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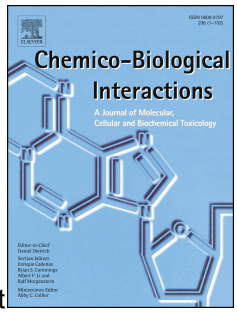

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Emerging Applications and Prospects of NFκB Decoy Oligodeoxynucleotides in Managing Respiratory Diseases

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Abstract

Chronic respiratory diseases like asthma and Chronic Obstructive Pulmonary Disease (COPD) have been a burden to society for an extended period. Currently, there are only preventative treatments in the form of mono- or multiple-drug therapy available to patients who need to utilise it daily. Hence, throughout the years there has been a substantial amount of research in understanding what causes inflammation in the context of these diseases. For example, the transcription factor NFκB has a pivotal role in causing chronic inflammation. Subsequent research has been exploring ways to block the activation of NFκB as a potential therapeutic strategy for many inflammatory diseases. One of the possible ways through which this is probable is the utilisation of decoy oligodeoxynucleotides, which are synthetic, short, single-stranded DNA fragments that mimic the consensus binding site of a targeted transcription factor, thereby functionally inactivating it. However, limitations to the implementation of decoy oligodeoxynucleotides include their rapid degradation by intracellular nucleases and the lack of targeted tissue specificity. An advantageous approach to overcome these limitations involves using nanoparticles as a vessel for drug delivery. In this review, all of those key elements will be explored as to how they come together as an application to treat chronic inflammation in respiratory diseases.

Keywords

NFκB; Decoy Oligodeoxynucleotides; Nanoparticles; COPD; Asthma; Inflammation

Introduction

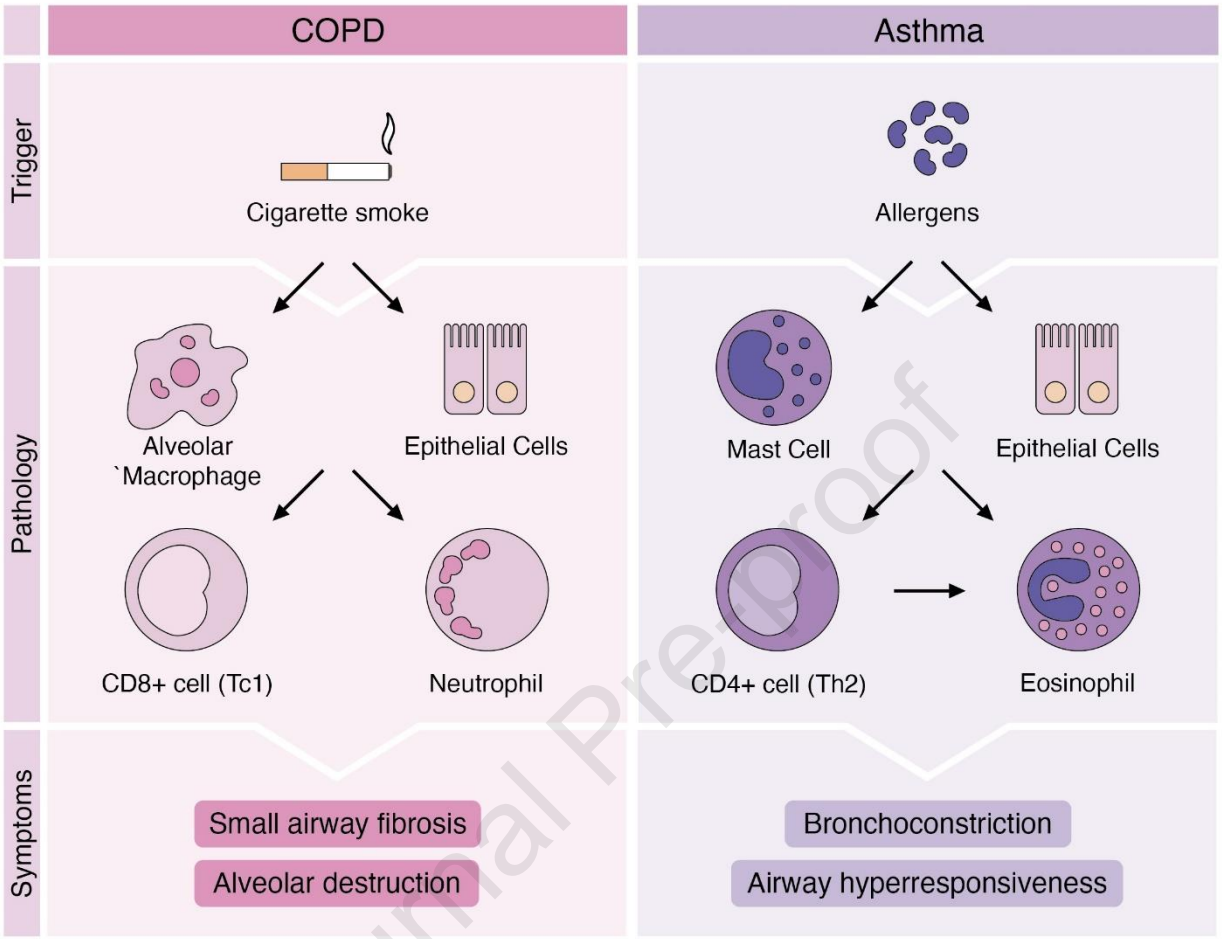
Both Asthma and COPD are respiratory diseases that cannot be cured but only adequately controlled, hence these respiratory diseases are considered a burden. Interestingly, the stark contrast in prevalence between 1990 and 2017 serves to illustrate this. It was estimated that 545 million people worldwide had one or more chronic respiratory disorders, as evidenced by the fact that there has been a 39.8% increase in these conditions from 1990 to 2017 (Labaki & Han, 2020). In Australia, COPD contributed to 43% of the total burden of all respiratory conditions, followed by asthma at 29% and upper respiratory tract infections at 20% (AIHW, 2017). Moreover, according to the Australian Institute of Health and Welfare, in 2011 asthma caused 1.5 per 100,000 deaths, and COPD caused 102 per 100,000 deaths. Generally speaking, an individual's airway tract will be impacted by respiratory illnesses. Asthma can be defined if there is a presence of the following: (i) respiratory symptoms and (ii) excessive variation in the patient's overall lung function. (National Asthma Council Australia, 2014). Asthma is one of the most prevalent respiratory conditions and has a variety of risk factors including air pollution, environmental allergens, aberrant immune reactions, and inherited susceptibilities. It is known that the pathophysiology of asthma is when one or more of the trigger factors listed here causes airway inflammation, which causes the hypersecretion of mucus, muscle airway constriction and bronchial hyperresponsiveness (Sinyor & Perez, 2022). Ultimately, this triggers the narrowing of the respiratory tract, leading to an individual having great difficulty breathing in and out of their nose as they will start coughing, wheezing, and having shortness of breath (Sinyor & Perez, 2022).

Prior to defining COPD, it is important to mention the differential factors between Asthma and COPD. These factors are visually highlighted in Figure 1; the cells that are involved in pulmonary inflammation of asthma include eosinophils, macrophages, mastocytes and CD4+ T cells, whereas for COPD, the cells involved are neutrophils, macrophages, CD8+ & CD4+ T cells, (Cukic *et al.,* 2012). Additionally, there's a difference in the key mediators that play a role in pulmonary inflammation between COPD and Asthma; COPD has Interleukin 8 (IL-8), Interleukin-1β (IL-1β), Interleukin-6 (IL-6), TNFα & Nitric Oxide (NO), (Cukic *et al.,* 2012). An interleukin is a type of cytokine that plays a role in the pathogenesis of the respiratory condition. Whereas mediators of asthma include eotaxin, IL-4, IL-5, IL-13, and NO, (Cukic *et al.,* 2012).

Asthma symptoms usually vary during the days and weeks, as opposed to COPD, where the symptoms are consistently present (AIHW, 2017). Additionally, COPD onset usually occurs in patients that are over the age of 45, and the severity of the symptoms will worsen over a period of time whereas asthma's onset usually occurs earlier in life. Furthermore, chronic risk factors for COPD include cigarette smoking, workplace gases and particulates, and outdoor air pollution. Hence, the pathophysiology of COPD has been identified as trigger factors such as the ones mentioned previously, which will act as the host factor amplifying lung inflammation through the stimulation of inflammatory cells such as T lymphocytes, neutrophils, and macrophages. This ultimately causes the pathological changes in the individual's respiratory tract, leading to symptoms such as constant shortness of breath, a chronic cough and overall chest tightness (MacNee, 2006). Moreover, acute respiratory tract infections (Ferkol & Schraufnagel, 2014) are another risk due to low vaccination rates, poor nutrition, and in particular, overcrowding. However, it is known that upper respiratory tract infections such as Influenza and COVID-19 worsen asthma and COPD symptoms and occasionally exacerbate these diseases. An exacerbation of asthma or COPD can be defined as instances of breathing difficulties in a patient with asthma and increased breathlessness, potentially brought on by rising levels in sputum production and coughing in a patient with COPD, (Viniol & Vogelmeier, 2018). It was determined that respiratory illnesses have had a significant impact on society.

In this review, it is highlighted how current treatments of COPD and asthma are not effective in dealing with the core issue of inflammation. Hence, there is a need to explore new ways of suppressing a key component of inflammation, Nuclear factor κB (NFκB), through the implementation of decoy ODN encapsulated in nanoparticles. Hence, in this context, this review discusses the current state of the research about the use of NFκB decoy ODNs as a potential treatment for inflammation in patients with asthma or COPD.

Figure 1. Visual schematic of the triggers, pathology and symptoms of COPD and Asthma.



Triggers for COPD and asthma are cigarette smoke allergens respectively. The Pathology for COPD and Asthma are macrophage, epithelial, CD8+ cells, neutrophil and Mast cell, Epithelial, CD4+ cells and Eosinophil. Lastly, the symptoms of COPD are small airway fibrosis and alveolar destruction, and the symptoms of Asthma are bronchoconstriction and airway hyperresponsiveness.

Current treatments of Asthma and COPD and their limitations

Asthma and COPD have become a renewed focus of interest, particularly post-COVID-19. Currently, the ways of treating COPD and Asthma are the baseline, daily implementation of various inhaled fixed-dose drugs to enable the individual not to experience the symptoms of chest tightness, wheezing, and shortness of breath. For example, a typical patient with a diagnosis of asthma will receive low, moderate, or high daily doses of inhaled corticosteroids (ICS), which help suppress the inflammation

through the inhibition of inflammatory gene expression and suppressing respiratory inflammatory cells, as well as switching on anti-inflammatory genes (Barnes, 2010). Hence, that same patient will receive low, moderate, or high daily doses of Long-acting Beta2 agonist (LABA). This agent is a bronchodilator hence, its main mode of action is to relax the respiratory muscles and encourage the greater opening of the airways (Newton & Giemycz, 2016). The addition of Long-acting muscarinic antagonist (LAMA) is only necessary if the patient has severe cases of Asthma, also known as stage 4 or 5 of therapy which is illustrated in **Figure 1** (Maselli *et al.,* 2019). A LAMA is also a bronchodilator that blocks bronchoconstriction from occurring by inhibiting acetylcholine binding to Muscarinic receptors (Naji & Gatling, 2022).

Moreover, a COPD patient will receive similar drug therapies such as ICS, LAMA or LABA depending on the stage of their disease (Maselli *et al.,* 2019). The current treatment options for both Asthma and COPD are summarised in **Table 1**, and they are reflective of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines for COPD and the Australian Therapeutic Guidelines (eTG) for Asthma. It is interesting to mention that a study conducted by Miravitlles et al., 2008, underlined that the most common treatment administered to patients with stable COPD is SABA and Asthma. This includes countries worldwide, such as Hong Kong, Spain, and Argentina, (Miravitlles *et al.,* 2008). It is important to note, although these treatment options have been used for decades worldwide, they still have an important limitation that needs to be pointed out, they are treatment options that prevent symptoms from occurring and allow to manage an exacerbation, they do not represent a cure option that fixes the underlying root cause of the Asthma or COPD. Furthermore, there are non-pharmacological management strategies that can be implemented for patients suffering from COPD and asthma. This includes pulmonary rehabilitation which is an eight-week education and exercise program that aims to teach individuals how to manage their condition safely as well as how to exercise without triggering an exacerbation (Hall *et al.,* 2017).

Additionally, tailored specifically to asthma, a patient with severe symptoms can undergo a bronchial thermoplasty. This is a treatment that utilises heat to shrink smooth muscles, which in turn will inhibit the pathophysiology of asthma and therefore decreases asthma symptoms (Hall *et al.,* 2017). Whereas for patients with COPD, a lung volume reduction surgery (LVRS) is an option that will improve the overall

exchange of gases in the aveolar space by making the bronchial muscles narrow more efficiently due to the decreasing hyperinflation that is as a result of a section of the lung being surgically removed (Clini & Ambrosino, 2008).

Table 1. Summarised medications for COPD and Asthma according to the GOLD and ETG guidelines in Australia.

	Asthma		**COPD**
Stage 1	SABA prn	**Stage 1**	SABA prn
Stage 2	Low dose ICS preventor + SABA prn	**Stage 2**	LAMA or LABA
Stage 3	Lose dose ICS + LABA + SABA prn	**Stage 3**	LAMA + LABA
Stage 4	Moderate dose ICS + LABA + SABA prn	**Stage 4**	LAMA + LABA + ICS
Stage 5	High dose ICS + LABA + SABA prn	**Stage 5**	

Additionally, there has been the implementation of other therapies for COPD and asthma that are outside the realm of LAMA, LABA or ICS. An example is represented by monoclonal antibodies, which have been used in the suppression of disease pathogenesis by targeting the inflammatory cytokines that mediate the pathophysiology of asthma and COPD. Examples include Omalizumab, which targets the IgE in Asthma, and Mepolizumab which targets IL-5 receptor in patients with COPD (Maselli *et al.,* 2019). Moreover, currently, a monoclonal antibody which is a particular protein that can connect to certain targets in the body and is created in a lab, called Tezepelumab which targets and inhibits Thymic Stromal lymphopoietin (TSLP) has been placed into the research spotlight (Kardas *et al.,* 2022). It has reportedly resulted in an improvement in lung functions and comparatively better asthma control due to the drug's ability to lower the levels of IgE, IL-5, and IL-13 as well as submucosal eosinophils. However, as underlined by both Kardas *et al.,* (2022) and Maselli *et al.,* (2019), the application of monoclonal antibodies (Mab) has significant limitations, including the fact that the majority of the Mab's are still in clinical trials, and some

patients have not received satisfactory results when it comes to asthma or COPD control and decreasing the number of exacerbations as well.

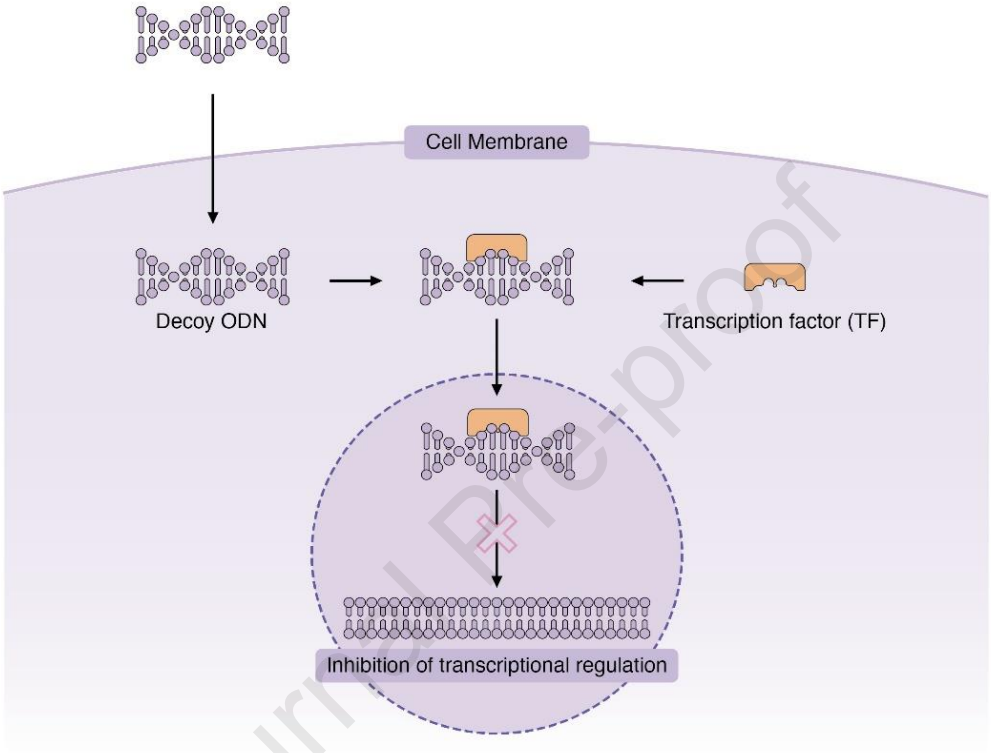
Another viable treatment option involves the employment of phosphodiesterase inhibitors (PDE) for COPD. Roflumilast has been administered at 500 µg a day for patients with COPD (Padda & Tripp, 2022). The mode of action of PDE-4 inhibitors explains why they are implemented, they are known to increase intracellular cyclic adenosine 3', 5'-monophosphate in airway smooth muscle and inflammatory cells to control lung inflammation and induce bronchodilation. (Padda & Tripp, 2022). Hence, this drug has been found effective in patients that have a forced expiratory volume in the first second (FEV1) of less than 50% or have more than two exacerbations a year, which corresponds to stages 3 and 4 of COPD. (Martinez *et al,* 2016). However, the drug is known to be a contraindication in patients that have any form of hypersensitivity, and it has a very narrow therapeutic window, which highlights the necessity of constantly monitoring the drug's serum concentration to give the patient the most effective dose without over-dosing and causing unnecessary side-effects (Phillips, 2020).

Oligodeoxynucleotides: An emerging option in mitigating respiratory diseases

Oligonucleotides (ODN) are known as short single or double-stranded DNA or RNA molecules that have a wide range of applications from research to drug design to genetic testing, (Hecker & Wagner, 2017). Additionally, as highlighted by Jolly *et al.,* (2016) there are six types of oligonucleotides each with distinct features and mechanisms of action. **Table 2** highlights the unique feature of oligonucleotides. It is important to differentiate between oligodeoxynucleotides. Decoy ODNs, in fact, are a subclass of short, artificially made artificially made double-stranded ODNs that mimic a particular transcription factor's DNA binding sequence. By binding a decoy ODN to the specific active transcription factor(s), these transcription factors are eliminated from the pool of active molecules that may bind to their original target sequence onto the genomic DNA, as underlined in Figure 2. (Ahmad *et al.,* 2013). This causes the targeted transcription factors to be selectively functionally inactivated which can lead to the inhibition of certain symptoms such as inflammation. (Ahmad *et al.,* 2013). Hence, by inactivating specific transcription factors decoy ODNs can cause the

modification or blockage of gene expression and that results in the alteration of protein expression as well, (Tehran *et al.,* 2019).

Figure 2. Visual schematic demonstrating of the inhibitory effect when implementing Decoy ODN to regulate transcription



These transcription factors are removed from the pool of active molecules that may bind to their original target sequence on the genomic DNA by attaching a decoy ODN to the particular active transcription factor(s).

Table 2. Summarised table of the different types of oligonucleotides and their distinctive features

	Structure	**Mechanism of Action (MOA)**	**Distinctive feature**
DecoyOligodeoxynucleotides	Known as a DNA that is single-stranded	Acts as a blockade for certain transcription factors	Has the ability to specifically target and inactivate transcription factors
CpG oligonucleotides	Known as a DNA that is Single stranded	Acts as the Stimulant of immune system	Its backbone is comprised of phosphonothioate
Antisense oligonucleotides	Known as a DNA or RNA that is single-stranded	Has a range of MOA included inhibition of transcription and miRNA and the degradation of mRNA	Capable of targeting the cause of development of the disease
Aptamer	Known as a DNA or RNA that is single-stranded	Causing protein function to be inhibited	Has the ability to bind to specific targets
siRNA	Known as a DNA that is double-stranded	Causes the degradation of mRNA	Works on mRNA only

miRNA	Known as a DNA that is double-stranded	Causes transcription to be inhibited due to mRNA degradation,	Works on mRNA only

The implementation of decoy oligodeoxynucleotides is still novel, with a substantial amount of research still necessary to fully grasp how this technology can be fully utilized in treating respiratory diseases. Currently, they have trialled an inhalational treatment option for people suffering from COPD and Asthma and it has been recorded that the use of decoy ODN via the inhalational route has had fewer side effects due to the direct targeting of the necessary transcription factor (Mehta *et al.,* 2022). ODNs have also been implemented in other fields such as breast cancer, where anti-sense oligonucleotides have been shown to increase apoptosis through successful inhibition of cell production (Nguyen *et al.,* 2021). Additional uses of decoy oligodeoxynucleotide include neurodegenerative disorders such as Huntington's disease, where the synthesis of the protein Huntingtin is inhibited by targeting the protein's mRNA (Scoles & Pulst, 2017).

Through both *in vivo* and *in vitro* studies, it has been acknowledged that decoy ODN has high efficacy and has proven to be a powerful tool that can be implemented to inhibit transcription factors (Tehran *et al.,* 2019). Some challenges and limitations are still present whilst utilizing decoy ODN as a tool with a clear therapeutic indication. Firstly, naked ODNs have very poor pharmacokinetic properties due to their increased susceptibility in biological fluids to various enzymes such as exonucleases and endonucleases that will contribute to the rapid degradation of ODN by decreasing their stability and their half-life (Wang *et al.,* 2018).

Additionally, when systemically administered to the patient, ODNs lack tissue specificity (Kannaujiya *et al.,* 2023). The physical characteristics of the ODN itself also contribute to its limitations. ODNs are very large molecules that have a significantly high density of negative charge. Those factors, in turn make the cellular uptake of the ODN difficult. Hence ODNs have poor cellular permeability (Hecker & Wagner., 2017). Additionally, ODNs can be easily damaged upon endocytosis (Juliano & Carver, 2015).

Hence, when the naked ODN is being uptaken into a cell the present lysosomes will cause the immediate breakdown of the ODN preventing its entrance into the nucleus of the cell where it exerts its effects (Mehta *et al,* 2022). Because of this, it is necessary to create effective delivery mechanisms for ODN decoy delivery to the intended location of the action such as the inhibition of NFκB to suppress inflammatory responses.

NFκB: a pivotal transcription factor regulating inflammation and immunity

Considering how successful decoy ODN are in inhibiting transcription factors making them a suitable tool to inhibit Nuclear Factor- κB. NFκB is defined as a vital transcription factor that plays a pivotal role in numerous biological processes (Park & Hong, 2016). These biological processes include the cell's ability to grow, survive and to properly develop. However, a key feature of NFκB is that it also plays a role in how the immune system responds, and it has been defined as the master regulator of inflammation, particularly in organs such as the lungs. (Park & Hong, 2016). Inflammation is defined as the body's protective response to tissue damage and to invasion of the host by a foreign organism. Hence, if such action occurs, the body will force an influx of immune cells and plasma-based proteins to the site of the infection (Park & Hong, 2016). It is known that not only does NFκB regulate inflammation it also acts as the causative factor of the activation, and therefore the differentiation of inflammatory T cells (Lawrence, 2009).

Moreover, there are five related subtypes of NFκB. These include NFκB1, NFκB2, RelA, RelB and C-Rel (Liu *et al.,* 2017) Hence, the activation of NFκB to result with inflammation or an immune response consists of two major pathways known as canonical and non-canonical; this is illustrated in Figure 3, (Liu *et al.,* 2017). To induce the canonical pathway, a variety of stimuli are needed in the form of B-cell, T-cell, ligands, and cytokine receptors (Zhang & Sun, 2015). The inducible degradation of I-B, which is caused by its site-specific phosphorylation by a multi-subunit I-B kinase (IKK) complex, is the main mechanism for canonical NFκB activation (Karin *et al.,* 2000). Whereas a non-canonical pathway can only be induced if there is a specific type of stimuli such as ligands and receptors from Tumor necrosis factor receptor

(TNFR) (Zhang & Sun, 2015). It is important to note that the non-canonical pathway is heavily reliant on the NFκB2 precursor protein p100 for activation of the pathway as opposed to involving the degradation of IκBa, (Sun, 2011). Additionally, the initiation of the p100 protein for processing occurs through the process of p100 phosphorylation due to the induction of NFκB inducing kinase (NIK) (Sun, 2011).

Hence, with this information there needs to be a differentiating factor between these two pathways. The functional difference is that the non-canonical pathway is only primarily involved in cooperating with the canonical pathway as a supplementing signalling axis only to help regulate specific pathways of the immune system (Sun, 2011) whereas the canonical pathway is functionally involved in the majority of the immune pathways, (Zhang & Sun, 2015).

As clearly illustrated in the paragraph above, NFκB is a pivotal regulator of inflammation, therefore, the inhibition of this transcription factor through the implementation of decoy-ODNs can potentially become a viable solution in dealing with a wide range of diseases that have an inflammatory component. Hence, due to NFκB being a transcription factor that has a pleiotropic effect on a variety of beneficial physiological functions, including cell and system homoeostasis, it is vital to mention that the implementation of nanoparticles to target NFκB can lead to off-target inhibition. This can lead to implications such as the inhibition of NFκB in other organs that are not part of the respiratory tract such as the liver, kidney, and heart, and can cause the suppression of physiological inflammation or immune responses that are vital for the health of the individual, (Locke & Anderson, 2011). However, to minimize those associated risks from occurring, three preventative measures can be put into place. First of all, it is possible to utilize formulations that specifically target inflammation in the respiratory system, including powders and other formulations that can be administered to the patient *via* inhalation instead of being taken orally, (Kumbhar *et al.,* 2021). In this context, the lungs represent a privileged site for the tissue-specific administration of therapeutics (Dhanjal *et al.,* 2022). Another possibility, which could be used in association with inhalational delivery, is represented by the targeted delivery of therapeutic moieties by virtue of the functionalization of drug delivery systems with antibodies targeting antigens expressed only in specific cell types within the lungs, (Wu *et al.,* 2015). Finally, a viable way to specifically target inflammation within the lungs includes the exploitation of the extracellular acidification that occurs

in the microenvironment of inflamed tissues, (Rajamaki *et al.,* 2013) through the use of acid-sensitive drug delivery systems, capable of releasing the therapeutic payload only in acidic environments, (Cohen *et al.,* 2011). These three preventative measures have great potential to substantially minimize the extent of off-target NF-kB inhibition, limiting the NF-kB inhibitory activity only in the organs or tissue where it is needed and, therefore, minimizing related adverse effects.

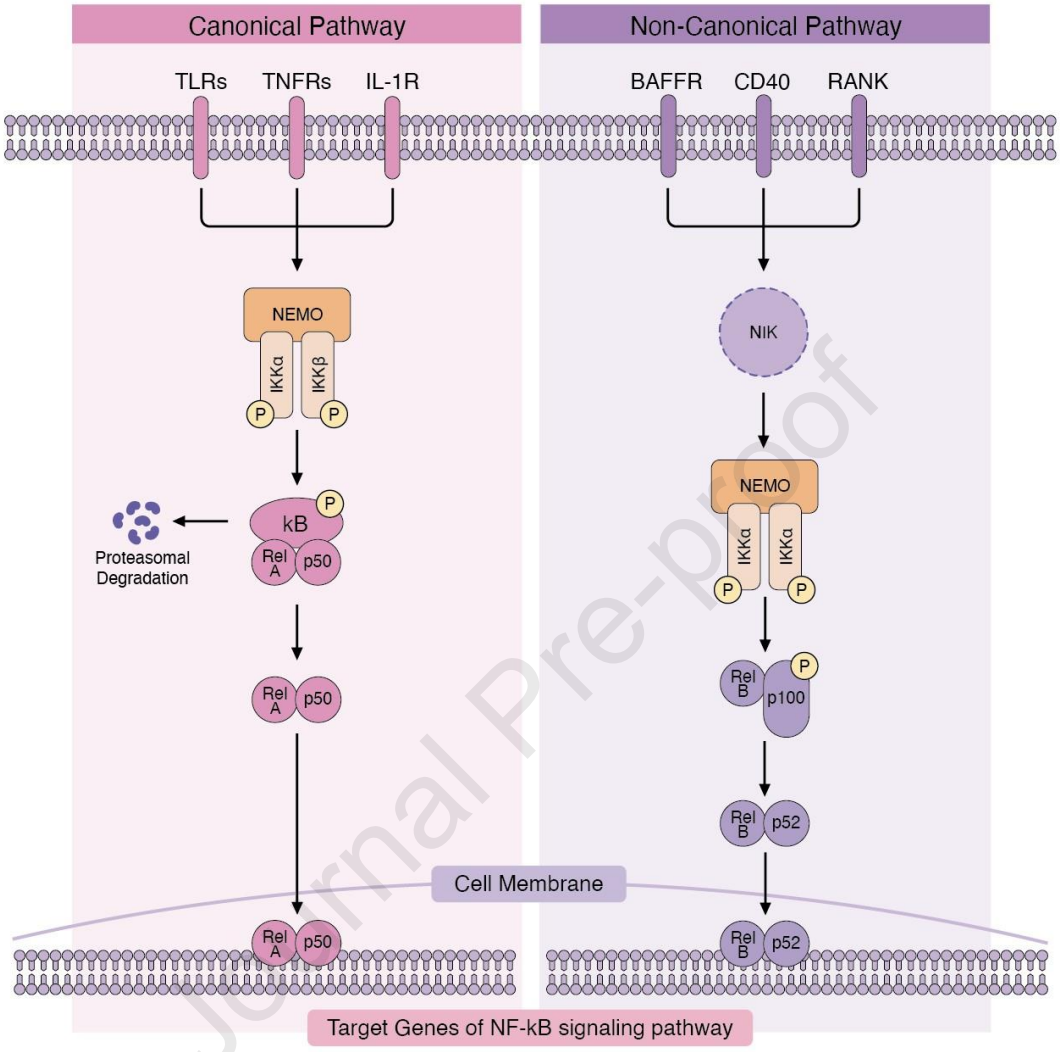
Examples of diseases having an inflammatory component include chronic respiratory conditions such as Asthma and COPD, whereby the upregulation of NFκB clearly contributes to the disease's pathogenesis and progression (Edwards *et al.,* 2009). Hence, there has been a significant increase in research and desire to understand what components can be implemented to target the NFκB signalling pathway as an option to treat these respiratory diseases. When it comes to patients with chronic asthma, NFκB has been reportedly overexpressed in participants bronchial tissue that had moderate or severe Asthma as opposed to the patients that did not (Hart *et al.,* 1998). Additionally, in the same type of patients, it has been noted that granulocyte-macrophage and colony-stimulating factors, which are all controlled by NFκB and play a part in chronic inflammation were all overexpressed, (Gagliadro, *et al.,* 2003). This enables the conclusion to be made that the constant NFκB activation results in patients having severe asthma due to chronic inflammation.

Smokers and COPD patients have been discovered to have elevated expression of the p65 protein, one of the primary elements of NFκB, notably in the bronchial epithelia. (Di Stefano *et al.,* 2002). Additionally, it is shown that the protein levels of p65 are substantially and consistently linked to airflow restrictions, suggesting that NFκB could play a crucial part in the aetiology of COPD (Di Stefano *et al.,* 2002). Another study that investigated the gene expression of NFκB elements in patients' whole blood found that patients with COPD had greater amounts of inflammatory molecules (IL-1, IL-8, and Cyclo-oxygenase enzyme 2 (COX-2), which are induced in inflammation, than those in the healthy group and overexpressed NF-B family genes (Zhou *et al.,* 2018). Lastly, subunits of p50 and p65 which are categorised as vital subunits of the NFκB signalling pathway are elevated in COPD patients and there has been a link formed between COPD patients and controls in terms of the recorded apoptosis numbers level of neutrophils, where COPD patients have reduced apoptosis of neutrophils in their sputum, (Brown *et al.,* 2008). Hence, all of these discussed

studies underline a common thread that chronic, dysregulated inflammation is frequently observed among individuals with COPD, and this is mediated at least partly by NFκB.

Besides the pivotal role that NFκB in regulating inflammatory responses. NFκB also plays a fundamental role as a mediator of virtually all cancer hallmarks where its activation causes cancer cell proliferation, and therefore cancer cell survival as well as other elements such as metastasis and cancer cell infiltration and therefore migration (Taniguchi & Karin, 2018). Hence, it has been found through various studies that NFκB is clearly involved in the pathogenesis of cancer, where the over-activation of NFκB and therefore the stimulation of the signalling pathway has been found in countless tumour tissues (Xia *et al.,* 2018). Hence, this underlines the multifaceted potential of NFκB inhibition as strategies to combat not only inflammatory diseases but also cancer.

Figure 3. Visual representation illustrating signalling pathways that activate NFκB



The major signalling pathways includes the Canonical and Non-canonical with highlights of the genes and proteins that have a cascading effect in activating other proteins for NFκB stimulation.

Application of nanoparticles in respiratory diseases

Nanoparticles can be a useful tool that can be employed for targeted drug delivery in order to overcome the above-mentioned limitations of nucleic acid therapies (Mitchell *et al.,* 2020). It is important to mention that there are certain preferential factors that

underline the advantages of using nanoparticles as drug delivery systems. These includes prolonged drug release, targeted delivery, patient compliance as well as increased effectiveness of commercial medications, overall lower toxicity, and low immunogenicity, (De Menezes *et al.,* 2020). As mentioned above, due to ODN and their clear pharmacokinetic and pharmacodynamic limitations, the utilization of nanoparticles' biggest positive characteristic of their minute size will allow for the transportation and then the sustained release of encapsulated ODN to any part of the human body, Kannaujiya *et al.,* 2023). Furthermore, utilising nanocarriers has several benefits, including improving the bioavailability and stability of the drug being implemented. This is particularly important for biological therapies as nanocarriers are advantageous to protect them from physiological processes that may cause their breakdown and deactivation (Mikušová & Mikuš *et al.,* 2021). When it comes to the implementation of nanoparticles in a respiratory setting the size and shape of the nanoparticle plays a pivotal in role whether the drug will be deposited in the bronchial airways, as the smaller the molecule the greater the chance of pulmonary deposition, (Löndahl, *et al.,* 2014). Moreover, historically speaking there has been the implementation of a number of different types of nanoparticles to successfully deliver ODN to suppress NFκB. These include spermine-functionalized acetalated dextran nanoparticles (Kannaujiya *et al.,* 2023), silver citrate nanoparticles, (Abdellatif et al., 2020), and nanogold particles (Khan & Khan, 2018).

In terms of the mechanism of action (MOA) by which nanoparticles enter cells, it has been observed that poly-lactide-co-glycolide (PLGA) nanoparticles have an impact on lung epithelial cells by widening the cell's tight junctions, (Shipuvona *et al.,* 2021). Hence, this enables for the PLGA nanoparticle and the contents inside of it to be absorbed through those openings, (Shipuvona *et al.,* 2021). It is crucial to remark that there are other studies that have suggested that the MOA through which nanoparticles enter cells is through transcytosis, a process whereby macromolecules are transported by vesicles from one end of a cell to another (Garcia-Castillo *et al.,* 2017). Other studies have underlined that nanoparticles are transported into the cell *via* a process called endocytosis, which is a process whereby the nanoparticle binds onto the plasma membrane or in specific case of lung cancer, the particle will bind to a folate receptor of the cell (Manzanares & Cena *et al.,* 2020). Nanoparticles can be broken down into three broad categories: lipid-based, polymeric, and inorganic

(Joudeh & Linke, 2022). Hence, throughout multiple studies, there is evidence that the utilization of nanoparticles for the delivery of ODN to remote regions of the pulmonary arteries results in the prevention of NFκB activation haltering the process of inflammation (Kimura *et al.,* 2018). However, it is important to note that nanoparticles have associated limitations. This includes using itself as a drug carrier, which may reduce the toxicity associated with the incorporated drug. Likewise, nanoparticles tend to release drugs gradually, (Omlor *et al,* 2015). Therefore, for cancer research studies, the concentration potentially will not be high enough to destroy the tumor. It is important to mention that nanoparticles have also been utilized as a delivery system in other respiratory diseases such as pulmonary fibrosis, tuberculosis, and lung cancers (Omlor *et al,* 2015).

NFκB inhibiting decoy ODNs used for the treatment of respiratory diseases

The first study in which decoy ODN to inhibit NFκB were used to prevent LPS-induced inflammation in the airways in rats was performed by De Stefano *et al.,* 2013. In this *in vivo* study, the animals were divided into two groups, group one was administered the naked decoy-ODN, and the other group was administered decoy ODN coated with a lipophilic protein nanoparticle (LPP). Afterwards, the two types of decoys ODN were released into the stimulated interstitial lung fluids of the participants of the experiment. The results of the study underlined the following aspects: the encapsulation of NFκB decoy ODN with LPP was able to inhibit NFκB transcriptional activity for up to 72 hours after LPS exposure, along with the associated increased in IL-6 and IL-8 gene expression and neutrophil recruitment. It is important to mention that the naked decoy ODN was able to also express similar effects compared to the LPP decoy-ODN, but this effect did not last over a six-hour period. In another study, Desmet *et al.,* (2004) also experimented with selectively blocking NFκB activity in respiratory immune cells that are involved with asthma and its effector phase. This study was categorised as an *in vivo* study with the utilisation of mice. Generally, the mice were first induced with an allergic respiratory disease through instillation of the allergen ovalbumin (OVA) in the airways and then administered with NFκB decoy ODN through intratracheal instillation at 28 days and 60 days after stimulation. Post instillation various tests were performed including cytology, cytokine assays and lung histology. Hence, the results

underlined the following firstly, the airway immune cells were effectively unclearly transfected when animals with OVA sensitisation were given NFκB decoy oligodeoxynucleotides intravenously, while epithelial lung cells and draining lymph node cells were not. This was further supported by the observation that OVA-induced respiratory tract NFκB expression was stopped upon administration of the ODN. Suppression of NFκB substantially lowered local mucus production, IL-5, IL-13, eotaxin, airway hyperresponsiveness, and allergic lung inflammation. IL-4 and OVA-specific IgE and IgG1 production were largely unaffected by this treatment. A study performed by Bezzerri *et al.,* (2008) also implemented decoy ODN in bronchial cells to inhibit the transcription of IL-8 by blocking NFκB. The focus of the paper was the exuberant increase in neutrophils in the bronchi of patients who suffer from chronic respiratory inflammation due to cystic fibrosis (CF). Hence, to reduce the overproduction of neutrophils an *in vitro* study was conducted by applying a decoy ODN to block NFκB and evaluating the expression of a variety of proteins that are involved in the promotion or upregulation of inflammatory responses such neutrophil type chemokines known as GRO-gamma and IL-8. That particular inflammatory response was caused by a common bacterial infection that occurs in patients with CF, known as *Pseudomonas aeruginosa.* The study's overarching results highlighted the following findings: evidently, the IL8 stimulating NFκB decoy ODN only was able to moderately inhibit the production of GRO-gamma and IL-8.

Li *et al.,* (2009) conducted an *in vivo* study utilising mice exposed to cigarette smoke in the long-term as a model of COPD. The aim of the study was to assess whether increased levels of lung inflammation, and respiratory dysfunction and any lung pathological modifications could be decreased or reversed by the treatment with NFκB decoy ODN. Hence, to deal with the lung inflammation, the researchers intratracheally administered NFκB decoy ODN. Therefore, the focus of the study was the levels of transfected alveolar macrophages. The results of the study enabled to conclude the following: compared with saline treated control mice, in the mice treated with decoy ODN the levels of macrophage inflammatory protein (MIP) were substantially reduced. Additionally, upon comparison of the same two groups of mice, the peak expiratory flow (PEF) study demonstrated that NFκB decoy ODN transfection substantially affected PEF, and bronchoalveolar lavage cytology revealed reduced macrophage infiltration upon treatment with NFκB decoy ODN. Lastly, it is important to point out

that the implementation of decoy ODN did not prevent any form of pathological changes in the respiratory tract of smoke-affected mice. In a successive study, Miyake *et al.,* (2017) aimed at assessing whether the inhibition of NFκB through the utilisation of standardised decoy ODN and simultaneous stimulation of signal transducer and activator of transcription 6 (STAT6) through the utilisation of chimeric decoy ODN was effective as a potential strategy for the prevention of exacerbation in asthma. To test their hypothesis, the authors used *in vivo* models of asthma obtained through the sensitisation of mice with methacholine and administered the ODNs intrathecally three days after sensitization. Additionally, a unique aspect of this study is the implementation of fluorescent dyes onto the transferred chimeric decoy ODN, which enabled researchers to visually inspect their positioning in the lungs, especially within macrophages and lymphocytes. The results of the study underlined the following points: the application of chimeric decoy ODNs or NFκB decoy ODNs prevented methacholine-induced hyperresponsiveness of the airways, with the former having a significantly greater effect compared to the latter. Additionally, it is important to note that only the introduction of chimeric decoy oligodeoxynucleotides had the capacity to have inhibitory effects on the production of IL-5, IL-13, and histamine.

Grisesenbach *et al.,* (2002) conducted an *in vivo* study where fluorescein labelled NFκB decoy ODN was tested on a model of CF obtained by exposing mice to bleomycin. Bleomycin is an antibiotic that is actually known to be poisonous to cells. It has been utilised to kill cells that are dividing at a rapid pace, especially in situations such as Hodgkin's lymphoma and cervical/ovarian cancer. It is important to note that decoy ODN was deposited into the cytoplasm of transfected conducted respiratory epithelial cells. These were the following findings of the paper: upon administration of decoy ODN at a dosage of 80 µg per mouse, the decoy was not detected in the nuclei of the cells that have been transfected, however they were visually seen in the cytoplasm within those same cells. Upon increasing the dosage of the decoy ODN to 500 µg per test subject, the decoy ODN failed to penetrate within the nucleus of the cells. Hence, due to the lack of nucleus penetration, there was no significant difference in the levels of IL-6 excretion in broncho-alveolar lavage fluid (BALF) and lung homogenates. All other cellular parameters that were tested within the study were not significantly different between the mice that were used as a control and the mice that

were administered bleomycin 5 days prior. Thus, it highlights that the implementation of the decoy ODN had no effect on inflammation.

As previously mentioned in this paper, NFκB plays a pivotal role not only as a mediator of inflammation but also in the development of cancer. As underlined by Kannaujiya et al., (2023), since its activation encourages phenomena like tumour cell proliferation and survival, NF-κB is an essential contributing factor to many cancer hallmarks. It is also thought to be the primary link between ongoing infections, persistent inflammation, and a higher probability of acquiring cancer (Gao et al., 2008). Kim et al., (2009) conducted an *in vivo* study using mice as their test subjects. The study aimed at looking how early acute lung injuries (ALI) that have been caused by lipopolysaccharide (LPS) would be affected by the intratracheal administration of NFκB Decoy ODN. Six hours after administering LPS, all inflammatory indicators such as NFκB, IL-6, and tumour necrosis factor (TNF)-alpha were at their highest peak. Hence, the results underlined the following: NFκB decoy ODN treatment significantly reduced the LPS-induced activity of NFκB in lung tissues. However, the histopathologic alterations defined by the ALI score and the indications of LPS-induced direct ALI, such as the presence of TNF-α and interleukin-6 in BALF, as well as myeloperoxidase activity in BALF, were not improved. This underlines that, overall, NFκB decoy ODN has actual little to no effect on LPS-induced models of ALI. The eighth paper written by Akeda et al., (2011) conducted a study to comprehend whether it is possible to block out metastasis in the lung through the process of transfection of decoy ODN onto NFκB within murine osteosarcoma cells that are part of the LM8 line of cells that had been cultured as a spheroid using beads made of alginate. The results of the paper underlined the following: the activation of NFκB signalling was consequently supressed due to the implementation of naked decoy ODN within LM8 cells. The secondary results of the study showed that the utilisation of decoy ODN did not affect the growth of the tumor itself, but it did however have an impact on successfully decreasing the levels and expression of intercellular adhesion molecule 1 (ICAM-1) and vascular endothelial growth factor (VEGF). The collation of different studies carried out with various oligonucleotides are collated in Table 3.

Table 3: Various Oligonucleotides driven *in-vitro* and *in-vivo* respiratory studies

Focused Respiratory condition	Type of study	Main outcomes of the study	Referenced article
Airway inflammation that occurs in CF patients	*In vitro*	IL8 stimulating NFκB decoy ODN only were able to moderately inhibit the production of GRO-gamma and IL-8.	Bezzerri *et al.,* 2008
Suppression of metastasis in lung tissue	*In vitro*	Decoy ODN did not affect the growth of the tumor itself, but successfully decreased the levels and expression of intercellular adhesion molecule 1 (CAM-1) and a vascular endothelial growth factor (VEGF).	Akeda *et al.,* 2011
Generalised airway inflammation	*In vivo*	The encapsulation of NFκB decoy ODN with LPP was able to inhibit NFκB transcriptional activity for up to 72 hours after LPS exposure, along with the associated elevated in IL-6 and IL-8 gene expression and neutrophil recruitment.	De Stefano *et al.,* 2013

Airway inflammation caused by Asthma	*In vivo*	OVA-induced respiratory tract NFκB expression was stopped upon administration of the NFκB decoy ODN. Suppression of NFκB substantially lowered local mucus production, IL-5, IL-13, eotaxin, airway hyperresponsiveness, and allergic lung inflammation	Desmet *et al.,* 2004
Airway inflammation caused by tobacco induced COPD	*In vivo*	NFκB decoy ODN transfection substantially affected PEF, and bronchoalveolar lavage cytology revealed reduced macrophage infiltration upon treatment with NFκB decoy ODN	Li *et al.,* 2009
Airway inflammation caused by Asthma	*In vivo*	The application of NFκB decoy ODN prevented methacholine-induced hyperresponsiveness in the airways.	Miyake *et al.,* 2017
Airway inflammation that occurs in CF patients	*In vivo*	Due to the lack of nucleus penetration, there was no difference in the levels of interleukin 6 excretion in Broncho-alveolar lavage fluid (BALF) and lung homogenates and all other cellular parameters that were tested within the	Grisesenbach *et al.,* 2002

		study between the mice that were used as a control and the NFκB decoy ODN-treated mice.	
Airway inflammation caused by acute lung injuries due LPS	*In vivo*	NFκB decoy ODN treatment significantly reduced the activity of NFκB in lung tissues. However, the histopathologic alterations defined by the ALI score and the indications of LPS-induced direct ALI, such as the presence of tumour necrosis factor and interleukin-6 in BALF and myeloperoxidase activity in BALF, were not improved.	Kim *et al.,* 2009

Conclusion and Future Prospects

For a long time, chronic respiratory illnesses like COPD and asthma have been a burden on society concerning the physical impact it has on individuals with the disease. This is due to the harsh symptoms such as shortness of breath, cough and wheeze that patient affected by the disease experience daily, (MacNee, 2006). It is important to mention that these diseases cause an important economic and healthcare burden that the governments must account for. Patients with asthma or COPD will most likely require daily use of preventative medicines. These are accessible in the form of mono or multi-drug therapy such as SABA, LABA, ICS & LAMA, (Australian Therapeutic Guidelines (eTG)). Due to these medications only acting as a preventor or a reliver therapies the patients' disease state is only being maintained but not cured. As a result, there has been substantial research over the years to comprehend what

triggers inflammation and how to suppress the inflammation from occurring. For instance, it's certain that the transcription factor NFκB plays a crucial part in the causation of chronic inflammation. NFκB can be called the master regulator of inflammation due to its pivotal role in the immune response, and there are five main sub-types of NFκB that exert partially overlapping functions. (Liu et al., 2017). Additionally, it has been established that there are two major NFκB signalling pathways, known as canonical and non-canonical, that mediate this inflammatory response, (Liu et al., 2017). Numerous research studies have looked into how to prevent the stimulation of NFκB through the implementation of various strategies that could inhibit the inflammatory response of NFκB. As of current, the most novel technique that has been utilized in *in vivo* and *in vitro* studies involves the use of decoy ODNs. Decoy ODNs are synthetic, short, single-stranded DNA fragments that imitate the consensus binding site of a particular transcription factor, (Ahmad et al., 2013). As mentioned in the present manuscript, there are drawbacks to using decoy oligodeoxynucleotides such as their timely destruction by intracellular nucleases and their lack of tissue selectivity when being administered to a specific part of the human body, (Juliano & Carver, 2015).

Therefore, with these drawbacks currently affecting the clinical application of decoy ODNs, there is an urgent need to implement nanoparticles for targeted drug delivery. The application of nanoparticles would provide positive characteristics due, for example, to their minute size which will enable the transportation and then the sustained release of encapsulated ODNs to any part of the human body. Other positive characteristics of nanoparticles includes the protection of ODN from degradation and the possibility to achieve tissue-specificity, (Mitchell et al., 2020).

As of current, it has been clear that by 2016 three FDA-approved drugs based on ODNs: Formiversen, Pegatanib, and Mipomersen, (Mehta *et al.,* 2022). However, these ODNs are administered as naked molecules, not encapsulated in nanoparticle systems (Mehta *et al.,* 2022). When it comes to the future perspectives of the implementation of nanoparticles as a drug delivery system, targeting NFκB to reduce inflammation in chronic respiratory diseases such as COPD and Asthma would enable patients to have a tool to combat those respiratory diseases effectively. Hence, this decreases the chance of exacerbations of the disease. Additionally, there is still a need

for further research as to how to find the perfect balance between targeting disease-causing factors such as inflammation yet not inhibiting other normal cellular responses from occurring. If there's success in the nanoparticle targeting technique, it can be then implemented in other parts of the body to target inflammation. Hence, as a concluding remark, as of current the ways of dealing with inflammation in respiratory diseases such as Asthma and COPD are all short-term solutions that are aimed at preventing the exacerbations from occurring and at relieving the diseases symptoms. Hence, the utilisation of decoy ODN to inhibit NFκB to prevent inflammatory process could be a solution to aid in the management of those respiratory diseases. In this context, the application of nanoparticle-based systems to improve the delivery of such constructs is advantageous as it allows to overcome many of the main barriers currently limiting the application of ODNs in the clinic.

Conflict of 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.

Abbreviation list

Acute lung injuries (ALI)

Ovalbumin (OVA)

Broncho-alveolar lavage fluid (BALF)

Chronic Obstructive Pulmonary Disease (COPD)

Cyclo-oxygenase enzyme 2 (COX-2)

Cystic fibrosis (CF)

Inhaled corticosteroids (ICS)

Intercellular adhesion molecule 1 (ICAM-1)

Tumor necrosis factor-alpha (TNF$-\alpha$)

Interleukin 8 (IL-8)

Interleukin-1β (IL-1β)

Interleukin-6 (IL-6)

Lipophilic protein nanoparticle (LPP).

Long-acting Beta2 agonist (LABA)

Long-acting muscarinic antagonist (LAMA)

Lung volume reduction surgery (LVRS)

Macrophage inflammatory protein (MIP)

Nitric Oxide (NO)

Nuclear factor κB (NFκB)

NFκB inducing kinase (NIK)

Oligonucleotides (ODN)

Peak expiratory flow (PEF)

Poly-lactide-co-glycolide (PLGA)

Signal transducer and activator of transcription 6 (STAT6)

Vascular endothelial growth factor (VEGF)

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- Inflammation and oxidative stress are key processes in chronic respiratory diseases
- NFkB is one of the principal transcription factors regulating these processes
- Blocking the function of NFkB is a viable strategy for counteracting inflammation
- Decoy oligonucleotides are novel tools to block transcription factors such as NFkB
- Using nanoparticles to deliver decoy oligos overcomes their inherent limitations

The authors of the literature review, ‘Emerging Applications and Prospects of NFκB Decoy Oligodeoxynucleotides in Managing Respiratory Diseases’ have no conflicts of interest to declare.