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Alternative approaches for the treatment of Asthma and COPD: Focus on Cell-based therapies, Epigenetics, and Gene silencing approaches

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Abstract

Despite many organized health initiatives and critically acclaimed guidelines for proper management of asthma therapy, there is still a large population of severe asthmatics having an uncontrolled disease. Severe persistent asthma, characterized by chronic airway inflammation, increased eosinophils and serum IgE is currently managed by using inhaled corticosteroids. It is quite challenging to get the best treatment guidelines for bronchial asthma in severe asthmatics, particularly in the presence of steroid resistance and the non-responsiveness to β-agonists. For that purpose, other methodologies are required to reverse the uncontrolled airway remodeling in steroid-resistant severe asthma. These advanced alternative approaches should be able to treat asthma symptoms and to improve the inflammatory conditions underlying characteristic pathological features of asthma. The current review focuses and summarizes the alternative approaches used in severe asthma patients. Agents targeting inflammatory cytokines, phosphodiesterase inhibitors, antibodies, oligonucleotides, stem cells, and target drug delivery using gene silencing, offer promise in treating severe asthma.

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Introduction

Asthma

Asthma is a chronic inflammatory disorder of the airways with a high global prevalence, affecting over 400 million people worldwide [1]. Airway obstruction and airway hyper-responsiveness are characteristic features of asthma, caused by multiple inflammatory reactions that induce numerous episodes of breathlessness and wheezing [2]. Essential inflammatory cells (activated Th2 lymphocytes, B cells, infiltrating eosinophils, degranulated mast cells, basophils), inflammatory mediators (IgE), cytokines, histamines, leukotrienes, and prostanoids) and structural cells of the airways (epithelial, airway smooth muscle, endothelial cells, and fibroblasts) play a vital role in the pathophysiology of asthma [3,4]. Of these, the T cells control the regulation of the inflammatory reactions accountable for the pathophysiology of asthma. Th-2 mediated responses are primarily active during the intermittent stages of asthma, while Th-1 mediated responses play a significant role in the severe advanced stages of asthma. Increased number of Th2 cells in the asthmatic airways induces the release of specific cytokines, interleukin IL-4, IL-5, IL-9, and IL-13, thereby stimulating eosinophilic inflammation and production of IgE cells [5]. These pathophysiological reactions are usually triggered by a variety of factors, including allergen exposure, seasonal variations, viral respiratory infections, or exercise intolerance [1]. The airway remodeling in asthma is proportional to the severity of the disease. It influences the structural and functional integrity of the large and small airways causing loss of epithelial cell integrity, fibrosis, goblet cell hyperplasia, and increased smooth muscle cell mass and airway vascularity [3-7].

Chronic Obstructive Pulmonary Diseases (COPD)

COPD is defined by persistent and irreversible airflow limitation, which is usually progressive and associated with abnormal inflammatory responses in the airways and lung to noxious particles or gases [2]. It has been estimated that COPD in 2030 will be the third foremost cause of mortalities worldwide [8,9]. The chronic airflow limitation in COPD may induce emphysema due to parenchymal tissue destruction and small airway fibrosis [2]. These structural changes and underlying inflammatory responses lead to loss of alveolar connections to small airways and decrease elastic lung recoil function. Inflammatory reactions in COPD are driven by a combination of macrophages, neutrophils, CD8+ T lymphocytes, dendritic cells, and B lymphocytes with neutrophils and B lymphocytes predominating in severe COPD cases [10,11]. Additional structural changes in COPD include an increased number of goblet cells and mucus hypersecretion, bronchiolar thickening, fibrosis, luminal inflammatory exudate, and obstructive bronchiolitis. The irreversible damage of respiratory bronchioles, and gas-exchanging air spaces (alveolar ducts, alveolar sacs, and alveoli) decreases the surface area of respiratory membrane available for gas transfer consequently reducing the amount of gases that can be transferred across in a given time, resulting in hypoxemia and hypercapnia [2,12]. Exacerbations constitute a further development of the inflammatory response in the airways of COPD subjects and may be activated by infection with microbes or by environmental impurities [13]. During COPD exacerbation, there is an increased inflation and air trapping, with a decreased rate of exhalation, thereby resulting in enhanced dyspnea [14]. There is also a deterioration of the ventilation-perfusion process, resulting in acute hypoxemia [2].

Role of Corticosteroids in Asthma and COPD

Corticosteroids are considered to be the most potent and effective anti-inflammatory medications currently available for the symptomatic control and maintenance of atopic or non-atopic asthma, as recommended by GINA guidelines [1]. Corticosteroids act by blocking the late-phase response to allergens, reduce airway hyper-responsiveness, and prevent inflammatory cell infiltration. Inhaled corticosteroids (ICS) are particularly beneficial in subjects with mild or moderate asthma [15]. ICS reduce the severity of symptoms, improve lung function, and decrease bronchial hyper-responsiveness (BHR), exacerbations, and airway wall remodeling. Besides, they also mitigate bronchial-epithelium abnormalities, bronchial inflammation, and inflammatory cell infiltration [16]. Corticosteroids exert their action through the cytosolic glucocorticoid receptor, which, after activation, is translocated into the nucleus where it either drives the expression of anti-inflammatory genes or limits the activity of nuclear factor kappaB (NF-κB), activator protein 1 (AP-1) or mitogen-activated protein kinase MAP kinase. A failure to respond to corticosteroid therapy may be caused by decreased expression and function of glucocorticoid receptors or elevated activation of inflammatory pathways [17]. ICS are first-line therapy for long-term control of persistent asthma, short courses of oral systemic corticosteroids are often used to gain prompt control of the disease when initiating long-term treatment for severe persistent asthma.

Bronchodilators are the foundation for the COPD treatment. The beta-2 (β2) adrenergic receptors located on the periphery of smooth muscle cells inside the bronchioles are directly activated by β2-agonists, thereby initiating changes in biochemical pathways resulting in an increased production of the cyclic adenosine monophosphate (cAMP) within cells. Elevated levels of cAMP trigger bronchodilation, whereas the reduced levels may result in bronchoconstriction in bronchioles. Activation of β2-adrenergic receptors with β2-agonists leads to smooth muscle relaxation, and bronchodilation [2,18]. Short-acting β2-agonists (e.g., salbutamol) are useful for 4 to 6 hours and are usually used "on-demand" by COPD patients to relieve acute symptoms. Long-acting β2-agonists (LABAs, e.g., formoterol and salmeterol) result in bronchodilation for at least 12 hours after a single dose. Long-acting bronchodilators are usually recommended for patients with moderate COPD. ICS are introduced later in the treatment plan, for subjects with acute COPD and medical history of relapses, and at higher doses, than prescribed for asthma [2]. Both the Gold Standard/International Guidelines treatment (GOLD) and the European Respiratory Society

(ERS)/American Thoracic Society (ATS) guidelines recommend the use of oral or parenteral corticosteroids for the treatment of exacerbations [19]. Data shows that these agents reduce the likelihood of treatment failure, shorten hospital stays, improve forced expiratory volume in 1 second (FEV1), ameliorate breathlessness, and balance blood gases [20]. After inhalation, the corticosteroids stimulate the glucocorticoid receptors, and reduce the expression of genes involved in inflammatory responses. Although ICS is recommended in patients with Stage III or Stage IV COPD, but if the patient experience repeated exacerbations, they do not appear to have any effect on inflammation in COPD. However, an improvement in overall health status has been reported [21].

Refractory Asthma and COPD

ICS are widely used in controlling the inflammatory reactions involved in airway hyper-responsiveness and are also recommended in patients with acute and long-lasting asthma. However, patients with severe persistent asthma continue to remain symptomatic, albeit the administration of high-dose ICS and LABA, and this places considerable restrictions on the management of poorly controlled asthma. Increasing the prescribed dose of ICS can result in systemic adverse effects. The effectiveness of ICS at low or moderate doses is usually improved if given along with long-acting β2 agonists, and such combinations are successfully used in the treatment/management of moderate-to-severe asthma in various tailored doses [22]. However, these combinations are not effective in all the asthmatic patients, as a sub-group of severe asthmatic patients (5-10%) do not respond to the usual corticosteroid therapy [23,24]. This group is the main contributor to the high health care expenditure (40–50% of medical care costs), as a result of increased and frequent hospitalization, and an enhanced and multiple usages of drugs and inhalers due to asthma exacerbations [25].

In asthma, corticosteroid resistance is identified by a lack of lung function improvement of more than 15% after treatment with 30-40 mg daily prednisolone for 2- weeks; however, it is scarce that a complete steroid resistance has been reported [21]. Refractory or corticosteroid resistant asthma is characterized by a failure to improve the forced expiratory volume in 1s by >=15% from a reference value of <=75% expected even after 14 days of administration with 40 mg prednisolone by mouth although representing >15% convertibility to an inhaled β2 agonist [26]. However, in COPD, ICS are found to provide minimal benefit, even if airway and lung inflammation was present. The treatment regimen used in COPD mostly involves similar drugs that are prescribed for asthma management, despite both diseases belong to two very different pathologies. ICS are not generally indicated as a monotherapy for COPD, but are mostly used in combination with regular long-acting bronchodilators usually being restricted to patients with severe COPD and repeated exacerbations [2]. Despite this, there is a widespread use of ICS in patients with the more moderate disease [27-30]. ICS treatment is used in two scenarios in COPD: in case of mixed asthma and COPD and COPD exacerbations, despite optimal bronchodilator treatment as this phenotype involves increased eosinophils similar to asthma [31]. In contrast to asthma, patients with COPD respond poorly to either ICS or oral corticosteroids because they were not found to be effective in inhibiting disease progression. However, a reduction in exacerbation frequency has been widely reported [32], but a significant decrease in inflammatory cells or mediator levels in the airways of patients with COPD has not been observed [33]. Therefore, functionally, corticosteroids do not seem to add much value in the treatment of COPD either in terms of improving lung function or controlling underlying inflammation [34]. This inadequate response in COPD and resistance in asthma has led to an increase in preclinical research and clinical studies for the advancement of other antiphlogistic drugs.

It is interesting to note that the corticosteroid resistance can also be found in other chronic inflammatory conditions [35]. Several mechanisms are thought to induce steroid resistance, including alterations in glucocorticoid receptors, abnormalities in histone acetylation, presence of lipocortin-1 antibodies, and pharmacokinetic abnormalities [36]. The addition of long-acting inhaled β2-agonists is believed to enhance the antiphlogistic effects of corticosteroids through cellular and molecular interactions [37]. However, this is relevant to the patients who have inherent defects in the nuclear localization of glucocorticoid receptors [38]. The unavoidable health care costs for the treatment of steroid-resistant patients who do not respond adequately to conventional and standard anti-inflammatory therapies is a required field for research not only in asthma but also in other chronic inflammatory diseases. The examination is increasingly being taken up in this area with a focus on alternative treatment options since the current regimen is not very advantageous in these subjects. The present review article attempts to understand some of the alternative treatment options for steroid unresponsive inflammatory diseases, including asthma and COPD. Cell-based therapies, epigenetic targets, and more importantly gene silencing approaches, will be discussed.

Methods

Literature search strategy and selection criteria

A logical search was carried out from PubMed national library of medicine (NLM), Web of Science, Google Scholar, ScienceDirect, Researchgate, and Google Web Browser by providing key terms "asthma, COPD, therapies, epigenetics, and gene silencing." To obtain a broad range of articles, we initially screened thousands of scientific manuscripts and gave particular attention to reports on clinical trials. The retrieved literature was further screened for the inclusion according to their contents. The reference lists of clinical trials were also screened, and relevant articles were included. The systematic review from the Global Initiative for Asthma (GINA) [1], Global Initiative for Chronic Obstructive Lung Diseases (GOLD) [2], and Expert Panel Report 3

(Guidelines for the Diagnosis and Management of Asthma) were also included [6]. A total of more than 109 peer-reviewed research articles were selected to write up this review.

Discussion

Stem Cell Therapy for Asthma and COPD

Stem cells, with their exceptional ability of self-replicating for an indefinite period, offer an enormous scope in the treatment of COPD [39,40]. Stem cells are associated with tissue homeostasis and restoration of the airways and may be localized in specific areas within the distal airways. Stem cells can be isolated from blood, bone marrow or adipose tissues (endothelial progenitor cells, mesenchymal stem cells) [41-43]. Among these, the mesenchymal stem cells (MSCs) are mostly used because of the ease of harvesting from the tissues and their extensive pleiotropic properties [44].

In severe asthma patients, asthma symptoms remain poorly controlled despite the use of corticosteroids in combination with bronchodilators. Stem cell therapy has been used in various diseases; however, studies in asthma are limited to animal models. Many studies have evaluated the effects of MSCs in murine asthma models by administering intravenous tail vein injection of human mesenchymal stem cells (hMSCs, up to 1×10^6). A reduction in eosinophilic inflammation was found following the MSCs administration before sensitization as well as after allergen challenge [45-49]. A decrease in Th2 cytokines IL-4 and IL-5 was reported in bronchoalveolar lavage fluid (BALF) [50]. A reduction in serum IgE or allergen-specific IgE was observed in ovalbumin (OVA) sensitized murine model of chronic asthma phenotype [51]. By using human bone marrow-derived MSCs (BM-MScs), in OVA-induced airway hyper-responsiveness, a decrease in BALF inflammatory cytokines such as IL-5, IL-13, IFNγ, and serum IgE levels was observed. Similar observations, along with decreased nasal inflammation, were also reported when mouse MSCs were used [52]. The hMSCs are multipotent cells in vitro that have been shown to reduce inflammation and can reverse airway remodeling when infused into an in vivo chronic asthma model.

Previously, it has been demonstrated that MSCs can produce IL-1Rα, which blocks macrophage activation through IL-1α, IL-1β, and TGF-β, which suppresses macrophage and T cell activation [53-55]. Due to the ability of MSCs to alter the immune system by decreasing inflammation and promoting wound healing, MSC-based therapy represents a potential therapy for asthma. Utilizing OVA-sensitization, an in vivo murine model of chronic asthma phenotype was produced, with and without hMSC infusion therapy. The hMSCs effectively influence the remodeling by reversing excessive amounts of extracellular matrix (ECM) deposition, more specifically by decreasing collagen I, collagen III, and changing the hyaluronan synthesis [56]. Diminished airway hyper-responsiveness, decreased eosinophilic inflammation, and Th2 to Th1 switching was also reported in OVA-induced asthma models [57]. Most of these studies reported histological improvements in lung tissues and clinically relevant reduction in airway hyper-responsiveness. However, more clinical studies are needed not only to assess the potential advantage of these cells in human subjects but also to prevent asthma exacerbations and further airway remodeling. Utilizing burrowing nanotubes, it was observed that MSCs expressing elevated Miro1 not only successfully reduced airway hyper-responsiveness to a greater extent but also decreased lung IL-5, IL-13, and a meager amount of inflammatory cell infiltration [58]. These MSCs also reduced collagen deposition and mucus hyper secretion in an OVA-induced murine asthma model. So far, MSCs indicate a decrease in eosinophilia and Th2 induced inflammation in allergic responses involved in asthma. However, the clinical efficacy of these studies needs to be established in asthma. In addition to using BM-MScs, other options such as using cells from adipose tissues, umbilical cord, or induced pluripotent stem cells (iPSCs) need to be evaluated entirely in both the preclinical and clinical studies in asthma [45,57,59].

The pathophysiological modifications, along with the enhanced inflammatory responses to stimuli associated with COPD, can cause endothelial cell destruction, accumulation of connective tissue, emphysema, and endothelial dysfunction. The pleiotropic properties of lung stem cells, in particular, the MSCs, can help in immunomodulation and controlling the inflammatory pathways in COPD. Adipose-derived stem cells were found to decrease emphysema through the production of hepatocyte growth factors [60,61]. Similarly, adipose stem cells also cause immunomodulation in inflammation paracrine pathways [62]. The value of endothelial progenitor cells in pulmonary vascular remodeling is currently being investigated in COPD, and the cytokines and growth factors that are involved in lung tissue repair are also under investigation [63]. BM-MSCs in a rat papain COPD model decreased the histologic injury and alveolar epithelial cell apoptosis after intravenous injection, and the vascular endothelial growth factor (VEGF) lung expression was also restored after intra-tracheal administration [64,65]. BM-MSCs in a mouse elastase model of COPD after intra-tracheal administration reduce the extent of collagen deposition and levels of inflammatory and profibrotic cytokines such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and IL-10 [66].

Based on the encouraging animal studies, it can be anticipated that stem cells therapy can be applied as a potential treatment for both asthma and COPD. However, the data from large-scale and reliable clinical trials is currently infrequent. More importantly, it is crucial to understand further the mechanism of injected cells within the tissue and the tools that induce lung regeneration in response to cytokines or growth factors. The safety and efficacy of systemically administered cells may have potential side effects of redifferentiation in the cell milieu or causing pulmonary emboli; therefore, these aspects need further studies [44].

Currently, two clinical trials are being conducted to test the safety and efficacy of MSC-based therapy for the treatment of asthmatic patients [67]. 1st clinical trial (entitled "Allogeneic Human Cells (hMSC) Via Intravenous Delivery in Patients With Mild Asthma

(ASTEC)") is ongoing at the Miller School of Medicine, the University of Miami under ClinicalTrials.gov identifier: NCT03137199 [68]. A 2nd study is being conducted by researchers from the Punta Pacifica Hospital of Panama City entitled (Safety and Feasibility Study of Intranasal Mesenchymal Trophic Factor (MTF) for Treatment of Asthma) under ClinicalTrials.gov Identifier: NCT02192736 [69]. Each patient participating in the study would be receiving MTF intranasally, and both of these studies are expected to be completed by the end of the year 2020.

Epigenetic Targets in Asthma and COPD

Changes in epigenetic regulation of gene expression is a highly conserved evolutionary process, and any adaptive changes in this mechanism can be the foundation for several pathological modifications in certain prolonged respiratory infections. Recognizing the epigenetic targets and developing strategies to prevent alterations in epigenetic mechanisms has been a core topic of research in many preclinical studies.

In patients with asthma, methylation of DNA has been described in some genes from post-mortem and lung biopsy samples [70]. DNA methylation was found to be altered in asthma and could be driven by allergen exposure, and this fact has been established in mouse asthma models. Increased alterations in global DNA methylation and hydroxymethylation was observed in lung tissue of house dust mite (HDM) sensitized mice.[71-73]. Additionally, mice deficient in DNA (cytosine-5)-methyltransferase 3A (DMT3A) in T cells showed higher eosinophil counts and an increased IL-13 secretion following OVA-sensitization [74]. Acute inhibition of the activity of DNA methyltransferases with 5-azacytidine was found to reduce airway hyperreactivity and inflammation in OVA-induced challenged mice [75]. Studies in asthmatic humans have shown decreased histone deacetylases (HDAC) and elevated histone acetyltransferases (HAT) activity with elevated histone H3 panacetylation causing enhanced expression of C-X-C Motif Chemokine Ligand 8 (CXCL8) in airway smooth muscle cells [76-79]. In addition to reduced histone H3 lysine 9 (H3K9) trimethylation, an increased expression of VEGF in airway smooth muscle cells was also reported [80]. Treatment of mice with a potent inhibitor of HDAC, trichostatin A, reduced airway hyper-responsiveness. However, questions about HDAC inhibitors in controlling inflammation are still unanswered [81]. Reduced HDAC activity in asthma might cause elevated expression of proinflammatory genes, and further work is needed to establish the role of histone acetylation in asthma. The development of clinically suitable and effective HAT inhibitors is currently at a preliminary stage and still needs to be further evaluated.

In COPD patients' similar mechanisms have been observed involving a combination of hypermethylation at some loci and hypomethylation at others [82,83]. Differentially expressed and regulated genes found in COPD are involved in PI3K/Akt and Nrf2 pathways [82]. Hyper-methylation of phosphatase and tensin homolog (*PTEN*) and Nrf2 genes at CpG islands cause decreased expression and activity of these genes. While the Nrf2 is involved in anti-inflammation, PTEN is a regulator of PI3K/AKT signaling, thus indirectly contributing to the inflammation and airway remodeling in COPD [84,85]. Hypomethylation causing enhanced expression of HDAC6 promoter has been linked to contributing to epithelial dysfunction in cigarette-smoke mediated COPD [86]. A reduction in HDACs 2,3,5,8 and an increase in expression of HDACs 4, 8 have been reported.

Furthermore, an increase in the acetylation of histones proportional to the disease severity was reported in inflammatory genes of COPD patients [87]. It has been shown previously that cigarette smoke can cause alterations in both HATs and HDACs, which in turn enhances NF-κB-dependent gene expression [88,89]. Increased histone acetylation of the CXCL8 gene regulated by NF-κB was mediated primarily due to variations in HDAC expressions [87].

A combination of alterations in acetylation of histones and the ensuing changes in levels of HAT and HDAC, including changes in histone methyltransferase (HMT) and histone demethylase (HDM) activities lead to alterations in gene expression profiles even in COPD. These combined mechanisms operating at the cellular level needs to be further confirmed in future studies. Since epigenetics plays a significant role in the pathogenesis of both asthma and COPD, any drug targets that inhibit these changes would be beneficial, especially in subjects with severe disease. However, clinical studies in this area had been limited, and there is a lack of information on the effectiveness of these drugs in the real-world scenario.

siRNA Technology Applications in Asthma and COPD

The concept of RNA interference (RNAi) has increasingly become an essential method for understanding gene functions. It plays a significant role in the therapeutic gene silencing of various inflammatory diseases. RNAi, as a natural gene silencing phenomenon, has a high degree of specificity and a potential to silence the genes [90]. The relatively new small-interfering RNAs (siRNA) technology involves the use of synthetic double-stranded RNA (dsRNA) to suppress target sequences through post-transcriptional gene silencing [91,92]. RNAi is a post-transcriptional event that causes sequence-specific gene silencing through the introduction of dsRNA, which is subsequently cleaved to smaller fragments to approximately 20 nucleotide short double-strand fragments called small interfering RNAs (siRNAs) [93]. An enzyme complex (Dicer), is involved in RNA unwinding, dsRNA binding, and ribonuclease activity, which is targeted to specific sequences and then cleaved [94,95]. The siRNA is finally unwound to two ssRNAs called passenger and guide strands. While the passenger strand is degraded, the guide strand is included in a RNA-induced protein silencing complex called RISC, which eventually causes the guide strand to pair with a sequence in mRNA molecules, causing

subsequent inhibition [96]. RNAi is a novel class of therapeutic agents that may have more rapid developments when matched with other traditional treatment options because of its naturally occurring ability to block any chosen gene of interest with high specificity [97].

Lung diseases, specifically asthma, COPD, and lung cancer, are currently the main focus for the implementation of siRNA based therapeutics not only due to their increasing prevalence but also due to the ease of delivery of siRNA through intra-tracheal, intra-pulmonary, or intra-nasal routes [98]. RNAi technology primarily focuses on blocking important mediators underlying disease pathogenesis. As mentioned previously, although ICS is practical in mild to moderate asthma for symptom control and reduction of exacerbations, their efficacy in severe asthma is debatable. Important cytokines (IL-4, IL-5, IL-13), cell receptors (IL-4R, and C-C chemokine receptor type 3 (CCR3), tyrosine kinases (Syk, Lyn), and transcription factors (STAT1, STAT6, GATA3, NF-κB) can form important targets for siRNA based therapeutics in severe asthma [99]. Many preliminary studies in cell models have shown the efficacy of siRNAs in asthma, and many animal studies have also demonstrated the positive results. For example, an anti-viral siRNA against IL-4 was found to be efficacious in reducing drug-induced asthma exacerbations in the mouse [100]. siRNAs targeted to suppressors of cytokine signaling (SOCS3) proteins delivered intra-nasally was found to inhibit asthma phenotype in mice [101]. One of the more recent development was a successful demonstration in phase I clinical trial of aerosolized Syk siRNA technology to inhibit inflammatory responses in asthma, this molecule called Excellair® is currently under phase II study [102]. These findings highlight the potential of siRNA technology in both preclinical and clinical studies.

A combination of PDE4 and PDE7 inhibition was found to potentially reduce inflammatory cell activation and cytokine release in the lungs. The molecule TPI 1100, comprising of two antisense oligonucleotides that target the PDE4B/4D and PDE 7A mRNA, was effective in reducing neutrophil influx and key cytokines in COPD [103]. In addition, the use of "Decoy" oligonucleotides that decoys for transcription factors of NF-κB can reduce its concentration in COPD, thus minimizing inflammatory processes [104,105]. Potential treatment options with oligonucleotides, including the antisense and the RNAi (siRNA and miRNA) technologies are relatively new in the field of respiratory medicine. However, significant problems with these techniques remain to be addressed, including the stability of siRNA or miRNA molecules post-delivery, the best delivery method, and safety during treatment.

The above treatment options are primarily aimed to block specific signal transduction pathways or proteins that are involved in inflammatory responses in asthma and COPD that are usually present in healthy cells and tissues but are frequently overexpressed/under-expressed in asthma and COPD. These revolutionary and targeted therapies either modify cell signaling events, block or activate targets or genes, and modulate cytokine functioning compared to the conventional therapies that have been used for the inflammatory diseases. However, the delivery of most of these therapies to specific sites of the body through the use of nanoparticles can allow these anti-inflammatory agents to avoid healthy cells/tissues and to get accumulated in the sites of inflammation to achieve a locally higher concentration while reducing the side effect on normal cells [106,107]. Besides, nanoparticles or nanocarriers can also help the drug/inhibitor against degradation and increase its half-life in the bloodstream allowing better bio-pharmacokinetic control in host tissues [108,109].

Conclusions and Future Outlook

An ever-increasing line of product options and various preclinical and clinical trials are currently focusing on controlling severe asthma and chronic inflammatory diseases. Complete understanding of the cellular and molecular mechanisms will help to manage corticosteroid resistant individuals appropriately. Recent substantial therapeutic advances offer considerable potential in managing and treating severe uncontrolled persistent asthma. A combination of epigenetics and gene silencing technologies with optimized delivery strategies and biosafety holds future promise for proper management and treatment of asthma, COPD, and various other diseases that have inflammatory pathophysiology.

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Authors' Contribution

S.A. performed a thorough review of the literature; S.A. and K.R. drafted the manuscript;
A.B.W. participated in the design of the study and provided oversight for the project.
All authors read and approved the final manuscript.

Competing Interest

The authors declare that they have no competing interests.

References

1. Boulet LP, FitzGerald JM, Reddel HK. The revised 2014 GINA strategy report: opportunities for change. Current Opinion in Pulmonary Medicine, (2015); 21(1): 1-7.
2. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. American Journal of Respiratory and Critical Care Medicine, (2013); 187(4): 347-365.
3. Fricker M, Qin L, Niessen N, Baines KJ, McDonald VM, et al. Relationship of sputum mast cells with clinical and inflammatory characteristics of asthma. Clin Exp Allergy, (2020); 15(10): 13609.
4. Potaczek DP, Miethe S, Schindler V, Alhamdan F, Garn H. Role of airway epithelial cells in the development of different asthma phenotypes. Cell Signal, (2020); 69(109523): 2.
5. Bhalla A, Mukherjee M, Nair P. Airway Eosinophilopoietic and Autoimmune Mechanisms of Eosinophilia in Severe Asthma. Immunology and Allergy Clinics of North America, (2018); 38(4): 639-654.
6. EPR-3. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007.

Journal of Allergy and Clinical Immunology, (2007); 120(5 Suppl): S94-138.
7. Halwani R, Al-Muhsen S, Hamid Q. Airway remodeling in asthma. Current Opinion in Pharmacology, (2010); 10(3): 236-245.
8. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, (2012); 380(9859): 2095-2128.
9. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLOS Medicine, (2006); 3(11): 0030442.
10. Caramori G, Casolari P, Giuffre S, Barczyk A, Adcock I, et al. COPD pathology in the small airways. Panminerva Medica, (2011); 53(1): 51-70.
11. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annual Review of Pathology, (2009); 4435-459.
12. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. European Respiratory Society, (2003); 22(4): 672-688.
13. Hurst JR, Wedzicha JA. The biology of a chronic obstructive pulmonary disease exacerbation. Clinics in Chest Medicine, (2007); 28(3): 525-536.
14. Parker CM, Voduc N, Aaron SD, Webb KA, O'Donnell DE. Physiological changes during symptom recovery from moderate exacerbations of COPD. European Respiratory Society, (2005); 26(3): 420-428.
15. Tattersfield AE, Knox AJ, Britton JR, Hall IP. Asthma. Lancet, (2002); 360(9342): 1313-1322.
16. Larj MJ, Bleecker ER. Therapeutic responses in asthma and COPD. Corticosteroids. Chest, (2004); 126(2 Suppl): 138S-149S; 159S-161S.
17. Adcock IM, Chou PC, Durham A, Ford P. Overcoming steroid unresponsiveness in airways disease. Biochemical Society Transactions, (2009); 37(Pt 4): 824-829.
18. Johnson M. The beta-adrenoceptor. American Journal of Respiratory and Critical Care Medicine, (1998); 158(5 Pt 3).
19. Celli B, ZuWallack R, Wang S, Kesten S. Improvement in resting inspiratory capacity and hyperinflation with tiotropium in COPD patients with increased static lung volumes. Chest, (2003); 124(5): 1743-1748.
20. Walters JA, Gibson PG, Wood-Baker R, Hannay M, Walters EH. Systemic corticosteroids for acute exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev, (2009); 21(1).
21. Barnes PJ. Corticosteroid resistance in airway disease. Proceedings of the American Thoracic Society, (2004); 1(3): 264-268.
22. Chung KF, Adcock IM. Combination therapy of long-acting beta2-adrenoceptor agonists and corticosteroids for asthma. Treatments in Respiratory Medicine, (2004); 3(5): 279-289.
23. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. American Journal of Respiratory and Critical Care Medicine, (2004); 170(8): 836-844.
24. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. Lancet. (2006) 26;368(9537):780-93.
25. Serra-Batlles J, Plaza V, Morejon E, Comella A, Brugues J. Costs of asthma according to the degree of severity. European Respiratory Society, (1998); 12(6): 1322-1326.
26. Barnes PJ. Inhaled glucocorticoids for asthma. The New England Journal of Medicine, (1995); 332(13): 868-875.
27. Jochmann A, Neubauer F, Miedinger D, Schafroth S, Tamm M, et al. General practitioner's adherence to the COPD GOLD guidelines: baseline data of the Swiss COPD Cohort Study. Swiss Medical Weekly, (2010); 9(140): 1-4.
28. Suissa S, Barnes PJ. Inhaled corticosteroids in COPD: the case against. European Respiratory Journal. (2009) 34(1):13-6. doi: 10.1183/09031936.00190908.
29. Jebrak G. [COPD routine management in France: are guidelines used in clinical practice?]. Revue des Maladies Respiratoires, (2010); 27(1): 11-18.
30. Lucas AE, Smeenk FW, Smeele IJ, van Schayck CP. Overtreatment with inhaled corticosteroids and diagnostic problems in primary care patients, an exploratory study. Family Practice, (2008); 25(2): 86-91.
31. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. American Journal of Respiratory and Critical Care Medicine, (2011); 184(6): 662-671.
32. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. The Journal of Allergy and Clinical Immunology, (2013); 131(3): 636-645.
33. Bourbeau J, Christodoulopoulos P, Maltais F, Yamauchi Y, Olivenstein R, et al. Effect of salmeterol/ fluticasone propionate on airway inflammation in COPD: a randomised controlled trial. Thorax, (2007); 62(11): 938-943.
34. Roche N, Marthan R, Berger P, Chambellan A, Chanez P, et al. Beyond corticosteroids: future prospects in the management of inflammation in COPD. European Respiratory Review, (2011); 20(121): 175-182.
35. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. Lancet, (2009); 373(9678): 1905-1917.
36. Culpitt SV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, et al. Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. American Journal of Respiratory and Critical Care Medicine, (1999); 160(5 Pt 1): 1635-1639.
37. Barnes PJ. Scientific rationale for inhaled combination therapy with long-acting beta2-agonists and corticosteroids. European Respiratory Journal, (2002); 19(1): 182-191.
38. Roth M, Johnson PR, Rudiger JJ, King GG, Ge Q, et al. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. Lancet, (2002); 360(9342): 1293-1299.
39. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, et al. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Molecular Medicine, (2000); 6(2): 88-95.
40. Srour N, Thebaud B. Stem cells in animal asthma models: a systematic review. Cytotherapy, (2014); 16(12): 1629-1642.
41. Kajstura J, Rota M, Hall SR, Hosoda T, D'Amario D, et al. Evidence for human lung stem cells. The New England Journal of Medicine, (2011); 364(19): 1795-1806.
42. Huertas A, Palange P. Circulating endothelial progenitor cells and chronic pulmonary diseases. European Respiratory Journal, (2011); 37(2): 426-431.
43. Rankin S. Mesenchymal stem cells. Thorax, (2012); 67(6): 565-566.
44. Tzouvelekis A, Ntolios P, Bouros D. Stem cell treatment for chronic lung diseases. Respiration, (2013); 85(3): 179-192.
45. Sun YQ, Deng MX, He J, Zeng QX, Wen W, et al. Human pluripotent stem cell-derived mesenchymal stem cells prevent allergic airway inflammation in mice. Stem Cells, (2012); 30(12): 2692-2699.
46. Wang CY, Chiou GY, Chien Y, Wu CC, Wu TC, et al. Induced pluripotent stem cells without c-Myc reduce airway responsiveness and allergic reaction in sensitized mice. Transplantation, (2013); 96(11): 958-965.
47. Firinci F, Karaman M, Baran Y, Bagriyanik A, Ayyildiz ZA, et al. Mesenchymal stem cells ameliorate the histopathological changes in a murine model of chronic asthma. International Immunopharmacology, (2011); 11(8): 1120-1126.
48. Kavanagh H, Mahon BP. Allogeneic mesenchymal stem cells prevent allergic airway inflammation by inducing murine regulatory T cells. Allergy, (2011); 66(4): 523-531.
49. Ge X, Bai C, Yang J, Lou G, Li Q, et al. Effect of mesenchymal stem cells on inhibiting airway remodeling and airway inflammation in chronic asthma. Journal of Cellular Biochemistry, (2013); 114(7): 1595-1605.
50. Goodwin M, Sueblinvong V, Eisenhauer P, Ziats NP, LeClair L, et al. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. Stem Cells, (2011); 29(7): 1137-1148.
51. Bonfield TL, Koloze M, Lennon DP, Zuchowski B, Yang SE, et al. Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. American Journal of Physiology-Lung Cellular and Molecular Physiology, (2010); 299(6): 3.
52. Cho KS, Roh HJ. Immunomodulatory effects of adipose-derived stem cells in airway allergic diseases. Current Stem Cell Research & Therapy, (2010); 5(2): 111-115.
53. Heldens GT, Blaney Davidson EN, Vitters EL, Schreurs BW, Piek E, et al. Catabolic factors and osteoarthritis-conditioned medium inhibit chondrogenesis of human mesenchymal stem cells. Tissue Engineering Part A, (2012); 18(1-2): 45-54.

54. Volarevic V, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. Autoimmunity, (2010); 43(4): 255-263.
55. Kunzmann S, Wright JR, Steinhilber W, Kramer BW, Blaser K, et al. TGF-beta1 in SP-A preparations influence immune suppressive properties of SP-A on human CD4+ T lymphocytes. American Journal of Physiology-Lung Cellular and Molecular Physiology, (2006); 291(4): 28.
56. Goldstein BD, Lauer ME, Caplan AI, Bonfield TL. Chronic asthma and Mesenchymal stem cells: Hyaluronan and airway remodeling. Journal of Inflammation, (2017); 14(18): 017-0165.
57. Park HK, Cho KS, Park HY, Shin DH, Kim YK, et al. Adipose-derived stromal cells inhibit allergic airway inflammation in mice. Stem Cells and Development, (2010); 19(11): 1811-1818.
58. Ahmad T, Mukherjee S, Pattnaik B, Kumar M, Singh S, et al. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. EMBO Journal, (2014); 33(9): 994-1010.
59. Mathias LJ, Khong SM, Spyroglou L, Payne NL, Siatskas C, et al. Alveolar macrophages are critical for the inhibition of allergic asthma by mesenchymal stromal cells. Journal of Immunology, (2013); 191(12): 5914-5924.
60. Shigemura N, Okumura M, Mizuno S, Imanishi Y, Matsuyama A, et al. Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. American Journal of Respiratory and Critical Care Medicine, (2006); 174(11): 1199-1205.
61. Shigemura N, Okumura M, Mizuno S, Imanishi Y, Nakamura T, et al. Autologous transplantation of adipose tissue-derived stromal cells ameliorates pulmonary emphysema. American Journal of Transplantation, (2006); 6(11): 2592-2600.
62. Schweitzer KS, Johnstone BH, Garrison J, Rush NI, Cooper S, et al. Adipose stem cell treatment in mice attenuates lung and systemic injury induced by cigarette smoking. American Journal of Respiratory and Critical Care Medicine, (2011); 183(2): 215-225.
63. Palange P, Testa U, Huertas A, Calabro L, Antonucci R, et al. Circulating haemopoietic and endothelial progenitor cells are decreased in COPD. European Respiratory Journal, (2006); 27(3): 529-541.
64. Zhen G, Liu H, Gu N, Zhang H, Xu Y, et al. Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. Frontiers in Bioscience, (2008); 133415-3422.
65. Zhen G, Xue Z, Zhao J, Gu N, Tang Z, et al. Mesenchymal stem cell transplantation increases expression of vascular endothelial growth factor in papain-induced emphysematous lungs and inhibits apoptosis of lung cells. Cytotherapy, (2010); 12(5): 605-614.
66. Katsha AM, Ohkouchi S, Xin H, Kanehira M, Sun R, et al. Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. Molecular Therapy, (2011); 19(1): 196-203.
67. Harrell CR, Sadikot R, Pascual J, Fellabaum C, Jankovic MG, et al. Mesenchymal Stem Cell-Based Therapy of Inflammatory Lung Diseases: Current Understanding and Future Perspectives. Stem Cells International, (2019); 2(4236973).
68. Glassberg M. Allogeneic Human Cells (hMSC) Via Intravenous Delivery in Patients With Mild Asthma (ASTEC). https://clinicaltrials.gov/ct2/show/NCT03137199. Accessed on 5 May 2020.
69. Biosciences T. Safety and Feasibility Study of Intranasal Mesenchymal Trophic Factor (MTF) for Treatment of Asthma. https://clinicaltrials.gov/ct2/show/NCT02192736. Accessed on 5 May 2020.
70. Stefanowicz D, Hackett TL, Garmaroudi FS, Gunther OP, Neumann S, et al. DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children. PLoS One, (2012); 7(9): 6.
71. Cheng RY, Shang Y, Limjunyawong N, Dao T, Das S, et al. Alterations of the lung methylome in allergic airway hyper-responsiveness. Environmental and Molecular Mutagenesis, (2014); 55(3): 244-255.
72. Shang Y, Das S, Rabold R, Sham JS, Mitzner W, et al. Epigenetic alterations by DNA methylation in house dust mite-induced airway hyperresponsiveness. American Journal of Respiratory Cell and Molecular Biology, (2013); 49(2): 279-287.
73. Verma M, Chattopadhyay BD, Paul BN. Epigenetic regulation of DNMT1 gene in mouse model of asthma disease. Molecular Biology Reports, (2013); 40(3): 2357-2368.
74. Yu Q, Zhou B, Zhang Y, Nguyen ET, Du J, et al. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. Proceedings of the National Academy of Sciences of the United States of America, (2012); 109(2): 541-546.
75. Wu CJ, Yang CY, Chen YH, Chen CM, Chen LC, et al. The DNA methylation inhibitor 5-azacytidine increases regulatory T cells and alleviates airway inflammation in ovalbumin-sensitized mice. International Archives of Allergy and Immunology, (2013); 160(4): 356-364.
76. Ito K, Lim S, Caramori G, Cosio B, Chung KF, et al. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. Proceedings of the National Academy of Sciences of the United States of America, 2002 Jun 25;99(13):8921-6.
77. Cosio BG, Mann B, Ito K, Jazrawi E, Barnes PJ, et al. Histone acetylase and deacetylase activity in alveolar macrophages and blood mononocytes in asthma. American Journal of Respiratory and Critical Care Medicine, (2004); 170(2): 141-147.
78. Hew M, Bhavsar P, Torrego A, Meah S, Khorasani N, et al. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. American Journal of Respiratory and Critical Care Medicine. 2006 Jul 15;174(2): 134-41.
79. John AE, Zhu YM, Brightling CE, Pang L, Knox AJ. Human airway smooth muscle cells from asthmatic individuals have CXCL8 hypersecretion due to increased NF-kappa B p65, C/EBP beta, and RNA polymerase II binding to the CXCL8 promoter. Journal of Immunology, (2009); 183(7): 4682-4692.
80. Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal histone methylation is responsible for increased vascular endothelial growth factor 165a secretion from airway smooth muscle cells in asthma. Journal of Immunology, (2012); 189(2): 819-831.
81. Banerjee A, Trivedi CM, Damera G, Jiang M, Jester W, et al. Trichostatin A abrogates airway constriction, but not inflammation, in murine and human asthma models. American Journal of Respiratory Cell and Molecular Biology, (2012); 46(2): 132-138.
82. Vucic EA, Chari R, Thu KL, Wilson IM, Cotton AM, et al. DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. American Journal of Respiratory Cell and Molecular Biology, (2014); 50(5): 912-922.
83. Qiu W, Baccarelli A, Carey VJ, Boutaoui N, Bacherman H, et al. Variable DNA methylation is associated with chronic obstructive pulmonary disease and lung function. American Journal of Respiratory and Critical Care Medicine, (2012); 185(4): 373-381.
84. Boutten A, Goven D, Artaud-Macari E, Boczkowski J, Bonay M. NRF2 targeting: a promising therapeutic strategy in chronic obstructive pulmonary disease. Trends in Molecular Medicine, (2011); 17(7): 363-371.
85. Bozinovski S, Vlahos R, Hansen M, Liu K, Anderson GP. Akt in the pathogenesis of COPD. International Journal of Chronic Obstructive Pulmonary Disease, (2006); 1(1): 31-38.
86. Lam HC, Cloonan SM, Bhashyam AR, Haspel JA, Singh A, et al. Histone deacetylase 6-mediated selective autophagy regulates COPD-associated cilia dysfunction. Journal of Clinical Investigation, (2013); 123(12): 5212-5230.
87. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. The New England Journal of Medicine, (2005); 352(19): 1967-1976.
88. Yang SR, Chida AS, Bauter MR, Shafiq N, Seweryniak K, et al. Cigarette smoke induces proinflammatory cytokine release by activation of NF-kappaB and posttranslational modifications of histone deacetylase in macrophages. American Journal of Physiology-Lung Cellular and Molecular Physiology, (2006); 291(1): 10.
89. Enesa K, Ito K, Luong le A, Thorbjornsen I, Phua C, et al. Hydrogen peroxide prolongs nuclear localization of NF-kappaB in activated cells by suppressing negative regulatory mechanisms. Journal of Biological Chemistry, (2008); 283(27): 18582-18590.
90. Bagasra O, Prilliman KR. RNA interference: the molecular immune system. Journal of Molecular Histology, (2004); 35(6): 545-553.
91. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. Nature Reviews Molecular Cell Biology, (2007); 8(1): 23-36.
92. Ali HM, Urbinati G, Raouane M, Massaad-Massade L. Significance and applications of nanoparticles in siRNA delivery for cancer therapy. Expert Review of Clinical Pharmacology, (2012); 5(4): 403-412.
93. Fuchs U, Damm-Welk C, Borkhardt A. Silencing of disease-related genes by small interfering RNAs. Current Molecular Medicine, (2004); 4(5): 507-517.

94. Denli AM, Hannon GJ RNAi: an ever-growing puzzle. Trends in Biochemical Sciences, 2003 Apr;28(4):196-201.
95. Dykxhoorn DM, Novina CD, Sharp PA Killing the messenger: short RNAs that silence gene expression. Nature Reviews Molecular Cell Biology, (2003); 4(6): 457-467.
96. Kupferschmidt K A lethal dose of RNA. Science, 2013; 341(6147): 732-3.
97. Bumcrot D, Manoharan M, Koteliansky V, Sah DW. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nature Chemical Biology, (2006); 2(12): 711-719.
98. Fujita Y, Takeshita F, Kuwano K, Ochiya T. RNAi Therapeutic Platforms for Lung Diseases. Pharmaceuticals, (2013); 6(2): 223-250.
99. Popescu FD. Antisense- and RNA interference-based therapeutic strategies in allergy. Journal of Cellular and Molecular Medicine, (2005); 9(4): 840-853.
100. Khaitov MR, Shilovskiy IP, Nikonova AA, Shershakova NN, Kamyshnikov OY, et al. Small interfering RNAs targeted to interleukin-4 and respiratory syncytial virus reduce airway inflammation in a mouse model of virus-induced asthma exacerbation. Human Gene Therapy, (2014); 25(7): 642-650.
101. Zafra MP, Mazzeo C, Gamez C, Rodriguez Marco A, de Zulueta A, et al. Gene silencing of SOCS3 by siRNA intranasal delivery inhibits asthma phenotype in mice. PLoS One, (2014); 9(3): e91996.
102. Ulanova M, Puttagunta L, Marcet-Palacios M, Duszyk M, Steinhoff U, et al. Syk tyrosine kinase participates in beta1-integrin signaling and inflammatory responses in airway epithelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology, (2005); 288(3): 19.
103. Seguin RM, Ferrari N. Emerging oligonucleotide therapies for asthma and chronic obstructive pulmonary disease. Expert Opinion on Investigational Drugs, (2009); 18(10): 1505-1517.
104. Edwards MR, Bartlett NW, Clarke D, Birrell M, Belvisi M, et al. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. Pharmacology & Therapeutics, (2009); 121(1): 1-13.
105. Gill JS, Zhu X, Moore MJ, Lu L, Yaszemski MJ, et al. Effects of NFkappaB decoy oligonucleotides released from biodegradable polymer microparticles on a glioblastoma cell line. Biomaterials, (2002); 23(13): 2773-2781.
106. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discovery Today, (2006); 11(17-18): 812-818.
107. Northfelt DW, Martin FJ, Working P, Volberding PA, Russell J, et al. Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumor localization, and safety in patients with AIDS-related Kaposi's sarcoma. The Journal of Clinical Pharmacology, (1996); 36(1): 55-63.
108. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nature Reviews Drug Discovery, (2008); 7(9): 771-782.
109. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, et al. Nanocarriers as an emerging platform for cancer therapy. Nature Nanotechnology, (2007); 2(12): 751-760.