Recent trends of NFκB decoy oligodeoxynucleotide-based nanotherapeutics in lung diseases

Meenu Mehta, Keshav Raj Paudel, Shakti Dhar Shukla, Venkata Sita Rama Raju Allam, Vinod Kumar Kannaujiya, Nisha Panth, Amlan Das, Vipan Kumar Parihar, Amlan Chakraborty, Md Khadem Ali, Niraj Kumar Jha, Dikaia Xenaki, Quian Peter Su, Peter Richard Wich, Jon Adams, Philip Michael Hansbro, Dinesh Kumar Chellappan, Brian Gregory George Oliver, Kamal Dua

PII: S0168-3659(21)00416-8

DOI: https://doi.org/10.1016/j.jconrel.2021.08.010

Reference: COREL 11062

To appear in: *Journal of Controlled Release*

Received date: 6 May 2021

Revised date: 4 August 2021

Accepted date: 5 August 2021

Please cite this article as: M. Mehta, K.R. Paudel, S.D. Shukla, et al., Recent trends of NFκB decoy oligodeoxynucleotide-based nanotherapeutics in lung diseases, *Journal of Controlled Release* (2021), https://doi.org/10.1016/j.jconrel.2021.08.010

Recent trends of NFκB decoy oligodeoxynucleotide-based nanotherapeutics in lung diseases

Meenu Mehta[1,2,3*], Keshav Raj Paudel[2,4*], Shakti Dhar Shukla[1,3], Venkata Sita Rama Raju Allam[5], Vinod Kumar Kannaujiya[6], Nisha Panth[2], Amlan Das[7], Vipan Kumar Parihar[8], Amlan Chakraborty[9], Md Khadem Ali[10,11], Niraj Kumar Jha[12], Dikaia Xenaki[13], Quian Peter Su[14], Peter Richard Wich[6,15], Jon Adams[3], Philip Michael Hansbro[2,4], Dinesh Kumar Chellappan[16], Brian Gregory George Oliver[4,13#], Kamal Dua[1,2,3#]

[1]Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, NSW 2007, Australia

[2]Centre for Inflammation, Centenary Institute, Sydney, NSW 2050, Australia

[3]Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, Australia.

[4]School of Life Sciences, University of Technology Sydney, Ultimo, NSW 2007, Australia

[5]Department of Medical Biochemistry and Microbiology, Uppsala University Sweden 75237

[6]School of Chemical Engineering, University of New South Wales, Sydney, NSW, 2052, Australia

[7]National Institute of Biomedical Genomics, Kalyani, Nadia-741235, WB, India

[8]Department of Radiation Oncology, NASA Specialized Center of Research, University of California, Irvine 92697, USA

[9]Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Melbourne, VIC 3800, Australia.

[10]Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Stanford University, Stanford, CA, USA

[11]Vera Moulton Wall Center for Pulmonary Vascular Disease, Stanford University, Stanford, CA, USA

[12]Department of Biotechnology, School of Engineering & Technology (SET), Sharda University, Greater Noida 201310, India

[13]Woolcock Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia

[14]School of Biomedical Engineering, Faculty of Engineering and Information Technology, University of Technology Sydney, Sydney, NSW 2007, Australia

[15]Australian Centre for NanoMedicine and Centre for Advanced Macromolecular Design, University of New South Wales, Sydney, NSW 2052, Australia

[16]Department of Life Sciences, School of Pharmacy, International Medical University, Bukit Jalil 57000, Kuala Lumpur, Malaysia

*Authors sharing first authorship in equal contribution

 # Corresponding authors:

1. Dr Kamal Dua, Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Ultimo NSW 2007, Australia, E-mail: Kamal.Dua@uts.edu.au

2. Dr Brian G Oliver, School of Life Sciences, University of Technology Sydney, Ultimo, NSW 2007, Australia, E-mail: Brian.Oliver@uts.edu.au

Abstract

Nuclear factor κB (NFκB) is a unique protein complex that plays a major role in lung inflammation and respiratory dysfunction. The NFκB signaling pathway, therefore becomes an avenue for the development of potential pharmacological interventions, especially in situations where chronic inflammation is often constitutively active and plays a key role in the pathogenesis and progression of the disease. NFκB decoy oligodeoxynucleotides (ODNs) are double-stranded and carry NFκB binding sequences. They prevent the formation of NFκB-mediated inflammatory cytokines and thus have been employed in the treatment of a variety of chronic inflammatory diseases. However, the systemic administration of naked decoy ODNs restricts their therapeutic effectiveness because of their poor pharmacokinetic profile, instability, degradation by cellular enzymes and their low cellular uptake. Both structural modification and nanotechnology have shown promising results in enhancing the pharmacokinetic profiles of potent therapeutic substances and have also shown great potential in the treatment of respiratory diseases such as asthma, chronic obstructive pulmonary disease and cystic fibrosis. In this review, we examine the contribution of NFκB activation in respiratory diseases and recent advancements in the therapeutic use of decoy ODNs. In addition, we also highlight the limitations and challenges in use of decoy ODNs as therapeutic molecules, cellular uptake of decoy ODNs, and the current need for novel delivery systems to provide efficient delivery of decoy ODNs. Furthermore, this review provides a common platform for discussion on the existence of decoy ODNs, as well as outlining perspectives on the latest generation of delivery systems that encapsulate decoy ODNs and target NFκB in respiratory diseases.

Keywords: Nuclear factor kappa B, Decoy oligodeoxynucleotide, Respiratory disorder, Novel delivery systems

1. Introduction

There are several pulmonary diseases including asthma, Chronic Obstructive Pulmonary Disease (COPD), idiopathic pulmonary fibrosis, acute lung injury/acute respiratory distress syndrome, lung infection and lung cancer [1]. Altogether, these diseases account for significant morbidity and mortality worldwide and impose tremendous economic and healthcare burden. The pulmonary microenvironment is nonsterile as it is constantly bombarded with a number of environmental antigens and allergens such as dust, soot, pollen and dander from animals. Yet, an effective immune system can mount an immune response to neutralize and remove the antigens that are potentially harmful to the lungs [2]. Generally, when pathogens/allergens enter into the respiratory tract, specialized antigen presenting cells (APCs) are recruited and a complicated cascade of intercellular communication occurs between APCs (e.g alveolar and interstitial macrophages, and dendritic cells) and effector T lymphocytes [3]. Activated dendritic cells can release a range of proinflammatory mediators, including IL-6 and TNF-α and then move to lymphoid organs to stimulate T cells and generate adaptive immunity [4-6].

The majority of respiratory diseases are characterised by chronic inflammation in the airways which occurs in response to toxicants (such as cigarette smoke, pollutants, carcinogens or chemicals), pathogens, allergens or tissue hypoxia (Figure 1). For example, ovalbumin is used to model allergic inflamation in the lungs of mices which results in the recruitment of eosinophils [7] while, tobacco cigarette smoke recruits macrophages and neutrophils in the lungs [8]. However, the ovalbumin-induced inflammation has a chronic inflammatory effect that worsens with severe airway remodelling that recruits pro-fibrotic macrophages characterised as M2 alveolar macrophages ($CD206^{+}F4/80^{+}$) [9]. These immune cells produce enormous quantities of pro-inflammatory cytokines, chemokines, enzymes, and proteins to facilitate the progression of airway inflammation [10].

Lipopolysaccharide (LPS), a potent endotoxin in gram-negative bacteria, may activate immune cells (such as dendritic cells and alveolar macrophages) during the course of respiratory tract infections via a special type of receptor known as toll-like receptors (TLRs) [11]. Inflammation can also be induced by hypoxia, for example acute hypoxia in rats with endotoxemia results in alveolar macrophages releasing TNF-, IL-1, IL-6, and TLR-4 [12]. The critical role of macrophages in controlling lung inflammation is exemplified by macrophage depletion in mice exposed to cigarette smoke. This results in reduced

inflammatory cell infiltration, ameliorated body weight loss and decreased inflammatory cytokines (IL-1β, IL-6, TNF-α) and the chemokine, monocyte chemoattractant protein-1 in bronchoalveolar lavage fluid (BALF) and reduced matrix metalloproteinase-3 protein expression in lung tissue [11].

Likewise, recruitment of neutrophils in lungs is governed by various mediators such as IL-8, IL-1β, TNF-α, and leukotriene B4 [12] while activation of lung neutrophils leads to overproduction of human neutrophil elastase (HNE) and myeloperoxidase (MPO) [13]. HNE and MPO are well-known mediators of bronchial inflammation that facilitate structural changes in the lungs such as, peribronchiolar fibrosis (associated with airway remodelling by the build-up of myofibroblasts) and alveolar destruction (emphysema) (Figure 1) [14-16]. Eosinophils are another type of immune cell that plays a role in the pathogenesis of airway inflammation. When activated, eosinophils produce proinflammatory cytokines, reactive oxygen species (ROS), arachidonic acid-derived mediators, complement proteins, matrix metalloproteases (MMPs), and cationic proteins, which cause cytotoxicity, irregular vascular permeability, and smooth muscle cell contraction. Eosinophils also release IL-4 and IL-13, amplify the T-helper-2 response, and in turn are recruited and activated by IL-4, IL-5, and IL-13 (Figure 1) [17-19]. While immune cells such as alveolar macrophages, neutrophils, and eosinophils play a significant role in airway inflammation, the role of bronchial epithelial cells on lung inflammation cannot be neglected. Broncho epithelial cells may undergo oxidative stress during bacterial infection and may release ROS and nitric oxide. Furthermore, they undergo apoptosis in response to a lipopolysaccharide (LPS) challenge [20] as well as an increased expression of oxidative stress genes (*NOX-4*, *NOX2B*) and decreased expression of antioxidant genes (*Nqo1*, *Gclc*) (Figure 1) [21].

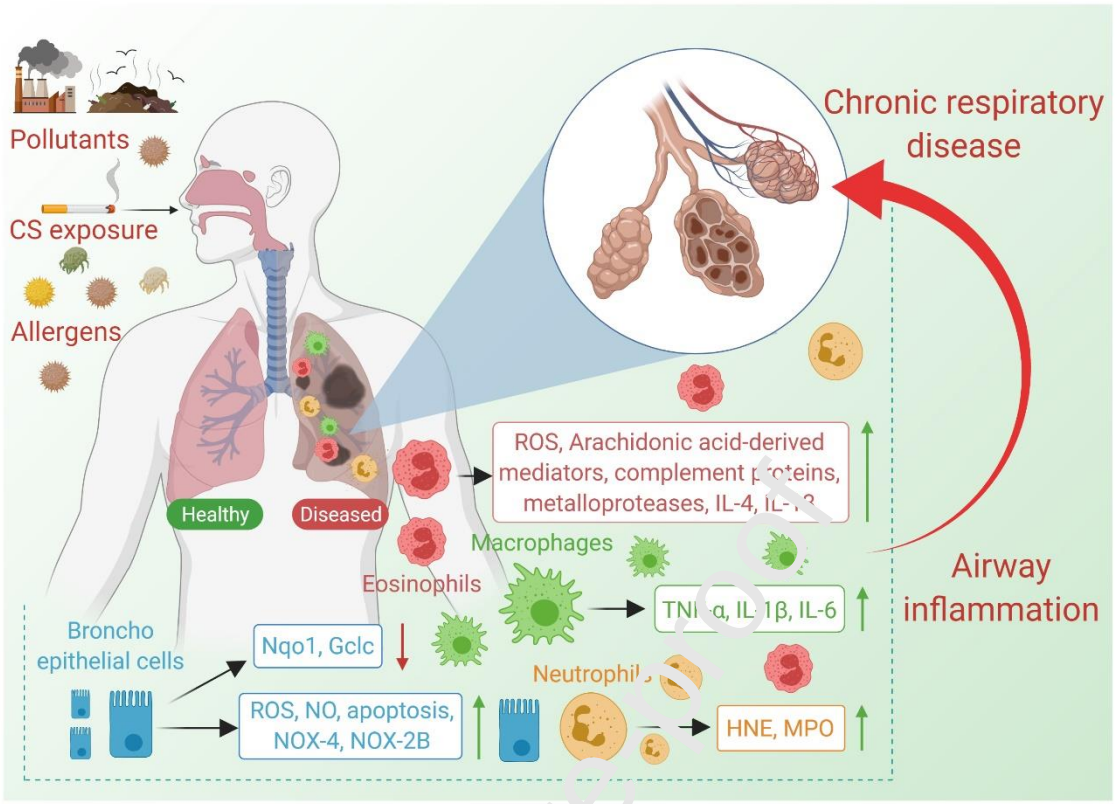

Figure 1: Involvement of various cells in the progression of chronic respiratory diseases

Toxic insults such as air/environmental pollution, cigarette smoke exposure and allergens stimulate/activate various cells to release several endogenous chemokines and cytokines that facilitate the progression of chronic respiratory diseases. Circulating immune cells infiltrate into the lungs in response to these toxic insults which are responsible for airway inflammation mediated by the release of inflammatory mediators. Stimulation of eosinophils results in the release of ROS, arachidonic acid-derived mediators, complement proteins, metalloproteinases, IL-4, and IL-13. Macrophage activation releases TNF-α, IL-1β, and IL-6. Neutrophil activation releases the enzymes, HNE and MPO. Exposure of broncho-epithelial cells to bacterial endotoxin releases ROS and NO; induces apoptosis and upregulates *NOX-4* and *NOX-2B* genes while downregulating *Nqo1* and *Gclc*. Altogether, alteration in the levels of these cytokines, chemokines, enzymes, and expressions of genes further lead to airway inflammation followed by progression to chronic respiratory disease. CS; cigarette smoke, ROS; reactive oxygen species, IL; interleukin, TNF-α; tumour necrosis factor-alpha, HNE: human neutrophil elastase, MPO; myeloperoxidase, NO; nitric oxide, *Nqo1*; NAD(P)H dehydrogenase (quinone) 1, *Gclc*; Glutamate-cysteine ligase catalytic subunit, *NOX-4*; NADPH oxidase 4, *NOX-2B*; NADPH oxidase-2B.

There are many cell signaling pathways involved in airway inflammation include, for example TLR signaling pathways which respond to pathogens, or cytokine signaling pathways which are activated in response to increased inflammation [22, 23] or oxidative stress pathways activated by tobacco cigarette smoke [24]. The common feature of all of these pathways is that gene transcription occurs in response to NFκB activation and

translocation to the nucleus. For example, after LPS recognition by TLR-4, a TLR4/MD-2 complex is formed which iniates the adapter protein MyD88 signaling. MyD88 activates a series of proteins, ultimately leading to the activation of the transcription factor NFκB. This leads to the production of inflammatory mediators such as interleukin IL-6, IL-1β, TNF-α and ROS resulting in lung injury, including pathological features of emphysema [3].

NFκB activation plays a critical role in all lung cells, ie both inflammatory and structural cells. The activation of NFκB in airway epithelial cells has been shown to be sufficient to enhance neutrophilic airway infiltration demonstrating that these cells are capable of triggering gene expression to activate an inflammatory signaling cascade [25]. A growing body of evidence suggests that NFκB activation is a critical signal for inflammatory response in a variety of pulmonary diseases [29-31]. As a result, there is a growing interest in developing newer advanced anti-inflammatory drugs capable of interfering with the NFκB activation pathway. NFκB decoy oligonucleotides (ODNs) are used to specifically block NFκB activation and have a similar sequence to the NFκB DNA binding site [32]. NFκB decoy ODNs have been shown to decrease the expression of inflammatory cytokines and to inhibit gene transactivation *in vitro* [26, 27] and *in vivo* [28, 29]. As potential therapeutics, these are relatively new in the respiratory domain and most of these are still in the early stages of development.

2. Biology of NFκB transcription factor and its role in the pathogenesis of respiratory diseases

Nuclear factor κB (NFκB), often referred to as the master regulator of inflammatory processes in the lungs (and other organs), plays a central role in the pathogenesis, progression, and severity of chronic respiratory diseases [30]. NFκB was first described more than 30 years ago as a transcription protein that binds to the immunoglobulin heavy chain and kappa light chain enhancers [31], and is now known to be a complex family of related protein complexes effecting as either homo- or hetero-dimers, all derived from a pool of five monomeric proteins (RelA, c-Rel, RelB, p50, and p52) [32]. NFκB is now implicated in the regulation of hundreds of genes involved in a variety of functions but not limited to, modulation of genes and regulators involved in chronic inflammation, cancer, and pathology. The specific and yet expanding processes modulated by NFκB include immune cell reception, expression of chemokines and cytokines, cell surface adhesion molecules, cell proliferation, transformation, apoptosis, angiogenesis, oxidative stress, cellular invasion, and

metastases [33]. Thus, understanding the biological implications of NFκB-modulated inflammatory and cellular responses in chronic respiratory diseases carry immense value in exploring novel, more effective therapeutic targets for treating these currently non-treatable chronic diseases.

Under a homeostatic environment, NFκB is rendered non-functional due to bondage with its natural inhibitor, I-kappa-B-alpha (IkBα) [34]. However, upon stimulation, the activation of NFκB could take place through two distinct pathways (Figure 2) [35]. The classical pathway involves TNF-α/IL-1/TLR/T-cell or B-cell binding receptor-induced phosphorylation of IκKB subunit of the IKK complex which in turn results in the ubiquitination and phosphorylation of two N-terminal serine residues of IκBα proteins [36]. The IKK complex is phosphorylated (at two serines in amino-terminus) Ikα and Ikβ, which are complexed with the IKKγ subunit, which is an NFκB essential modulator [NEMO]), a regulatory subunit of the NFκB complex. This is followed by the phosphorylation of IκB by the activated IKK complex primarily through IKKβ. This leads to polyubiquitination of lysine 48 (K48)-linked at adjacent lysine residues which is initiated by the ubiquitin E3 ligase complex Skp1/Cul1/F-box protein-β-TrCp. This subsequently results in the proteolysis of NFκB-bound IκB at the 26S proteasome. Free NFκB dimers (most commonly the p50/p65 heterodimer) then translocate to the nucleus, where they bind NFκB DNA sites and activate gene transcription (Figure 2) [37].

Alternative activation of NFκB involves the activation of NFκB–inducing kinase (NIK), which results in the processing of NFκB2 from the full-length precursor protein, p100 to the activated p52 monomer. This process subsequently leads to the formation of activated p52:RelB complexes. The latter complex is transcriptionally active and results in the regulation of inflammation, especially by modulating the adaptive immune system (Figure 2) [38].

One of the hallmark features of chronic, non-communicable respiratory diseases is the persistent and aberrant inflammation in the lungs that could become systemic if the disease state is prolonged [39, 40]. It is now widely accepted that NFκB indeed plays a central role in the modulation of inflammation in chronic respiratory diseases (CRDs), such as asthma, COPD and CF [41]. Thus, targeting the components of the NFκB signaling pathway could be a putative therapeutic approach in the management of patients with these currently non-treatable diseases (Figure 2).

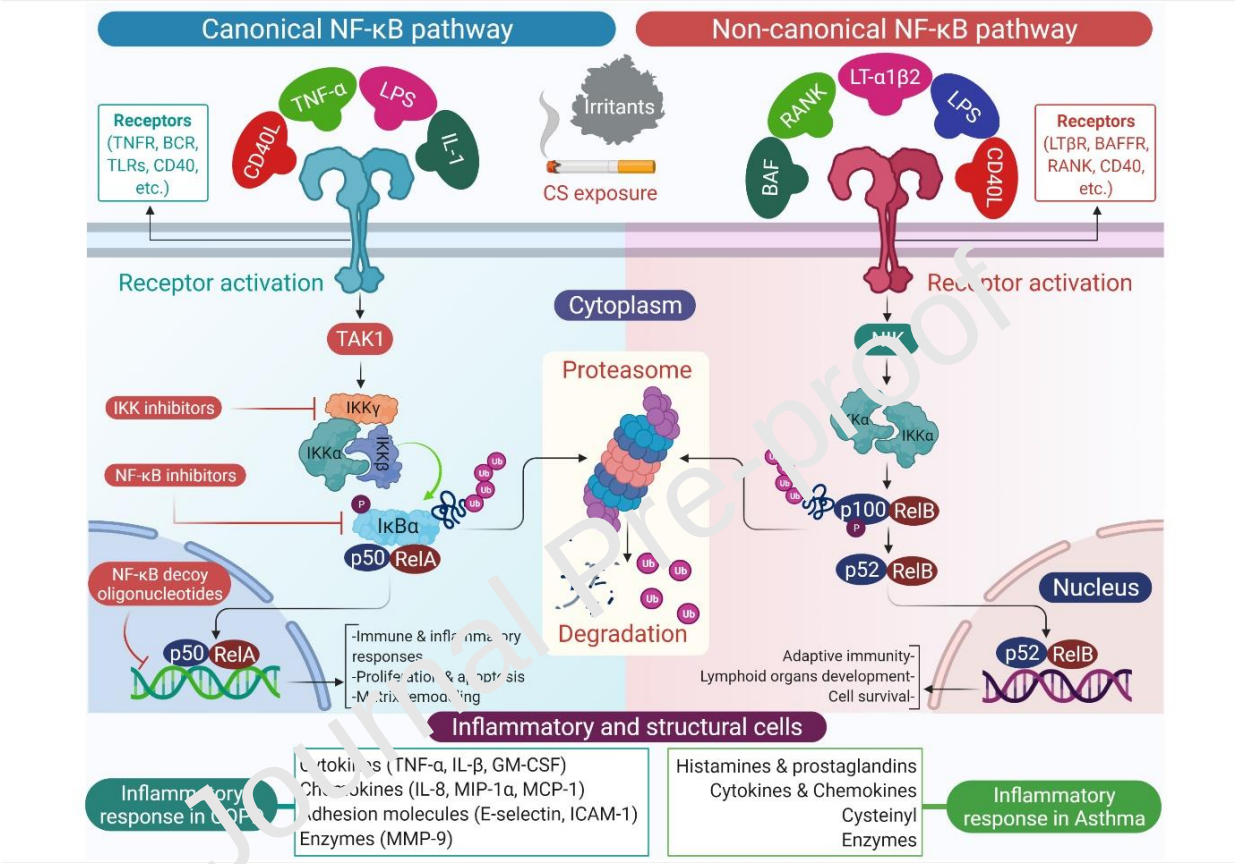

Figure 2: NFκB activation by classical (canonical) and alternate (non-canonical) pathways. Several receptors mediate the activation of the canonical pathway, including tumour necrosis factor receptor (TNFR), Toll-like receptors (TLRs), CD-40L or B-cell receptors (BCRs) when triggered by various stimuli, such as cigarette smoke, pollutants, or bacterial lipopolysaccharides (LPS). This pathway involves a number of NFκB components that act in tandem to activate the upregulation of inflammatory and other functional genes in structural and immune cells. Similarly, various receptors, including lymphotoxin-β receptor (LTβR) and the B-cell activating factor receptor (BAFFR) are triggered thereby activating a series of NFκB components that subsequently result in heightened inflammation and pathological features that are observed in patients with chronic respiratory diseases. The NFκB-mediated inflammation and pathology could be targeted by specific inhibitors at various stages of activation cascade (indicated by red block arrows).

2.1. The role of NFκB in asthma

The role of NFκB and its activation in asthma has been consistently studied over two decades. Hart *et al*., extracted the nuclear protein from bronchial biopsies and subsequently isolated the cells from induced sputum, where they reported overexpression of NFκB in patients with asthma when compared with healthy volunteers [42]. In addition, Gagliardo *et al*., found that peripheral eosinophils obtained from moderate and severe asthma patients showed significantly higher expression of granulocyte-macrophage colony-stimulating factor, interleukin-8 (both are regulated by NFκB), suggesting that the persistent inflammation especially in severe asthma may be the result of persistent NFκB activation [43]. An exploratory study assessing peripheral blood samples from patients with allergic asthma reported that NFκB was indeed associated with reduced eosinophil apoptosis [44]. This could result in aberrant inflammation in asthma which could result in worsening of symptoms and hospitalisations. Ather *et al*., employed a transgenic murine model of allergic asthma and showed the significant role that NFκB played in modifying the immune response which resembled allergic asthma, including airway hyperresponsiveness to methacholine, increased eosinophil number, mucus hypersecretion as well as higher levels of antigen-specific IgE and IgG1 in serum [45]. Another study conducted in a murine model of asthma demonstrated that the inhibition of NFκB by pyrrolidine dithiocarbamate (*via* intraperitoneal injection) had reduced the airway constriction as measured by the Penh value, airway smooth muscle (ASM) area and collagen deposition, as well as a reduction in systemic inflammation [46]. Collectively, it may be concluded that therapeutic targeting of NFκB activation or the downstream signaling would benefit patients with asthma.

2.2. The role of NFκB in COPD

The expression of p65 protein, one of the major constituents of NFκB, has been found to be upregulated in smokers and patients with COPD, particularly in bronchial epithelia [47]. Moreover, the protein levels of p65 are found to be significantly and positively correlated with the airflow limitation, thus, asserting a potential key role of NFκB in the pathogenesis of COPD [47]. In addition, the levels of p50 and p65, the crucial subunits of activated NFκB signaling pathway, are usually increased in COPD and are associated with a significant reduction in sputum neutrophil apoptosis in COPD when compared to healthy controls [48]. This could lead to the dysregulated and persistent inflammation generally observed in COPD patients. Another study which assessed the gene expression of NFκB constituents in the

whole blood of patients reported an overexpression of NFκB family genes, as well as higher levels of inflammatory molecules (IL-1β, IL-8 and COX-2) in patients with COPD compared to subjects in the healthy group [49]. This underlines that regulation of inflammation probably occurs at a systemic level in smoking-related COPD. However, one study showed that cigarette smoke-induced inflammation may be independent of the NFκB pathway [50]. This could be attributed to the cigarette-smoke related induction of cellular or tissue hypoxia, which in turn affects NFκB activation [51]. In an experimental murine model of cigarette smoke-induced COPD, Yu *et al*., assessed the effects of orally administered (twice daily, 1 hour prior to cigarette smoke exposure) isoliquiritigenin (a flavonoid derived from the root of liquorice), and found that isoliquiritigenin significantly down-regulated the expression of the NFκB signaling pathway constituents induced by cigarette smoke [52]. Similarly, in a mouse model of cigarette smoke extract induced emphysema, the protein arginine methyltransferase 6 suppressed NFκB activation and inflammation, as well as improved the lung morphometry [53]. Notably, the persistently activated state of NFκB in smoking-related COPD could lead to the development of lung cancer by providing a pro-tumourigenic microenvironment through regulation of alternatively activated macrophages and/or through regulatory T-cells [54]. Taken together, the exact role and utility of NFκB as a therapeutic target in smoking-related COPD should be further investigated in both experimental *in vitro* cell line models and experimental *in vivo* models for in depth details and clarity.

2.3. The role of NFκB in cystic fibrosis (CF)

In individuals with CF, the mutation in cystic fibrosis transmembrane conductance regulator (CFTR) genes results in persistent stimulation of NFκB, thereby maintaining chronic inflammation and further aggravating lung pathology [33]. Utilising transfected cell lines, Weber *et al*., reported that mutant DeltaF508 CFTR resulted in a multi-fold increase in the activation of NFκB, which in turn, had increased the expression of pro-inflammatory IL-8 [55]. IL-8 is a chemoattractant and results in airway neutrophilia, thus, further aggravating the disease pathology/symptoms [56]. Another seminal study investigated the interaction of *Pseudomonas aeruginosa* (a major bacterial pathogen in CF) and various respiratory epithelial cells. The findings suggested that bacterial pili act as primary stimulants for the activation of NFκB and subsequent expression of IL-8 [57]. This seems logical as the bacteria in the airways persistently release lipopolysaccharides which then interact with TLRs, thereby activating NFκB, subsequently leading to a perpetual cycle of increased but dysregulated inflammation, infection, and lung damage [58].

2.4. The role of NFκB in pulmonary arterial hypertension (PAH)

Pulmonary arterial hypertension (PAH) is a progressive and life-threatening disease resulting from the restricted flow of blood through the pulmonary arterial circulation. During PAH, the blood vessels in the lungs are narrowed, blocked, or even destroyed, resulting in restricted blood flow through the lungs, which causes increased blood pressure in the lung arteries. The increased circulatory resistance results in an overload in the right ventricle (RV) and may lead to hyperplasia, hypertrophy, and fibrosis [59, 60]. These conditions may eventually lead to heart failure and death in PAH patients [59, 60]. Hemodynamically, PAH is characterized by an increased pulmonary artery capillary wedge pressure of ≤ 15 mmHg, mean pulmonary arterial pressure > 20 mmHg, and pulmonary vascular resistance of ≥ 3 Wood Units [61]. Pathophysiologically, the key manifestations of PAH include pulmonary vascular remodelling (neointima formation, medial hypertrophy/hyperplasia, plexiform lesions), endothelial dysfunction, vasoconstriction, metabolic dysfunction, perivascular inflammation, and adventitial and intimal fibrosis [62]. Several reports indicate the role of NFκB in the pathogenesis of PAH [63-66]. PAH is generally characterized by an excessive proliferation of vascular cells leading to pulmonary vascular remodelling. Inflammatory responses are known to play an important role in the pathophysiology of this disease [67]. The transcription factor of NFκB is known to regulate a wide array of inflammatory cytokines including TNF-α, IL-1β, IL-6, and cyclooxygenase-2 to name a few, which may act as a stimulus for vascular remodelling [38]. NFκB has been known to be activated in the monocrotaline (MCT)-induced pulmonary hypertension. Inhibition with either club (clara) cell-10 promoter driving IκBα mutant plasmid or nanoparticle-mediated delivery of nuclear factor NFκB decoy has resulted in the significant amelioration of the PAH features [65, 68]. In a recently published report, Liu *et al*., have shown that mesenchymal stem cell therapy may ameliorate hypoxia-induced PAH by activating P53 and NFκB signaling *via* TNF-α secretion [66]. HIF-1α acts as a key regulator of oxygen homeostasis and hypoxia in the lung. Aberrant HIF-1α activation was reported in PAH leading to the proliferation of pulmonary arterial smooth muscle cells (PASMCs) and vascular remodelling [69]. Furthermore, HIF1α heterozygous mice with inactivated HIF1α or PASMCs depleted for HIF1α, exhibited reduced pulmonary vascular remodelling and PAH under hypoxic condition [70, 71]. Interestingly, BelAiba *et al*., showed that the transcription factor, NFκB binds directly to the promoter region of HIF-1α resulting in its transcriptional activation in hypoxia-exposed PASMCs [72]. Thus, it could be

concluded that NFκB may regulate several signaling cascades such as cytokine induction or activation of HIF-1α in the pathogenesis of PAH.

3. Decoy Oligonucleotides

Oligonucleotides are characterized as polynucleic acid chains which depending upon their application and source consist of different functional groups [73]. Oligonucleotide therapy comprises antisense, RNAi (siRNA and miRNA), immunomodulatory, aptamers and decoy approaches [74]. In 1978, Zamecnik and Stephenson first suggested the use of DNA antisense oligonucleotide (AON), which modulated the expression of a target gene by binding to mRNA and inhibiting translation [75]. From this initial concept, a variety of RNA-targeting techniques in mammalian cells have emerged, including siRNA [76] and miRNA [77]. Apart from targeting RNA, another technique through which oligonucleotides can be employed as therapeutics is via protein binding. Oligonucleotides with nonmethylated cytosine phosphate Guanosine (CpG) motifs (CpG DNA) bind to toll-like receptor 9 (TLR9), which causes activation of intracellular signaling, leading to an immunological response [78, 79]. Aptamers are short DNA or RNA oligonucleotides or peptides that acquire a particular and persistent 3D structure *in vivo*, providing a precise tight binding to secreted protein targets to limit its activity [80].

Decoy ODNs are recently discovered promising therapeutic agents that are double stranded synthetic oligonucleotide molecules. They contain a specific binding sequence which can attach to a specific transcription factor and thereby alter their activity, leading to variations in gene expression [81]. They have shown exceptional efficacy in the management of various pathological conditions. The reason behind their potent pharmacological activity is their ability to modulate the expressions of various genes in such diseases [82].

Gene therapy based on the applications of ODNs provides an alternative to existing therapeutic approaches for the prevention/treatment of various pathological conditions namely, renal diseases [83], cancers [84, 85], cystic fibrosis (CF) [86], asthma and COPD [87]. Recent advances in the fields of molecular and cellular biology have made it possible to transfer target genes of interest into somatic cells. The technology that involves transferring genes of interest through several viral vectors has progressed significantly in recent decades. In addition, the advances in molecular biology have also established accurate methods to inhibit target gene expression. The transfer of cis-element double-stranded ODNs (= decoy) is now considered as a promising tool for gene therapy [83].

The administration of decoy ODNs offers several advantages over gene transfer strategies and traditional therapeutic approaches. For example, the administration of decoy ODN's overcomes technical problems linked with procuring and altering transfected genetic construct's expression. Moreover, for an efficient decoy therapy, ascertainment of the consensus sequences recognized by transcription factors within the promoter region of the gene alone is sufficient. Once the sequences are established, it is possible to investigate the efficacy of these ODNs in a short period of time (a couple of weeks), compared to the longer period (years) which is required for the development of small lead therapeutic candidates or anti-sense agents [82, 88]. At present, small interfering RNAs and short hairpin RNAs are the widely applied constructs for gene therapy, and they are effective in downregulating the activity of transcription factors [89]. However, decoy ODNs have their own constraints. For example Griesenbach *et al.,* have shown that cytoplasmic deposition of NFκB decoy oligonucleotides is insufficient to inhibit bleomycin-induced pulmonary inflammation [90]. This becomes apparent when the decoy ODNs are involved in the modulation of extensive cellular restrictions that result in adverse effects/toxicities. Moreover, these limitations are alike with non-selective drugs used in the treatment of chronic inflammatory diseases. Therefore, the most effective method to solve such limitations is to specifically target selective transcription factors that are comparatively specific in regulating cellular signaling pathway, for example, STAT3 in cancer and NFκB in inflammation [91, 92].

In addition, the use of drug delivery systems targeting tissues has recently become possible by the expeditiously growing field of nanotechnology and thus could be a potential solution to overcome the associated limitations. Another limitation of ODNs is their decreased uptake by cells due to their negative charges and larger sizes [93]. Therefore, *in vivo* administration of these ODNs is another potential challenge to overcome before considering them for clinical use.

In comparison to nude plasmid DNA, ODNs are smaller in size. However, ODNs cannot boast of the benefits provided by viral delivery systems, both for cellular uptake and for cytoplasmic to nuclear translocation to exert their biological activities. Although few of these ODNs are internalized by cells through endocytosis, the majority of them may degrade due to lysosomal activity [94]. Considering their positive aspects, various other approaches have been established to improve the uptake of ODNs and to bypass the endosome-lysosome degradation pathway [94, 95]. Among these approaches, cationic liposomes [96], covalent linkers [97], lipid-viral particle complexes [98], and non-covalent carriers have shown

promising results [94]. Additionally, the revolution of advanced nano-drug delivery methods has introduced multiple advantages in solving these limitations. In our review, we will particularly highlight NFκB decoy ODNs targeting various respiratory diseases/airway inflammation (Table 1). Researchers have reported the successful use of NFκB decoy ODNs to suppress expression of inflammatory cytokines such as IL-6 & IL-8 in bronchial cells [99, 100]. Luhrmann and his team investigated the effectiveness of decoy ODNs against STAT transcription factors and reported remarkable reduction of eosinophils and T lymphocytes in the BALF. Moreover, they had also reported reduction in the CD4+ and CD8+ lymphocyte number in the lung tissue along with reduced CD40 protein expression in the lung tissue [101]. Yu *et al.,* reported that intratracheal administration of NFκB ODNs decreased macrophage infiltration in airways. Furthermore, macrophage inflammatory protein 1-α and monocyte chemoattractant protein-1 (MCP-1) were also inhibited in lung homogenates. In contrast, there was a drastic increase in TNF-α and pro-MMP-9 levels in BALF of mice administered with NFκB ODNs [102].

A novel therapeutic approach was reported in another study which prevented the development of septic lung failure by transfecting decoy NFκB ODNs, thereby inhibiting gene expression of the major molecules necessary to enhance pulmonary permeability [103]. Matsuda *et al.,* explored the possibility of preventing acute lung damage by introducing synthetic double stranded oligodeoxynucleotides with the suppression of pulmonary expression of multiple genes in the cecal ligation and puncture septic mouse model. Their findings have revealed notable reduction of sepsis-induced gene overexpression involving iNOS, COX-2, platelet-activating factor receptor, histamine H1-receptor, and bradykinin B1 and B2 receptors in lung tissues [104]. Decoy NFκB ODNs reduce NFκB reverse the suppression of serum IL-10 and IL-13 in the early stage severe lung injury in experimental rabbits [105]. In addition, NFκB ODNs transfection have shown efficacy in inhibiting lung injury during allograft rejection [106].

Table 1: Decoy ODNs in respiratory diseases

Decoy ODNs	Experimental model	Findings	Reference
NFκB	LPS induced rat model of airway inflammation	Prevented infiltration of neutrophils and inhibited the expression of NFκB related genes	[107]
	LPS-stimulated airway epithelial cells	Inhibited IL-8 and MUC2 expressions	[108]
	Bleomycin-induced mice model of pulmonary inflammation	No changes in IL-6 secretion in BALF	[90]
	Mice model of acute lung injury induced by cecal ligation and puncture-induced sepsis	Notably reduced the sepsis-induced gene overexpression involving iNOS, COX-2, platelet-activating factor receptor, histamine H_1-receptor, and bradykinin B_1 and B_2 receptors in lung tissues	
	Rabbits with severe lung contusion	Decreased the expression of NFκB, IL-10 and IL-13	[105]
	Lung transplantation carried in pairs of Brown Norway and Lewis rats	In the donor's lung, the decoy ODNs transfection inhibited the activation of NFκB in the allograft and resulted in the amelioration of lung injury during allograft rejection.	[106]
	Chronic cigarette smoke-exposed mice model of lung inflammation	Intratracheal administration of NFκB ODNs decreased macrophage infiltration in airways, inhibited macrophage inflammatory protein 1-α and MCP-1 in lung homogenates In contrast, there was a drastic increase in TNF-α and pro-MMP-9 levels in BALF of mice administered with NFκB ODNs	
	Mice model of septic lungs induced by intravenous injection of 10 mg/kg *Escherichia coli* endotoxin	NFκB ODNs induced decreased levels of plasma histamine, whereas, in the lung tissue ODNs inhibited gene and protein expressions of histidine decarboxylase, histamine H (1) receptors, and iNOS	[103]
	LPS-stimulated cystic fibrosis bronchial cells	NFκB Decoy ODNs inhibited the expression of IL-6 and IL-8	[99]
	IB3-1 (cystic fibrosis bronchial cells)	NFκB Decoy ODNs inhibited transcription of IL-8 in cells	[100]
STAT-1 and STAT-3	Ovalbumin induced Brown Norway rat model of allergic asthma	Single-dose administration of the STAT decoy ODNs resulted in a remarkable reduction of eosinophils and T lymphocytes in the BALF, CD4+ and CD8+ lymphocytes number in the lung tissue and CD40 protein expression in the lung tissue	[101]

LPS: lipopolysaccharide, BALF: broncho alveolar lavage fluid, MS ODNs: microsatellite DNA mimicking oligodeoxynucleotides, MUC: mucin, STAT: Signal transducer and activator of transcription, iNOS: inducible nitric oxide synthase, COX-2: cyclooxygenase-2, MCP-1: monocyte chemoattractant protein-1

4. Limitations and challenges of using decoy ODNs as therapeutic molecules

Although, decoy ODNs therapy is a versatile therapeutic tool for researchers when compared to other gene therapy strategies, certain challenges limit its therapeutic efficiency. The limitations are majorly attributed to both pharmacokinetic and pharmacodynamic properties. One of the key pharmacological limitations which hampers the therapeutic use of decoy ODNs is the targeting of multi-functional transcription factor which is involved in the transcription of various genes that may further intricate various cellular homeostatic activities [109, 110]. Thus, altering the function of the transcription factor using ODNs therapy may result in severe toxic effects [88].

The involvement of various transcription factors like NFκB, activator protein (AP)-1, CCAAT/enhancer-binding protein (C/EBP), and cAMP response element-binding protein (CREB) that are involved in the transcription of the IL-6 gene for the production of IL-6, that activates a single gene has also proved to be a limitation for decoy ODN therapy [111, 112]. Another major challenge in implementing the use of decoy ODNs clinically is its delivery to local and specific tissues/organs, as most of the decoy ODNs are delivered via the systemic route and it is difficult to construct the sequence of the decoy ODNs that are tissue-specific to enhance the biological effect [113].

Poor pharmacokinetic properties of naked decoy ODNs are considered as major hurdles for their use in clinical settings, as decoy ODNs are highly susceptible to intracellular nucleases, both exonuclease and endonuclease enzymes that exist in the biological fluids. These nucleases involve in the rapid degradation of ODNs to further reduce their stability and half-life. Another problem that persists with naked decoy ODNs is their degradation by endocytosis, a cellular uptake mechanism involved in the transfer of these molecules into the cells. The ingested active decoy ODNs *via* endocytosis into the cells are sequestered in the lysosomes where they undergo rapid degradation in the presence of lysosomal enzymes which make them less available to reach the nucleus to produce their effects (Figure 3) [88, 114]. Furthermore, the large molecular size, hydrophilic nature with high negative charges are other barriers of using the decoy ODNs that limit their cellular uptake and reduce their therapeutic efficacy [88, 114].

5. Current need of novel delivery systems for efficient delivery of decoy ODNs

Various strategies have been explored to enhance the cellular uptake of decoy ODNs and to prevent their degradation by various cellular enzymes. Different structural modifications in the native decoy ODNs have been implemented to increase the resistance of ODNs against degradation by various nucleases and to enhance their stability and bioavailability. One such structural modification includes the replacement of the phosphodiester backbone with a phosphorothioate group in the native decoy ODNs. This may enhance their stability by preventing its degradation by different intracellular and extracellular nucleases [115, 116]. However, the major concerns associated with the use of phosphorothioate-substituted ODNs are non-specificity and immune activation which may further induce toxicity and side effects *in vivo* [113]. Similarly, structural modifications of decoy ODNs with the addition of peptide nucleic acid, a non-charged achiral oligonucleotide mimic has also been investigated [117] to enhance the stability against various proteases and DNAases [118]. However, these structural modifications have exhibited poor binding efficacy to the DNA binding proteins [119]. The addition of the locked nucleic acids (LNAs) to the naïve decoy ODNs is another strategy to prevent their degradation as LNAs contain a methylene bridge connecting the 2′-oxygen with the 4′-carbon of the ribose ring that enhances thermal stability and increases resistance against the exonuclease of the ODNs [114, 119]. However, the reduced affinity of NF-κB for its target sequence has been observed when the nucleotides are been replaced with the LNAs [120]. Another approach contributing towards structural modification is the use of dumbbell-shaped or ribbon-shaped or hairpin shaped decoy ODNs as these possess both thermal stability as well as exonuclease resistance (Figure 3) [115].

Although, structural modifications of the decoy ODNs have shown promising results in increasing the stability and bioavailability, the concerns with respect to non-specificity and reduced affinity against the target sequences further render them unsuccessful to be implemented as a therapeutic strategy for the treatment of various diseases. Therefore, to advance the therapeutic efficacy of the decoy ODNs, alternative or additional structural modifications are highly needed. Combining novel drug delivery systems with structural modifications could be an efficient strategy to deliver the decoy ODNs effectively with improved cellular uptake and increased stability [82].

Various novel drug delivery approaches have been currently used to envelope these decoy ODNs. Based on their diversity, these are further characterized into viral and non-viral delivery systems [115]. Viral delivery systems, for instance, decoy ODNs encapsulated in plasmids or adenovirus vectors are commonly used as carriers in gene therapy. However, limitations like immunogenicity, poor selectivity, poor efficacy and intestinal mutagenesis have further led to the emergence of newer approaches to develop various non-viral drug delivery systems [121]. Non-viral delivery systems include microspheres [122], microbubbles [123], cationic liposomes [124, 125], nanospheres and nanoparticles [126] which provide an effective delivery platform for transferring the decoy ODNs into the cells and tissues.

Cellular uptake mechanisms of decoy ODNs

Endocytosis

Naked ODNs enter into the cell via endocytosis which results in the formation of early endosomes. These endosomes fused with lysosomes to become late endosomes. The naked ODNs mostly degrade in the presence of lysosomal enzymes that cause the release of reduced concentrations of ODNs into the cytoplasm. The cytoplasmic naked ODNs further undergo rapid degradation in the presence of 3'exonucleases which prevents the entry of ODNs into the nucleus. Structurally modified ODNs enter into the cell *via* endocytosis as similar to naked ODNs. However, they become resistant to the exo- and endo-nucleases which further enter the nucleus and inhibit the transcription of different inflammatory genes.

Membrane fusion

The majority of the decoy ODNs carriers have cellular uptake *via* membrane fusion [127] and/or receptor-mediated endocytosis [128]. Membrane fusion uptake of the carriers primarily comprises of the nanocarrier with the inactivated Sendai virus containing two fusogenic proteins on its viral coat or the recombinant fusogenic proteins. They can facilitate the internalization of the targeted decoy ODNs *via* enabling the fusion process through the cell membrane which bypasses endocytosis and endo-lysosomal degradation (Figure 3) [127, 129].

Receptor mediated endocytosis

Receptor-mediated endocytosis is the most common cellular uptake mechanistic pathway mediated by various decoy ODNs delivering carriers. Internalization of the decoy ODNs *via* endocytosis occurs through different endocytic pathways including clathrin, caveolin or

dynamin mediated pathways [130]. After internalization *via* endocytosis, the carriers loaded with the decoy ODNs immediately escape from the endosomes to prevent them from degradation. The first mechanism is *via* membrane destabilization through direct contact of the lipid layers of the carriers with the endosomal membrane (Figure 3) [131]. The alternate mechanism is through proton sponge, for example, cationic polymer-based carrier systems such as, polyethyleneimine or poly(amidoamine). These promote the swelling of the endosome *via* increasing their osmotic pressure during the acidification process which eventually results in the release of these carries into the cytoplasm (Figure 3) [88, 121]. Photochemical internalization is another endosomal escape pathway in which carriers are loaded with the photosensitizers that are activated in the presence of light to generate the reactive oxygen species. The generated ROS disrupt the endosomal membrane to release the decoy ODNs loaded carriers into the cytosol (Figure 3) [132] and inhibits the transcription process of various inflammatory components by binding to the transcription protein.

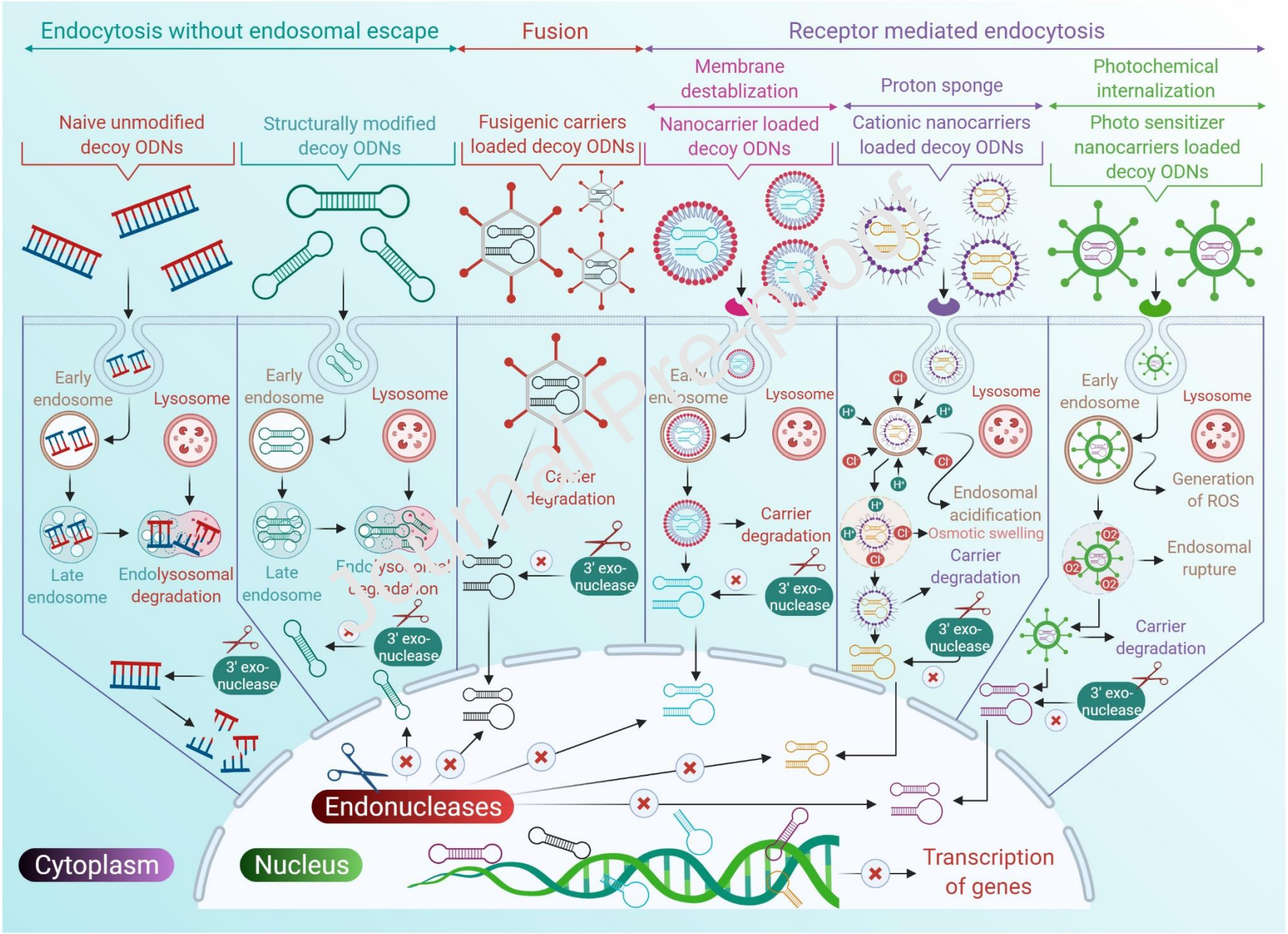
Endocytosis without endosomal escape
Fusion
Receptor mediated endocytosis
Naive unmodified decoy ODNs
Structurally modified decoy ODNs
Fusigenic carriers loaded decoy ODNs
Membrane destablization
Nanocarrier loaded decoy ODNs
Proton sponge
Cationic nanocarriers loaded decoy ODNs
Photochemical internalization
Photo sensitizer nanocarriers loaded decoy ODNs
Early endosome
Lysosome
Late endosome
Endolysosomal degradation
3' exo-nuclease
Carrier degradation
Endosomal acidification
Osmotic swelling
Generation of ROS
Endosomal rupture
Endonucleases
Cytoplasm
Nucleus
Transcription of genes

Figure 3: Mechanisms involved in the cellular uptake of the naked ODNs, structural modified ODNs and carrier loaded ODNs.
Naked ODNs enter into the cell via endocytosis which results in the formation of the early endosomes. These endosomes undergo various steps and are finally fused with the lysosomes to become late endosomes. The naked ODNs mostly degrade in the presence of lysosomal enzymes that result in the release of reduced concentrations of ODNs into the cytoplasm. The cytoplasmic naked ODNs further undergo rapid degradation in the presence of the 3'exonucleases and prevent the entry of the ODNs into the nucleus. Structurally modified ODNs enter into the cell *via* endocytosis as similar to naked ODNs. However, they become resistant to the exo- and endo-nucleases which further enter the nucleus and inhibit the transcription of different inflammatory genes. Fusigenic carriers loaded with the ODNs directly enter into the cells *via* a fusion process by bypassing endocytosis and thus release the structurally modified ODNs into the cytoplasm and nucleus which are again resistant to the exo- and endo-nucleases to inhibit the transcription of inflammatory genes. Nanocarriers loaded with the ODNs enter the cell *via* receptor-mediated endocytosis through the formation of the endosomes around the carriers. However, due to the physical and chemical characteristics of the nanocarriers, they undergo endosomal escape *via* different mechanisms which involve chemical destabilization, proton sponge and photochemical internalization which inhibits the formation of the late endosomes. This helps in preventing the degradation of the ODNs which are further released into the cytoplasm and then enter the nucleus to be involved in the inhibition of the gene transcription.

6. Novel carriers used for NFκB decoy ODNs

Rapid advancements in the field of nanomedicine have provided new insights to improve the effectiveness of inhalation therapy in pulmonary diseases [133]. Generally, integrating nanotechnology with the development of drug delivery systems enables the effective delivery of the active drug to the target tissue and thus prevents any adverse effects on normal tissues and cells [134, 135]. These systems can encapsulate hydrophobic drugs while protecting them from degradation, leading to improved clinical outcomes with lower drug concentrations [136]. A variety of nanocarriers, both natural and synthetic, have recently been developed for successful decoy ODNs delivery, such as polymeric nanoparticles, lipid-based nanoparticles, and liposomes to name a few. Several of them have shown impressive outcomes both *in vivo* and *in vitro* models (Table 2) [68, 99, 131].

6.1 Novel carriers used for NFκB decoy ODNs in different inflammatory diseases

Poly (D,L-lactide-co-glycolide) (PLGA) microspheres have been shown to prolong encapsulated drug release and improve the stability of ODNs in biological systems [137]. On the other hand, it has been proven that chitosan based nanoparticles are rapidly absorbed by cells. As a result, these two polymers or their mixtures have therefore been widely employed to deliver different ODNs decoys [138]. Wardwell *et al.*, used *in-vitro* models to assess the anti-inflammatory efficacy of NFκB decoy ODNs coated chitosan-based nanoparticles in rheumatoid arthritis. These nanoparticles showed enhanced cellular uptake and significantly reduced the expression of pro-inflammatory cytokines such as, IL-6 and IL-8 [126].

Hattor *et al*., and their colleagues developed folate linked lipid nanoparticles for the delivery of NFκB decoy ODNs in murine macrophages for the selective targeting of NFκB pathway. As the RAW264.7 macrophages have LPS-induced expression of folic acid receptors, incorporating folic acid into nanoparticles is a good effort to improve cell selectivity of nanoparticles. Findings revealed selective cellular uptake of NFκB decoy ODNs by murine macrophages and they suppressed inflammatory processes in rheumatoid arthritis at lower doses [139]. Another study showed the effect of NFκB pathway inactivation particularly in Kupffer cells using gelatin nanoparticles. These nanoparticles have shown selective uptake by Kupffer cells and suppression of NFκB pathway improved survival and effective reduction in liver injury [140]. Sugar-containing cationic liposomes were also used to deliver NFκB decoys [141] to splenic macrophages and Kupffer cells. The primary goal of these targeted

therapies was to suppress immunological responses while also decreasing adeno-virus-induced hepatotoxicity [142].

Buchnan and his team developed unique liposomes named as echogenic liposomes for the delivery of NFκB decoy ODNs, which encapsulated gas and drug together and are capable of releasing payload using ultrasounds. Three important advantages were achieved by applying this strategy. Firstly, the liposomal packaging substantially protected the ODNs against degradation. Secondly, while using ultrasound, drug cargos can be released conveniently and in a desired way. Thirdly, the ultrasonic gas bubble cavitation dramatically enhances the transfer of genes and medicines into the walls of the arteries [143].

Table 2: NFκB decoy ODN-loaded novel drug delivery systems in different inflammatory diseases

S. No.	Nanocarrier used	Target disease	Outcomes of the study	Reference
1.	Poly(D,L-lactide-co-glycolide) (PLGA) microspheres	Chronic inflammation	• Prolonged the release profile and increased the cellular uptake • Inhibited the activation of NFκB at a dose 80 times lower than naked DNA • Increased the stability in biological fluids	[137]
2.	Chitosan modified PLGA nanospheres	Inflammatory bowel disease	• Enhanced the oral bioavailability • Improved the mucoadhesiveness • Enhanced the cellular uptake • Protected NFκB ODNs against acidic medium	[138]
3.	Chitosan modified nanoparticles	Rheumatoid arthritis	• Significantly reduced the expression of proinflammatory cytokines such as, IL-6 & IL-8 • Enhanced the cellular uptake • Easily evaded reticuloendothelial system and showed decreased immunogenicity	
4.	Echogenic liposomes	Cardiovascular disorders	• Liposomal bilayer protected the ODNs from degradation • Drug cargo release was controlled through ultrasound exposure such as higher release of ODNs from the bolus of high amplitude pulses, while low amplitudes were applied for sustained release	[143]
5.	Folate linked lipid-based nanoparticles	Rheumatoid arthritis	• Showed selective cellular uptake of NFκB decoy ODN by murine macrophages • Suppressed inflammatory processes in rheumatoid arthritis at lower doses	
6.	Dendrimers	Hepatitis	• Showed higher stability • Significantly reduced serum concentration of inflammation-related protein and cytokines	[144]
7.	Gelatin nanoparticles	Fulminant hepatitis and ischaemia–	• Caused selective uptake by Kupffer cells and suppressed NFκB pathway	[140]

		reperfusion	• Improved survival and effective reduction in liver injury	
8.	Sugar modified cationic liposomes	Adenovirus vector-induced innate immune responses	• Reduced the serum levels of interleukin (IL)-12 (main inflammatory cytokine-induced by adenovirus vectors • Reduced the hepatotoxicity	[142]
9.	Cationic liposomes	Liver failure	• Significantly suppressed the TNF-α production	[141]

6.2 Novel carriers used for NFκB decoy ODNs in respiratory diseases

Nanoparticles

Nanoparticles, a form of colloidal drug delivery system, consists of particles ranging from 10-1000nm [145]. The small size of the nanoparticles enables them to act as drug delivery vehicles, rendering them capable to reach any part of the human body [74]. A study by Kimura *et al*., has showed that nanoparticle-mediated delivery of nuclear factor kappa B (NFκB) decoy ODNs prevented NFκB activation, hence reduced inflammation, proliferation and airway remodelling in a rat model of monocrotaline-induced pulmonary hypertension. They demonstrated that these nanoparticles reach the distal regions of the lung and were found in alveolar macrophages and small pulmonary arteries for about 14 days after single instillation. In this study, the authors hypothesised that the cellular absorption of the nanoparticles may result in the slow release of encapsulated NFκB decoy ODNs into the cytoplasm by hydrolysing the polymeric structure of the nanoparticles. This would protect the encapsulated decoys from intracellular degradation before reaching the nuclear target and may optimise the decoy's inhibitory function [68].

Polymeric nanoparticles

Polymeric nanoparticles have received much attention among other types of nanocarriers due to their biodegradability and superior biocompatibility [146]. Moreover, ODNs encapsulated within these nanoparticles could be considered as a powerful approach for enhancing the ODNs long-term release by protecting against *in vivo* degradation after administration [147].

Wardwell *et al*., used an *in vitro* model of cystic fibrosis based on the IB3-1 cell line which has a CFTR gene mutation to demonstrate the anti-inflammatory activity of chitosan modified NFκB decoy ODN nanoparticles. Although, there have been no significant changes in the secretion of pro-inflammatory mediators for cystic fibrosis when treated with naked decoy ODNs and scrambled ODN-coated nanoparticles, NFκB decoy ODN nanoparticles significantly reduced the secretion of IL-6 and IL-8 from epithelial cells, especially when the time period of treatment was increased [148]. Furthermore, large porous PLGA particles have been developed as an inhalable dry powder in order to measure the inhalable efficacy of NFκB decoy ODNs in cystic fibrosis *in vivo* treatment. Results showed that these particles prevented NFκB transcriptions and associated gene expression. Consequently, they reduced chronic inflammation in the lungs of patients with cystic fibrosis [99].

Stefano and co-workers prepared poly (lactic-co-glycolic) acid encapsulated NFκB decoy ODNs with large porous particles to enhance the delivery in pulmonary systems. These porous particles containing decoy ODNs were designed to meet the aerodynamic requirements necessary for pulmonary delivery, including the achievement of efficient loading of decoy ODNs, maintenance of their release and structural integrity in pulmonary fluids. The findings have shown that the broncho alveolar neutrophil infiltration caused by lipopolysaccharides decreased for up to 72 hours by a single intratracheal infiltration of decoy ODN large porous particles, whereas the naked decoy ODNs were only able to inhibit it for 6 hours. The inhibition of neutrophil infiltrate was associated with decreased binding activity of NFκB/DNA and decreased expression of IL-6, IL-8, and mRNA mucin-2 in pulmonary homogenates [107].

Angela *et al*., hypothesised that developed PLGA-based large porous particles with branched poly-(ethylenimine) (PEI) could enhance decoy ODN delivery to the lungs, particularly for the treatment of *Pseudomonas aeruginosa* infections in the lungs [108]. The synergistic effect of free PEI or PEI released from large porous particles showed *in vitro* antimicrobial activity of tobramycin and aztreonam against *Pseudomonas aeruginosa* which was discovered after understanding the role of PEI on the technical properties of PLGA-based particles for delivery of decoy ODNs [108]. Cytotoxicity studies were conducted on A549 cells and findings clearly demonstrated that aerosolization properties of the dry powders were promising and enabled both decoy ODNs and PEI to be released *in vitro* for a longer duration of time. Encapsulation of PEI leads to a two-fold reduction in the minimum inhibitory concentration of aztreonam with a reduction in the cytotoxicity of PEI. The formed particles remained in the lung for about 14 days following intratracheal administration in rats. This provided a long and simultaneous release of decoy ODNs and PEI. PEI was necessary for the development of a decoy ODNs delivery system which could act in the mucoepidermoid lung epithelial cells. In addition, it controlled lung inflammation and mucin production [108]. The study concluded that decoy ODNs may serve as anti-inflammatory agents, where PEI can improve the therapeutic efficacy of inhaled antibiotics that are widely used to treat concurrent respiratory infections [149].

Lipid based drug delivery systems

Lipid-based delivery systems are widely used to deliver various types of proteins, drugs and DNA. Among them liposomes are increasingly being employed to deliver ODNs due to their capability to improve the resistance of ODNs to nucleases, increase the circulation half-life of

ODNs, and therefore improve the absorption and efficiency of ODNs in target cells [143, 150]. The selective alveolar macrophage targeting by the use of nanoparticles modified with sugar is of interest since the mannose receptor on cell surface can identify the mannose and fucose moieties. Wijagkanalan *et al*., evaluated the impact of mannosylated cationic liposomes on the alveolar macrophage-targeted NFκB decoy in a LPS induced lung inflammation model after intratracheal administration. When compared with naked NFκB decoy, the cationic liposome containing NFκB decoy complex was predominantly delivered to alveolar macrophages for its respective migration of NFκB decoy in the cytoplasm. This showed significant suppression of TNF- α, IL-1β, CINC-1 release, neutrophil infiltration and NFκB activation .

De Rosa *et al*., evaluated the potential of a liposomal formulation containing cationic lipid (2,3-didodecyloxypropyl) (2-hydroxyethyl) dimethylammonium bromide (DE) in terms of their delivery of double-stranded anti-NFκB decoys to LPS-stimulated RAW 264.7 macrophages and their potency in suppressing NFκB activation. Cholesterol was used as helper lipids in the liposome formulation. Liposomes were conjugated with ODNs at various charge ratios, either alone or in combination with helpers. DE/cholesterol mixed liposomes complexed with ODNs at charge ratios of 8 showed the highest inhibition rate of NO development, the highest suppression of iNO protein expression, and the lowest binding of NFκB to DNA. Furthermore, when DE/cholesterol liposomes were applied at maximum charge ratios, confocal microscopy showed a high cellular uptake of ODNs .

Another approach for further enhancing the pulmonary delivery of NFκB decoy ODNs after systemic administration was to deliver them *via* a group of nano-vectors formed by mixing protamines, cationic liposomes and DNA under optimised conditions. This study found that NFκB decoy ODNs, especially single-stranded ODNs, significantly increased transgenic expression and inhibited TNF-α induction in cationic vector-mediated systemic gene transmission when co-delivered with a plasmid DNA .

Table 3: NFκB decoy ODN-loaded novel drug delivery systems in respiratory diseases

S. No.	Nanocarrier used	Advantages	Disadvantages	Reference
1.	Nanoparticles	• Sustained release of encapsulated decoy ODNs • Enhanced cellular uptake	-	[68]

		• Increase stability in biological fluids		
2.	PLGA based large porous particles	• Sustained release • Larger size and a porous structure (i.e. low density) were expected to facilitate deposition of drug to the lung in the deep airways	-	[107]
3.	PLGA-based large porous particles with PEI	• Sustain the drug release profile, • Improve localisation in specific areas of the lungs, • Facilitate transport over the mucus layer and improve uptake by epithelial cells	• In case of DNA PLGA has slow release rate • Low encapsulation efficiency	[108, 149]
4.	Cationic liposomes	• Biodegradable, • Biocompatibile, • Low immunogenicity, • Easy to prepare • High ODN cellular uptake	• To achieve transfection, large volumes of cationic lipids must be used, which may have toxic and adverse consequences.	[151]
5.	Mannosylated cationic liposome	• Physically stable • Selectively delivered to alveolar macrophages • Low toxicity	-	[152]
6.	Cationic liposomes	• Improve cellular uptake • Higher transfection efficiency • Lower toxicity • Increased physical stability	• Tendency of cationic lipids complex to aggregate in the presence of serum recommends their application for local delivery only	[124]

7. Clinical studies of oligonucleotides on respiratory diseases

Several clinical trials involving oligonucleotides have been completed successfully to assess their potential in the treatment of respiratory diseases. These trials included preclinical, Phase I, and Phase II trials. These trials used CpG, antisense, decoy, and siRNA-based therapeutics to target various receptors, protein signaling pathways, and receptors involved in the pathophysiology of respiratory diseases such as asthma, COPD, cystic fibrosis, and lung cancer (table 4). Some of these trials found no additional benefits, while others found promising results when compared to the placebo control. A phase I trial (first-in-human study) found AZD1419 [an inhaled toll like receptor (TLR)-9 agonist] to be safe and tolerable in healthy subjects. Target engagement in the lung was achieved at all dose levels tested and the maximum tolerated dose was found to be 15.4 mg suggesting it was suitable for further therapeutic development for the treatment of asthma [153], resulting in a phase IIa clinical trial of AZD1419 in adults with eosinophilic asthma. Thirteen weeks of daily single dose of AZD1419 followed by a 40 weeks observation period showed that AZD1419 induced a T-helper cell type-1 kind of interferon response with a sustained reduction in markers of type 2 inflammation. However, no notable changes were observed in terms asthma control as compared to the placebo treated group. AZD1419 was safe and tolerable even when there was no improvement in asthma control although there was a reduction in type 2 inflammation markers [154]. Another CpG type drugs are CYT003 and QbG10 (a TLR-9 agonist). An interesting approach to immunostimulation uses encapsulated CpG ODN within virus-like particles [155]. This technology uses the virus-like particle Qb, a recombinantly produced empty virus shell to deliver an A-class CpG ODN termed CYT003-QbG10 (Cytos Biotechnology). CpGs packaged into virus-like particles were shown to have increased stability. In contrast to free CpGs, packaged CpGs prevented splenomegaly, normally associated with immunostimulation in mice, but no effect was observed in their immunostimulatory capacity. Early Phase I/II clinical trial results in allergic patients with mild asthma showed that CYT003-QbG10 administered subcutaneously was well tolerated. The findings revealed almost complete tolerance to the allergen with symptoms of rhinitis and allergic asthma significantly reduced [156].

A double-blind phase IIa trail studied the safety and efficacy of subcutaneous CYT003 therapy (0.3, 1, or 2 mg) in 365 subjects with persistent moderate-to-severe asthma. Although CTY003 was well tolerated there was treatment related injection site reaction as an adverse side effect. However, there was no remarkable difference between the CYT003 and placebo groups at week 12 suggesting no additional benefit with CYT003 in patients with asthma

[157]. Bacteriophage Qbeta-derived virus-like particles with CpG-motif G10 inside (QbG10) is another CpG based oligonucleotide and TLR9 agonist tested for its tolerability, safety and efficacy in 63 mild-to-moderate persistent allergic asthma patients in a phase II trial. The QbG10 group (n=33) and placebo group (n=30) received 7 separate injections and clinical parameters such as fraction of exhaled nitric oxide, forced expiratory volume (FEV)-1, and blood eosinophils were examined over a period of 12 weeks. Two thirds of the patients treated with QbG10 has stable asthma e compared with placebo at 12 weeks. FEV1 was significantly worsened in the placebo group, while it was stable in the QbG10 group. Treatment related adverse effects were observed as reactions at injection site post-administration of QbG10 [158]. TPI ASM8 is another antisense oligonucleotide containing two modified phosphorothioate. One targets the common beta chain (βc) of the IL-3/IL-5/GM-CSF receptors while the other targets the chemokine receptor (CCR)-3. An open-label phase II study was performed in 14 stable, allergic mild asthmatic subjects. After 4 days of treatment with TPI ASM8 (4mg twice a day or 8mg once a day) and placebo, subjects underwent allergen challenges. TPI ASM8 was found to be well tolerated and was safe in asthma patients. The findings revealed that TPI ASF8 blocked CCR3 and βc expression and notably reduced airway eosinophilia after allergen challenge [159]. TPI ASM8 also downregulates the allergen-induced increase in beta(c) mRNA and CCR3 mRNA in cells derived from sputum as well as airway responses in patients with mild asthma [160]. A preclinical study suggested that 2'-deoxy-2'-Fluoro-β-D-Arabinonucleic acid (FANA), a phosphodiesterase type IV (PDE4) inhibitor containing antisense oligonucleotide can be a potential therapy for COPD. Treatment of FANA to mice that were exposed to 1 week of cigarette smoke resulted in a remarkable reduction of neutrophil count, KC and pro-MMP-9 enzymatic level in bronchoalveolar lavage fluid**.** Furthermore, the reduction of neutrophil count was found to be correlated with reduction in mRNA expression of PDE4B, PDE4D, and PDE7A [161]. EPI-2010 formulated by Epigenesis Pharmaceuticals is an inhaled antisense oligonucleotide that targeted the mRNA of adenosine A1 receptors. Adenosine regulates tissue function by activating four G-protein Coupled Receptors (GPCRs): A1, A2A, A2B and A3 [162]. In clinical studies, EPI-2010 was shown to be well tolerable in healthy subjects and in mild asthmatic subjects. In mild asthmatics, a single nebulized dose of EPI-2010 (50 μg/kg) resulted in a drastic reduction of need-for-rescue bronchodilator and reduced asthma symptom scores [163]. AIR645 is a second-generation antisense oligonucleotide that targets the common α chain of IL-4 and IL-13 receptors. Administration of AIR645 by nebulization in a murine model of asthma attenuated antigen-induced increased airway

eosinophil and neutrophil, mucus overproduction, airway hyperresponsiveness (AHR), and reduced target protein expression on epithelial cells and pulmonary antigen presenting cells [164]. A phase I trial of AIR645 in 72 healthy subjects and 8 patients with controlled asthma showed that nebulized AIR645 was well tolerated and confirmed the low systemic exposure of AON following delivery to the lung as plasma levels were only detected after the 30 mg dose. AIR645 was calculated to have a half-life in sputum of ~ 5 days independent of dose level, reaffirming the possibility of a once weekly treatment for asthma [165]. ATL1102 is another second-generation antisense oligonucleotide that targets CD49d, a subunit of the adhesion molecule VLA-4 (very late antigen-4). Adhesion molecules are implicated in several facets of asthma, from leukocyte migration, exocytosis, cytokine production and respiratory burst. VLA-4 is involved in the recruitment of eosinophils and T cells [166]. Preclinical studies in an asthma model in mice demonstrated that VLA-4 knock down reduced AHR, eosinophil recruitment, inflammation and mucus production [167].

Antisense oligonucleotides have also been tested in cystic fibrosis (CF) and lung cancer. Eluforsen is designed to restore the function of cystic fibrosis transmembrane conductance regulator (CFTR) protein in the lung epithelium. A phase 1b dose escalation study evaluated the safety, tolerability and efficacy of nebulized eluforsen in CF patients. It was observed that nebulized eluforsen (50 mg, 3 times/week for 4 weeks) was safe, tolebrable and showed improvement in CF questionnaire revised respiratory symptom score [168]. AZD9150 is an antisense oligonucleotide that inhibits the signal transducer and activator of transcription (STAT)-3. AZD9150 reduced STAT3 expression in a wide range of preclinical models to exhibit antitumor potential in lung cancer models. The preclinical antitumor activity of AZD9150 was translated into patients with highly treatment-refractory lymphoma and non-small cell lung cancer (NCSLC) in a phase 1 study [169]. Another antisense oligonucleotide tested against lung cancer was apatorsen which targets the heat shock protein (HSP) 27 mRNA. A phase II trial evaluated the efficacy of carboplatin and pemetrexed combined with apatorsen or placebo in metastatic NSCLC patients. Although, patients showed good tolerability with the combination therapy, it failed to improve clinical outcomes [170]. Lung tumour cells exhibit chemoresistance mediated by the inhibition of tumour cell apoptosis. Survivin is a candidate for antiapoptotic protein that inhibits chemotherapy-induced apoptosis. LY2181308 is an antisense oligonucleotide that inhibits survivin. A phase 2 study of LY2181308 investigated if blocking survivin expression increased docetaxel-mediated apoptosis in NSCLC patients. This was done by comparing the activity of the LY2181308

and docetaxel combination with only docetaxel. Patients (N = 162) at stage IIIB/IV of NSCLC were intravenously administered LY2181308 (750 mg, weekly) and docetaxel (75 mg/m^2, day 1) or docetaxel alone every 3 weeks. However, there was significant improvement in antitumor activity (measured as change in tumour size) between two groups. Since there were no remarkable changes among two groups for progression-free survival (PFS), both groups were combined. Using the combined groups, change in tumour size was correlated with PFS, suggesting its use in early decision-making in phase II studies [171].

AVT-01 (Avontec), is a short double-stranded ODN that targets STAT-1, a transcription factor to control inflammation. In a preclinical model of asthma induced by ovalbumin-sensitization and challenge showed that intra-nasally administered AVT-01 resulted in significant decrease of BALF eosinophil and lymphocyte counts, and IL-5 level as compared to controls [172]. In the AVT-01 treatment group, there was also a decrease in CD40 expression in peri-bronchial infiltrates and in vascular cell adhesion molecule 1 on vascular endothelial cells. Moreover, the progression of AHR after ovalbumin challenge was also reduced by AVT-01. In the clinical trial, AVT-01 was found to be safe and tolerable when administered as single nebulized dose of 3 and 10 mg in healthy subjects. A single dose of AVT-01 administered to asthma patients reduced AHR following AMP challenge [173]. In the Phase IIa follow-up clinical study, AVT-01 was administered once a day for a week and it was found to be safe and tolerable but did not show statistically significant effect on AHR. Excellair™ (ZaBeCor, Bala Cynwyd, PA, USA) is an inhaled siRNA-based oligonucleotide designed to silence spleen tyrosine kinase (Syk). In a preclinical model (using rats), aerosolized Syk antisense was able to significantly suppress Syk expression, inflammation mediator (nitric oxide, TNF-a, IL-1b) release from alveolar macrophages, and pulmonary inflammation which was measured by counting BALF cells [174]. Excellair has received approval to initiate Phase I clinical trials in humans, but its current status is unknown.

Table 4. Clinical studies of oligonucleotide therapeutics on respiratory diseases.

Drug	**Type**	**Target**	**Disease**	**Clinical trial phase**
AZD1419	CpG	TLR9	Asthma	Phase I, Phase II
CYT003	CpG	TLR9	Asthma	Phase IIb
QbG10	CpG	TLR9	Asthma	Phase II

TPI ASM8	Antisense	CCR3 receptor, b-chain of IL-3, IL-5 and GM-CSF receptors	Asthma	Phase II
FANA	Antisense	PDE4D, 4B and 7A	COPD	Preclinical
EPI 2010	Antisense	Adenosine A1R	Asthma	Phase II
AIR645	Antisense	IL-4Rα	Asthma	Phase I
ALT1102	Antisense	VLA-4	Asthma	Preclinical
Eluforsen	Antisense	CFTR	Cystic fibrosis	Phase Ib
AZD9150	Antisense	STAT-3	Lung cancer	Preclinical
Apatorsen	Antisense	HSP 27 mRNA	Lung cancer	Phase II
LY2181308	Antisense	Survivin	Lung cancer	Phase II
AVT-01	Decoy	STAT-1	Asthma	Phase II
Excellair	siRNA	Syk kinase	Asthma	Phase I

CpG: Cytosine-phosphate-guanosine; TLR: Toll-like receptor, COPD: Chronic obstructive pulmonary disease; FANA: 2'-deoxy-2'-Fluoro-β-D-Arabinonucleic Acid CCR3: chemokine receptor 3, IL: interleukin, GM-CSF: granulocyte-macrophage colony-stimulating factor, PDE4D: Phosphodiesterase 4D, VLA-4: Integrin α4β1 (very late antigen-4), CFTR: cystic fibrosis transmembrane conductance regulator, STAT: signal transducer and activator of transcription, HSP: heat shock protein, siRNA: small interfering RNA, Syk: spleen tyrosine kinase.

8. Future prospects and conclusion

A promising breakthrough in ODN-based research was achieved in 1978 with the discovery of 13-mer DNA-based ODNs that could inhibit protein expression *in vitro* [175]. Twenty years later, fomiversen became the first approved ODN-based therapeutic drug followed by the approval of pegaptanib and mipomersen in 2016 [176]. Currently, there is an upward trend in terms of research progress with ODN-based drugs, with 11 ODN-based drugs being approved [177]. There are various "drug-like" potential applications of ODN transcription factors including NFκB decoy ODNs targeting different diseases. Utilising advanced drug delivery systems for the delivery of decoy ODNs nanotherapeutics can entirely change the perspective of therapies targeting respiratory diseases. In this aspect, the strategies to prevent NFκB signal transduction by the application of NFκB decoy ODNs could be a promising tool to combat chronic inflammation more effectively in respiratory diseases. Nanomedicine

offers an exceptional opportunity to improve existing treatments and create new therapeutic solutions for previously deemed difficult or impossible to treat respiratory diseases. Targeted delivery systems are mainly limited to optimizing activity only in specifically targeted cells. On the other hand, the applications of various methods to increase the efficacy and prolongation of the half-life of decoy ODNs seem critical in approaching clinical application. In addition, the lack of *in vivo* toxicity assessments and the selective distribution of these particles in the body appears to be a key question that needs to be addressed in future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors would like to thank Graduate School of Health (GSH), University of Technology Sydney for GSH Seed grant 2021. Meenu Mehta is supported by the International Research Training Program Scholarship (IRTP), Australia. Keshav Raj Paudel is supported by a fellowship from Prevent Cancer Foundation (PCF) and the International Association for the Study of Lung Cancer (IASLC) foundation, USA. Kamal Dua is supported by a project grant from Sydney Partnership for Health, Education, Research and Enterprise (SPHERE) for the TRIPLE I CAG Secondment/Exchange grant.

References

[1] D.K. Chellappan, L.W. Yee, K.Y. Xuan, K. Kunalan, L.C. Rou, L.S. Jean, L.Y. Ying, L.X. Wie, J. Chellian, M. Mehta, S. Satija, S.K. Singh, M. Gulati, H. Dureja, M.W. Da Silva, M.M. Tambuwala, G. Gupta, K.R. Paudel, R. Wadhwa, P.M. Hansbro, K. Dua, Targeting neutrophils using novel drug delivery systems in chronic respiratory diseases, 81 (2020) 419-436.
[2] R.P. Dickson, J.R. Erb-Downward, G.B. Huffnagle, Homeostasis and its disruption in the lung microbiome, Am J Physiol Lung Cell Mol Physiol, 309 (2015) L1047-L1055.
[3] A. Chakraborty, J.C. Boer, C. Selomulya, M. Plebanski, S.G. Royce, Insights into endotoxin-mediated lung inflammation and future treatment strategies, Expert review of respiratory medicine, 12 (2018) 941-955.
[4] T.-m. Kim, K.R. Paudel, D.-W. Kim, Eriobotrya japonica leaf extract attenuates airway inflammation in ovalbumin-induced mice model of asthma, Journal of Ethnopharmacology, 253 (2020) 112082.
[5] L.H. Chin, C.M. Hon, D.K. Chellappan, J. Chellian, T. Madheswaran, F. Zeeshan, R. Awasthi, A.A.A. Aljabali, M.M. Tambuwala, H. Dureja, P. Negi, D.N. Kapoor, R. Goyal, K.R. Paudel, S. Satija, G. Gupta, A. Hsu, P. Wark, M. Mehta, R. Wadhwa, P.M. Hansbro, K. Dua, Molecular mechanisms of action of naringenin in chronic airway diseases, European Journal of Pharmacology, 879 (2020) 173139.
[6] P.C. Cook, A.S. MacDonald, Dendritic cells in lung immunopathology, Semin Immunopathol, 38 (2016) 449-460.
[7] P.S. Foster, S. Maltby, H.F. Rosenberg, H.L. Tay, S.P. Hogan, A.M. Collison, M. Yang, G.E. Kaiko, P.M. Hansbro, R.K. Kumar, J. Mattes, Modeling T(H) 2 responses and airway inflammation to understand fundamental mechanisms regulating the pathogenesis of asthma, Immunological reviews, 278 (2017) 20-40.
[8] E.L. Beckett, R.L. Stevens, A.G. Jarnicki, R.Y. Kim, I. Hanish, N.G. Hansbro, A. Deane, S. Keely, J.C. Horvat, M. Yang, B.G. Oliver, N. van Rooijen, M.D. Inman, R. Adachi, R.J. Soberman, S. Hamadi, P.A. Wark, P.S. Foster, P.M. Hansbro, A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis, J Allergy Clin Immunol, 131 (2013) 752-762.
[9] A. Chakraborty, A.A. Pinar, M. Lam, L.E. Bourke, S.G. Royce, C. Selomulya, C.S. Samuel, Pulmonary myeloid cell uptake of biodegradable nanoparticles conjugated with an anti-fibrotic agent provides a novel strategy for treating chronic allergic airways disease, Biomaterials, 273 (2021) 120796.
[10] K.R. Paudel, V. Dharwal, V.K. Patel, I. Galvao, R. Wadhwa, V. Malyla, S.S. Shen, K.F. Budden, N.G. Hansbro, A. Vaughan, I.A. Yang, M.R.J. Kohonen-Corish, M. Bebawy, K. Dua, P.M. Hansbro, Role of Lung Microbiome in Innate Immune Response Associated With Chronic Lung Diseases, Front Med (Lausanne), 7 (2020) 554-554.
[11] D. Lim, W. Kim, C. Lee, H. Bae, J. Kim, Macrophage Depletion Protects against Cigarette Smoke-Induced Inflammatory Response in the Mouse Colon and Lung, Frontiers in physiology, 9 (2018) 47-47.
[12] R.W. Chapman, M. Minnicozzi, C.S. Celly, J.E. Phillips, T.T. Kung, R.W. Hipkin, X. Fan, D. Rindgen, G. Deno, R. Bond, W. Gonsiorek, M.M. Billah, J.S. Fine, J.A. Hey, A novel, orally active CXCR1/2 receptor antagonist, Sch527123, inhibits neutrophil recruitment, mucus production, and goblet cell hyperplasia in animal models of pulmonary inflammation, J Pharmacol Exp Ther, 322 (2007) 486-493.
[13] N. Borregaard, O.E. Sørensen, K. Theilgaard-Mönch, Neutrophil granules: a library of innate immunity proteins, Trends in immunology, 28 (2007) 340-345.
[14] Y. Gernez, R. Tirouvanziam, P. Chanez, Neutrophils in chronic inflammatory airway diseases: can we target them and how?, European Respiratory Journal, 35 (2010) 467.
[15] H. Ilumets, P.H. Rytilä, A.R. Sovijärvi, T. Tervahartiala, M. Myllärniemi, T.A. Sorsa, V.L. Kinnula, Transient elevation of neutrophil proteinases in induced sputum during COPD exacerbation, Scandinavian journal of clinical and laboratory investigation, 68 (2008) 618-623.
[16] S.J. Weiss, Tissue destruction by neutrophils, N Engl J Med, 320 (1989) 365-376.

[17] S.P. Hogan, H.F. Rosenberg, R. Moqbel, S. Phipps, P.S. Foster, P. Lacy, A.B. Kay, M.E. Rothenberg, Eosinophils: biological properties and role in health and disease, Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 38 (2008) 709-750.
[18] M.E. Rothenberg, S.P. Hogan, The eosinophil, Annu Rev Immunol, 24 (2006) 147-174.
[19] C. Blanchard, M.E. Rothenberg, Biology of the eosinophil, Advances in immunology, 101 (2009) 81-121.
[20] K.R. Paudel, R. Wadhwa, M. Mehta, D.K. Chellappan, P.M. Hansbro, K. Dua, Rutin loaded liquid crystalline nanoparticles inhibit lipopolysaccharide induced oxidative stress and apoptosis in bronchial epithelial cells in vitro, Toxicology in Vitro, 68 (2020) 104961.
[21] M. Mehta, K.R. Paudel, S.D. Shukla, M.D. Shastri, S. Satija, S.K. Singh, M. Gulati, H. Dureja, F.C. Zacconi, P.M. Hansbro, D.K. Chellappan, K. Dua, Rutin-loaded liquid crystalline nanoparticles attenuate oxidative stress in bronchial epithelial cells: a PCR validation, 13 (2021) 543-549.
[22] M. Mehta, D.S. Dhanjal, K.R. Paudel, B. Singh, G. Gupta, S. Rajeshkumar, L. Thangavelu, M.M. Tambuwala, H.A. Bakshi, D.K. Chellappan, P. Pandey, H. Dureja, N.B. Charbe, S.K. Singh, S.D. Shukla, S. Nammi, A.A. Aljabali, P.R. Wich, P.M. Hansbro, S. Satija, K. Dua, Cellular signaling pathways mediating the pathogenesis of chronic inflammatory respiratory diseases: an update, Inflammopharmacology, 28 (2020) 795-817.
[23] M. Mehta, K.R. Paudel, N. Panth, D. Xenaki, R. Macloughlin, B.G. Oliver, R. Lobenberg, P.M. Hansbro, D.K. Chellappan, K. Dua, Drug delivery advances in mitigating inflammation via matrix metalloproteinases in respiratory diseases, 16 (2021) 437-452.
[24] W. Domej, K. Oettl, W. Renner, Oxidative stress and free radicals in COPD--implications and relevance for treatment, Int J Chron Obstruct Pulmon Dis, 9 (2014) 1207-1224.
[25] T. Liu, L. Zhang, D. Joo, S.-C. Sun, NF-κB signaling in inflammation, Signal transduction and targeted therapy, 2 (2017) 17023.
[26] H.W. Sharma, J.R. Perez, K. Higgins-Sochaski, R. Hsiao, R. Narayanan, Transcription factor decoy approach to decipher the role of NF-kappa B in oncogenesis, Anticancer Res, 16 (1996) 61-69.
[27] M. Borgatti, V. Bezzerri, I. Mancini, F. Nicolis, M. Dechecchi, P. Rizzotti, I. Lampronti, R. Gambari, G.J.M.B. Cabrini, Silencing of genes coding for transcription factors: biological effects of decoy oligonucleotides on cystic fibrosis bronchial epithelial cells, 20 (2008) 79-83.
[28] Y.-T. Li, B. He, Y.-Z. Wang, J.J.R.r. Wang, Effects of intratracheal administration of nuclear factor-kappaB decoy oligodeoxynucleotides on long-term cigarette smoke-induced lung inflammation and pathology in mice, 10 (2009) 1-14.
[29] N. Matsuda, Y. Hattori, Y. Takashi, J. Nishihira, S. Jesmin, M. Kobayashi, S.J.A.J.o.P.-L.C. Gando, M. Physiology, Therapeutic effect of in vivo transfection of transcription factor decoy to NF-κB on septic lung in mice, 287 (2004) L1248-L1255.
[30] M. Schuliga, NF-kappaB Signaling in Chronic Inflammatory Airway Disease, Biomolecules, 5 (2015) 1266-1283.
[31] R. Sen, D. Baltimore, In vitro transcription of immunoglobulin genes in a B-cell extract: effects of enhancer and promoter sequences, Mol Cell Biol, 7 (1987) 1989-1994.
[32] S.T. Smale, Dimer-specific regulatory mechanisms within the NF-κB family of transcription factors, Immunol Rev, 246 (2012) 193-204.
[33] Q. Zhang, M.J. Lenardo, D. Baltimore, 30 Years of NF-κB: A Blossoming of Relevance to Human Pathobiology, Cell, 168 (2017) 37-57.
[34] J.D. Wardyn, A.H. Ponsford, C.M. Sanderson, Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways, Biochem Soc Trans, 43 (2015) 621-626.
[35] G. Bonizzi, M. Karin, The two NF-kappaB activation pathways and their role in innate and adaptive immunity, Trends Immunol, 25 (2004) 280-288.
[36] M. Karin, Y. Ben-Neriah, Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity, Annu Rev Immunol, 18 (2000) 621-663.
[37] M.S. Hayden, S. Ghosh, Signaling to NF-kappaB, Genes Dev, 18 (2004) 2195-2224.

[38] T. Liu, L. Zhang, D. Joo, S.-C. Sun, NF-κB signaling in inflammation, Signal Transduction and Targeted Therapy, 2 (2017) 17023.
[39] S.D. Shukla, K. Swaroop Vanka, A. Chavelier, M.D. Shastri, M.M. Tambuwala, H.A. Bakshi, K. Pabreja, M.Q. Mahmood, R.F. O'Toole, Chapter 1 - Chronic respiratory diseases: An introduction and need for novel drug delivery approaches, in: K. Dua, P.M. Hansbro, R. Wadhwa, M. Haghi, L.G. Pont, K.A. Williams (Eds.) Targeting Chronic Inflammatory Lung Diseases Using Advanced Drug Delivery Systems, Academic Press, 2020, pp. 1-31.
[40] J.G. Wright, J.W. Christman, The Role of Nuclear Factor Kappa B in the Pathogenesis of Pulmonary Diseases: Implications for Therapy, American Journal of Respiratory Medicine, 2 (2003) 211-219.
[41] M. Schuliga, NF-kappaB Signaling in Chronic Inflammatory Airway Disease, Biomolecules, 5 (2015) 1266-1283.
[42] L.A. Hart, V.L. Krishnan, I.M. Adcock, P.J. Barnes, K.F. Chung, Activation and localization of transcription factor, nuclear factor-kappaB, in asthma, Am J Respir Crit Care Med, 158 (1998) 1585-1592.
[43] R. Gagliardo, P. Chanez, M. Mathieu, A. Bruno, G. Costanzo, C. Gougat, I. Vachier, J. Bousquet, G. Bonsignore, A.M. Vignola, Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma, Am J Respir Crit Care Med, 168 (2003) 1190-1198.
[44] D. Hoppenot, K. Malakauskas, S. Lavinskiene, R. Sakalauskas, p-STAT6, PU.1, and NF-κB are involved in allergen-induced late-phase airway inflammation in asthma patients, BMC Pulmonary Medicine, 15 (2015) 122.
[45] J.L. Ather, S.R. Hodgkins, Y.M. Janssen-Heininger, M.E. Poynter, Airway epithelial NF-κB activation promotes allergic sensitization to an innocuous inhaled antigen, Am J Respir Cell Mol Biol, 44 (2011) 631-638.
[46] C. Qiu, J. Li, J. Zhang, Q. He, L. Wang, X. Weng, M. Guan, Modulation of the airway smooth muscle phenotype in a murine asthma model and effects of nuclear factor-κB inhibition, Journal of Asthma, 56 (2019) 1247-1256.
[47] A. Di Stefano, G. Caramori, T. Oates, A. Capelli, M. Lusuardi, I. Gnemmi, F. Ioli, K.F. Chung, C.F. Donner, P.J. Barnes, I.M. Adcock, Increased expression of nuclear factor-κB in bronchial biopsies from smokers and patients with COPD, European Respiratory Journal, 20 (2002) 556-563.
[48] V. Brown, J.S. Elborn, J. Bradley, M. Ennis, Dysregulated apoptosis and NFκB expression in COPD subjects, Respiratory Research, 10 (2009) 24.
[49] L. Zhou, Y. Liu, X. Chen, S. Wang, H. Liu, T. Zhang, Y. Zhang, Q. Xu, X. Han, Y. Zhao, X. Song, L. Ye, Over-expression of nuclear factor-κB family genes and inflammatory molecules is related to chronic obstructive pulmonary disease, Int J Chron Obstruct Pulmon Dis, 13 (2018) 2131-2138.
[50] J.M.D. Rastrick, C.S. Stevenson, S. Eltom, M. Grace, M. Davies, I. Kilty, S.M. Evans, M. Pasparakis, M.C. Catley, T. Lawrence, I.M. Adcock, M.G. Belvisi, M.A. Birrell, Cigarette Smoke Induced Airway Inflammation Is Independent of NF-κB Signaling, PLOS ONE, 8 (2013) e54128.
[51] S.D. Shukla, E.H. Walters, J.L. Simpson, S. Keely, P.A.B. Wark, R.F. O'Toole, P.M. Hansbro, Hypoxia-inducible factor and bacterial infections in chronic obstructive pulmonary disease, Respirology, 25 (2020) 53-63.
[52] D. Yu, X. Liu, G. Zhang, Z. Ming, T. Wang, Isoliquiritigenin Inhibits Cigarette Smoke-Induced COPD by Attenuating Inflammation and Oxidative Stress via the Regulation of the Nrf2 and NF-κB Signaling Pathways, Frontiers in Pharmacology, 9 (2018).
[53] X. He, T. Li, L. Luo, H. Zeng, Y. Chen, S. Cai, PRMT6 mediates inflammation via activation of the NF-κB/ p65 pathway on a cigarette smoke extract-induced murine emphysema model, Tobacco Induced Diseases, 18 (2020).
[54] R. Zaynagetdinov, T.P. Sherrill, L.A. Gleaves, P. Hunt, W. Han, A.G. McLoed, J.A. Saxon, H. Tanjore, P.M. Gulleman, L.R. Young, T.S. Blackwell, Chronic NF-κB activation links COPD and lung cancer through generation of an immunosuppressive microenvironment in the lungs, Oncotarget, 7 (2016) 5470-5482.

[55] A.J. Weber, G. Soong, R. Bryan, S. Saba, A. Prince, Activation of NF-kappaB in airway epithelial cells is dependent on CFTR trafficking and Cl- channel function, Am J Physiol Lung Cell Mol Physiol, 281 (2001) L71-78.
[56] S.L. Kunkel, T. Standiford, K. Kasahara, R.M. Strieter, Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung, Exp Lung Res, 17 (1991) 17-23.
[57] E. DiMango, A.J. Ratner, R. Bryan, S. Tabibi, A. Prince, Activation of NF-kappaB by adherent Pseudomonas aeruginosa in normal and cystic fibrosis respiratory epithelial cells, J Clin Invest, 101 (1998) 2598-2605.
[58] S. Hippenstiel, B. Opitz, B. Schmeck, N. Suttorp, Lung epithelium as a sentinel and effector system in pneumonia--molecular mechanisms of pathogen recognition and signal transduction, Respir Res, 7 (2006) 97.
[59] J.J. Ryan, S.L. Archer, The right ventricle in pulmonary arterial hypertension: disorders of metabolism, angiogenesis and adrenergic signaling in right ventricular failure, Circ Res, 115 (2014) 176-188.
[60] S.J. Shah, Pulmonary hypertension, Jama, 308 (2012) 1366-1374.
[61] G. Simonneau, D. Montani, D.S. Celermajer, C.P. Denton, M.A. Gatzoulis, M. Krowka, P.G. Williams, R. Souza, Haemodynamic definitions and updated clinical classification of pulmonary hypertension, European Respiratory Journal, 53 (2019) 1801913.
[62] M. Spaczyńska, S.F. Rocha, E. Oliver, Pharmacology of Pulmonary Arterial Hypertension: An Overview of Current and Emerging Therapies, ACS Pharmacology & Translational Science, 3 (2020) 598-612.
[63] S. Hosokawa, G. Haraguchi, A. Sasaki, H. Arai, S. Muto, A. Itai, S. Doi, S. Mizutani, M. Isobe, Pathophysiological roles of nuclear factor kappaB (NF-kB) in pulmonary arterial hypertension: effects of synthetic selective NF-kB inhibitor IMD-0354, Cardiovascular research, 99 (2013) 35-43.
[64] L.C. Price, G. Caramori, F. Perros, C. Meng, M. Gambaryan, P. Dorfmuller, D. Montani, P. Casolari, J. Zhu, K. Dimopoulos, D. Shao, B. Girerd, S. Wort, A. Proudfoot, M. Griffiths, A. Papi, M. Humbert, I.M. Adcock, S.J. Wort, Nuclear factor κ-B is activated in the pulmonary vessels of patients with end-stage idiopathic pulmonary arterial hypertension, PloS one, 8 (2013) e75415-e75415.
[65] L. Li, C. Wei, I.K. Kim, Y. Janssen-Heininger, S. Gupta, Inhibition of nuclear factor-κB in the lungs prevents monocrotaline-induced pulmonary hypertension in mice, Hypertension (Dallas, Tex. : 1979), 63 (2014) 1260-1269.
[66] J. Liu, J. Li, C. Xie, L. Xuan, B. Yang, MSCs attenuate hypoxia induced pulmonary hypertension by activating P53 and NF-kB signaling pathway through TNFα secretion, Biochem Biophys Res Commun, 532 (2020) 400-405.
[67] R. Kumar, B. Graham, How does inflammation contribute to pulmonary hypertension?, The European respiratory journal, 51 (2018) 1702403.
[68] S. Kimura, K. Egashira, L. Chen, K. Nakano, E. Iwata, M. Miyagawa, H. Tsujimoto, K. Hara, R. Morishita, K. Sueishi, R. Tominaga, K. Sunagawa, Nanoparticle-mediated delivery of nuclear factor kappaB decoy into lungs ameliorates monocrotaline-induced pulmonary arterial hypertension, Hypertension (Dallas, Tex. : 1979), 53 (2009) 877-883.
[69] G.L. Semenza, Hypoxia-inducible factors in physiology and medicine, Cell, 148 (2012) 399-408.
[70] A.Y. Yu, L.A. Shimoda, N.V. Iyer, D.L. Huso, X. Sun, R. McWilliams, T. Beaty, J.S. Sham, C.M. Wiener, J.T. Sylvester, G.L. Semenza, Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha, The Journal of clinical investigation, 103 (1999) 691-696.
[71] M.K. Ball, G.B. Waypa, P.T. Mungai, J.M. Nielsen, L. Czech, V.J. Dudley, L. Beussink, R.W. Dettman, S.K. Berkelhamer, R.H. Steinhorn, S.J. Shah, P.T. Schumacker, Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1α, American journal of respiratory and critical care medicine, 189 (2014) 314-324.
[72] R.S. Belaiba, S. Bonello, C. Zähringer, S. Schmidt, J. Hess, T. Kietzmann, A. Görlach, Hypoxia up-regulates hypoxia-inducible factor-1alpha transcription by involving phosphatidylinositol 3-kinase

and nuclear factor kappaB in pulmonary artery smooth muscle cells, Molecular biology of the cell, 18 (2007) 4691-4697.
[73] N. Ferrari, R. Seguin, P. Renzi, Oligonucleotides: a multi-targeted approach for the treatment of respiratory diseases, Future Med Chem, 3 (2011) 1647-1662.
[74] M. Mehta, Deeksha, D. Tewari, G. Gupta, R. Awasthi, H. Singh, P. Pandey, D.K. Chellappan, R. Wadhwa, T. Collet, P.M. Hansbro, S.R. Kumar, L. Thangavelu, P. Negi, K. Dua, S. Satija, Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases, Chemico-Biological Interactions, 308 (2019) 206-215.
[75] P.C. Zamecnik, M.L.J.P.o.t.N.A.o.S. Stephenson, Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide, 75 (1978) 280-284.
[76] H. Lu, H. Zhu, Effect of siRNA-mediated gene silencing of transketolase on A549 lung cancer cells, Oncol Lett, 14 (2017) 5906-5912.
[77] L. Chen, X. He, Y. Xie, Y. Huang, D.W. Wolff, P.W. Abel, Y. Tu, Up-regulated miR-133a orchestrates epithelial-mesenchymal transition of airway epithelial cells, Scientific Reports, 8 (2018) 15543.
[78] H. Shirota, D. Tross, D.M.J.V. Klinman, CpG oligonucleotides as cancer vaccine adjuvants, 3 (2015) 390-407.
[79] A. Bielinska, R. Shivdasani, L. Zhang, G. Nabel, Regulation of gene expression with double-stranded phosphorothioate oligonucleotides, 250 (1990) 997-1000.
[80] K.-M. Song, S. Lee, C.J.S. Ban, Aptamers and their biological applications, 12 (2012) 612-631.
[81] M. Zaki Ahmad, S. Akhter, N. Mallik, M. Anwar, W. Tabassum, F.J.C.d.d.t. Jalees Ahmad, Application of decoy oligonucleotides as novel therapeutic strategy: a contemporary overview, 10 (2013) 71-84.
[82] M.J. Mann, Transcription factor decoys: a new model for disease intervention, Ann N Y Acad Sci, 1058 (2005) 128-139.
[83] N. Tomita, N. Kashihara, R. Morishita, Transcription factor decoy oligonucleotide-based therapeutic strategy for renal disease, Clinical and experimental nephrology, 11 (2007) 7-17.
[84] M.M. Tehran, S. Rezaei, A. Jalili, S.H. Aghaee-Bakhtiari, A. Sahebkar, Decoy oligodeoxynucleotide technology: an emerging paradigm for breast cancer treatment, Drug Discov Today, 25 (2020) 195-200.
[85] Y.S. Cho-Chung, Y.G. Park, M. Nesterova, Y.N. Lee, Y.S. Cho, CRE-decoy oligonucleotide-inhibition of gene expression and tumor growth, Mol Cell Biochem, 212 (2000) 29-34.
[86] R. Gambari, M. Borgatti, V. Bezzerri, E. Nicolis, I. Lampronti, M.C. Dechecchi, I. Mancini, A. Tamanini, G. Cabrini, Decoy oligodeoxyribonucleotides and peptide nucleic acids-DNA chimeras targeting nuclear factor kappa-B: inhibition of IL-8 gene expression in cystic fibrosis cells infected with Pseudomonas aeruginosa, Biochem Pharmacol, 80 (2010) 1887-1894.
[87] R.M. Séguin, N. Ferrari, Emerging oligonucleotide therapies for asthma and chronic obstructive pulmonary disease, Expert opinion on investigational drugs, 18 (2009) 1505-1517.
[88] L. Farahmand, B. Darvishi, A.K. Majidzadeh, Suppression of chronic inflammation with engineered nanomaterials delivering nuclear factor κB transcription factor decoy oligodeoxynucleotides, Drug Deliv, 24 (2017) 1249-1261.
[89] Y. Deng, C.C. Wang, K.W. Choy, Q. Du, J. Chen, Q. Wang, L. Li, T.K. Chung, T. Tang, Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies, Gene, 538 (2014) 217-227.
[90] U. Griesenbach, R.L. Cassady, R.J. Cain, R.M. duBois, D.M. Geddes, E.W. Alton, Cytoplasmic deposition of NFkappaB decoy oligonucleotides is insufficient to inhibit bleomycin-induced pulmonary inflammation, Gene Ther, 9 (2002) 1109-1115.
[91] D. De Stefano, G. De Rosa, R. Carnuccio, NFkappaB decoy oligonucleotides, Current opinion in molecular therapeutics, 12 (2010) 203-213.
[92] X. Sun, J. Zhang, STAT3 Decoy ODN Therapy for Cancer, Methods Mol Biol, 1317 (2015) 167-183.

[93] R. Morishita, N. Tomita, Y. Kaneda, T. Ogihara, Molecular therapy to inhibit NFkappaB activation by transcription factor decoy oligonucleotides, Curr Opin Pharmacol, 4 (2004) 139-146.
[94] M.Z. Ahmad, S. Akhter, N. Mallik, M. Anwar, W. Tabassum, F.J. Ahmad, Application of decoy oligonucleotides as novel therapeutic strategy: a contemporary overview, Current drug discovery technologies, 10 (2013) 71-84.
[95] A.R. Kirtane, J. Panyam, Polymer nanoparticles: Weighing up gene delivery, Nat Nanotechnol, 8 (2013) 805-806.
[96] T. Serikawa, H. Kikuchi, T. Oite, K. Tanaka, Enhancement of gene expression efficiency using cationic liposomes on ovarian cancer cells, Drug Deliv, 15 (2008) 523-529.
[97] Y. Liu, F. Quan, J. Wang, X. Bai, [Nuclear localization of oligonucleotides decoy effect on nuclear factor-kappaB activity], Sheng wu gong cheng xue bao = Chinese journal of biotechnology, 26 (2010) 1683-1689.
[98] Y. Wang, L. Miao, A. Satterlee, L. Huang, Delivery of oligonucleotides with lipid nanoparticles, Adv Drug Deliv Rev, 87 (2015) 68-80.
[99] D. De Stefano, F. Ungaro, C. Giovino, A. Polimeno, F. Quaglia, R. Carnuccio, Sustained inhibition of IL-6 and IL-8 expression by decoy ODN to NF-κB delivered through respirable large porous particles in LPS-stimulated cystic fibrosis bronchial cells, The journal of gene medicine, 13 (2011) 200-208.
[100] V. Bezzerri, M. Borgatti, E. Nicolis, I. Lampronti, M.C. Dechecchi, I. Mancini, P. Rizzotti, R. Gambari, G. Cabrini, Transcription factor oligodeoxynucleotides to NF-kappaB inhibit transcription of IL-8 in bronchial cells, Am J Respir Cell Mol Biol, 39 (2008) 95-96.
[101] A. Lührmann, T. Tschernig, H. von der Leyen, M. Hocker, R. Pabst, A.H. Wagner, Decoy oligodeoxynucleotide against STAT transcription factors decreases allergic inflammation in a rat asthma model, Exp Lung Res, 36 (2010) 85-93.
[102] Y.-T. Li, B. He, Y.-Z. Wang, J. Wang, Effects of intratracheal administration of nuclear factor-kappaB decoy oligodeoxynucleotides on long-term cigarette smoke-induced lung inflammation and pathology in mice, Respiratory Research, 10 (2009) 79.
[103] N. Matsuda, Y. Hattori, Y. Takahashi, J. Nishihira, S. Jesmin, M. Kobayashi, S. Gando, Therapeutic effect of in vivo transfection of transcription factor decoy to NF-kappaB on septic lung in mice, Am J Physiol Lung Cell Mol Physiol, 287 (2004) L1248-1255.
[104] N. Matsuda, Y. Hattori, S. Jesmin, S. Gando, Nuclear factor-kappaB decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis, Mol Pharmacol, 67 (2005) 1018-1025.
[105] D.Q. Fang, T. Yu, L. Wan, [Effect of nuclear factor-kappa B decoy oligodeoxynucleotides on IL-10, IL-13 and nuclear factor-kappa B protein expressions in rabbits with severe lung contusion], Nan fang yi ke da xue xue bao = Journal of Southern Medical University, 30 (2010) 2284-2287.
[106] K. Ohmori, S. Takeda, S. Miyoshi, M. Minami, S. Nakane, M. Ohta, Y. Sawa, H. Matsuda, Attenuation of lung injury in allograft rejection using NF-kappaB decoy transfection-novel strategy for use in lung transplantation, European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery, 27 (2005) 23-27.
[107] D. De Stefano, C. Coletta, R. Bianca, L. Falcone, I. d'Angelo, F. Ungaro, F. Quaglia, R. Carnuccio, R. Sorrentino, A decoy oligonucleotide to NF-κB delivered through inhalable particles prevents LPS-induced rat airway inflammation, Am J Respir Cell Mol Biol, 49 (2013) 288-295.
[108] F. Ungaro, D. De Stefano, C. Giovino, A. Masuccio, A. Miro, R. Sorrentino, R. Carnuccio, F. Quaglia, PEI-engineered respirable particles delivering a decoy oligonucleotide to NF-κB: inhibiting MUC2 expression in LPS-stimulated airway epithelial cells, PLoS One, 7 (2012) e46457.
[109] M.J. Mann, V.J. Dzau, Therapeutic applications of transcription factor decoy oligonucleotides, J Clin Invest, 106 (2000) 1071-1075.
[110] L. Sampieri, P. Di Giusto, C. Alvarez, CREB3 Transcription Factors: ER-Golgi Stress Transducers as Hubs for Cellular Homeostasis, Frontiers in Cell and Developmental Biology, 7 (2019).

[111] Y. Luo, S.G. Zheng, Hall of Fame among Pro-inflammatory Cytokines: Interleukin-6 Gene and Its Transcriptional Regulation Mechanisms, Front Immunol, 7 (2016).
[112] W. Wongchana, T. Palaga, Direct regulation of interleukin-6 expression by Notch signaling in macrophages, Cellular & Molecular Immunology, 9 (2012) 155-162.
[113] R. Morishita, J. Higaki, N. Tomita, T. Ogihara, Application of Transcription Factor “Decoy” Strategy as Means of Gene Therapy and Study of Gene Expression in Cardiovascular Disease, Circulation Research, 82 (1998) 1023-1028.
[114] D. De Stefano, Oligonucleotides decoy to NF-kappaB: becoming a reality?, Discovery medicine, 12 (2011) 97-105.
[115] M. Hecker, A.H. Wagner, Transcription factor decoy technology: A therapeutic update, Biochem Pharmacol, 144 (2017) 29-34.
[116] S.D. Putney, S.J. Benkovic, P.R. Schimmel, A DNA fragment with an alpha-phosphorothioate nucleotide at one end is asymmetrically blocked from digestion by exonuclease III and can be replicated in vivo, Proceedings of the National Academy of Sciences, 78 (1981) 7350.
[117] C. Mischiati, M. Borgatti, N. Bianchi, C. Rutigliano, M. Tomassetti, G. Feriotto, R. Gambari, Interaction of the human NF-kappaB p52 transcription factor with DNA-PNA hybrids mimicking the NF-kappaB binding sites of the human immunodeficiency virus type 1 promoter, J Biol Chem, 274 (1999) 33114-33122.
[118] V.V. Demidov, V.N. Potaman, M.D. Frank-Kamenetskii, M. Egholm, O. Buchard, S.H. Sönnichsen, P.E. Nielsen, Stability of peptide nucleic acids in human serum and cellular extracts, Biochem Pharmacol, 48 (1994) 1310-1313.
[119] R. Crinelli, M. Bianchi, L. Gentilini, M. Magnani, Design and characterization of decoy oligonucleotides containing locked nucleic acids, Nucleic Acids Res, 30 (2002) 2435-2443.
[120] R. Crinelli, M. Bianchi, L. Gentilini, L. Palma, M.D. Sørensen, T. Bryld, R.B. Babu, K. Arar, J. Wengel, M. Magnani, Transcription factor decoy oligonucleotides modified with locked nucleic acids: an in vitro study to reconcile biostability with binding affinity, Nucleic Acids Res, 32 (2004) 1874-1885.
[121] C. Roma-Rodrigues, L. Rivas-García, P.V. Baptista, A.R. Fernandes, Gene Therapy in Cancer Treatment: Why Go Nano?, Pharmaceutics, 12 (2020) 233.
[122] D. De Stefano, G. De Rosa, M.C. Maiuri, F. Ungaro, F. Quaglia, T. Iuvone, M.P. Cinelli, M.I. La Rotonda, R. Carnuccio, Oligonucleotide decoy to NF-κB slowly released from PLGA microspheres reduces chronic inflammation in rat, Pharmacological Research, 60 (2009) 33-40.
[123] H. Yamaguchi, Y. Ishida, J. Hasomichi, J.-i. Suzuki, R. Usumi-Fujita, Y. Shimizu, S. Kaneko, T. Ono, A new approach to transfect NF-κB decoy oligodeoxynucleotides into the periodontal tissue using the ultrasound-microbubble method, International Journal of Oral Science, 9 (2017) 80-86.
[124] G. De Rosa, D.D. Stefano, V. Laguardia, S. Arpicco, V. Simeon, R. Carnuccio, E. Fattal, Novel cationic liposome formulation for the delivery of an oligonucleotide decoy to NF-κB into activated macrophages, European Journal of Pharmaceutics and Biopharmaceutics, 70 (2008) 7-18.
[125] A. Nishimura, K. Akeda, T. Matsubara, K. Kusuzaki, A. Matsumine, K. Masuda, T. Gemba, A. Uchida, A. Sudo, Transfection of NF-κB decoy oligodeoxynucleotide suppresses pulmonary metastasis by murine osteosarcoma, Cancer Gene Therapy, 18 (2011) 250-259.
[126] P.R. Wardwell, M.B. Forstner, R.A. Bader, Investigation of the cytokine response to NF-κB decoy oligonucleotide coated polysaccharide based nanoparticles in rheumatoid arthritis in vitro models, Arthritis research & therapy, 17 (2015) 310.
[127] Y. Ma, X. Zhang, X. Xu, L. Shen, Y. Yao, Z. Yang, P. Liu, STAT3 Decoy Oligodeoxynucleotides-Loaded Solid Lipid Nanoparticles Induce Cell Death and Inhibit Invasion in Ovarian Cancer Cells, PloS one, 10 (2015) e0124924-e0124924.
[128] M.M. Tehran, S. Rezaei, A. Jalili, S.H. Aghaee-Bakhtiari, A. Sahebkar, Decoy oligodeoxynucleotide technology: an emerging paradigm for breast cancer treatment, Drug Discovery Today, 25 (2020) 195-200.
[129] J. Dzau Victor, Transcription Factor Decoy, Circulation Research, 90 (2002) 1234-1236.

[130] R.L. Juliano, The delivery of therapeutic oligonucleotides, Nucleic Acids Res, 44 (2016) 6518-6548.
[131] C.R. Dass, Liposome-mediated delivery of oligodeoxynucleotides in vivo, Drug Deliv, 9 (2002) 169-180.
[132] A. Ahmad, J.M. Khan, S. Haque, Strategies in the design of endosomolytic agents for facilitating endosomal escape in nanoparticles, Biochimie, 160 (2019) 61-75.
[133] A.K. Thakur, D.K. Chellappan, K. Dua, M. Mehta, S. Satija, I. Singh, Patented therapeutic drug delivery strategies for targeting pulmonary diseases, Expert Opin Ther Pat, 30 (2020) 375-387.
[134] P.Q. Ng, L.S.C. Ling, J. Chellian, T. Madheswaran, J. Panneerselvam, A.P. Kunnath, G. Gupta, S. Satija, M. Mehta, P.M. Hansbro, T. Collet, K. Dua, D.K. Chellappan, Applications of Nanocarriers as Drug Delivery Vehicles for Active Phytoconstituents, Curr Pharm Des, 26 (2020) 4580-4590.
[135] K. Dua, R. Wadhwa, G. Singhvi, V. Rapalli, S.D. Shukla, M.D. Shastri, G. Gupta, S. Satija, M. Mehta, N. Khurana, R. Awasthi, P.K. Maurya, L. Thangavelu, R. S, M.M. Tambuwala, T. Collet, P.M. Hansbro, D.K. Chellappan, The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress, Drug Dev Res, 80 (2019) 714-730.
[136] M. Mehta, D.K. Chellappan, P.R. Wich, N.G. Hansbro, P.M. Hansbro, K. Dua, miRNA nanotherapeutics: potential and challenges in respiratory disorders, Future Med Chem, 12 (2020) 987-990.
[137] D. De Stefano, G. De Rosa, M.C. Maiuri, F. Ungaro, F. Quaglia, T. Iuvone, M.P. Cinelli, M.I. La Rotonda, R. Carnuccio, Oligonucleotide decoy to NF-kappaB slowly released from PLGA microspheres reduces chronic inflammation in rat, Pharmacol Res, 60 (2009) 33-40.
[138] K. Tahara, S. Samura, K. Tsuji, H. Yamamoto, Y. Tsukada, Y. Bando, H. Tsujimoto, R. Morishita, Y. Kawashima, Oral nuclear factor-κB decoy oligonucleotides delivery system with chitosan modified poly(D,L-lactide-co-glycolide) nanospheres for inflammatory bowel disease, Biomaterials, 32 (2011) 870-878.
[139] Y. Hattori, M. Sakaguchi, Y. Maitani, Folate-linked lipid-based nanoparticles deliver a NFkappaB decoy into activated murine macrophage-like RAW264.7 cells, Biol Pharm Bull, 29 (2006) 1516-1520.
[140] F. Hoffmann, G. Sass, J. Zillies, S. Zahler, G. Tiegs, A. Hartkorn, S. Fuchs, J. Wagner, G. Winter, C. Coester, A.L. Gerbes, A.M. Vollmar, A novel technique for selective NF-kappaB inhibition in Kupffer cells: contrary effects in fulminant hepatitis and ischaemia-reperfusion, Gut, 58 (2009) 1670-1678.
[141] Y. Higuchi, S. Kawakami, M. Oka, F. Yamashita, M. Hashida, Suppression of TNFalpha production in LPS induced liver failure in mice after intravenous injection of cationic liposomes/NFkappaB decoy complex, Pharmazie, 61 (2006) 144-147.
[142] H. Huang, F. Sakurai, Y. Higuchi, S. Kawakami, M. Hashida, K. Kawabata, H. Mizuguchi, Suppressive effects of sugar-modified cationic liposome/NF-kappaB decoy complexes on adenovirus vector-induced innate immune responses, J Control Release, 133 (2009) 139-145.
[143] K.D. Buchanan, S.-L. Huang, H. Kim, D.D. McPherson, R.C. MacDonald, Encapsulation of NF-kappaB decoy oligonucleotides within echogenic liposomes and ultrasound-triggered release, J Control Release, 141 (2010) 193-198.
[144] Y. Sugao, K. Watanabe, Y. Higuchi, R. Kurihara, S. Kawakami, M. Hashida, Y. Katayama, T. Niidome, NFkappaB decoy delivery using dendritic poly(l-lysine) for treatment of endotoxin-induced hepatitis in mice, Bioorganic & medicinal chemistry, 17 (2009) 4990-4995.
[145] A.J. Omlor, J. Nguyen, R. Bals, Q.T. Dinh, Nanotechnology in respiratory medicine, Respiratory Research, 16 (2015) 64.
[146] A. Cano, M. Ettcheto, M. Espina, A. López-Machado, Y. Cajal, F. Rabanal, E. Sánchez-López, A. Camins, M.L. García, E.B. Souto, State-of-the-art polymeric nanoparticles as promising therapeutic tools against human bacterial infections, Journal of Nanobiotechnology, 18 (2020) 156.
[147] K. Tahara, S. Samura, K. Tsuji, H. Yamamoto, Y. Tsukada, Y. Bando, H. Tsujimoto, R. Morishita, Y. Kawashima, Oral nuclear factor-κB decoy oligonucleotides delivery system with chitosan modified poly(d,l-lactide-co-glycolide) nanospheres for inflammatory bowel disease, Biomaterials, 32 (2011) 870-878.

[148] P.R. Wardwell, R.A. Bader, Immunomodulation of cystic fibrosis epithelial cells via NF-κB decoy oligonucleotide-coated polysaccharide nanoparticles, Journal of biomedical materials research. Part A, 103 (2015) 1622-1631.
[149] I. d'Angelo, B. Perfetto, G. Costabile, V. Ambrosini, P. Caputo, A. Miro, R. d'Emmanuele di Villa Bianca, R. Sorrentino, G. Donnarumma, F. Quaglia, F. Ungaro, Large Porous Particles for Sustained Release of a Decoy Oligonucelotide and Poly(ethylenimine): Potential for Combined Therapy of Chronic Pseudomonas aeruginosa Lung Infections, Biomacromolecules, 17 (2016) 1561-1571.
[150] C.R.J.D.d. Dass, Liposome-mediated delivery of oligodeoxynucleotides in vivo, 9 (2002) 169-180.
[151] G. De Rosa, D. De Stefano, V. Laguardia, S. Arpicco, V. Simeon, R. Carnuccio, E. Fattal, Novel cationic liposome formulation for the delivery of an oligonucleotide decoy to NF-kappaB into activated macrophages, European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V, 70 (2008) 7-18.
[152] W. Wijagkanalan, S. Kawakami, Y. Higuchi, F. Yamashita, M. Hashida, Intratracheally instilled mannosylated cationic liposome/NFκB decoy complexes for effective prevention of LPS-induced lung inflammation, Journal of Controlled Release, 149 (2011) 42-50.
[153] S. Jackson, A.F. Candia, S. Delaney, S. Floettmann, C. Wong, L.D. Campbell, S. Kell, J. Lum, E.M. Hessel, P. Traquina, M. McHale, I. Robinson, J. Bell, R. Fuhr, D. Keeling, R.L. Coffman, First-in-Human Study With the Inhaled TLR9 Oligonucleotide Agonist AZD1419 Results in Interferon Responses in the Lung, and Is Safe and Well-Tolerated, Clinical pharmacology and therapeutics, 104 (2018) 335-345.
[154] I. Psallidas, V. Backer, P. Kuna, R. Palmér, S. Necander, M. Aurell, K. Korsback, Z. Taib, M. Hashemi, P. Gustafson, S. Asimus, S. Delaney, K. Pardali, F. Jiang, J. Almquist, S. Jackson, R.L. Coffman, D. Keeling, T. Sethi, A Phase 2a, Double-Blind, Placebo-controlled Randomized Trial of Inhaled TLR9 Agonist AZD1419 in Asthma, Am J Respir Crit Care Med, 203 (2021) 296-306.
[155] T. Storni, C. Ruedl, K. Schwarz, R.A. Schwendener, W.A. Renner, M.F. Bachmann, Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects, J Immunol, 172 (2004) 1777-1785.
[156] G. Senti, P. Johansen, S. Haug, C. Bull, S. Gottschaller, P. Müller, T. Pfister, P. Maurer, M.F. Bachmann, N. Graf, T.M. Kündig, Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial, Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 39 (2009) 562-570.
[157] T.B. Casale, J. Cole, E. Beck, S.F. Vogelmeier, J. Willers, C. Lassen, A. Hammann-Haenni, L. Trokan, P. Saudan, M.E. Wechsler, CYT003, a TLR9 agonist, in persistent allergic asthma - a randomized placebo-controlled Phase 2b study, Allergy, 70 (2015) 1160-1168.
[158] K.M. Beeh, F. Kanniess, F. Wagner, C. Schilder, I. Naudts, A. Hammann-Haenni, J. Willers, H. Stocker, P. Mueller, M.F. Bachmann, W.A. Renner, The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma, J Allergy Clin Immunol, 131 (2013) 866-874.
[159] H. Imaoka, H. Campbell, I. Babirad, R.M. Watson, M. Mistry, R. Sehmi, G.M. Gauvreau, TPI ASM8 reduces eosinophil progenitors in sputum after allergen challenge, Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 41 (2011) 1740-1746.
[160] G.M. Gauvreau, L.P. Boulet, D.W. Cockcroft, A. Baatjes, J. Cote, F. Deschesnes, B. Davis, T. Strinich, K. Howie, M. Duong, R.M. Watson, P.M. Renzi, P.M. O'Byrne, Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses, Am J Respir Crit Care Med, 177 (2008) 952-958.
[161] M. Fortin, H. D'Anjou, M.E. Higgins, J. Gougeon, P. Aubé, K. Moktefi, S. Mouissi, S. Séguin, R. Séguin, P.M. Renzi, L. Paquet, N. Ferrari, A multi-target antisense approach against PDE4 and PDE7 reduces smoke-induced lung inflammation in mice, Respir Res, 10 (2009) 39.
[162] G. Haskó, J. Linden, B. Cronstein, P. Pacher, Adenosine receptors: therapeutic aspects for inflammatory and immune diseases, Nature reviews. Drug discovery, 7 (2008) 759-770.

[163] H.A. Ball, A. Sandrasagra, L. Tang, M. Van Scott, J. Wild, J.W. Nyce, Clinical potential of respirable antisense oligonucleotides (RASONs) in asthma, American journal of pharmacogenomics : genomics-related research in drug development and clinical practice, 3 (2003) 97-106.
[164] J.G. Karras, J.R. Crosby, M. Guha, D. Tung, D.A. Miller, W.A. Gaarde, R.S. Geary, B.P. Monia, S.A. Gregory, Anti-inflammatory activity of inhaled IL-4 receptor-alpha antisense oligonucleotide in mice, Am J Respir Cell Mol Biol, 36 (2007) 276-285.
[165] Randomized, Double-Blind, Placebo Controlled First in Human Study of Inhaled AIR645, an IL-4Rα Oligonucleotide, in Healthy Volunteers, in: B92. NEW TREATMENT APPROACHES FOR ASTHMA AND ALLERGY, pp. A3640.
[166] D.A. Medina-Tato, M.L. Watson, S.G. Ward, Leukocyte navigation mechanisms as targets in airway diseases, Drug Discov Today, 11 (2006) 866-879.
[167] F.-D. Popescu, F. Popescu, A review of antisense therapeutic interventions for molecular biological targets in asthma, Biologics, 1 (2007) 271-283.
[168] P. Drevinek, T. Pressler, M. Cipolli, K. De Boeck, C. Schwarz, F. Bouisset, M. Boff, N. Henig, N. Paquette-Lamontagne, S. Montgomery, J. Perquin, N. Tomkinson, W. den Hollander, J.S. Elborn, Antisense oligonucleotide eluforsen is safe and improves respiratory symptoms in F508DEL cystic fibrosis, Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society, 19 (2020) 99-107.
[169] D. Hong, R. Kurzrock, Y. Kim, R. Woessner, A. Younes, J. Nemunaitis, N. Fowler, T. Zhou, J. Schmidt, M. Jo, S.J. Lee, M. Yamashita, S.G. Hughes, L. Fayad, S. Piha-Paul, M.V. Nadella, M. Mohseni, D. Lawson, C. Reimer, D.C. Blakey, X. Xiao, J. Hsu, A. Reventos, B.P. Monia, A.R. MacLeod, AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer, Sci Transl Med, 7 (2015) 314ra185.
[170] D.R. Spigel, D.L. Shipley, D.M. Waterhouse, S.F. Jones, P.J. Ward, K.C. Shih, B. Hemphill, M. McCleod, R.C. Whorf, R.D. Page, J. Stilwill, T. Meehan, C. Jacobs, H.A. Burris, 3rd, J.D. Hainsworth, A Randomized, Double-Blinded, Phase II Trial of Carboplatin and Pemetrexed with or without Apatorsen (OGX-427) in Patients with Previously Untreated Stage IV Non-Squamous-Non-Small-Cell Lung Cancer: The SPRUCE Trial, Oncologist, 24 (2019) e1409-e1416.
[171] R. Natale, F. Blackhall, D. Kowalski, K. Ramlau, G. Bepler, F. Grossi, C. Lerchenmüller, M. Pinder-Schenck, J. Mezger, S. Danson, S.M. Sedgeel, Y. Summers, S. Callies, V. André, M. Das, M. Lahn, D. Talbot, Evaluation of antitumor activity using change in tumor size of the survivin antisense oligonucleotide LY2181308 in combination with docetaxel for second-line treatment of patients with non-small-cell lung cancer: a randomized open-label phase II study, J Thorac Oncol, 9 (2014) 1704-1708.
[172] D. Quarcoo, S. Weixler, D. Groneberg, R. Joachim, B. Ahrens, A.H. Wagner, M. Hecker, E. Hamelmann, Inhibition of signal transducer and activator of transcription 1 attenuates allergen-induced airway inflammation and hyperreactivity, J Allergy Clin Immunol, 114 (2004) 288-295.
[173] R.M. Séguin, N.J.E.o.o.i.d. Ferrari, Emerging oligonucleotide therapies for asthma and chronic obstructive pulmonary disease, 18 (2009) 1505-1517.
[174] G.R. Stenton, M.K. Kim, O. Nohara, C.F. Chen, N. Hirji, F.L. Wills, M. Gilchrist, P.H. Hwang, J.G. Park, W. Finlay, R.L. Jones, A.D. Befus, A.D. Schreiber, Aerosolized Syk antisense suppresses Syk expression, mediator release from macrophages, and pulmonary inflammation, J Immunol, 164 (2000) 3790-3797.
[175] P.C. Zamecnik, M.L. Stephenson, Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide, Proc Natl Acad Sci U S A, 75 (1978) 280-284.
[176] S.M. Hammond, A. Aartsma-Rus, S. Alves, S.E. Borgos, R.A.M. Buijsen, R.W.J. Collin, G. Covello, M.A. Denti, L.R. Desviat, L. Echevarría, C. Foged, G. Gaina, A. Garanto, A.T. Goyenvalle, M. Guzowska, I. Holodnuka, D.R. Jones, S. Krause, T. Lehto, M. Montolio, W. Van Roon-Mom, V. Arechavala-Gomeza, Delivery of oligonucleotide-based therapeutics: challenges and opportunities, EMBO Mol Med, (2021) e13243.

[177] A. Aartsma-Rus, D.R. Corey, The 10th Oligonucleotide Therapy Approved: Golodirsen for Duchenne Muscular Dystrophy, Nucleic Acid Ther, 30 (2020) 67-70.