Foundation Launceston General Hospital, Rebecca L. Cooper Medical Research Foundation, Thoracic Society of Australia & New Zealand (TSANZ) Boehringer Ingelheim COPD Research Award, Lung Foundation Australia and Cancer Council Tasmania. PMH is supported National Health and Medical Research Council (NHMRC) of Australia Principal Research Fellowship. PS is supported by Rebecca L. Cooper Medical Research Foundation.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

All authors contributed to the writing of this manuscript and approved the final version.

Figure 1. New potential therapies targeting pathogen-host binding and immune signalling pathways involved in infectious exacerbations of COPD. Bacterial and viral pathogens inhaled from the upper respiratory tract or the environment exploit upregulated host cell receptors for attachment via their cognate ligands. Pathogen adhesion to host receptors causes further upregulation of some host receptors which facilitates chronic colonization and invasion of COPD airways. Epithelial binding triggers immune defences which are mitigated by the invading pathogen at various steps in the immune signalling pathway. Avoidance of immune clearance by a dysfunctional immune response allows pathogens to further facilitate chronic colonisation and cause acute exacerbations that contribute to progression of COPD. The additive effects of macrophage dysfunction, pathogen persistence and oxidative stress also contribute to disease progression through thickening of the airway wall. Potential therapeutic agents/inhibitors of pathogen host-cell attachment and the altered immune response are demonstrated in orange. NTHi, nontypeable *Haemophilus influeanzae;* RSV, respiratory syncytial virus; HRV, human rhinovirus; ChoP, phosphorylcholine; gG, glycoprotein G; gF, glycoprotein F; HA, haemagglutinin; OMP, outer membrane protein; UspA1, ubiquitous surface protein A1; EBP, entry-blocking peptide; Mab, monoclonal neutralising antibody; CEACAM, anti-carcinoembryonic antigen cell adhesion molecule 1; ICAM-1, Intracellular adhesion molecule 1; PAFR, platelet-activating factor receptor; GAG, glycosaminoglycan; SA, sialic acid; RV, resveratrol; NS1, non-structural protein; IFN, interferon; IFITM, interferon-inducible transmembrane; PTEN, Phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; SOCS, suppressor of cytokine signalling; PKC, protein kinase C; PKR, protein kinase R; EGFR, epidermal Growth Factor receptor; TLR, toll-lik receptor; PDE, Phosphodiesterase; COPD, chronic obstructive pulmonary disease; IL-8, interleukin 8.

Table 1: Current therapies and clinical trials in COPD

Reference	Drug names/ combinations and target	COPD condition and stage	Clinical trial (Phase)	Study details and patient numbers	Outcomes and conclusions
Triple *versus* dual therapies with combination of inhaled corticosteroids, LABA and LAMA					
Halpin et al FULFIL study [452]	Once daily triple therapy (ICS+LABA+LAMA) fluticasone furoate/umeclidinium/vilanterol compared to twice-daily dual therapy (ICS+LABA) budesonide/formoterol	Moderate to severe	Phase III clinical trials	A total of 2240 patients participated and were sub-grouped. 1121 patients received triple therapy while 1119 received dual therapy	Once daily triple therapy showed reductions in moderate- severe exacerbation rates compared with twice-daily dual therapy. The effectiveness of the therapy was regardless of disease severity or exacerbation history or prior COPD medication
Lipson et al IMPACT study [88]	Once daily triple therapy (ICS+LABA+LAMA) fluticasone furoate umeclidinium/vilanterol compared to fluticasone dual therapy (ICS + ultra LABA) furoate–vilanterol and dual bronchodilator (LAMA+ ultra LABA) umeclidinium–vilanterol	Moderate to severe	Phase III clinical trials	A total 10,355 patients of which 4151 received triple therapy and the rest were administered dual therapy	Once-daily triple therapy reduced moderate or severe COPD exacerbations and improved lung function and quality of life than both dual therapy.
Singh et al TRILOGY Study [453]	Once daily triple therapy (ICS+LABA+LAMA) with single inhaler containing ultrafine beclometasone dipropionate, formoterol fumarate, and glycopyrronium bromide compared to twice daily (ICS+LABA) beclometasone dipropionate and formoterol fumarate	Moderate to severe	Randomised, double-blinded active-controlled trial done in 159 sites across 14 countries	A total of 1367 patients of which 687 were on triple therapy and 680 on dual therapy	Triple therapy showed greater benefit than dual therapy in reduction of moderate to severe exacerbation and quality of life. Triple therapy effectively increased bronchodilation increased in patients with severe exacerbations however, no changes in dyspnea was observed

Papi et al TRIBUTE study [454]	Once daily triple therapy (ICS+LABA+LAMA) beclometasone dipropionate/formoterol fumarate compared to (LABA+LAMA) indacaterol and glycopyrronium	Moderate to Severe	Phase 3b clinical trials done at 187 sites across 17 countries	A total of 1532 patients of which 764 were on triple therapy and 768 on dual therapy	The triple therapy again was found to be effective in reducing the rate of moderate-to-severe COPD exacerbations than dual bronchodilator combination, without increasing the risk of pneumonia
Dual/monotherapy involving ICS/LABA/LAMA comparative clinical studies					
Wedzicha et al FLAME study [455]	Dual therapy with LABA indacaterol / LAMA glycopyrronium once daily compared to LABA salmeterol + ICS fluticasone twice daily	Moderate to severe	A multicentre randomized, double-blind, double-dummy, parallel-group, non-inferiority trial	A total of 3362 patients underwent randomization; 1680 were assigned to the indacaterol–glycopyrronium group, and 1682 to the salmeterol–fluticasone group	Indacaterol–glycopyrronium was consistently more effective than salmeterol–fluticasone in preventing exacerbations and was associated with no detectable increase in adverse events
Anzueto et al [456]	Comparison of dual therapy (LABA+LAMA) indacaterol and glycopyrronium(IND+GLY) and monotherapy (LAMA) tiotropiun (TIO) (SHINE study)and dual therapy (LABA+ICS) salmeterol/fluticasone (SFC) (LANTERN study)	Moderate to severe	Multi-centre, Phase III randomized clinical trial as part of IGNITE program	A total of 954 patients for SHINE study of which 474 administered (IND+GLY) and 480 TIO monotherapy. In LANTERN study 1,263 patients 630 (IND+GLY) and 633 with SFC	Dual bronchodilator therapy with IND+GLY offered significant benefits over treatment with a single-agent LAMA or a LABA/ICS both in terms of the incidence and time to clinically important deterioration (CID).
Kardos et al FAVOR study [457]	Comparison of dual therapy (LABA+LAMA) indacaterol and glycopyrronium (IND+GLY) and monotherapy (LAMA) tiotropium	Moderate to severe	This randomized, open-label, multicenter, crossover study was conducted at 18	Total of 176 patients 88 in each arm	The combination of LABA/LAMA improved lung function in COPD patients compared to monotherapy with Tiotropium

			centres in Germany		
Roflumilast (PDE4 inhibitor) in COPD clinical studies					
Martinez et al RESPOND study [458]	Roflumilast compared with placebo	Moderate to Severe	Phase IV multicentre, randomized, double-blind, placebo-controlled	A total of 2,354 COPD patients were randomized 1,178 treated with roflumilast and 1,176 placebo	The study found roflumilast effective in reducing moderate to severe exacerbation and was more pronounced in patients with history of COPD-related hospitalization and those with frequent COPD exacerbations
Martinez et al REACT study [459]	Roflumilast compared with placebo	Moderate to Severe	Phase III/IV multi-center, randomized, double-blind, placebo-controlled	1945 participants, 973 to the roflumilast group and 972 placebos	Roflumilast effectively reduces exacerbations and hospital admissions in patients with severe chronic obstructive pulmonary disease and severe exacerbations.
Rabe KF et al ROBERT study [460]	Roflumilast compared with placebo	Moderate to severe and chronic bronchitis	Double-blind, placebo-controlled trial done at 18 sites in five countries	158 patients were randomly assigned: 79 to the roflumilast group, and 79 to the placebo group	16 weeks of treatment with roflumilast did not affect the number of CD8 cells in bronchial submucosa compared with placebo but reduced the eosinophil biopsy numbers
Statins in COPD clinical trials					
Balaguer et al [461]	Simvastatin compared with placebo	Moderate to severe	Randomised control trial	18 eligible participants. 9 in each arm	No significant change was observed on pulmonary/ systemic inflammation and lung function in simvastatin treated COPD patients compared to placebo.

REFERENCES

1. Agrawal, A. and T.F. Murphy, *Haemophilus influenzae infections in the H. influenzae type b conjugate vaccine era.* Journal of clinical microbiology, 2011. **49**(11): p. 3728-3732.
2. Global Strategy for the Diagnosis, M.a.P.o.C. *Global Initiative for Chronic Obstructive Lung Disease (GOLD)*. 2017 25 Sept 2017; Available from: Available from: http://goldcopd.org.
3. King, P., *Haemophilus influenzae and the lung (Haemophilus and the lung).* Clinical and translational medicine, 2012. **1**(1): p. 1.
4. Cruz, A.A., *Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach*. 2007: World Health Organization.
5. Diaz-Guzman, E. and D.M. Mannino, *Epidemiology and prevalence of chronic obstructive pulmonary disease.* Clinics in chest medicine, 2014. **35**(1): p. 7-16.
6. Pickering, J.L., et al., *Haemophilus haemolyticus Interaction with Host Cells Is Different to Nontypeable Haemophilus influenzae and Prevents NTHi Association with Epithelial Cells.* Frontiers in cellular and infection microbiology, 2016. **6**.
7. Toews, G., *Impact of bacterial infections on airway diseases.* European Respiratory Review, 2005. **14**(95): p. 62-68.
8. Caramori, G., et al., *Molecular pathogenesis of cigarette smoking–induced stable COPD.* Annals of the new York Academy of Sciences, 2015. **1340**(1): p. 55-64.
9. Cottin, V., et al., *Burden of Idiopathic Pulmonary Fibrosis Progression: A 5-Year Longitudinal Follow-Up Study.* PloS one, 2017. **12**(1): p. e0166462.
10. Leung, J.M., et al., *The role of acute and chronic respiratory colonization and infections in the pathogenesis of COPD.* Respirology, 2017. **22**(4): p. 634-650.
11. Starkey, M.R., et al., *Murine models of infectious exacerbations of airway inflammation.* Current opinion in pharmacology, 2013. **13**(3): p. 337-344.
12. Sapey, E. and R. Stockley, *COPD exacerbations· 2: Aetiology.* Thorax, 2006. **61**(3): p. 250-258.
13. Erkan, L., et al., *Role of bacteria in acute exacerbations of chronic obstructive pulmonary disease.* International journal of chronic obstructive pulmonary disease, 2008. **3**(3): p. 463.
14. Hansbro, P.M., et al., *Mechanisms and treatments for severe, steroid-resistant allergic airway disease and asthma.* Immunological reviews, 2017. **278**(1): p. 41-62.
15. Sethi, S., *Infection as a comorbidity of COPD.* European Respiratory Journal, 2010. **35**(6): p. 1209-1215.
16. King, P., *Haemophilus influenzae and the lung (Haemophilus and the lung).* Clinical and translational medicine, 2012. **1**(1): p. 10.
17. Morey, P., et al., *Evidence for a non-replicative intracellular stage of nontypable Haemophilus influenzae in epithelial cells.* Microbiology, 2011. **157**(1): p. 234-250.
18. Simpson, J.L., et al., *COPD is characterized by increased detection of H aemophilus influenzae, S treptococcus pneumoniae and a deficiency of B acillus species.* Respirology, 2016. **21**(4): p. 697-704.
19. Matsumoto, K. and H. Inoue, *Viral infections in asthma and COPD.* Respiratory investigation, 2014. **52**(2): p. 92-100.
20. Sykes, A., P. Mallia, and S.L. Johnston, *Diagnosis of pathogens in exacerbations of chronic obstructive pulmonary disease.* Proceedings of the American Thoracic Society, 2007. **4**(8): p. 642-646.
21. Greenberg, S.B., et al., *Respiratory viral infections in adults with and without chronic obstructive pulmonary disease.* American Journal of Respiratory and Critical Care Medicine, 2000. **162**(1): p. 167-173.
22. Wedzicha, J.A., *Role of viruses in exacerbations of chronic obstructive pulmonary disease.* Proceedings of the American Thoracic Society, 2004. **1**(2): p. 115-120.
23. Mohan, A., et al., *Prevalence of viral infection detected by PCR and RT-PCR in patients with acute exacerbation of COPD: a systematic review.* Respirology, 2010. **15**(3): p. 536-542.

24. Seemungal, T., et al., *Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease.* European Respiratory Journal, 2000. **16**(4): p. 677-683.
25. Wang, H., et al., *Resolving viral-induced secondary bacterial infection in COPD: a concise review.* Frontiers in immunology, 2018. **9**: p. 2345.
26. Eapen, M.S., et al., *Profiling cellular and inflammatory changes in the airway wall of mild to moderate COPD.* Respirology, 2017. **22**(6): p. 1125-1132.
27. Chotirmall, S.H., et al., *Microbiomes in respiratory health and disease: An Asia-Pacific perspective.* Respirology, 2017. **22**(2): p. 240-250.
28. Shukla, S.D., et al., *Microbiome effects on immunity, health and disease in the lung.* Clinical & translational immunology, 2017. **6**(3): p. e133.
29. Budden, K.F., et al., *Emerging pathogenic links between microbiota and the gut–lung axis.* Nature Reviews Microbiology, 2017. **15**(1): p. 55.
30. Morey, P., et al., *Evidence for a non-replicative intracellular stage of nontypable Haemophilus influenzae in epithelial cells.* Microbiology, 2011. **157**(1): p. 234-250.
31. Chen-Yu Hsu, A., et al., *Targeting PI3K-p110α suppresses influenza virus infection in chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2015. **191**(9): p. 1012-1023.
32. Kedzierski, L., et al., *Suppressor of cytokine signaling (SOCS) 5 ameliorates influenza infection via inhibition of EGFR signaling.* Elife, 2017. **6**: p. e20444.
33. Tay, H.L., et al., *Antagonism of miR-328 increases the antimicrobial function of macrophages and neutrophils and rapid clearance of non-typeable Haemophilus influenzae (NTHi) from infected lung.* PLoS pathogens, 2015. **11**(4): p. e1004549.
34. Desai, H., et al., *Bacterial colonization increases daily symptoms in patients with chronic obstructive pulmonary disease.* Annals of the American Thoracic Society, 2014. **11**(3): p. 303-309.
35. Wilson, R., et al., *Antibiotics for treatment and prevention of exacerbations of chronic obstructive pulmonary disease.* Journal of Infection, 2013. **67**(6): p. 497-515.
36. Ni, W., et al., *Prophylactic use of macrolide antibiotics for the prevention of chronic obstructive pulmonary disease exacerbation: a meta-analysis.* PloS one, 2015. **10**(3): p. e0121257.
37. Vogelmeier, C.F., et al., *Global strategy for the diagnosis, management and prevention of chronic obstructive lung disease 2017 report.* Respirology, 2017. **22**(3): p. 575-601.
38. Woodruff, P.G., et al., *Current concepts in targeting chronic obstructive pulmonary disease pharmacotherapy: making progress towards personalised management.* The Lancet, 2015. **385**(9979): p. 1789-1798.
39. Agusti, A., et al., *Precision medicine in COPD exacerbations.* The Lancet Respiratory Medicine, 2018. **6**(9): p. 657-659.
40. Shaw, J.G., et al., *Biomarkers of progression of chronic obstructive pulmonary disease (COPD).* Journal of thoracic disease, 2014. **6**(11): p. 1532.
41. Chen, Y.-W.R., J.M. Leung, and D.D. Sin, *A systematic review of diagnostic biomarkers of COPD exacerbation.* PLoS One, 2016. **11**(7): p. e0158843.
42. Cascini, S., et al., *Inhaled Corticosteroid Use in Chronic Obstructive Pulmonary Disease and Risk of Pneumonia: A Nested Case-Control Population-based Study in Lazio (Italy)—The OUTPUL Study.* COPD: Journal of Chronic Obstructive Pulmonary Disease, 2017. **14**(3): p. 311-317.
43. Eapen, M.S., et al., *Chronic Obstructive Pulmonary Disease and Lung Cancer: Underlying Pathophysiology and New Therapeutic Modalities.* Drugs, 2018. **78**(16): p. 1717-1740.
44. Oba, Y., S.T. Sarva, and S. Dias, *Efficacy and safety of long-acting β-agonist/long-acting muscarinic antagonist combinations in COPD: a network meta-analysis.* Thorax, 2015: p. thoraxjnl-2014-206732.

45. Casaburi, R., et al., *The spirometric efficacy of once-daily dosing with tiotropium in stable COPD: a 13-week multicenter trial.* Chest, 2000. **118**(5): p. 1294-1302.
46. Kew, K.M., C. Mavergames, and J.A. Walters, *Long-acting beta2-agonists for chronic obstructive pulmonary disease.* The Cochrane Library, 2013.
47. Burge, P.S., et al., *Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial.* Bmj, 2000. **320**(7245): p. 1297-1303.
48. van der Molen, T. and M. Cazzola, *Beyond lung function in COPD management: effectiveness of LABA/LAMA combination therapy on patient-centred outcomes.* Primary Care Respiratory Journal, 2012. **21**: p. 101-108.
49. Montuschi, P., et al., *Pharmacological treatment of chronic obstructive pulmonary disease: from evidence-based medicine to phenotyping.* Drug discovery today, 2014. **19**(12): p. 1928-1935.
50. Singh, D., et al., *Current controversies in the pharmacological treatment of chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2016. **194**(5): p. 541-549.
51. Hansbro, P.M., et al., *Th2 cytokine antagonists: potential treatments for severe asthma.* Expert Opinion on Investigational Drugs, 2013. **22**(1): p. 49-69.
52. Ye, Q., X.-O. He, and A. D'Urzo, *A Review on the Safety and Efficacy of Inhaled Corticosteroids in the Management of Asthma.* Pulmonary Therapy, 2017. **3**(1): p. 1-18.
53. D'Urzo, A., et al., *A re-evaluation of the role of inhaled corticosteroids in the management of patients with chronic obstructive pulmonary disease.* Expert opinion on pharmacotherapy, 2015. **16**(12): p. 1845-1860.
54. Mapel, D., et al., *Pneumonia among COPD patients using inhaled corticosteroids and long-acting bronchodilators.* Prim Care Respir J, 2010. **19**(2): p. 109-17.
55. Culpitt, S., J. Nightingale, and P. Barnes. *Effect of fluticasone propionate on induced sputum matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with COPD*. in *AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE*. 1999. AMER LUNG ASSOC 1740 BROADWAY, NEW YORK, NY 10019 USA.
56. Keatings, V.M., et al., *Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD.* American journal of respiratory and critical care medicine, 1997. **155**(2): p. 542-548.
57. Barnes, P.J., *Inhaled corticosteroids are not beneficial in chronic obstructive pulmonary disease*. 2000.
58. Vestbo, J., et al., *Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial.* The Lancet, 1999. **353**(9167): p. 1819-1823.
59. Bourbeau, J., M.Y. Rouleau, and S. Boucher, *Randomised controlled trial of inhaled corticosteroids in patients with chronic obstructive pulmonary disease.* Thorax, 1998. **53**(6): p. 477-482.
60. Group, L.H.S.R., *Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease.* N Engl J Med, 2000. **2000**(343): p. 1902-1909.
61. Weir, D., et al., *A double-blind placebo-controlled study of the effect of inhaled beclomethasone dipropionate for 2 years in patients with nonasthmatic chronic obstructive pulmonary disease.* Clinical & Experimental Allergy, 1999. **29**(s2): p. 125-128.
62. Paggiaro, P.L., et al., *Multicentre randomised placebo-controlled trial of inhaled fluticasone propionate in patients with chronic obstructive pulmonary disease.* The Lancet, 1998. **351**(9105): p. 773-780.
63. Suissa, S., *Statistical treatment of exacerbations in therapeutic trials of chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2006. **173**(8): p. 842-846.

64. Szafranski, W., et al., *Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease.* European Respiratory Journal, 2003. **21**(1): p. 74-81.
65. Calverley, P., et al., *Maintenance therapy with budesonide and formoterol in chronicobstructive pulmonary disease.* European Respiratory Journal, 2003. **22**(6): p. 912-919.
66. Karbasi-Afshar, R., J. Aslani, and M. Ghanei, *Efficacy and safety of inhaler steroids in COPD patients: Systematic review and meta-analysis of randomized placebo-controlled trials.* Caspian journal of internal medicine, 2014. **5**(3): p. 130.
67. Falk, J.A., O.A. Minai, and Z. Mosenifar, *Inhaled and systemic corticosteroids in chronic obstructive pulmonary disease.* Proceedings of the American Thoracic Society, 2008. **5**(4): p. 506-512.
68. Glaab, T., C. Vogelmeier, and R. Buhl, *Outcome measures in chronic obstructive pulmonary disease (COPD): strengths and limitations.* Respiratory research, 2010. **11**(1): p. 79.
69. Ernst, P., et al., *Inhaled corticosteroid use in chronic obstructive pulmonary disease and the risk of hospitalization for pneumonia.* American journal of respiratory and critical care medicine, 2007. **176**(2): p. 162-166.
70. Vedel-Krogh, S., et al., *Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study.* American journal of respiratory and critical care medicine, 2016. **193**(9): p. 965-974.
71. Brusselle, G., et al., *Blood eosinophil levels as a biomarker in COPD.* Respiratory Medicine, 2018. **138**: p. 21-31.
72. Cheng, S.-L., *Blood eosinophils and inhaled corticosteroids in patients with COPD: systematic review and meta-analysis.* International journal of chronic obstructive pulmonary disease, 2018. **13**: p. 2775.
73. Ho, J., et al., *Eosinophilia and clinical outcome of chronic obstructive pulmonary disease: a meta-analysis.* Scientific reports, 2017. **7**(1): p. 13451.
74. Pascoe, S., et al., *Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials.* The lancet Respiratory medicine, 2015. **3**(6): p. 435-442.
75. Pavord, I.D., et al., *Blood eosinophils and inhaled corticosteroid/long-acting β-2 agonist efficacy in COPD.* Thorax, 2016. **71**(2): p. 118-125.
76. Global Strategy for the Diagnosis, M.a.P.o.C. *Global Initiative for Chronic Obstructive Lung Disease (GOLD)*. 2019 14 Nov 2018; Available from: Available from: http://goldcopd.org.
77. Sohal, S.S., *Inhaled corticosteroids and increased microbial load in COPD: potential role of epithelial adhesion molecules.* Eur Respir J, 2018. **51**(2).
78. Eapen, M.S., et al., *Dysfunctional Immunity and Microbial Adhesion Molecules in Smoking-induced Pneumonia.* Am J Respir Crit Care Med, 2019. **199**(2): p. 250-251.
79. Eapen, M.S., et al., *Airway inflammation in chronic obstructive pulmonary disease (COPD): a true paradox.* Expert review of respiratory medicine, 2017. **11**(10): p. 827-839.
80. Sohal, S.S., et al., *Airway inflammation and inhaled corticosteroids in COPD.* Eur Respir J, 2017. **49**(6).
81. Sohal, S.S., *Fluticasone propionate and increased risk of pneumonia in COPD: is it PAFR-dependent?* Int J Chron Obstruct Pulmon Dis, 2017. **12**: p. 3425-3427.
82. Sohal, S.S., et al., *Changes in Airway Histone Deacetylase2 in Smokers and COPD with Inhaled Corticosteroids: A Randomized Controlled Trial.* PLoS One, 2013. **8**(5): p. e64833.
83. Soltani, A., et al., *Inhaled corticosteroid normalizes some but not all airway vascular remodeling in COPD.* Int J Chron Obstruct Pulmon Dis, 2016. **11**: p. 2359-2367.
84. Eapen, M.S., et al., *Abnormal M1/M2 macrophage phenotype profiles in the small airway wall and lumen in smokers and chronic obstructive pulmonary disease (COPD).* Sci Rep, 2017. **7**(1): p. 13392.

85. Eapen, M.S., et al., *Dysfunctional Immunity and Microbial Adhesion Molecules in Smoking-Induced Pneumonia.* Am J Respir Crit Care Med, 2018.
86. Iannella, H., C. Luna, and G. Waterer, *Inhaled corticosteroids and the increased risk of pneumonia: what's new? A 2015 updated review.* Therapeutic advances in respiratory disease, 2016. **10**(3): p. 235-255.
87. Chen, D., et al., *Observational study of inhaled corticosteroids on outcomes for COPD patients with pneumonia.* American journal of respiratory and critical care medicine, 2011. **184**(3): p. 312-316.
88. Lipson, D.A., et al., *Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD.* New England Journal of Medicine, 2018. **378**(18): p. 1671-1680.
89. Rodriguez-Roisin, R., et al., *Withdrawal Of Inhaled Corticosteroids And Exacerbations Of COPD: The Wisdom Phenotyping Substudy.* Management. **5**: p. 6.
90. Stolberg, V.R., et al., *Glucocorticoid-augmented efferocytosis inhibits pulmonary pneumococcal clearance in mice by reducing alveolar macrophage bactericidal function.* The Journal of Immunology, 2015. **195**(1): p. 174-184.
91. Patterson, C.M., et al., *Inhaled fluticasone propionate impairs pulmonary clearance of Klebsiella pneumoniae in mice.* Respiratory research, 2012. **13**(1): p. 40.
92. Singanayagam, A., et al., *Corticosteroid suppression of antiviral immunity increases bacterial loads and mucus production in COPD exacerbations.* Nature communications, 2018. **9**(1): p. 2229.
93. Vedel-Krogh, S., et al., *Blood eosinophil count and risk of pneumonia hospitalisations in individuals with COPD.* European Respiratory Journal, 2018. **51**(5): p. 1800120.
94. Varricchi, G., et al., *Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs.* Current opinion in allergy and clinical immunology, 2016. **16**(2): p. 186.
95. Long, G. and J. Wall, *Precision medicine in COPD: review of mepolizumab for eosinophilic COPD.* Breathe, 2018. **14**(4): p. 338-341.
96. Brightling, C.E., et al., *Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study.* The Lancet Respiratory Medicine, 2014. **2**(11): p. 891-901.
97. Tashkin, D.P. and M.E. Wechsler, *Role of eosinophils in airway inflammation of chronic obstructive pulmonary disease.* International journal of chronic obstructive pulmonary disease, 2018. **13**: p. 335.
98. Kim, V.L., et al., *Impact and associations of eosinophilic inflammation in COPD: analysis of the AERIS cohort.* European Respiratory Journal, 2017. **50**(4): p. 1700853.
99. Kolsum, U., et al., *Blood and sputum eosinophils in COPD; relationship with bacterial load.* Respiratory research, 2017. **18**(1): p. 88.
100. Janson, C., et al., *Scientific rationale for the possible inhaled corticosteroid intraclass difference in the risk of pneumonia in COPD.* International journal of chronic obstructive pulmonary disease, 2017. **12**: p. 3055.
101. Miravitlles, M. and A. Anzueto, *Antibiotic prophylaxis in COPD: Why, when, and for whom?* Pulmonary pharmacology & therapeutics, 2015. **32**: p. 119-123.
102. Maddi, S., et al., *ampicillin resistance in Haemophilus influenzae from COPD patients in the UK.* International journal of chronic obstructive pulmonary disease, 2017. **12**: p. 1507.
103. Albert, R.K., et al., *Azithromycin for prevention of exacerbations of COPD.* New England Journal of Medicine, 2011. **365**(8): p. 689-698.
104. Parameswaran, G.I. and S. Sethi, *Long-term macrolide therapy in chronic obstructive pulmonary disease.* Canadian Medical Association Journal, 2014. **186**(15): p. 1148-1152.
105. Herath, S.C. and P. Poole, *Prophylactic antibiotic therapy for chronic obstructive pulmonary disease (COPD).* The Cochrane Library, 2013.

106. Simpson, J.L., et al., *The effect of azithromycin in adults with stable neutrophilic COPD: a double blind randomised, placebo controlled trial.* PLoS One, 2014. **9**(8): p. e105609.
107. Baines, K.J., et al., *Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD.* ERJ open research, 2018. **4**(4): p. 00031-2018.
108. Simpson, J.L., et al., *Influence of age, past smoking, and disease severity on TLR2, neutrophilic inflammation, and MMP-9 levels in COPD.* Mediators of inflammation, 2013. **2013**.
109. Sriram, K.B., et al., *Nontypeable Haemophilus influenzae and chronic obstructive pulmonary disease: a review for clinicians.* Critical Reviews in Microbiology, 2017: p. 1-18.
110. Cipolla, D., J. Froehlich, and I. Gonda, *Emerging opportunities for inhaled antibiotic therapy.* J Antimicrob, 2015. **1**: p. 104.
111. Pettigrew, M.M., et al., *Effect of fluoroquinolones and macrolides on eradication and resistance of Haemophilus influenzae in chronic obstructive pulmonary disease.* Antimicrobial agents and chemotherapy, 2016. **60**(7): p. 4151-4158.
112. Desai, H., et al., *Antibiotic resistance in sputum isolates of Streptococcus pneumoniae in chronic obstructive pulmonary disease is related to antibiotic exposure.* COPD: Journal of Chronic Obstructive Pulmonary Disease, 2010. **7**(5): p. 337-344.
113. Sethi, S., et al., *Pulsed moxifloxacin for the prevention of exacerbations of chronic obstructive pulmonary disease: a randomized controlled trial.* Respiratory research, 2010. **11**(1): p. 10.
114. Puig, C., et al., *Molecular characterization of fluoroquinolone resistance in nontypeable Haemophilus influenzae clinical isolates.* Antimicrobial agents and chemotherapy, 2015. **59**(1): p. 461-466.
115. Vila, J., et al., *Increase in Quinolone Resistance in aHaemophilus influenzae Strain Isolated from a Patient with Recurrent Respiratory Infections Treated with Ofloxacin.* Antimicrobial agents and chemotherapy, 1999. **43**(1): p. 161-162.
116. Bastida, T., *Levofloxacin treatment failure in Haemophilus influenzae pneumonia.* 2003.
117. Brill, S.E., et al., *Effects of different antibiotic classes on airway bacteria in stable COPD using culture and molecular techniques: a randomised controlled trial.* Thorax, 2015: p. thoraxjnl-2015-207194.
118. Moreno, D., J. Barroso, and A. Garcia, *Vaccines for patients with COPD.* Recent patents on inflammation & allergy drug discovery, 2015. **9**(1): p. 23-30.
119. Walters, J.A., et al., *Pneumococcal vaccines for preventing pneumonia in chronic obstructive pulmonary disease.* The Cochrane Library, 2017.
120. Alfageme, I., et al., *Clinical efficacy of anti-pneumococcal vaccination in patients with COPD.* Thorax, 2006. **61**(3): p. 189-195.
121. Bonten, M.J., et al., *Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults.* New England Journal of Medicine, 2015. **372**(12): p. 1114-1125.
122. Bekkat-Berkani, R., et al., *Seasonal influenza vaccination in patients with COPD: a systematic literature review.* BMC pulmonary medicine, 2017. **17**(1): p. 79.
123. Kopsaftis, Z., R. Wood-Baker, and P. Poole, *Influenza vaccine for chronic obstructive pulmonary disease (COPD).* Cochrane Database of Systematic Reviews, 2018(6).
124. Poole, P., et al., *Influenza vaccine for patients with chronic obstructive pulmonary disease.* The Cochrane Library, 2006.
125. HSU, A.C.Y., et al., *Innate immunity to influenza in chronic airways diseases.* Respirology, 2012. **17**(8): p. 1166-1175.
126. Nath, K.D., et al., *Clinical factors associated with the humoral immune response to influenza vaccination in chronic obstructive pulmonary disease.* International journal of chronic obstructive pulmonary disease, 2014. **9**: p. 51.
127. Burel, J.G., et al., *Evaluation of immune responses to influenza vaccination in chronic obstructive pulmonary disease.* Journal of Vaccines and Vaccination, 2013. **4**(6).

128. Parpaleix, A., et al., *Inefficiency Of Influenza Vaccination In COPD Patients Is Associated With Defect Of B Cell Differentiation And Decrease Of IFNγ CD4+ T Cells Production*, in *C107. IMMUNITY, EXACERBATION AND IMMUNE CELL FUNCTION IN COPD*. 2016, American Thoracic Society. p. A6340-A6340.
129. Wong, S.M., et al., *Defining the Binding Region in Factor H to Develop a Therapeutic Factor H-Fc Fusion Protein against Non-Typeable Haemophilus influenzae.* Frontiers in cellular and infection microbiology, 2016. **6**.
130. Mastromarino, P., et al., *Resveratrol inhibits rhinovirus replication and expression of inflammatory mediators in nasal epithelia.* Antiviral research, 2015. **123**: p. 15-21.
131. Garofalo, R.P., D. Kolli, and A. Casola, *Respiratory syncytial virus infection: mechanisms of redox control and novel therapeutic opportunities.* Antioxidants & redox signaling, 2013. **18**(2): p. 186-217.
132. Vandini, S., C. Biagi, and M. Lanari, *Respiratory syncytial virus: the influence of serotype and genotype variability on clinical course of infection.* International journal of molecular sciences, 2017. **18**(8): p. 1717.
133. Thibaut, H.J., et al., *Toward antiviral therapy/prophylaxis for rhinovirus-induced exacerbations of chronic obstructive pulmonary disease: challenges, opportunities, and strategies.* Reviews in medical virology, 2016. **26**(1): p. 21-33.
134. Gunawardana, N., et al., *Experimental rhinovirus infection in COPD: implications for antiviral therapies.* Antiviral research, 2014. **102**: p. 95-105.
135. Muratore, G., et al., *Small molecule inhibitors of influenza A and B viruses that act by disrupting subunit interactions of the viral polymerase.* Proceedings of the National Academy of Sciences, 2012. **109**(16): p. 6247-6252.
136. Palamara, A.T., et al., *Inhibition of influenza A virus replication by resveratrol.* Journal of Infectious Diseases, 2005. **191**(10): p. 1719-1729.
137. Hayden, F.G., et al., *Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenzavirus infections.* New England Journal of Medicine, 1997. **337**(13): p. 874-880.
138. Ludwig, S., et al., *Influenza-virus-induced signaling cascades: targets for antiviral therapy?* Trends in molecular medicine, 2003. **9**(2): p. 46-52.
139. Peters Jr, P.H., et al., *Study of the impact of oseltamivir on the risk for pneumonia and other outcomes of influenza, 2000–2005.* The Medscape Journal of Medicine, 2008. **10**(6): p. 131.
140. Hedrick, J.A., et al., *Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age: a randomized controlled trial.* The Pediatric infectious disease journal, 2000. **19**(5): p. 410-417.
141. Monto, A.S., A. Webster, and O. Keene, *Randomized, placebo-controlled studies of inhaled zanamivir in the treatment of influenza A and B: pooled efficacy analysis.* Journal of Antimicrobial Chemotherapy, 1999. **44**(suppl_2): p. 23-29.
142. Murphy, K.R., et al., *Efficacy and safety of inhaled zanamivir for the treatment of influenza in patients with asthma or chronic obstructive pulmonary disease.* Clinical Drug Investigation, 2000. **20**(5): p. 337-349.
143. Mallia, P. and S.L. Johnston, *Influenza infection and COPD.* International journal of chronic obstructive pulmonary disease, 2007. **2**(1): p. 55.
144. Williamson, J.C. and P.S. Pegram, *Neuraminidase inhibitors in patients with underlying airways disease.* American Journal of Respiratory Medicine, 2002. **1**(2): p. 85-90.
145. Kaiser, L., et al., *Impact of oseltamivir treatment on influenza-related lower respiratory tract complications and hospitalizations.* Archives of internal medicine, 2003. **163**(14): p. 1667-1672.
146. McKimm-Breschkin, J., et al., *Neuraminidase sequence analysis and susceptibilities of influenza virus clinical isolates to zanamivir and oseltamivir.* Antimicrobial agents and chemotherapy, 2003. **47**(7): p. 2264-2272.

147. Abed, Y. and G. Boivin. *A Review of Clinical Influenza A and B Infections With Reduced Susceptibility to Both Oseltamivir and Zanamivir*. in *Open forum infectious diseases*. 2017. Oxford University Press.
148. Gubareva, L.V., et al., *Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus.* The Journal of infectious diseases, 1998. **178**(5): p. 1257-1262.
149. Moscona, A., *Global transmission of oseltamivir-resistant influenza.* New England Journal of Medicine, 2009. **360**(10): p. 953-956.
150. Popov, A.F., et al., *Combined therapy of influenza with antiviral drugs with a different mechanism of action in comparison with monotherapy.* Journal of Pharmaceutical Sciences and Research, 2018. **10**(2): p. 357-360.
151. de Mello, C.P.P., et al., *Oseltamivir-zanamivir combination therapy suppresses drug-resistant H1N1 influenza A viruses in the hollow fiber infection model (HFIM) system.* European Journal of Pharmaceutical Sciences, 2018. **111**: p. 443-449.
152. Shi, L., et al., *Antiviral activity of arbidol against influenza A virus, respiratory syncytial virus, rhinovirus, coxsackie virus and adenovirus in vitro and in vivo.* Archives of virology, 2007. **152**(8): p. 1447-1455.
153. Tamura, D., et al., *Emergence of multidrug-resistant influenza A (H1N1) pdm09 virus variants in an immunocompromised child treated with oseltamivir and zanamivir.* The Journal of infectious diseases, 2015. **212**(8): p. 1209-1213.
154. May Wang, Q. and S.-H. Chen, *Human rhinovirus 3C protease as a potential target for the development of antiviral agents.* Current Protein and Peptide Science, 2007. **8**(1): p. 19-27.
155. Casanova, V., et al., *Antiviral therapeutic approaches for human rhinovirus infections.* Future virology, 2018. **13**(07): p. 505-518.
156. Safka, K.A. and R.A. McIvor, *Non-pharmacological management of chronic obstructive pulmonary disease.* The Ulster medical journal, 2015. **84**(1): p. 13.
157. Bellamy, D., et al., *International Primary Care Respiratory Group (IPCRG) Guidelines: management of chronic obstructive pulmonary disease (COPD).* Primary Care Respiratory Journal, 2006. **15**(1): p. 48.
158. Anthonisen, N.R., et al., *Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1: the Lung Health Study.* Jama, 1994. **272**(19): p. 1497-1505.
159. Pelkonen, M., et al., *Pulmonary function, smoking cessation and 30 year mortality in middle aged Finnish men.* Thorax, 2000. **55**(9): p. 746-750.
160. Scanlon, P.D., et al., *Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease: the Lung Health Study.* American Journal of Respiratory and Critical Care Medicine, 2000. **161**(2): p. 381-390.
161. Anthonisen, N.R., J.E. Connett, and R.P. Murray, *Smoking and lung function of Lung Health Study participants after 11 years.* American journal of respiratory and critical care medicine, 2002. **166**(5): p. 675-679.
162. Tashkin, D.P. and R.P. Murray, *Smoking cessation in chronic obstructive pulmonary disease.* Respiratory medicine, 2009. **103**(7): p. 963-974.
163. Tashkin, D.P. *Smoking cessation in chronic obstructive pulmonary disease*. in *Seminars in respiratory and critical care medicine*. 2015. Thieme Medical Publishers.
164. Rennard, S.I. and D.M. Daughton, *Smoking cessation.* Clinics in chest medicine, 2014. **35**(1): p. 165-176.
165. Group, T.L.-T.O.T.T.R., *A Randomized Trial of Long-Term Oxygen for COPD with Moderate Desaturation.* New England Journal of Medicine, 2016. **375**(17): p. 1617-1627.
166. Chaouat, A., *A randomized trial of nocturnal oxygen therapy in chronic obstructive pulmonary disease patients.* The European respiratory journal. **14**(5).

167. Górecka, D., *Effect of long-term oxygen therapy on survival in patients with chronic obstructive pulmonary disease with moderate hypoxaemia.* Thorax. **52**(8): p. 674-679.
168. Yusen, R.D., et al., *The Long-Term Oxygen Treatment Trial for Chronic Obstructive Pulmonary Disease: Rationale, Design, and Lessons Learned.* Ann Am Thorac Soc, 2018. **15**(1): p. 89-101.
169. Staudt, M.R., et al., *Altered lung biology of healthy never smokers following acute inhalation of E-cigarettes.* Respiratory research, 2018. **19**(1): p. 78.
170. Sherwood, C.L. and S. Boitano, *Airway epithelial cell exposure to distinct e-cigarette liquid flavorings reveals toxicity thresholds and activation of CFTR by the chocolate flavoring 2, 5-dimethypyrazine.* Respiratory research, 2016. **17**(1): p. 57.
171. Cervellati, F., et al., *Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells.* Toxicology in Vitro, 2014. **28**(5): p. 999-1005.
172. Higham, A., et al., *Electronic cigarette exposure triggers neutrophil inflammatory responses.* Respiratory research, 2016. **17**(1): p. 56.
173. Lerner, C.A., et al., *Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung.* PloS one, 2015. **10**(2): p. e0116732.
174. Sussan, T.E., et al., *Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model.* PloS one, 2015. **10**(2): p. e0116861.
175. Lee, H.-W., et al., *E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells.* Proceedings of the National Academy of Sciences, 2018. **115**(7): p. E1560-E1569.
176. Hsu, A.C.-Y., et al., *Impaired antiviral stress granule and IFN-β enhanceosome formation enhances susceptibility to influenza infection in chronic obstructive pulmonary disease epithelium.* American journal of respiratory cell and molecular biology, 2016. **55**(1): p. 117-127.
177. Miyashita, L., et al., *E-cigarette vapour enhances pneumococcal adherence to airway epithelial cells.* Eur Respir J, 2018. **51**(2).
178. Giebelen, I.A., et al., *Stimulation of acetylcholine receptors impairs host defence during pneumococcal pneumonia.* European Respiratory Journal, 2009. **33**(2): p. 375-381.
179. Matsunaga, K., et al., *Involvement of nicotinic acetylcholine receptors in suppression of antimicrobial activity and cytokine responses of alveolar macrophages to Legionella pneumophila infection by nicotine.* The Journal of Immunology, 2001. **167**(11): p. 6518-6524.
180. Wongtrakool, C., et al., *In utero nicotine exposure promotes M2 activation in neonatal mouse alveolar macrophages.* Pediatric research, 2012. **72**(2): p. 147.
181. Radek, K.A., et al., *Neuroendocrine nicotinic receptor activation increases susceptibility to bacterial infections by suppressing antimicrobial peptide production.* Cell host & microbe, 2010. **7**(4): p. 277-289.
182. Mahapatra, S.K., et al., *Alteration of immune functions and Th1/Th2 cytokine balance in nicotine-induced murine macrophages: immunomodulatory role of eugenol and N-acetylcysteine.* International Immunopharmacology, 2011. **11**(4): p. 485-495.
183. Varughese, S., et al., *Effects of theatrical smokes and fogs on respiratory health in the entertainment industry.* American journal of industrial medicine, 2005. **47**(5): p. 411-418.
184. Sohal, S.S., et al., *IQOS exposure impairs human airway cell homeostasis: direct comparison with traditional cigarette and e-cigarette.* ERJ Open Res, 2019. **5**(1).
185. Hakansson, A., C. Orihuela, and D. Bogaert, *Bacterial-host interactions: physiology and pathophysiology of respiratory infection.* Physiological reviews, 2018. **98**(2): p. 781-811.
186. King, P.T. and R. Sharma, *The lung immune response to nontypeable Haemophilus influenzae (lung immunity to NTHi).* Journal of immunology research, 2015. **2015**.
187. Shukla, S.D., et al., *Platelet activating factor receptor: gateway for bacterial chronic airway infection in chronic obstructive pulmonary disease and potential therapeutic target.* Expert review of respiratory medicine, 2015. **9**(4): p. 473-485.

188. Fillon, S., et al., *Platelet-activating factor receptor and innate immunity: uptake of gram-positive bacterial cell wall into host cells and cell-specific pathophysiology.* The Journal of Immunology, 2006. **177**(9): p. 6182-6191.
189. Chao, W. and M.S. Olson, *Platelet-activating factor: receptors and signal transduction.* Biochemical Journal, 1993. **292**(3): p. 617-629.
190. Kelesidis, T., et al., *The role of platelet-activating factor in chronic inflammation, immune activation, and comorbidities associated with HIV infection.* AIDS reviews, 2015. **17**(4): p. 191.
191. Duell, B.L., Y.C. Su, and K. Riesbeck, *Host–pathogen interactions of nontypeable Haemophilus influenzae: from commensal to pathogen.* FEBS letters, 2016. **590**(21): p. 3840-3853.
192. Clark, S.E. and J.N. Weiser, *Microbial modulation of host immunity with the small molecule phosphorylcholine.* Infection and immunity, 2013. **81**(2): p. 392-401.
193. Geme, J.W.S., *Molecular and cellular determinants of non-typeable Haemophilus influenzae adherence and invasion.* Cellular microbiology, 2002. **4**(4): p. 191-200.
194. Swords, W.E., et al., *Non-typeable Haemophilus influenzae adhere to and invade human bronchial epithelial cells via an interaction of lipooligosaccharide with the PAF receptor.* Molecular microbiology, 2000. **37**(1): p. 13-27.
195. Grigg, J., et al., *Cigarette smoke and platelet-activating factor receptor dependent adhesion of Streptococcus pneumoniae to lower airway cells.* Thorax, 2012. **67**(10): p. 908-913.
196. Shukla, S.D., et al., *An antagonist of the platelet-activating factor receptor inhibits adherence of both nontypeable Haemophilus influenzae and Streptococcus pneumoniae to cultured human bronchial epithelial cells exposed to cigarette smoke.* International journal of chronic obstructive pulmonary disease, 2016. **11**: p. 1647.
197. Shukla, S.D., et al., *Platelet-activating factor receptor (PAFr) is upregulated in small airways and alveoli of smokers and COPD patients.* Respirology, 2016. **21**(3): p. 504-510.
198. Shukla, S.D., et al., *Airway epithelial platelet-activating factor receptor expression is markedly upregulated in chronic obstructive pulmonary disease.* International journal of chronic obstructive pulmonary disease, 2014. **9**: p. 853.
199. Sohal, S.S., et al., *Potential Mechanisms of Microbial Pathogens in Idiopathic Interstitial Lung Disease.* Chest, 2017. **152**(4): p. 899-900.
200. Miyashita, L., R. Suri, and J. Grigg, *The Effect Of E-cigarettes (EC) On Nasal Platelet Activating Factor Receptor (PAFR) Expression*, in *A16. WHY SHOULD WE BE CONCERNED ABOUT E-CIGARETTES?* 2017, Am Thoracic Soc. p. A1029-A1029.
201. Clementi, C.F., A.P. Håkansson, and T.F. Murphy, *Internalization and trafficking of nontypeable Haemophilus influenzae in human respiratory epithelial cells and roles of IgA1 proteases for optimal invasion and persistence.* Infection and immunity, 2014. **82**(1): p. 433-444.
202. Avadhanula, V., et al., *Nontypeable Haemophilus influenzae adheres to intercellular adhesion molecule 1 (ICAM-1) on respiratory epithelial cells and upregulates ICAM-1 expression.* Infection and immunity, 2006. **74**(2): p. 830-838.
203. Roy, J., M. Audette, and M.J. Tremblay, *Intercellular Adhesion Molecule-1 (ICAM-1) Gene Expression in Human T Cells Is Regulated by Phosphotyrosyl Phosphatase Activity INVOLVEMENT OF NF-κB, Ets, AND PALINDROMIC INTERFERON-γ-RESPONSIVE ELEMENT-BINDING SITES.* Journal of Biological Chemistry, 2001. **276**(18): p. 14553-14561.
204. Staunton, D.E., et al., *A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses.* Cell, 1989. **56**(5): p. 849-853.
205. Novotny, L.A. and L.O. Bakaletz, *Intercellular adhesion molecule 1 serves as a primary cognate receptor for the Type IV pilus of nontypeable Haemophilus influenzae.* Cellular microbiology, 2016. **18**(8): p. 1043-1055.

206. Ledford, R.M., et al., *VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds.* Journal of virology, 2004. **78**(7): p. 3663-3674.
207. Bianco, A., et al., *Expression of intercellular adhesion molecule-1 (ICAM-1) in nasal epithelial cells of atopic subjects: a mechanism for increased rhinovirus infection?* Clinical & Experimental Immunology, 2000. **121**(2): p. 339-345.
208. Shukla, S.D., et al., *The main rhinovirus respiratory tract adhesion site (ICAM-1) is upregulated in smokers and patients with chronic airflow limitation (CAL).* Respiratory research, 2017. **18**(1): p. 6.
209. Sajjan, U.S., et al., *H. influenzae potentiates airway epithelial cell responses to rhinovirus by increasing ICAM-1 and TLR3 expression.* The FASEB journal, 2006. **20**(12): p. 2121-2123.
210. Mukhopadhyay, S., et al., *Intercellular adhesion molecule-1 as a drug target in asthma and rhinitis.* Respirology, 2014. **19**(4): p. 508-513.
211. Traub, S., et al., *An anti-human ICAM-1 antibody inhibits rhinovirus-induced exacerbations of lung inflammation.* PLoS pathogens, 2013. **9**(8): p. e1003520.
212. Palmenberg, A.C. and J.E. Gern, *Classification and evolution of human rhinoviruses.* Rhinoviruses: Methods and Protocols, 2015: p. 1-10.
213. Jurcisek, J.A., et al., *The PilA protein of non-typeable Haemophilus influenzae plays a role in biofilm formation, adherence to epithelial cells and colonization of the mammalian upper respiratory tract.* Molecular microbiology, 2007. **65**(5): p. 1288-1299.
214. Jiang, Z., et al., *Fimbria-Mediated Enhanced Attachment of NontypeableHaemophilus influenzae to Respiratory Syncytial Virus-Infected Respiratory Epithelial Cells.* Infection and immunity, 1999. **67**(1): p. 187-192.
215. Novotny, L., J. Clements, and L. Bakaletz, *Transcutaneous immunization as preventative and therapeutic regimens to protect against experimental otitis media due to nontypeable Haemophilus influenzae.* Mucosal immunology, 2011. **4**(4): p. 456-467.
216. Slevogt, H., et al., *CEACAM1 inhibits Toll-like receptor 2–triggered antibacterial responses of human pulmonary epithelial cells.* Nature immunology, 2008. **9**(11): p. 1270-1278.
217. Klaile, E., et al., *Carcinoembryonic antigen (CEA)-related cell adhesion molecules are co-expressed in the human lung and their expression can be modulated in bronchial epithelial cells by non-typable Haemophilus influenzae, Moraxella catarrhalis, TLR3, and type I and II interferons.* Respiratory research, 2013. **14**(1): p. 85.
218. Kc, R., et al., *Temporal upregulation of host surface receptors provides a window of opportunity for bacterial adhesion and disease.* Microbiology, 2017. **163**(4): p. 421-430.
219. Vitenshtein, A., et al., *CEACAM1-mediated inhibition of virus production.* Cell reports, 2016. **15**(11): p. 2331-2339.
220. Khairnar, V., et al., *CEACAM1 induces B-cell survival and is essential for protective antiviral antibody production.* Nature communications, 2015. **6**: p. 6217.
221. Khairnar, V., et al., *CEACAM1 promotes CD8+ T cell responses and improves control of a chronic viral infection.* Nature communications, 2018. **9**(1): p. 2561.
222. Blau, D.M., et al., *Targeted disruption of the Ceacam1 (MHVR) gene leads to reduced susceptibility of mice to mouse hepatitis virus infection.* Journal of virology, 2001. **75**(17): p. 8173-8186.
223. Traving, C. and R. Schauer, *Structure, function and metabolism of sialic acids.* Cellular and Molecular Life Sciences CMLS, 1998. **54**(12): p. 1330-1349.
224. Rathod, S., et al., *Comparative analysis of salivary sialic acid levels in patients with chronic obstructive pulmonary disease and chronic periodontitis patients: A biochemical study.* Indian Journal of Dental Research, 2018. **29**(1): p. 22.
225. Pinnelli, V.B.K., *Elevated levels of serum sialic acid and C-reactive protein: markers of systemic inflammation in patients with chronic obstructive pulmonary disease.* International Journal of Research in Medical Sciences, 2016. **4**(4): p. 1209.

226. Fukuyama, S. and Y. Kawaoka, *The pathogenesis of influenza virus infections: the contributions of virus and host factors.* Current opinion in immunology, 2011. **23**(4): p. 481-486.
227. Lakadamyali, M., M.J. Rust, and X. Zhuang, *Endocytosis of influenza viruses.* Microbes and infection, 2004. **6**(10): p. 929-936.
228. Rust, M.J., et al., *Assembly of endocytic machinery around individual influenza viruses during viral entry.* Nature Structural and Molecular Biology, 2004. **11**(6): p. 567.
229. Xiao, S., et al., *Pentacyclic triterpenes grafted on CD cores to interfere with influenza virus entry: A dramatic multivalent effect.* Biomaterials, 2016. **78**: p. 74-85.
230. Kadam, R.U. and I.A. Wilson, *A small-molecule fragment that emulates binding of receptor and broadly neutralizing antibodies to influenza A hemagglutinin.* Proceedings of the National Academy of Sciences, 2018: p. 201801999.
231. Du, L., et al., *Identification and structural characterization of a broadly neutralizing antibody targeting a novel conserved epitope on the influenza virus H5N1 hemagglutinin.* Journal of virology, 2013. **87**(4): p. 2215-2225.
232. Wu, W., et al., *New influenza A virus entry inhibitors derived from the viral fusion peptides.* PloS one, 2015. **10**(9): p. e0138426.
233. Basu, A., et al., *New small molecule entry inhibitors targeting hemagglutinin-mediated influenza a virus fusion.* Journal of virology, 2014. **88**(3): p. 1447-1460.
234. Liu, S., et al., *CL-385319 inhibits H5N1 avian influenza A virus infection by blocking viral entry.* European journal of pharmacology, 2011. **660**(2-3): p. 460-467.
235. Marjuki, H., et al., *Membrane accumulation of influenza a virus hemagglutinin triggers nuclear export of the viral genome via PKCα mediated activation of ERK signaling.* Journal of Biological Chemistry, 2006.
236. Lu, R., P. Müller, and K.M. Downard, *Molecular basis of influenza hemagglutinin inhibition with an entry-blocker peptide by computational docking and mass spectrometry.* Antiviral Chemistry and Chemotherapy, 2015. **24**(3-4): p. 109-117.
237. Skalickova, S., et al., *Perspective of use of antiviral peptides against influenza virus.* Viruses, 2015. **7**(10): p. 5428-5442.
238. Blaising, J., S.J. Polyak, and E.-I. Pécheur, *Arbidol as a broad-spectrum antiviral: an update.* Antiviral research, 2014. **107**: p. 84-94.
239. Kadam, R.U. and I.A. Wilson, *Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol.* Proceedings of the National Academy of Sciences, 2017. **114**(2): p. 206-214.
240. Tikhonova, E., et al., *Study of effectiveness of antiviral drugs (umifenovir, triazavirin) against acute respiratory viral infections.* Kazan medical journal, 2018. **99**(2): p. 215-223.
241. Titova, O., et al., *Afficacy of Arbidol in the prevention of virus-induced exacerbations of bronchial asthma and chronic obstructive pulmonary disease.* Therapeutic Archive, 2018. **90**(8).
242. Leneva, I., et al., *Virus susceptibility and clinical effectiveness of anti-influenza drugs during the 2010–2011 influenza season in Russia.* International Journal of Infectious Diseases, 2016. **43**: p. 77-84.
243. Mc Mahon, A. and I. Martin-Loeches, *The pharmacological management of severe influenza infection–'existing and emerging therapies'.* Expert review of clinical pharmacology, 2017. **10**(1): p. 81-95.
244. Laursen, N.S. and I.A. Wilson, *Broadly neutralizing antibodies against influenza viruses.* Antiviral research, 2013. **98**(3): p. 476-483.
245. Throsby, M., et al., *Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells.* PloS one, 2008. **3**(12): p. e3942.

246. Ekiert, D.C., et al., *A highly conserved neutralizing epitope on group 2 influenza A viruses.* Science, 2011: p. 1204839.
247. Yasugi, M., et al., *Human monoclonal antibodies broadly neutralizing against influenza B virus.* PLoS pathogens, 2013. **9**(2): p. e1003150.
248. Corti, D., et al., *A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins.* Science, 2011: p. 1205669.
249. Sui, J., et al., *Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses.* Nature structural & molecular biology, 2009. **16**(3): p. 265.
250. Zhu, X., et al., *Hemagglutinin homologue from H17N10 bat influenza virus exhibits divergent receptor-binding and pH-dependent fusion activities.* Proceedings of the National Academy of Sciences, 2013. **110**(4): p. 1458-1463.
251. Tong, S., et al., *New world bats harbor diverse influenza A viruses.* PLoS pathogens, 2013. **9**(10): p. e1003657.
252. Zhao, X., et al., *The NS1 gene from bat-derived influenza-like virus H17N10 can be rescued in influenza A PR8 backbone.* Journal of General Virology, 2016. **97**(8): p. 1797-1806.
253. Juozapaitis, M., et al., *An infectious bat-derived chimeric influenza virus harbouring the entry machinery of an influenza A virus.* Nature communications, 2014. **5**: p. 4448.
254. Metcalfe, H., et al., *Effects of cigarette smoke on Toll-like receptor (TLR) activation of chronic obstructive pulmonary disease (COPD) macrophages.* Clinical & Experimental Immunology, 2014. **176**(3): p. 461-472.
255. Martinez, F.O. and S. Gordon, *The M1 and M2 paradigm of macrophage activation: time for reassessment.* F1000prime reports, 2014. **6**.
256. Thorburn, A.N., et al., *TLR2, TLR4 and MyD88 mediate allergic airway disease (AAD) and Streptococcus pneumoniae-induced suppression of AAD.* PloS one, 2016. **11**(6): p. e0156402.
257. Yamasaki, K. and S.F.v. Eeden, *Lung Macrophage Phenotypes and Functional Responses: Role in the Pathogenesis of COPD.* International journal of molecular sciences, 2018. **19**(2): p. 582.
258. Eapen, M.S., et al., *Abnormal M1/M2 macrophage phenotype profiles in the small airway wall and lumen in smokers and chronic obstructive pulmonary disease (COPD).* Scientific reports, 2017. **7**(1): p. 13392.
259. Rath, M., et al., *Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages.* Frontiers in immunology, 2014. **5**: p. 532.
260. Berenson, C.S., et al., *Impaired phagocytosis of nontypeable Haemophilus influenzae by human alveolar macrophages in chronic obstructive pulmonary disease.* The Journal of infectious diseases, 2006. **194**(10): p. 1375-1384.
261. Hodge, S., et al., *Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells.* Immunology and cell biology, 2003. **81**(4): p. 289.
262. Hodge, S., et al., *Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease.* American journal of respiratory cell and molecular biology, 2007. **37**(6): p. 748-755.
263. Morimoto, K., et al., *Lovastatin enhances clearance of apoptotic cells (efferocytosis) with implications for chronic obstructive pulmonary disease.* The Journal of Immunology, 2006. **176**(12): p. 7657-7665.
264. Bain, W.G., et al., *Low-dose oxygen enhances macrophage-derived bacterial clearance following cigarette smoke exposure.* Journal of immunology research, 2016. **2016**.
265. Serban, K.A., et al., *Alpha-1 antitrypsin supplementation improves alveolar macrophages efferocytosis and phagocytosis following cigarette smoke exposure.* PloS one, 2017. **12**(4): p. e0176073.
266. Martorana, P.A., et al., *Effect of roflumilast on inflammatory cells in the lungs of cigarette smoke-exposed mice.* BMC pulmonary medicine, 2008. **8**(1): p. 17.

267. Hodge, S. and P.N. Reynolds, *Low-dose azithromycin improves phagocytosis of bacteria by both alveolar and monocyte-derived macrophagesin chronic obstructive pulmonary disease subjects.* Respirology, 2012. **17**(5): p. 802-807.
268. Bozinovski, S., D. Anthony, and R. Vlahos, *Targeting pro-resolution pathways to combat chronic inflammation in COPD.* Journal of thoracic disease, 2014. **6**(11): p. 1548.
269. Buckley, C.D., D.W. Gilroy, and C.N. Serhan, *Proresolving lipid mediators and mechanisms in the resolution of acute inflammation.* Immunity, 2014. **40**(3): p. 315-327.
270. Arora, S., et al., *Influenza A virus enhances its propagation through the modulation of Annexin-A1 dependent endosomal trafficking and apoptosis.* Cell death and differentiation, 2016. **23**(7): p. 1243.
271. Sugimoto, M.A., et al., *Annexin A1 and the resolution of inflammation: modulation of neutrophil recruitment, apoptosis, and clearance.* Journal of immunology research, 2016. **2016**.
272. Wang, X., et al., *Resolution of inflammation is altered in Alzheimer's disease.* Alzheimer's & Dementia, 2015. **11**(1): p. 40-50. e2.
273. Serhan, C.N., N. Chiang, and J. Dalli. *The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution*. in *Seminars in immunology*. 2015. Elsevier.
274. Pedersen, J., et al., *Inflammatory pathways of importance for management of inflammatory bowel disease.* World journal of gastroenterology: WJG, 2014. **20**(1): p. 64.
275. Chen, Y.-C., et al., *Defective formyl peptide receptor 2/3 and annexin A1 expressions associated with M2a polarization of blood immune cells in patients with chronic obstructive pulmonary disease.* Journal of translational medicine, 2018. **16**(1): p. 69.
276. Lai, T., et al., *Annexin A1 is elevated in patients with COPD and affects lung fibroblast function.* International journal of chronic obstructive pulmonary disease, 2018. **13**: p. 473.
277. Henson, P.M., G.P. Cosgrove, and R.W. Vandivier, *State of the art. Apoptosis and cell homeostasis in chronic obstructive pulmonary disease.* Proceedings of the American Thoracic Society, 2006. **3**(6): p. 512-516.
278. Morimoto, K., W.J. Janssen, and M. Terada, *Defective efferocytosis by alveolar macrophages in IPF patients.* Respiratory medicine, 2012. **106**(12): p. 1800-1803.
279. Bezemer, G.F., et al., *Dual role of Toll-like receptors in asthma and chronic obstructive pulmonary disease.* Pharmacological reviews, 2012. **64**(2): p. 337-358.
280. Pomerenke, A., et al., *Characterization of TLR-induced inflammatory responses in COPD and control lung tissue explants.* International journal of chronic obstructive pulmonary disease, 2016. **11**: p. 2409.
281. Lea, S.R., et al., *The effects of repeated Toll-like receptors 2 and 4 stimulation in COPD alveolar macrophages.* International journal of chronic obstructive pulmonary disease, 2018. **13**: p. 771.
282. Chen, H., et al., *Tobacco smoking inhibits expression of proinflammatory cytokines and activation of IL-1R-associated kinase, p38, and NF-κB in alveolar macrophages stimulated with TLR2 and TLR4 agonists.* The Journal of Immunology, 2007. **179**(9): p. 6097-6106.
283. Kent, L., et al., *Cigarette smoke extract induced cytokine and chemokine gene expression changes in COPD macrophages.* Cytokine, 2008. **42**(2): p. 205-216.
284. Armstrong, J., C. Sargent, and D. Singh, *Glucocorticoid sensitivity of lipopolysaccharide-stimulated chronic obstructive pulmonary disease alveolar macrophages.* Clinical & Experimental Immunology, 2009. **158**(1): p. 74-83.
285. Brown, G., et al., *Cigarette smoking decreases interleukin 1 release by human alveolar macrophages.* American Journal of Physiology-Cell Physiology, 1989. **256**(2): p. C260-C264.
286. Soliman, D.M. and H. Twigg 3rd, *Cigarette smoking decreases bioactive interleukin-6 secretion by alveolar macrophages.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 1992. **263**(4): p. L471-L478.

287. Ohta, T., et al., *Cigarette smoking decreases interleukin-8 secretion by human alveolar macrophages.* Respiratory medicine, 1998. **92**(7): p. 922-927.
288. Berenson, C.S., et al., *Impaired alveolar macrophage response to Haemophilus antigens in chronic obstructive lung disease.* American journal of respiratory and critical care medicine, 2006. **174**(1): p. 31-40.
289. Lappalainen, U., et al., *Interleukin-1β causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung.* American journal of respiratory cell and molecular biology, 2005. **32**(4): p. 311-318.
290. Kraft, R., et al., *Predictive value of IL-8 for sepsis and severe infections after burn injury-A clinical study.* Shock (Augusta, Ga.), 2015. **43**(3): p. 222.
291. Basith, S., et al., *Toll-like receptor modulators: a patent review (2006–2010).* Expert opinion on therapeutic patents, 2011. **21**(6): p. 927-944.
292. Thorburn, A.N. and P.M. Hansbro, *Harnessing regulatory T cells to suppress asthma: from potential to therapy.* American journal of respiratory cell and molecular biology, 2010. **43**(5): p. 511-519.
293. To, E.E., et al., *Intranasal and epicutaneous administration of Toll-like receptor 7 (TLR7) agonists provides protection against influenza A virus-induced morbidity in mice.* Scientific reports, 2019. **9**(1): p. 2366.
294. Duong, C., et al., *Glutathione peroxidase-1 protects against cigarette smoke-induced lung inflammation in mice.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2010. **299**(3): p. L425-L433.
295. Vlahos, R., J. Stambas, and S. Selemidis, *Suppressing production of reactive oxygen species (ROS) for influenza A virus therapy.* Trends in Pharmacological Sciences, 2012. **33**(1): p. 3-8.
296. Bernardo, I., S. Bozinovski, and R. Vlahos, *Targeting oxidant-dependent mechanisms for the treatment of COPD and its comorbidities.* Pharmacology & therapeutics, 2015. **155**: p. 60-79.
297. Kirkham, P.A. and P.J. Barnes, *Oxidative stress in COPD.* Chest, 2013. **144**(1): p. 266-273.
298. Oostwoud, L., et al., *Apocynin and ebselen reduce influenza A virus-induced lung inflammation in cigarette smoke-exposed mice.* Scientific reports, 2016. **6**: p. 20983.
299. Vlahos, R. and S. Bozinovski, *Glutathione peroxidase-1 as a novel therapeutic target for COPD.* Redox Report, 2013. **18**(4): p. 142-149.
300. Hogg, J., *Why does airway inflammation persist after the smoking stops?* 2006, BMJ Publishing Group Ltd.
301. Vlahos, R., et al., *Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation.* PLoS pathogens, 2011. **7**(2): p. e1001271.
302. To, E.E., et al., *Novel endosomal NOX2 oxidase inhibitor ameliorates pandemic influenza A virus-induced lung inflammation in mice.* Respirology, 2019.
303. Vlahos, R. and S. Bozinovski, *Role of alveolar macrophages in chronic obstructive pulmonary disease.* Frontiers in immunology, 2014. **5**: p. 435.
304. Bedard, K. and K.-H. Krause, *The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology.* Physiological reviews, 2007. **87**(1): p. 245-313.
305. To, E.E., et al., *Endosomal NOX2 oxidase exacerbates virus pathogenicity and is a target for antiviral therapy.* Nature communications, 2017. **8**(1): p. 69.
306. Russell, R., et al., *Bronchial epithelial gene expression of NOX isoforms are related to important clinical characteristics in COPD*. 2016, Eur Respiratory Soc.
307. Downs, C.A., et al., *Cigarette smoke extract is a Nox agonist and regulates ENaC in alveolar type 2 cells.* 2016.
308. Rastogi, R., et al., *NOX activation by subunit interaction and underlying mechanisms in disease.* Frontiers in Cellular Neuroscience, 2017. **10**: p. 301.
309. Diebold, B.A., et al., *NOX2 as a target for drug development: indications, possible complications, and progress.* Antioxidants & redox signaling, 2015. **23**(5): p. 375-405.

310. Kuhns, D.B., et al., *Residual NADPH oxidase and survival in chronic granulomatous disease.* New England Journal of Medicine, 2010. **363**(27): p. 2600-2610.
311. Guo, X., et al., *NOX4 expression and distal arteriolar remodeling correlate with pulmonary hypertension in COPD.* BMC pulmonary medicine, 2018. **18**(1): p. 111.
312. Liu, X., et al., *The expression of NOX4 in smooth muscles of small airway correlates with the disease severity of COPD.* BioMed research international, 2016. **2016**.
313. Vlahos, R. and S. Selemidis, *NADPH oxidases as novel pharmacologic targets against influenza A virus infection.* Molecular pharmacology, 2014. **86**(6): p. 747-759.
314. Lubos, E., J. Loscalzo, and D.E. Handy, *Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities.* Antioxidants & redox signaling, 2011. **15**(7): p. 1957-1997.
315. Dahl, M., et al., *Superoxide dismutase 3 polymorphism associated with reduced lung function in two large populations.* American journal of respiratory and critical care medicine, 2008. **178**(9): p. 906-912.
316. Kluchova, Z., et al., *The association between oxidative stress and obstructive lung impairment in patients with COPD.* Physiological Research, 2007. **56**(1).
317. Regan, E.A., et al., *Smoking and COPD increase sputum levels of extracellular superoxide dismutase.* Free Radical Biology and Medicine, 2011. **51**(3): p. 726-732.
318. Yatmaz, S., et al., *Glutathione peroxidase-1 reduces influenza A virus–induced lung inflammation.* American journal of respiratory cell and molecular biology, 2013. **48**(1): p. 17-26.
319. Yao, H., et al., *Extracellular superoxide dismutase protects against pulmonary emphysema by attenuating oxidative fragmentation of ECM.* Proceedings of the National Academy of Sciences, 2010. **107**(35): p. 15571-15576.
320. Juul, K., et al., *Genetically increased antioxidative protection and decreased chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2006. **173**(8): p. 858-864.
321. Bellinghausen, C., et al., *Viral–bacterial interactions in the respiratory tract.* Journal of General Virology, 2016. **97**(12): p. 3089-3102.
322. Dickson, R.P., F.J. Martinez, and G.B. Huffnagle, *The role of the microbiome in exacerbations of chronic lung diseases.* The Lancet, 2014. **384**(9944): p. 691-702.
323. Marsland, B.J., A. Trompette, and E.S. Gollwitzer, *The gut–lung axis in respiratory disease.* Annals of the American Thoracic Society, 2015. **12**(Supplement 2): p. S150-S156.
324. Kim, H.J., et al., *The microbiome of the lung and its extracellular vesicles in nonsmokers, healthy smokers and COPD patients.* Experimental & molecular medicine, 2017. **49**(4): p. e316.
325. Huang, Y.J. and H.A. Boushey, *The sputum microbiome in chronic obstructive pulmonary disease exacerbations.* Annals of the American Thoracic Society, 2015. **12**(Supplement 2): p. S176-S180.
326. Wang, Z., et al., *Lung microbiome dynamics in chronic obstructive pulmonary disease exacerbations.* European Respiratory Journal, 2016: p. ERJ-01406-2015.
327. Mayhew, D., et al., *Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations.* Thorax, 2018: p. thoraxjnl-2017-210408.
328. Garcia-Nuñez, M., et al., *Severity-Related Changes of Bronchial Microbiome in Copd.* Journal of clinical microbiology, 2014: p. JCM. 01967-14.
329. de Steenhuijsen Piters, W.A., E.A. Sanders, and D. Bogaert, *The role of the local microbial ecosystem in respiratory health and disease.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2015. **370**(1675): p. 20140294.

330. Huang, Y.J., et al., *A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations.* OMICS A Journal of Integrative Biology, 2010. **14**(1): p. 9-59.
331. Segal, L.N., et al., *Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation.* Microbiome, 2013. **1**(1): p. 19.
332. Sze, M.A., et al., *Host response to the lung microbiome in chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2015. **192**(4): p. 438-445.
333. Gao, Z., et al., *Human pharyngeal microbiome may play a protective role in respiratory tract infections.* Genomics, proteomics & bioinformatics, 2014. **12**(3): p. 144-150.
334. El-Solh, A.A., *Association between pneumonia and oral care in nursing home residents.* Lung, 2011. **189**(3): p. 173.
335. Tilahun, B., et al., *Nasal colonization and lower respiratory tract infections with methicillin-resistant Staphylococcus aureus.* American Journal of Critical Care, 2015. **24**(1): p. 8-12.
336. Sassone-Corsi, M. and M. Raffatellu, *No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens.* The Journal of Immunology, 2015. **194**(9): p. 4081-4087.
337. Sze, M.A., et al., *The lung tissue microbiome in chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2012. **185**(10): p. 1073-1080.
338. Heinrich, A., et al., *Moraxella catarrhalis decreases antiviral innate immune responses by down-regulation of TLR3 via inhibition of p53 in human bronchial epithelial cells.* Faseb j, 2016. **30**(6): p. 2426-34.
339. Tuvim, M.J., et al., *Augmented lung inflammation protects against influenza A pneumonia.* PLoS One, 2009. **4**(1): p. e4176.
340. Wang, J., et al., *Bacterial colonization dampens influenza-mediated acute lung injury via induction of M2 alveolar macrophages.* Nature communications, 2013. **4**: p. 2106.
341. Drake, M.G., et al., *Toll-Like Receptor–2/6 and Toll-Like Receptor–9 Agonists Suppress Viral Replication but Not Airway Hyperreactivity in Guinea Pigs.* American journal of respiratory cell and molecular biology, 2013. **48**(6): p. 790-796.
342. Ichinohe, T., et al., *Microbiota regulates immune defense against respiratory tract influenza A virus infection.* Proc Natl Acad Sci U S A, 2011. **108**(13): p. 5354-9.
343. Mortaz, E., et al., *Probiotics in the management of lung diseases.* Mediators of inflammation, 2013. **2013**.
344. Tang, Y., et al., *Increased numbers of NK cells, NKT-like cells, and NK inhibitory receptors in peripheral blood of patients with chronic obstructive pulmonary disease.* Clinical and Developmental Immunology, 2013. **2013**.
345. Culley, F.J., *Natural killer cells in infection and inflammation of the lung.* Immunology, 2009. **128**(2): p. 151-163.
346. Prieto, A., et al., *Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (inmunoferon).* American journal of respiratory and critical care medicine, 2001. **163**(7): p. 1578-1583.
347. Cloonan, S.M., et al., *The "iron"-y of iron overload and iron deficiency in chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2017. **196**(9): p. 1103-1112.
348. Choby, J.E. and E.P. Skaar, *Heme synthesis and acquisition in bacterial pathogens.* Journal of molecular biology, 2016. **428**(17): p. 3408-3428.
349. Sgheiza, V., et al., *Covalent bonding of heme to protein prevents heme capture by nontypeable Haemophilus influenzae.* FEBS open bio, 2017. **7**(11): p. 1778-1783.
350. Szelestey, B.R., et al., *Haemophilus responses to nutritional immunity: epigenetic and morphological contribution to biofilm architecture, invasion, persistence and disease severity.* PLoS pathogens, 2013. **9**(10): p. e1003709.

351. Ali, M.K., et al., *Role of iron in the pathogenesis of respiratory disease.* The international journal of biochemistry & cell biology, 2017. **88**: p. 181-195.
352. Ghio, A.J., et al., *Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect.* American journal of respiratory and critical care medicine, 2008. **178**(11): p. 1130-1138.
353. Ito, Y., et al., *Influenza induces IL-8 and GM-CSF secretion by human alveolar epithelial cells through HGF/c-Met and TGF-α/EGFR signaling.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2015. **308**(11): p. L1178-L1188.
354. Wang, W., et al., *Inhibition of influenza a virus infection by Fucoidan targeting viral neuraminidase and cellular EGFR pathway.* Scientific reports, 2017. **7**: p. 40760.
355. Liu, K., et al., *Epidermal growth factor receptor signaling to Erk1/2 and STATs control the intensity of the epithelial inflammatory responses to rhinovirus infection.* Journal of Biological Chemistry, 2008. **283**(15): p. 9977-9985.
356. Khiroya, H. and A.M. Turner, *The role of iron in pulmonary pathology.* Multidisciplinary respiratory medicine, 2015. **10**(1): p. 34.
357. Sohn, Y.-S., et al., *Redistribution of accumulated cell iron: a modality of chelation with therapeutic implications.* Blood, 2008. **111**(3): p. 1690-1699.
358. Filosa, A., et al., *Long-term treatment with deferiprone enhances left ventricular ejection function when compared to deferoxamine in patients with thalassemia major.* Blood Cells, Molecules, and Diseases, 2013. **51**(2): p. 85-88.
359. Liu, Z.D. and R.C. Hider, *Design of iron chelators with therapeutic application.* Coordination Chemistry Reviews, 2002. **232**(1-2): p. 151-171.
360. White, D.C. and S. Granick, *Hemin biosynthesis in Haemophilus.* Journal of bacteriology, 1963. **85**(4): p. 842-850.
361. Morton, D.J., et al., *Reduced severity of middle ear infection caused by nontypeable Haemophilus influenzae lacking the hemoglobin/hemoglobin–haptoglobin binding proteins (Hgp) in a chinchilla model of otitis media.* Microbial pathogenesis, 2004. **36**(1): p. 25-33.
362. Hariadi, N.I., et al., *Comparative profile of heme acquisition genes in disease-causing and colonizing nontypeable Haemophilus influenzae and Haemophilus haemolyticus.* Journal of clinical microbiology, 2015. **53**(7): p. 2132-2137.
363. Morton, D.J., et al., *The heme-binding protein (HbpA) of Haemophilus influenzae as a virulence determinant.* International Journal of Medical Microbiology, 2009. **299**(7): p. 479-488.
364. Seale, T.W., et al., *Complex role of hemoglobin and hemoglobin-haptoglobin binding proteins in Haemophilus influenzae virulence in the infant rat model of invasive infection.* Infection and immunity, 2006. **74**(11): p. 6213-6225.
365. Ahearn, C.P., M.C. Gallo, and T.F. Murphy, *Insights on persistent airway infection by non-typeable Haemophilus influenzae in chronic obstructive pulmonary disease.* Pathogens and Disease, 2017. **75**(4).
366. Stites, S.W., et al., *Increased concentrations of iron and isoferritins in the lower respiratory tract of patients with stable cystic fibrosis.* American journal of respiratory and critical care medicine, 1999. **160**(3): p. 796-801.
367. Murphy, T.F., et al., *Haemophilus haemolyticus: a human respiratory tract commensal to be distinguished from Haemophilus influenzae.* The Journal of infectious diseases, 2007. **195**(1): p. 81-89.
368. Latham, R.D., et al., *An isolate of Haemophilus haemolyticus produces a bacteriocin-like substance that inhibits the growth of nontypeable Haemophilus influenzae.* International Journal of Antimicrobial Agents, 2017. **49**(4): p. 503-506.
369. Barnes, P.J., *Kinases as novel therapeutic targets in asthma and chronic obstructive pulmonary disease.* Pharmacological reviews, 2016. **68**(3): p. 788-815.

370. Gross, N.J. and P.J. Barnes, *New therapies for asthma and chronic obstructive pulmonary disease.* American journal of respiratory and critical care medicine, 2017. **195**(2): p. 159-166.
371. Monick, M.M., et al., *Respiratory syncytial virus infection results in activation of multiple protein kinase C isoforms leading to activation of mitogen-activated protein kinase.* The Journal of Immunology, 2001. **166**(4): p. 2681-2687.
372. Constantinescu, S.N., C.D. Cernescu, and L.M. Popescu, *Effects of protein kinase C inhibitors on viral entry and infectivity.* FEBS letters, 1991. **292**(1-2): p. 31-33.
373. Root, C.N., et al., *Entry of influenza viruses into cells is inhibited by a highly specific protein kinase C inhibitor.* Journal of General Virology, 2000. **81**(11): p. 2697-2705.
374. Sieczkarski, S.B., H.A. Brown, and G.R. Whittaker, *Role of protein kinase C βII in influenza virus entry via late endosomes.* Journal of virology, 2003. **77**(1): p. 460-469.
375. San-Juan-Vergara, H., et al., *Protein kinase C-α activity is required for respiratory syncytial virus fusion to human bronchial epithelial cells.* Journal of virology, 2004. **78**(24): p. 13717-13726.
376. Park, M.S., et al., *Towards the Application of Human Defensins as Antivirals.* Biomolecules & therapeutics, 2018. **26**(3): p. 242.
377. Salvatore, M., et al., *β-defensin inhibits influenza virus replication by cell-mediated mechanism (s).* Journal of Infectious Diseases, 2007. **196**(6): p. 835-843.
378. Kota, S., et al., *Role of human beta defensin-2 during tumor necrosis factor-α/NF-κB mediated innate anti-viral response against human respiratory syncytial virus.* Journal of Biological Chemistry, 2008.
379. Li, W., et al., *Construction of eukaryotic expression vector with mBD1-mBD3 fusion genes and exploring its activity against influenza A virus.* Viruses, 2014. **6**(3): p. 1237-1252.
380. Mallia, P., et al., *Rhinovirus infection induces degradation of antimicrobial peptides and secondary bacterial infection in chronic obstructive pulmonary disease.* American Journal of Respiratory and Critical Care Medicine, 2012. **186**(11): p. 1117-1124.
381. Baines, K.J., et al., *Airway β-defensin-1 protein is elevated in COPD and severe asthma.* Mediators of inflammation, 2015. **2015**.
382. Andresen, E., et al., *Increased expression of beta-defensin 1 (DEFB1) in chronic obstructive pulmonary disease.* PLoS One, 2011. **6**(7): p. e21898.
383. Pace, E., et al., *Beta defensin-2 is reduced in central but not in distal airways of smoker COPD patients.* PLoS One, 2012. **7**(3): p. e33601.
384. Hiemstra, P., S. Van Wetering, and J. Stolk, *Neutrophil serine proteinases and defensins in chronic obstructive pulmonary disease: effects on pulmonary epithelium.* European Respiratory Journal, 1998. **12**(5): p. 1200-1208.
385. Machado, L.R. and B. Ottolini, *An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses.* Frontiers in immunology, 2015. **6**: p. 115.
386. Suarez-Carmona, M., et al., *Defensins:"Simple" antimicrobial peptides or broad-spectrum molecules?* Cytokine & growth factor reviews, 2015. **26**(3): p. 361-370.
387. Rapista, A., et al., *Human defensins 5 and 6 enhance HIV-1 infectivity through promoting HIV attachment.* Retrovirology, 2011. **8**(1): p. 45.
388. Abba, Y., et al., *Antiviral activity of resveratrol against human and animal viruses.* Advances in virology, 2015. **2015**.
389. Slater, S.J., et al., *Inhibition of protein kinase C by resveratrol.* Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2003. **1637**(1): p. 59-69.
390. Dauber, B. and T. Wolff, *Activation of the antiviral kinase PKR and viral countermeasures.* Viruses, 2009. **1**(3): p. 523-544.
391. Bergmann, M., et al., *Influenza virus NS1 protein counteracts PKR-mediated inhibition of replication.* Journal of virology, 2000. **74**(13): p. 6203-6206.

392. Hale, B.G., et al., *The multifunctional NS1 protein of influenza A viruses.* Journal of general virology, 2008. **89**(10): p. 2359-2376.
393. Min, J.-Y., et al., *A site on the influenza A virus NS1 protein mediates both inhibition of PKR activation and temporal regulation of viral RNA synthesis.* Virology, 2007. **363**(1): p. 236-243.
394. Dauber, B., J. Schneider, and T. Wolff, *Double-stranded RNA binding of influenza B virus nonstructural NS1 protein inhibits protein kinase R but is not essential to antagonize production of alpha/beta interferon.* Journal of virology, 2006. **80**(23): p. 11667-11677.
395. Li, S., et al., *Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA.* Virology, 2006. **349**(1): p. 13-21.
396. Zhu, Q., et al., *A naturally occurring deletion in its NS gene contributes to the attenuation of an H5N1 swine influenza virus in chickens.* Journal of virology, 2008. **82**(1): p. 220-228.
397. Steel, J., et al., *Live attenuated influenza viruses containing NS1 truncations as vaccine candidates against H5N1 highly pathogenic avian influenza.* Journal of virology, 2009. **83**(4): p. 1742-1753.
398. Bergmann, M., et al., *A genetically engineered influenza A virus with ras-dependent oncolytic properties.* Cancer research, 2001. **61**(22): p. 8188-8193.
399. Twu, K.Y., et al., *The CPSF30 binding site on the NS1A protein of influenza A virus is a potential antiviral target.* Journal of virology, 2006. **80**(8): p. 3957-3965.
400. Das, K., et al., *Structural basis for suppression of a host antiviral response by influenza A virus.* Proceedings of the National Academy of Sciences, 2008. **105**(35): p. 13093-13098.
401. Basu, D., et al., *Novel influenza virus NS1 antagonists block replication and restore innate immune function.* Journal of virology, 2009. **83**(4): p. 1881-1891.
402. Kleinpeter, A.B., et al., *Structural analyses reveal the mechanism of inhibition of influenza virus NS1 by two antiviral compounds.* Journal of Biological Chemistry, 2018. **293**(38): p. 14659-14668.
403. Sajjan, U., et al., *Elastase-and LPS-exposed mice display altered responses to rhinovirus infection.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2009. **297**(5): p. L931-L944.
404. Engel, D.A., *The influenza virus NS1 protein as a therapeutic target.* Antiviral research, 2013. **99**(3): p. 409-416.
405. Mallia, P., et al., *Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation.* American journal of respiratory and critical care medicine, 2011. **183**(6): p. 734-742.
406. Melén, K., et al., *Nuclear and nucleolar targeting of influenza A virus NS1 protein: striking differences between different virus subtypes.* Journal of virology, 2007. **81**(11): p. 5995-6006.
407. Kuo, R.-L., et al., *Influenza A virus strains that circulate in humans differ in the ability of their NS1 proteins to block the activation of IRF3 and interferon-β transcription.* Virology, 2010. **408**(2): p. 146-158.
408. Mok, B.W.-Y., et al., *The role of nuclear NS1 protein in highly pathogenic H5N1 influenza viruses.* Microbes and infection, 2017. **19**(12): p. 587-596.
409. Zielecki, F., et al., *Virulence determinants of avian H5N1 influenza A virus in mammalian and avian hosts: role of the C-terminal ESEV motif in the viral NS1 protein.* Journal of virology, 2010. **84**(20): p. 10708-10718.
410. Hsu, A.C.-Y., et al., *Human influenza is more effective than avian influenza at antiviral suppression in airway cells.* American journal of respiratory cell and molecular biology, 2011. **44**(6): p. 906-913.
411. Klinkhammer, J., et al., *IFN-λ prevents influenza virus spread from the upper airways to the lungs and limits virus transmission.* eLife, 2018. **7**: p. e33354.
412. Kugel, D., et al., *Intranasal administration of alpha interferon reduces seasonal influenza A virus morbidity in ferrets.* Journal of virology, 2009. **83**(8): p. 3843-3851.

413. Matzinger, S.R., et al., *Exogenous IFN-alpha administration reduces influenza A virus replication in the lower respiratory tract of rhesus macaques.* PLoS One, 2011. **6**(12): p. e29255.
414. Steel, J., et al., *Transmission of pandemic H1N1 influenza virus and impact of prior exposure to seasonal strains or interferon treatment.* Journal of virology, 2010. **84**(1): p. 21-26.
415. Baines, K.J., et al., *Novel immune genes associated with excessive inflammatory and antiviral responses to rhinovirus in COPD.* Respiratory research, 2013. **14**(1): p. 15.
416. Hayden, F.G., *Advances in antivirals for non-influenza respiratory virus infections.* Influenza and other respiratory viruses, 2013. **7**: p. 36-43.
417. Brass, A.L., et al., *The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus.* Cell, 2009. **139**(7): p. 1243-1254.
418. Feeley, E.M., et al., *IFITM3 inhibits influenza A virus infection by preventing cytosolic entry.* PLoS pathogens, 2011. **7**(10): p. e1002337.
419. Bailey, C.C., et al., *IFITM-family proteins: the cell's first line of antiviral defense.* Annual review of virology, 2014. **1**: p. 261-283.
420. Davidson, S., et al., *Pathogenic potential of interferon αβ in acute influenza infection.* Nature communications, 2014. **5**: p. 3864.
421. Sun, K. and D.W. Metzger, *Inhibition of pulmonary antibacterial defense by interferon-γ during recovery from influenza infection.* Nature medicine, 2008. **14**(5): p. 558.
422. Ehrhardt, C. and S. Ludwig, *A new player in a deadly game: influenza viruses and the PI3K/Akt signalling pathway.* Cellular microbiology, 2009. **11**(6): p. 863-871.
423. Shin, Y.-K., et al., *Effect of the phosphatidylinositol 3-kinase/Akt pathway on influenza A virus propagation.* Journal of General Virology, 2007. **88**(3): p. 942-950.
424. Ehrhardt, C., et al., *Bivalent role of the phosphatidylinositol-3-kinase (PI3K) during influenza virus infection and host cell defence.* Cellular microbiology, 2006. **8**(8): p. 1336-1348.
425. Lu, X., et al., *The PI3K/Akt pathway inhibits influenza A virus-induced Bax-mediated apoptosis by negatively regulating the JNK pathway via ASK1.* Journal of General Virology, 2010. **91**(6): p. 1439-1449.
426. Ehrhardt, C., et al., *Influenza A virus NS1 protein activates the PI3K/Akt pathway to mediate antiapoptotic signaling responses.* Journal of virology, 2007. **81**(7): p. 3058-3067.
427. Shin, Y.-K., et al., *SH3 binding motif 1 in influenza A virus NS1 protein is essential for PI3K/Akt signaling pathway activation.* Journal of virology, 2007. **81**(23): p. 12730-12739.
428. Shin, Y.-K., et al., *Influenza A virus NS1 protein activates the phosphatidylinositol 3-kinase (PI3K)/Akt pathway by direct interaction with the p85 subunit of PI3K.* Journal of General Virology, 2007. **88**(1): p. 13-18.
429. Hale, B.G., et al., *Influenza A virus NS1 protein binds p85β and activates phosphatidylinositol-3-kinase signaling.* Proceedings of the National Academy of Sciences, 2006. **103**(38): p. 14194-14199.
430. Lee, J., J.H. Park, and J.-Y. Min, *A naturally truncated NS1 protein of influenza A virus impairs its interferon-antagonizing activity and thereby confers attenuation in vitro.* Archives of virology, 2017. **162**(1): p. 13-21.
431. Renda, T., et al., *Increased activation of p38 MAPK in COPD.* European Respiratory Journal, 2008. **31**(1): p. 62-69.
432. Yanagisawa, S., et al., *Decreased phosphatase PTEN amplifies PI3K signaling and enhances proinflammatory cytokine release in COPD.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2017. **313**(2): p. L230-L239.
433. Nobs, S.P., et al., *PI3Kγ is critical for dendritic cell-mediated CD8+ T cell priming and viral clearance during influenza virus infection.* PLOS pathogens, 2016. **12**(3): p. e1005508.
434. Wardle, E.N., *Guide to signal pathways in immune cells*. 2009: Springer Science & Business Media.

435. Burgel, P. and J. Nadel, *Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium.* Thorax, 2004. **59**(11): p. 992-996.
436. Bergin, D.A., et al., *Activation of the epidermal growth factor receptor (EGFR) by a novel metalloprotease pathway.* Journal of biological chemistry, 2008. **283**(46): p. 31736-31744.
437. Zhang, J. and C. Bai, *The Significance of Serum Interleukin-8 in Acute Exacerbations of Chronic Obstructive Pulmonary Disease.* Tanaffos, 2018. **17**(1): p. 13.
438. Zhang, J. and C. Bai, *Elevated serum IL-8: a biomarker indicating exacerbation-prone COPD*. 2017, Eur Respiratory Soc.
439. Ganesan, S., et al., *Aberrantly activated EGFR contributes to enhanced IL-8 expression in COPD airways epithelial cells via regulation of nuclear FoxO3A.* Thorax, 2013. **68**(2): p. 131-141.
440. O'donnell, R., et al., *Expression of ErbB receptors and mucins in the airways of long term current smokers.* Thorax, 2004. **59**(12): p. 1032-1040.
441. Zhang, X., et al., *Increased interleukin (IL)-8 and decreased IL-17 production in chronic obstructive pulmonary disease (COPD) provoked by cigarette smoke.* Cytokine, 2011. **56**(3): p. 717-725.
442. Tyner, J.W., et al., *Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals.* The Journal of clinical investigation, 2006. **116**(2): p. 309-321.
443. Stolarczyk, M. and B.J. Scholte, *The EGFR-ADAM17 Axis in Chronic Obstructive Pulmonary Disease and Cystic Fibrosis Lung Pathology.* Mediators of inflammation, 2018. **2018**.
444. Jiang, J., et al., *Regulation of viral infection-induced airway remodeling cytokine production by the TLR3-EGFR signaling pathway in human bronchial epithelial cells.* COPD: Journal of Chronic Obstructive Pulmonary Disease, 2016. **13**(6): p. 750-755.
445. Ueki, I.F., et al., *Respiratory virus–induced EGFR activation suppresses IRF1-dependent interferon λ and antiviral defense in airway epithelium.* Journal of Experimental Medicine, 2013. **210**(10): p. 1929-1936.
446. N'Guessan, P.D., et al., *The Moraxella catarrhalis-induced pro-inflammatory immune response is enhanced by the activation of the epidermal growth factor receptor in human pulmonary epithelial cells.* Biochemical and biophysical research communications, 2014. **450**(2): p. 1038-1044.
447. Kawaguchi, Y., et al., *Targeting EGFR and HER-2 with cetuximab-and trastuzumab-mediated immunotherapy in oesophageal squamous cell carcinoma.* British journal of cancer, 2007. **97**(4): p. 494.
448. Larbouret, C., et al., *Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts.* Annals of Oncology, 2009. **21**(1): p. 98-103.
449. Kario, E., et al., *Suppressors of cytokine signaling 4 and 5 regulate epidermal growth factor receptor signaling.* Journal of Biological Chemistry, 2005. **280**(8): p. 7038-7048.
450. Negishi, H., et al., *Supplementation of Elderly Japanese Men and Women with Fucoidan from Seaweed Increases Immune Responses to Seasonal Influenza Vaccination, 2.* The Journal of nutrition, 2013. **143**(11): p. 1794-1798.
451. Lee, N.Y., et al., *Inhibitory effects of fucoidan on activation of epidermal growth factor receptor and cell transformation in JB6 Cl41 cells.* Food and chemical Toxicology, 2008. **46**(5): p. 1793-1800.
452. Halpin, D.M.G., et al., *Single-inhaler triple therapy in symptomatic COPD patients: FULFIL subgroup analyses.* ERJ Open Research, 2018. **4**(2): p. 00119-2017.
453. Singh, D., et al., *Single inhaler triple therapy versus inhaled corticosteroid plus long-acting β2-agonist therapy for chronic obstructive pulmonary disease (TRILOGY): a double-blind, parallel group, randomised controlled trial.* The Lancet, 2016. **388**(10048): p. 963-973.

454. Papi, A., et al., *Extrafine inhaled triple therapy versus dual bronchodilator therapy in chronic obstructive pulmonary disease (TRIBUTE): a double-blind, parallel group, randomised controlled trial.* The Lancet, 2018. **391**(10125): p. 1076-1084.
455. Wedzicha, J.A., et al., *Indacaterol–Glycopyrronium versus Salmeterol–Fluticasone for COPD.* New England Journal of Medicine, 2016. **374**(23): p. 2222-2234.
456. Anzueto, A.R., et al., *The effect of indacaterol/glycopyrronium versus tiotropium or salmeterol/fluticasone on the prevention of clinically important deterioration in COPD.* International Journal of Chronic Obstructive Pulmonary Disease, 2017. **12**: p. 1325-1337.
457. Kardos, P. and I. Hagedorn-Peinz, *The impact of indacaterol/glycopyrronium fixed-dose combination versus tiotropium monotherapy on lung function and treatment preference: a randomized crossover study – the FAVOR study.* International Journal of Chronic Obstructive Pulmonary Disease, 2018. **13**: p. 69-77.
458. Martinez, F.J., et al., *Effect of Roflumilast and Inhaled Corticosteroid/Long-Acting beta2-Agonist on Chronic Obstructive Pulmonary Disease Exacerbations (RE(2)SPOND). A Randomized Clinical Trial.* Am J Respir Crit Care Med, 2016. **194**(5): p. 559-67.
459. Martinez, F.J., et al., *Articles: Effect of roflumilast on exacerbations in patients with severe chronic obstructive pulmonary disease uncontrolled by combination therapy (REACT): a multicentre randomised controlled trial.* The Lancet, 2015. **385**: p. 857-866.
460. Rabe, K.F., et al., *Anti-inflammatory effects of roflumilast in chronic obstructive pulmonary disease (ROBERT): a 16-week, randomised, placebo-controlled trial.* Lancet Respir Med, 2018.
461. Balaguer, C., et al., *Effects of simvastatin in chronic obstructive pulmonary disease: Results of a pilot, randomized, placebo-controlled clinical trial.* Contemporary Clinical Trials Communications, 2016. **2**: p. 91-96.