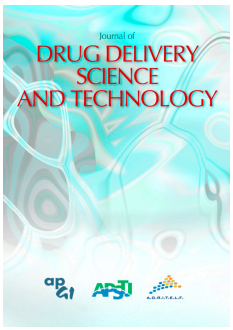


Tackling the cytokine storm using advanced drug delivery in allergic airway disease

Vyoma K. Patel, Sukriti Vishwas, Rajan Kumar, Gabriele De Rubis, Shakti D. Shukla, Keshav Raj Paudel, Bikash Manandhar, Thakur Gurjeet Singh, Dinesh Kumar Chellappan, Monica Gulati, Indu Pal Kaur, Venkata Sita Rama Raju Allam, Philip M. Hansbro, Brian G. Oliver, Ronan MacLoughlin, Sachin Kumar Singh, Kamal Dua

PII: S1773-2247(23)00218-6

DOI: https://doi.org/10.1016/j.jddst.2023.104366

Reference: JDDST 104366

To appear in: *Journal of Drug Delivery Science and Technology*

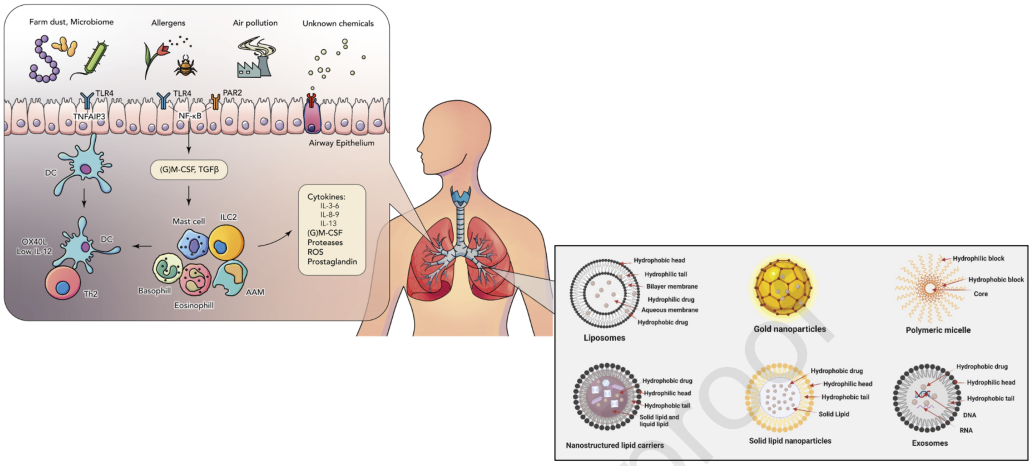
Received Date: 14 November 2022

Revised Date: 6 March 2023

Accepted Date: 12 March 2023

Please cite this article as: V.K. Patel, S. Vishwas, R. Kumar, G. De Rubis, S.D. Shukla, K.R. Paudel, B. Manandhar, T.G. Singh, D.K. Chellappan, M. Gulati, I.P. Kaur, V.S.R.R. Allam, P.M. Hansbro, B.G. Oliver, R. MacLoughlin, S.K. Singh, K. Dua, Tackling the cytokine storm using advanced drug delivery in allergic airway disease, *Journal of Drug Delivery Science and Technology* (2023), doi: https://doi.org/10.1016/j.jddst.2023.104366.

Graphical abstract



Tackling the cytokine storm using advanced drug delivery in allergic airway disease

Vyoma K Patel[a, b, c,] Sukriti Vishwas[d], Rajan Kumar[d], Gabriele De Rubis[a,b], Shakti D Shukla[a,b], Keshav Raj Paudel[e], Bikash Manandhar[a,b], Thakur Gurjeet Singh[f], Dinesh Kumar Chellappan[g], Monica Gulati[b,d], Indu Pal Kaur[h], Venkata Sita Rama Raju Allam[i], Philip M Hansbro[e], Brian G Oliver[j,k], Ronan MacLoughlin[l,m,n], Sachin Kumar Singh[b,d*], Kamal Dua[a,b,o *]

a. Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, P.O. Box 123 Broadway, Ultimo, NSW 2007, Australia

b. Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, NSW 2007, Australia

c. School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW 2052, Australia

d. School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

e. Centre for Inflammation, Centenary Institute and University of Technology Sydney, School of Life Sciences, Faculty of Science, Sydney, NSW 2007, Australia

f. Chitkara college of Pharmacy, Chitkara University, Punjab 140401, India

g. Department of Life Sciences, School of Pharmacy, International Medical University, Bukit Jalil, Kuala Lumpur 57000, Malaysia

h. University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, 160014, India

i. Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

j. School of Life Sciences, Faculty of Science, University of Technology Sydney, Ultimo, NSW, Australia

k. Woolcock Institute of Medical Research, University of Sydney, Sydney, NSW, Australia

l. IDA Business Park, Dangan, H91 HE94, Galway, Ireland

m. School of Pharmacy & Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin, D02 YN77, Ireland

n. School of Pharmacy & Pharmaceutical Sciences, Trinity College, Dublin, D02 PN40, Ireland

o. Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, 248007, India

***Correspondence:**

Dr. Kamal Dua
Email: Kamal.Dua@uts.edu.au
Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, P.O. Box 123 Broadway, Ultimo, NSW 2007, Australia; Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, NSW 2007, Australia; Woolcock Institute of Medical Research, University of Sydney, Sydney, NSW, Australia

Dr. Sachin Kumar Singh
Email: sachin.16030@lpu.co.in
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Highlights

- Cytokine storm is one of the key epidemiological and pathophysiological features of asthma
- The ongoing developments in gene therapy, drug delivery systems and clinical trials may unravel the complexity of asthma

Declarations

Ethics Approval – Not applicable

Consent to participate – Not applicable

Consent to for publication – Not applicable

Abstract

Asthma is one of the leading causes of mortality worldwide presenting a huge socio-economic burden with rising morbidity and mortality rates. It is a chronic inflammatory airway disease that is eminent with multiple epidemiological and pathophysiological features such as over production of pro-inflammatory cytokines that triggers an uncontrolled aberrant inflammatory response known as 'cytokine storm'. This phenomenon interferes with the signalling and production of cytokines over time leading to the progression of disease and the development of complications that lead to fatal consequences in many individuals. Targeting this overproduction and signalling of cytokines may prove a promising approach to develop novel cytokine specific therapies in the treatment of asthma. This review discusses on the various pharmacological strategies, recent advancements in drug delivery systems and significant findings from clinical trials that may have a potential to outweigh the limitations of the current therapies in the treatment of asthma.

Key words: asthma, cytokines, drug delivery systems, clinical trials

1. Introduction

The prevalence and burden of chronic respiratory diseases (CRDs), especially asthma and chronic obstructive pulmonary disease (COPD), are constantly accelerating worldwide. Notably, the report on the global burden of disease highlights that about 545 million individuals develop a chronic respiratory condition on an average. This accounts for approximately 3.9 million deaths annually [1]. Moreover, over the past years, CRDs have accounted towards a huge proportion of disability-adjusted life years globally, with an 18% increase [1]. In Australia, both COPD and asthma, along with lung cancer feature in the top ten leading causes of diseases [2]. In 2020-21, the prevalence of asthma and COPD were reported to be 10.7% and 1.5% respectively among individuals of all ages [3]. Understandably, the prevalence of COPD has now increased to 5.2% in people aged >75 years. In Australia, asthma-related deaths remain exceptionally high (417 deaths in 2020) when compared worldwide. Both asthma and COPD are categorised as chronic respiratory diseases that require lifelong patient-centric management of symptoms resulting in an enormous health as well as economic burden on patient as well as on the healthcare system [2].

Asthma is a rather common but heterogeneous chronic condition, and unfortunately, it is often inadequately controlled in most patients [4]. For example, only 1 in 3 Australians has a scripted asthma action plan or uses asthma-related medications recurrently [5]. This further complicates the disease presentation and management, and often leads to accelerated progression of disease. Hence, there is a critical need for novel strategies for managing asthma. Furthermore, asthma affects individuals of all ages, although the presentation of disease may vary depending upon the exposure to allergens, racial background, gender, diet, and other indicators such as socio-economic status [6]. Asthma may present differently in individuals and is categorised based on clinical symptoms and immunological characteristics of the disease. The symptoms of asthma

include shortness of breath, chest tightness, coughing and wheezing. Allergic asthma exacerbates in response to exposure to non-specific environmental stimuli, including infectious (microbes) and non-infectious (mould, dust mite, and pollen) triggers, in addition to host genetic makeup [7]. These intrinsic and extrinsic triggers lead to aberrant inflammatory response that may result in the exacerbation of asthma symptoms, resulting in severe and deleterious consequences such as hospitalisation and increased risk of morbidity. Mild-to-moderate asthma is primarily characterised by a T-helper cell type-2-mediated inflammation developing due to heightened responses to common allergens. The major cell types involved in type-2 asthma are mast cells, Th2 cells, basophils, eosinophils, group 2 innate lymphoid cells (ILC2s), and IgE-producing B cells [8]. Moreover, prolonged exposure to these common allergens and resulting persistent inflammation further leads to myofibroblast infiltration, leading to remodeling of the airway epithelium. This results in increased mucus secretion, hypertrophy of airway smooth muscle, and significant increase in collagen deposition [8]. This causes the airways to become increasingly more responsive to aeroallergen provocation, and this leads to the characteristic 'wheezing' and obstructed airflow. An extensive amount of scientific research has progressed the understanding of the underlying inflammatory mechanisms and the development of new therapies in asthma management.

Asthma can be categorised into different phenotypes, depending on the observable characteristics, which primarily relies on the clinical presentation of the disease; or endotypes, depending on the distinct underlying disease mechanisms that are taken into consideration [9]. These are rather complex and represent the heterogenous and varying outcomes of host-environment interactions [7]. Based on the cells activated during inflammation, asthma can be categorised into neutrophilic, eosinophilic, or paucigranulocytic (in which no increased numbers of neutrophils or eosinophils are observed). A combination of clinical symptoms and cellular and molecular, are utilised to categorise the asthma sub-types, as well as to determine

the treatment strategies for individuals with asthma. In this review, we summarise the key inflammatory pathways activated in asthma, and we explore the potential approaches, including recent drug delivery techniques, to mitigate the inflammation-induced lung damage in asthma.

2. Pathophysiology of asthma

The pathogenesis of asthma involves the interaction between inflammation and remodeling of the airways of the lungs, resulting in airway hyperresponsiveness (AHR) [10]. AHR is the major feature of asthma, leading to an exaggerated bronchoconstrictor response to various environmental stimuli such as allergens (infectious and non-infectious), farm dust, microbiomes, air pollutants, and other chemicals. The hyperresponsiveness of the bronchial airways alters the integrity of the inner wall of the lung epithelium, which is maintained by the contraction and relaxation of smooth muscles and elastic fibers, in response to the inflammatory mediators, thus resulting in excessive narrowing of the airways [11]. Asthma diminishes the lung function in an accelerated manner, which in severe and chronic cases, causes an irreversible airflow obstruction via airway remodeling. AHR involves multiple and complex mechanisms, including increased histamine production from mast cells, inflammation, loss of airway contractility, and increased thickness of the inner wall, causing difficulty in breathing [8, 12]. The interplay between innate and adaptive cells and mediators in type 2 inflammation underpins the pathophysiology of asthma as discussed below (Figure 1). Moreover, airway inflammation is a characteristic feature of asthma and involves an early phase of initiation involving sensitisation of immunoglobulin E (IgE), released by plasma cells, to environmental allergens and pollutants. The serum IgE is elevated and binds to mast cells and basophils [8, 13]. Mast cells then de-granulate to release cytokines such as histamine, prostaglandins, and leukotrienes, which in turn causes the contraction of airway smooth muscle resulting in airway constriction [7, 8].

Th2 lymphocytes play an important role in the exacerbation of airway inflammation [14]. The antigen-presenting dendritic cells in the airways process the allergens into small peptides and presents them to naïve T cells via major histocompatibility complex (MHC) class II molecules [14]. This leads to differentiation of the T cells to Th2 phenotype, which produce a series of pro-inflammatory cytokines such as interleukin (IL)-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, as well as granulocyte macrophage-colony stimulating factor (GM-CSF), proteases, reactive oxygen species (ROS) and prostaglandins. This phenomenon is also known as 'cytokine storm' [8, 15-18]. These cytokines subsequently stimulate localisation of dendritic cells (DCs), type 2 innate lymphoid cells (ILC2s), basophils, eosinophils, mast cells, and alternatively activated macrophages (AAMs) that promote adaptive Th2 immunity via induction of ox40L (is also known as tumour necrosis factor super family member 4 and is ligand for ox40 that is expressed by many antigen-presenting cells such as T cells, natural killer (NK) cells and lymphocytes)[19] and suppression of IL-12, particularly in DCs [20-22]. This leads to bronchoconstriction and inflammation. Notably, a prior exposure to allergens can dampen the epithelial cell response leading to an upregulation of tumour necrosis factor acute induced protein-3 (TNFAIP-3), which further suppresses the activity of nuclear factor-κB (NF-κB) in epithelial cells (Figure 1). This aberrant inflammatory response eventually leads to airway remodeling - a complex clinical feature of asthma that involves long term disruption and modification of airway architecture. This significantly contributes to AHR and lung function decline [23, 24]. Airway remodeling is characterised by epithelial damage, cilial dysfunction, extracellular matrix deposition, and increased epithelial-to-mesenchymal transition (EMT), causing permanent airflow obstruction [8, 23-26]. This further leads to inefficient adhesion of bronchial epithelial cells to the airway wall thus, leading to infiltration of airway smooth muscle cells and thickening of the airway walls due to migration of bronchial epithelial cells [27]. At this stage, further release of a wider range of cytokines such as TNF-α, IL1-β, IL-6, IL-13 IL-17

and ROS via interactions between eosinophils and mast cells further amplifies inflammatory response and oxidative stress. This leads to an irreversible airflow obstruction and lung function decline [8, 26, 28]. Although the pathophysiology of asthma, airway inflammation and remodeling, including subsequent cytokine release, have been well documented in the literature, this dogma is predominantly translated from only murine models of asthma. Nevertheless, further research is essential to determine the effects of inhibiting the cytokine response, being that is a key player in both airway inflammation and remodeling in asthma. Targeting the release of this wider repertoire of cytokines may have the potential to dramatically improve symptoms and disease burden in asthmatic patients.

3. Current therapies to treat asthma and their limitations

Although there is no proper cure for asthma, the overall aim is to effectively manage and control the symptoms that are associated with the disease, such as inflammation, exacerbations, airway narrowing, mucus production that are associated with the disease, and to improve the quality of life of the patients [29]. The current treatment strategies employed in the treatment of asthma are mainly focussed on reducing symptoms for the two key clinical features of asthma explicitly, inflammation and bronchoconstriction via anti-inflammatory agents (commonly, inhaled corticosteroids) and bronchodilators. Targeting inflammation is a key success factor to the long-term management of asthma, and identifying various inflammatory targets implied with the disease is fundamental. Treatment strategies against these targets to surrogate the inflammation result in better outcomes when compared to targeting bronchodilation [30]. The conventional anti-inflammatory therapy that is widely recommended by the global clinical guidelines includes the use of the inhaled corticosteroids (ICS) [31]. ICS therapy is a key treatment for asthmatics that reduces an inflammation either by inducing apoptosis or by reducing infiltration of the inflammatory cells such as eosinophils, mast cells

and T cells thereby preventing the exacerbations and improving the lung function to further reduce morbidity and mortality associated to asthma [32, 33].

Although ICS are effective in the treatment of asthma, unwanted adverse effects associated with long-term treatment include weight gain, development of cataract and glaucoma, osteoporosis, hyperglycaemia, and drug-associated diabetes, and skin diseases associated with the long-term treatment limit the use of ICS [32]. In addition to the adverse effects, the compliance with ICS is very poor as this therapy does not cure asthma, and the symptoms usually persist upon treatment discontinuation. Interestingly, about 5-10% asthmatics respond poorly to this conventional therapy due to altered and unstable expression of glucocorticoid receptor and nuclear translocation failure of the corticoids-receptor complex that ultimately leads to steroid resistance, highlighting the necessity to develop alternative anti-inflammatory therapies that can treat the disease effectively. Moreover, oxidative stress in severe asthma further mediates various mechanisms including impairment of the histone deacetylase-2 (HDAC2) and activation of p38 mitogen activated protein kinase (MAPK), and this which results in the amplification of the inflammatory response and the reduction of the anti-inflammatory response to the steroids [34].

Targeting bronchoconstriction is another significant strategy in asthma treatment, and bronchodilators are highly necessary to relieve bronchoconstriction, enabling to relax the smooth muscle especially in acute episodes of asthma. Bronchodilators including the fast-acting β2-agonists, such as albuterol, short-acting anticholinergics, such as ipratropium bromide, and long acting β2-agonists (LABAs) such as salmeterol and formoterol, are currently used either alone or in combination with the ICS for the effective management of asthma [31, 35]. Although advancements in research have led to the development of ultra-long acting β2-agonists such as indacaterol, carmoterol, milveterol, poor adherence and associated adverse

effects such as sympathomimetic effects i.e., anxiety, tremors, tachycardia, and cholinergic blocking effects i.e., xerostomia, limit the usage of this class of drugs [32, 36].

The use of biologics represents another approach for the treatment of asthma associated with allergic conditions. Blockade of IgE with omalizumab and targeting IL-5 cytokine with antibodies such as mepolizumab, resilizumab and benralizumab, and IL-4 cytokine with duplizumab acts on various pathways to prevent IgE binding to receptors on mast cells. Moreover, blockade of IL-5 cytokine from binding to its receptor and activating apoptosis of eosinophils are reported to be reasonably effective particularly in cases of severe asthma [31, 37, 38]. Several experiments have been trailed by the administration of the Th1 cytokines like interferon (IF)- α, β, γ, and IL-12 to suppress the Th2 asthmatic responses. However, due to adverse effects and the lack of efficacy, this strategy has not progressed further [39]. The existing biologics mainly targets the Th2 asthmatic response, although there are other Th2 lower asthmatic endotypes that are not fully characterised. Biologics targeting these endotypes that are specific to the airway neutrophilia were proven to be unsuccessful. Therefore, the current research aim to target the wider repertoire of the cytokines such as TNF-α, IL1-β, IL-6, IL-17 which may affect airway neutrophilia of the Th2 lower asthmatic endotypes [40]. Furthermore, the crossover and overlap of different pathways in asthma and the high cost of the treatment limit the effective usage of these biologics effectively in asthma treatment [37].

Alternative therapeutic approaches include the use of leukotriene modifiers such as montelukast and the 5-lipoxygenase inhibitor zileuton that act as anti-inflammatory and bronchodilators have also been recommended for use in combination with LABAs or ICS to treat asthma [33]. Similarly, phosphodiesterase inhibitors such as theophylline and roflumilast have also shown beneficial effects in inhibiting inflammation in murine models and clinical studies [31, 32]. Furthermore, inhibiting the kinases particularly, phosphoinositide-3-kinase (PI3K) - that regulates the expression of chemotactic and inflammatory genes (PI3Kγ), and

reduces the corticosteroid response (PI3Kδ) – may also aid as an alternative to the ICS therapy [31, 33]. However, these inhibitors possess a narrow therapeutic index and hold potential adverse effects that are associated with multiple inhibitory homeostatic pathways. Finally, the use of nutraceuticals as supplements has also shown some efficacy in the treatment of asthma. However, poor drug distribution and lack of efficacy have further led researchers to find alternate strategies to these therapies [41, 42]. Despite the development of various therapeutic strategies, a better understanding of the asthma pathogenesis is highly essential to develop alternative therapies that can selectively target mechanistic pathways using novel drug delivery methods with minimal adverse effects and enhanced efficacy.

4. Pharmacological strategies and recent advancements in nano-drug delivery for targeting asthma

Nano-drug delivery systems are the latest trends in drug delivery science that are rapidly growing in the present era. Particles in the nanoscale range are used for diagnosis as well as to deliver therapeutic agents to the targeted sites in a controlled manner. The major benefit of nanotechnology is that it allows to treat chronic diseases could be treated though site-specific, and target-oriented delivery systems. Nanotechnology is used to deliver several therapeutic moieties including biological agents, chemotherapeutic agents, and immunotherapeutic agents to treat various diseases [43].

Nanoparticles (NPs) as therapeutic agents provide more benefits than conventional drugs in the management of respiratory diseases. These benefits of NPs include uniform drug distribution, dissolution rate, macromolecule delivery, enhanced solubility, sustained release, appropriate cell internalisation, and targeted delivery of drug molecules to the required site. With the help of targeted delivery, local drug concentration could be improved in the lungs, and this may enhance the therapeutic efficacy of drugs in lungs for the treatment of asthma [44, 45].

The pulmonary route is advantageous for locally acting drugs and bioactives that are used to treat lung diseases because it reduces adverse effects associated with systemic administration. It is also a possible route for the administration of drugs to achieve systemic effects, since it offers a large surface area for drug absorption, a weak epithelial barrier, and efficient blood circulation, resulting in the direct and rapid entrance of medications into the bloodstream [46]. Pulmonary administration is crucial for the efficient delivery of drugs to the lungs as NPs travel a considerable distance from the nasal canal to the lung alveoli. Throughout this process of drug delivery, NPs should overcome several obstacles, including pulmonary barriers [47]. Various type of nano delivery systems developed to treat asthma are shown in Figure 2A and the mechanistic illustration of pulmonary drug targeting using nano-formulations into the lung are presented in Figure 2B and 2C.

A list of a key studies implicating role and development of various NPs in treatment of asthma is summarised in Table 1.

4.1 Inorganic metallic NPs

Inorganic metallic NPs have been reported as very good therapeutic, diagnostic, sensor, and imaging agents for the treatment of asthma. There are various types of inorganic metallic NPs including gold, platinum, zinc, cerium, and silver NPs [48, 49]. Recently, these materials have been extensively utilised in biomedical applications, primarily for cell and biomolecule labelling. Metallic NPs may be employed as antioxidants due to their capacity to lower ROS levels and reduce their potential. Since oxidative stress plays a key role in progression of asthma and metallic NPs such as gold and silver NPs conjugated with antioxidants have been shown to enhance drug absorption at the target site and thus, reduce the progression of asthma [44, 49].

Interestingly, a study conducted by Serra *et al.*, reported that gold NPs administered intranasally in a mice model of asthma attenuated glucocorticoid (GC) resistance in asthma via administration through intranasal route in a mice model. For the induction of asthma, ovalbumin was administered through intranasal route once a week for nine weeks. Moreover, another study has reported that gold NPs can attenuate the levels of biomarkers such as nuclear factor erythroid 2-related factor 2 (NRF2), histone deacetylase 2 (HDAC2), thiobarbituric acid reactive substances (TBARS), and PI3Kδ, which play a critical role in mediating oxidative stress. These findings indicate that the gold NPs possess a potential to reduce the level of oxidative stress and inflammation by attenuating these asthmatic biomarkers [50]. The mechanistic pathways of gold NPs against lung inflammation especially in asthma are presented in Figure 3.

Interestingly, studies have shown that gold NPs exhibited good cellular permeability and reached the target site rapidly due to their small particle size, 2-3nm [51, 52]. Indeed, Barreto *et al.*, demonstrated the efficacy of gold NPs administered via intranasal route in asthmatic mice and showed that gold NPs reduced airway hyper-reactivity, lung remodeling, and inflammation upon induction with ovalbumin [53]. Park *et al.*, reported that the intranasal delivery of silver NPs to treat asthma in ovalbumin-induced asthmatic mice led to increase in the levels of various cytokines such as IL-5, IL-6, IL-13, and nuclear factor kappa light chain enhancer of activated B cells (NFκB) as well as ROS. By enhancing the levels of these potential biomarkers, ovalbumin increased the degree of inflammation and oxidative stress. Various biochemical parameters have been analysed to evaluate the antioxidant and anti-inflammatory effects of silver NPs against ovalbumin-induced oxidative stress. The results showed that silver NPs reduced the levels of IL-5, IL-6, IL-13, NFκB and ROS. [54]. Jang *et al.*, developed silver NPs and analysed their inhibitory effects on mucus hypersecretion in allergic airway inflammation in female BALBc mice [55]. The findings showed that intranasal administration

of silver NPs significantly reduced the elevated levels of VEGF, PI3K, hypoxia-inducible factor (HIF)-1, and phosphorylated-*Akt* levels, as well as mucosal glycoprotein expression (Muc5ac) in lung tissues [55].

4.2 Polymeric NPs

In recent years, polymeric NPs have gained substantial attention owing to their compact size [56, 57]. Properties of polymeric NPs are categorised based on their capability for controlled release, ability to shield biologically active drugs and other chemicals from the environment, and their capacity to enhance bioavailability and therapeutic index of conjugated drug [58]. Various studies have reported that polymeric NPs alone, as well as those conjugated with drugs can enhance drug delivery to the pulmonary route and increase drug potency.

A study conducted in 2022 by Ullah *et al*., reported an enhanced pulmonary delivery of montelukast when loaded into polymeric NPs. The optimised polymeric NPs prepared by the ionic gelation were reported to have average particle size range, zeta potential, and polydispersity index (PDI) of 220 nm to 383 nm, - 11 mV to 22 mV, and 0.50, respectively. To evaluate physical and chemical properties as well as purity of the developed polymeric NPs, differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) studies were performed. Polymeric NPs significantly increased potency and pulmonary delivery of montelukast conjugated with them as that of raw montelukast [59]. Another study conducted in 2021 by Jin *et al*., showed that NPs loaded with berberine exhibited a beneficial effect in a house dust mite (HDM) model of asthma in mice. NPs were prepared with polylactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG) that were coated with platelet membranes (PM). These NPs were then administered intranasally, and the resulting therapeutic outcome was evaluated. The formed NPs without PM were found to be of 280 nm in size, and after coating with PM, the size of NPs increased up to 400 nm. The surface charge of the NPs

was found to be 0 mV and -23 mV in case of NPs with and without PM coating, respectively. The berberine loading percentage significantly increased from 8.1% to 81.02% after encapsulation and coating resulted in a sustained release of berberine. The cellular uptake of the NPs was evaluated on the A549 human adenocarcinoma cell line and was observed that, when the cell line was in normal conditions, there was no difference between the uptake of the uncoated and PM coated NPs. However, when the cell line was treated with house dust mite (HDM), the uptake of PM-coated NPs was higher than uncoated NPs. Further analysis showed that PM-coated NPs enhanced the expression of cytokine IL-12 and decreased the expression of IL-13 and IL-4, demonstrating the role of PM coating in treating inflammation [60].

Matsuo *et al.*, showed the beneficial effect of polymeric NPs, prepared using poly (D, L-lactic acid (PLA) homopolymers, polyethylene glycol (PEG), diethanolamine, acetone, and a blend of PLA-PEG, and conjugated with betamethasone (BP), in a murine model of asthma. The evaluation of particle size and drug loading was performed using dynamic light scattering (DLS) method and high-performance liquid chromatography (HPLC). Importantly, it was revealed that BP conjugated with NPs was detectable in the lungs upto a week from the day of administration compared to BP alone that was undetectable after 24 hours post-administration. Importantly, the findings from this study demonstrated that BP conjugated NPs significantly reduced the inflammation and the release of IL-13 and IL-14 upon intravenous administration when compared with BP alone [61].

4.3 Chitosan-based NPs

Chitosan-based NPs are highly biodegradable, biocompatible, and stable in nature, that are soluble in aqueous acidic solutions and can regulate the release of active substances [62]. Chitosan is a mucopolysaccharide found abundantly in the cell walls of several fungal species [63]. Wang *et al.*, reported the successful development and evaluation of the chitosan-based

385 NPs of baicalein and their efficacy in altering the pathology of asthma in ovalbumin-induced
386 asthma mouse model. This study used glyceryl monooleate, P407, anhydrous ethanol, gelucire
387 44/14, and trimethyl chitosan for the development of the NPs. The particle size of the developed
388 NPs was reported to be 285 ± 25 nm with negative zeta potential of -10.5 mV, whereas drug
389 loading and encapsulation efficiency were 74.2% and 96.1%, respectively, with enhanced drug
390 release in drug-loaded NPs compared to encapsulated NPs. While evaluating the efficacy of
391 these NPs against asthma, it was observed that both loaded and encapsulated NPs significantly
392 increased IL-12 levels and decreased IL-5 levels when compared to the non-treatment group
393 [64]. Dhayanandamoorthy *et al*., have reported anti-asthmatic effects of hyaluronic acid-
394 decorated and ferulic acid-loaded chitosan NPs (HAFACNPs) against ovalbumin-induced
395 asthma in mice. Intranasal administration of HAFACNPs aerosols significantly attenuated the
396 level of various cytokines such as IL-5, IL-13, TNF-α, and IFN-γ in addition to the attenuation
397 of airway resistance, eosinophil infiltration and serum IgE levels [65].
398
399 ***4.4 Dendrimers***
400 Dendrimers are radially symmetric, homogeneous, monodispersed systems that are structurally
401 similar in appearance as that of tree branches. They consist of a core structure that is made up
402 of a central atom or group of atoms [66]. Branches of dendrimers also known as "dendrons,"
403 are developed from this core structure by several chemical processes [66].
404 Nasr *et al*., successfully developed, and characterised beclomethasone dipropionate (BDP)-
405 loaded polyamidoamine (PAMAM) dendrimers. To characterise the optimised BDP-loaded
406 dendrimers, drug solubility in various buffers having different pH and *in vitro* drug release
407 were evaluated. Three different nebulisers: Aeroneb Pro® (actively vibrating mesh), Pari LC®
408 Sprint (air-jet), and Omron Micro Air® (passively vibrating-mesh) were used to evaluate
409 aerosol formulation properties. Drug release study revealed that only 35% drug was released

410 from the dendrimer loaded with the drug after 8 hours successfully representing a sustained
411 release pattern of the drug from the formulation.
412 Importantly, dendrimers are classified based on their growth process and generation number,
413 such as G0.5, G1, G2, G3, G4, and G5 [67]. When a generation 3 (G3) was used as a model
414 dendrimer it was observed that the amount of BDP complexed with the dendrimers was
415 increased upon increasing the pH from 5 to 7.4, and the maximum solubility was found at pH
416 9.8. In contrast, there was no difference in the solubility of the uncoated BDP upon change in
417 pH. Moreover, it was also observed that the generation of dendrimer has no impact on the
418 aerosol's output, however, higher aerosol output was observed in Pari LC® Sprint nebuliser
419 compared to the Aeroneb Pro device, but this was not statistically significant ($p > 0.05$) [68].
420
421 ***4.5 Microspheres***
422 Microspheres are very small particles that are spherical in shape and have an average particle
423 size lies in the range of 1 to 100 microns. These can be prepared by several techniques such as
424 coacervation, coprecipitation, solvent dispersion, emulsification, and spray freeze-drying
425 technique [69, 70].
426 A study conducted by Nagaraja *et al.*, reported the successful development of microspheres of
427 salbutamol using spray drying. The microspheres (developed with PLGA and PEG) loaded
428 with salbutamol were administered through the intravenous route and its anti-asthma potential
429 was evaluated on A547 cell line. The percentage yield and encapsulation efficiency of
430 microspheres were found to be 86% ± 0.4% and 72% ± 0.8%, respectively. The particle size of
431 the optimised microspheres was found to be 8.24 µm. The developed salbutamol microspheres
432 showed sustained release action as compared to salbutamol alone. Interestingly, MTT assay
433 demonstrated that salbutamol entrapped in the microsphere was less toxic compared to
434 salbutamol alone, thus implying the relative safety of the developed NPs [70].

435

436 ***4.6 Liposomes***

437 Liposomes are commonly used drug delivery systems that employ microscopic spherical-
438 shaped vesicles composed of cholesterol and non-toxic natural phospholipids [71]. Liposomes
439 possess several biomedical applications owing to their nano size and hydrophobicity [71, 72].
440 A study conducted by Ng *et al*., demonstrated significant anti-inflammatory activity of
441 liposomes in a lipopolysaccharide (LPS)-induced *in vitro* asthma model that employed the BCi-
442 NS1.1 minimally immortalized human airway epithelial cell line. These liposomes were
443 prepared by lipid hydration technique and were loaded with curcumin. The optimised
444 liposomes showed an average vesicle size and zeta potential of 271.3 ± 3.06 nm and -61.0 mV,
445 respectively. During biochemical studies, various inflammation parameters were analysed.
446 Curcumin-loaded liposomes significantly attenuated IL-6, IL-8, IL-1β, and TNFα levels as
447 compared to naïve curcumin. Curcumin-loaded liposomes were found to be five times more
448 potent than naïve curcumin [73].
449 Xiao *et al*., reported bergenin-loaded cationic liposomes (designed by thin film dispersion
450 technique) for the treatment of asthma. Optimised liposomes showed a vesicle size and zeta
451 potential of 158.33 ± 5.88 nm, and + 24.51 ± 0.51 mV respectively. The bergenin-loaded
452 liposomes showed 3.33-fold more potential when compared with naïve bergenin. This study
453 reported that bergenin loaded liposomes minimised inflammatory activities and enhanced the
454 balance between T helper 1 cytokines and T helper 2 cytokines [74, 75].

455

456 ***4.7 Polymeric micelles (PMs)***

457 PMs are one of the most viable nanocarriers for delivery of a drug through the pulmonary route.
458 The small size of PMs increases the solubility of lipophilic drugs and aids in preventing
459 pulmonary macrophage clearance [76]. Importantly, PMs can improve the drug release

properties and facilitate lung targeting. The alteration of polymers with ligands targeting receptors on the surface of alveoli could promote absorption of medicines, diffusion via the epithelium, and cellular uptake, thereby enhancing their bioavailability. Eventually, the administered drug doses may be decreased by effectively targeting the drug to lungs, which significantly increases patient compliance [77].

Peng and co-workers have formulated anti-high affinity immunoglobulin epsilon receptor subunit gamma (FcRI) fragment antigen binding (Fab)-loaded celastrol micelles against allergic inflammation. *In vitro* analysis revealed that the drug-loaded micellar formulation exhibited greater cellular uptake and cytotoxicity towards the human basophil cell line KU812. During the *in vivo* study, it was observed that the celastrol micelles got accumulated in the lungs and caused a significant decrease in IgE, histamine, Th2 cytokines such as IL-4, IL-5, TNF-α, as well as eosinophil infiltration and mucus formation [78]. Yoo *et al*., have developed hydroxybenzyl alcohol incorporated polyoxalate (HPOX) micelles, prepared by using conventional single emulsion technique, against airway inflammatory diseases in ovalbumin-induced asthmatic rats. Importantly, intratracheal administration of HPOX micelles reduced not only the action of inflammatory cells but also, demonstrated antioxidant and anti-inflammatory action as they reduced levels of ROS, IL-1β, COX-2, and inducible nitric oxide synthetase (iNOS) in lungs [79].

4.8 Exosomes

Exosomes are small extracellular vesicles that are released by virtually every cell type and possess an excellent capacity to deliver drugs at a target site rapidly. In addition, exosomes also have several benefits over other inorganic and organic carriers, including high transmission efficiency, and minimal immunogenicity [80-83].

Various studies have shown that intranasal administration of M2 macrophage-derived exosomes (M2Φ-Exos) in ovalbumin-induced asthmatic mice inhibited the expression of various cytokines such as IL6, IL1β, TNF-α and monocyte chemoattractant protein-1 (MCP-1)[84]. Another study conducted by Shang *et al*., reported the anti-inflammatory effects of adipose-derived stem cell (ADSC)-exosomes administered intravenously for the treatment of asthma in mice via intravenous route. The results of biochemical studies revealed that adipose-derived stem cell (ADSC)-exosomes significantly reduced iNOS, TNF-, and IFN- expression, simultaneously increasing the expression of anti-inflammatory cytokine IL-10 [85].

4.9 Solid lipid NPs (SLNs)

SLNs are novel drug delivery systems that are generally spherical in shape, with an average diameter of 10 to 1000 nm. SLNs are categorised as colloidal NPs that contain a lipid drug carrier (prepared by solid lipid, surfactants, and water.) They provide good drug loading capacity and remarkable physical stability [86, 87]. SLNs have several biomedical applications as they enhance the drug solubility and bioavailability. They offer controlled drug release, improve drug targeting, and offer flexibility of administration through numerous routes, including parenteral, oral, pulmonary, and topical [87].

Lv *et al*., reported the therapeutic benefits of rhynchophylline-loaded within SLNs. The purpose of developing this system was to enhance the therapeutic efficacy of rhynchophylline for the treatment of allergic asthma. SLNs were prepared by solvent injection method and showed an average particle size, zeta potential and drug entrapment efficiency of 62.06 ± 1.62 nm, -6.53 ± 0.04 mV, and 82.6 ± 1.8%, respectively. On days 0, 14, 28, and 42, mice were subcutaneously injected with 20 µg of ovalbumin mixed with 1 mg aluminium hydroxide. From days 21 to 42, mice were administered with aerosol of 1% ovalbumin (w/v) for the induction of allergic asthma. Rhynchophylline-loaded within SLNs at the dose of 20 mg/kg significantly

attenuated the levels of airway remodeling markers such as mucus gland hyperplasia and collagen deposition, oxidative stress, and airway inflammation. By inhibiting the p38 signalling pathway, it also increased the expression of suppressor of cytokine signalling 1. These findings suggested that rhynchophylline loaded SLNs showed improved response compared with the free drug [88]. Li et al., developed curcumin loaded SLNs in the form dry powder inhaler (DPI) for the treatment of asthma in mice. Curcumin loaded SLNs were prepared by microemulsion method and solidification was done by spray-drying techniques. Ovalbumin was administrated to produce asthma in mice. Curcumin SLNs DPI showed a sustained-release action during *in vitro* release studies and there was no evidence for acute toxicity of this formulation found in mice. Curcumin's SLNs DPI significantly ameliorated the airway inflammatory response of the airway and the severity of pulmonary congestion in mice [89].

4.10 Nanostructured lipid carriers (NLCs)

The second generation of SLNs are reported as NLCs. NLCs are preferred over other lipid formulations due to their increased physical stability, drug loading capacity, enhanced oral bioavailability, and modulated drug release profile. Interestingly, Gadhe *et al*., have reported the anti-asthmatic potential of montelukast-loaded NLCs, where NLCs were prepared by the melt emulsification homogenization technique and exhibited an average particle size and encapsulation efficiency of 181.4 ± 6.5 nm and 96.13 ± 0.98%, respectively. Montelukast-loaded NLCs enhanced the oral bioavailability by 1.43-fold as compared to naïve montelukast [90].

5. Gene therapy for asthma

As the existing therapeutic approaches for asthma management are outdated, and a considerable patient population is developing steroid-resistant asthma, researchers are now

focusing on the promising aspect of gene therapy for asthma [91]. *In vitro* studies have demonstrated that the usage of the decoy oligodeoxynucleotide (ODN) inhibiting NFκB can attenuate airway inflammation by inhibiting the expression of LPS-induced cytokines such as IL-6 and IL-8 in IB3-1 human bronchial epithelial cells [92, 93]. In an ovalbumin-induced rat asthma model, the ODN specific for signal transducer and activator of transcription (STAT) family members 1 and 3 were found to decrease the characteristic features of allergic inflammation as revealed by a decrease in the bronchoalveolar lavage fluid (BALF) count of eosinophils and T lymphocytes. Similarly, the protein expression of CD40 in the lung tissue was significantly attenuated by STAT ODN [94].

Using the nanotechnology approach, it is now possible to formulate gene-loaded nanotherapeutics such as NFκB ODN to target chronic respiratory diseases, including asthma [95]. Ungaro *et al*., developed a sustained release decoy ODN to NFκB using poly(lactic-co-glycolic) acid (PLGA), and an “adjuvant" hydrophilic polymer polyethylenemine (PEI) as a carrier. These respirable particles delivering ODN to NFκB were tested for their efficacy to inhibit the expression of cytokines IL-8 and mucus-secreting gene *MUC2* in LPS-induced airway epithelial cells. It was observed that ODN to NFκB results in an extended inhibition of IL-8 and *MUC2* expression *in vitro*, compared to the naked decoy ODN, in human bronchial epithelial cells and human epithelial pulmonary cells, respectively [96].

Nanoparticle-based thymulin gene therapy can reverse the primary features of allergic asthma in a mice model induced by ovalbumin[97]. A single dose intratracheal administration of plasmids encoding biologically active thymulin analog showed enhanced penetration of the airway mucus barrier[97]. After three weeks of thymulin treatment, it was observed that the chronic airway inflammation measured in terms of BALF eosinophil and lymphocytes counts, immune cells recruiting chemokines such as CCL11, CXCL1, CCL17, and pro-and anti-inflammatory mediators such as IL-4, IL-13, IL-10 were significantly decreased compared to

559 only ovalbumin (without thymulin treatment) group. Similarly, the airway remodeling factors
560 such as vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-
561 β) were significantly decreased. Likewise, the dysregulated levels of lymphocyte mediators
562 such as IL-13 and IL-10 and macrophage polarization marker Foxp3 and Arginase were
563 normalised by a single dose of thymulin-loaded nanoparticles. Furthermore, in a methacholine
564 challenge assessment, more pronounced airway resistance and dynamic compliance were
565 observed in asthmatic mice lungs while improved in the thymulin nanoparticles group [97].
566 Another study conducted by Gavitt *et al.* investigated the GATA binding protein 3 (GATA3)
567 peptide DNAzyme nucleic acid nanocapsules (pep Dz-NANs) for *in vivo* efficacy in asthma.
568 In this study, HDM antigen was administered intranasally for five days/week for five weeks to
569 induce allergic asthma in mice and intranasal pep Dz-NANs at a dose of 25nM, 125nM,
570 250nM, and 1250nM. On the site of airway inflammation, the high level of matrix
571 metalloproteinase-9 (MMP9), a proteolytic enzyme can cleave the pep Dz NAN to individual
572 DNAzyme-surfactant conjugates for intracellular gene regulation of *GATA3*. Interestingly,
573 mice treated with pep Dz-NANs reduced the severity of HDM-induced allergic lung
574 inflammation as shown by a decrease in BALF eosinophil count. This study thus suggests that
575 peptide-based GATA3 Dz-NANs could be a promising approach to decrease the severity of
576 asthma symptoms [98].
577 Kumar *et al*., showed that in an ovalbumin-induced mice model of allergic asthma, the
578 chitosan-based interferon-γ (IFN-γ) plasmid DNA nanoparticles can reduce the inflammation
579 and airway reactivity [99]. Mechanistically, it was demonstrated that intranasal administration
580 of 10 μg chitosan IFN-γ nanoparticles before intraperitoneal injection of ovalbumin decreased
581 the antigen-presenting capacity of dendritic cells isolated from lung and lymph nodes, as
582 evident from the reduction in CD80 and CD86 expression. Likewise, chitosan IFN-γ
583 nanoparticles remarkably reduced the population of $CD11c^{+}b^{+}$ dendritic cells in lymph nodes,

584 indicating that endogenous IFN-γ expression may immunomodulate dendritic cell migration
585 and activation. Furthermore, there was a reduction of IFN-γ production and apoptosis of
586 ovalbumin-specific $CD8^+$ T cells (isolated from mice) cultured *in vitro* in the presence of
587 ovalbumin [100].

588 Researchers have established that c-kit, a proto-oncogene in dendritic cells, plays a crucial role
589 in the development of allergic asthma by regulating T helper cell differentiation [101]. Wu *et*
590 *al*., found that intranasal administration of siRNA nanoparticles targeting c-kit can suppress
591 the features of allergic asthma in an ovalbumin-induced mouse model. In this study, the
592 intranasal administration of 35μg/day of siRNA for 3 consecutive days (Day 21-23 after
593 ovalbumin sensitisation) significantly downregulated the expression of the c-*kit* gene and
594 suppressed mucus secretion and eosinophil infiltration in the BALF. Additionally, c-*kit* siRNA
595 inhibited the generation of stem cell factor (a ligand of c-kit), IL-4, and IL-5, while there were
596 no changes in interferon-γ (IFN-γ) levels, suggesting the therapeutic potential of siRNA-based
597 nanoparticles to manage allergic asthma [102]. Taken together, these studies have clearly
598 highlighted the immense beneficial biological activity of gene therapy for the effective
599 management of asthma, which could be a promising alternative to current treatment options
600 such as β_2 agonists and ICS.

601

602 **6. Clinical trials and studies using advanced drug delivery systems to target asthma**

603 Despite the growing body of evidence showcasing the enormous potential of advanced drug
604 delivery systems in pulmonary diseases, most of the clinical studies so far have focused on the
605 treatment of malignancies [43], and a limited number of clinical studies are currently in
606 progress or have completed to assess the efficacy of these drug delivery systems against
607 asthma. The clinical studies identified are summarised in Table 2.

Interestingly, a clinical study showed that liposomes possess a potential to be used as an advanced delivery system to entrap and deliver *D. pteronyssinus* allergens that are used as antigen vaccination approaches against asthma [103]. In this case, liposome encapsulation of the antigen was used to exploit the depot effect and the increased antigen delivery to lymphatic vessels and lymph nodes that are characteristic of liposome-based formulations administered through subcutaneous injections [103]. This vaccination approach proved successful in protecting mild asthma patients from the worsening of symptoms that usually occurs upon dust mite exposure [103].

Notably, a phase II clinical trial demonstrated safety, efficacy, and tolerability of QbG10 (i.e., bacteriophage Qbeta-derived virus-like particle loaded with the bacterial oligonucleotide CpG-motif G10), administered subcutaneously (NCT00890734). QbG10 is identified to stimulate the immune system towards a protective Th1-mediated response *via* stimulation of toll-like receptor (TLR-9). This study showed that treatment with QbG10 achieved continued control of asthma upon steroid reduction in patients under treatment with moderate or high doses of inhalational steroids [45].

Allergic bronchopulmonary aspergillosis (ABPA) is an infrequent complication of ailments such as chronic asthma and cystic fibrosis [46]. Notably, Godet *et al*., reported that inhalational treatment with nebulised liposomal amphotericin B (LAmB) was effective and induced durable improvements in a patient with uncontrolled ABPA [47]. Despite this finding, a Phase II clinical trial (NCT02273661) performed by the same research group failed to show a reduced risk of severe ABPA clinical exacerbations upon maintenance therapy with nebulised liposomal amphotericin-B (Ambisome®) [104]. Nevertheless, some positive secondary outcomes such as significant reduction of immunoglobulin-E and *Aspergillus* precipitins were achieved in the treatment group, providing a platform for further investigation in the field. [104].

Arafa *et al*., had successfully developed niosome-based nanovesicles entrapping salbutamol sulphate (SS) for the treatment of asthma with potential for packaging into a metered dose inhaler (MDI) meeting the US Pharmacopoeia aerosol guidelines [105]. The relative bioavailability of this formulation was compared with that of a classical SS MDI formula in a Phase I clinical trial (NCT03059017). Administration of the niosome-based formulation resulted in sustained, controlled pulmonary release of SS to maintain therapeutic drug levels [49].

7. Conclusion

Over the last few years our understanding of the pathophysiology of asthma, especially the role of cytokines, has undergone a paradigm shift. Primarily, asthma is recognised as a Th2-mediated cell disorder, and this dogma has been predominantly translated from mouse models of asthma that led to development of several type-2 targeted therapeutic agents specifically to reduce frequency of exacerbations in patients who are on conventional therapy. However, not all patients demonstrate improvement in symptoms, and this is due to the heterogeneity of cytokine pathways and mechanisms that drive the disease. This has led to the development of cytokine targeting drug delivery systems as well as clinical trials as we discussed in this review. However, despite the growing body of research that illustrates enormous potential of advanced drug delivery systems in pulmonary diseases, most of the clinical research so far has focused on the treatment of malignancies, and very few clinical studies are currently in progress or completed to assess the efficacy of these drug delivery systems against asthma that have a pleotropic effect on specific cytokines. The bottlenecks associated with the clinical translation of advanced drug delivery systems are manufacturing of such systems in bulk quantity, biological barriers, safety, government regulated protocols, and overall cost-effectiveness as compared to currently available treatments. The maintenance of the integrity of nanoparticles

in terms of their size, homogeneity and release behaviour with batch-to-batch consistency and reproducibility during scale-up has always been a challenging task. Despite, industries have taken several initiatives in this area with approaches such as six sigma (i.e., process that uses statistics and data analysis to analyse and reduce errors in medication non-adherence [106]) and quality by design, for understanding of key manufacturing attributes affecting physicochemical properties of formulations however, a complete control on the variables still requires further elucidation.

To overcome the biological barriers such as inter-individual heterogeneity of tissue barriers, immune system, cellular uptake, and intracellular trafficking in repose to disease, a good correlation between disease pathology and inter-individual heterogeneity is crucial. The understanding of physicochemical behaviour of advanced drug delivery systems *in vivo* and strategies for overcoming biological barriers for achieving better targeting of drugs to diseased tissue and restriction in their build-up in non-specific organs should be tailored. Unfortunately, limited attention has been paid towards correlations between behaviour of advanced drug delivery systems and patients' biology. In our opinion, this could also be one of the prime reasons for the failure of such formulations during clinical trials. These biological barriers could be an important deterrent for pharmaceutical industries for investing on advanced drug delivery systems. Hence, it is important to comprehensively evaluate the preclinical data in terms of therapeutic efficacy, safety, biodistribution, and pharmacokinetics in appropriate animal models and correlate them with human physiology. Furthermore, for achieving reproducible results, the studies should be done on multiple animal models rather than depending on the trial done on a single animal model. This is very important as the animals decipher only correlation with human physiology. Using multiple animal models to understand the safety and efficacy of the formulation will reduce the chances of failure of formulation's performance during correlation of preclinical and clinical data.

Advanced drug delivery systems are always associated with clinical toxicities. The sponsors must ensure the safety of developed delivery systems prior to their clinical use. A careful monitoring of excipients used and their safety, as well as the stability of the formulations during their production should be performed. Such practices would assist the sponsors in reducing the risk of product failure due to regulatory objections related to their toxicity or instability. The knowledge of biological activity and toxicity associated with active pharmaceutical ingredients, excipients, and their advanced drug delivery, as well as their interaction with biological components, helps in predicting the toxicity of formulations. Furthermore, the influence of drug release rate on target and off-target concentrations of bioavailable drug could also help in predicting their safety and toxicity. In addition, conducting specialized toxicity studies in suitable animal models can provide additional in the assessment of both short-term and long-term toxicity.

Nevertheless, ongoing developments in gene therapy, drug delivery systems and clinical trials will further unravel the complexity of asthma leading to improved therapies that can target specific or multiple cytokine pathways underlying pathophysiological asthma traits.

Declaration of interest

None

705 **Table 1 Role of different NPs in targeting and drug delivery for asthma**

No.	Formulation	Route of administrations	Animals or/and cell lines	Inducing agent	Outcomes	References
			Inorganic metallic NPs			
1.	PEGylated and Citrated gold NPs	Intranasal	Mice	Ovalbumin	• It provided anti-inflammatory effects by attenuating IL-1β • Inhibited airway hyperreactivity and inflammatory infiltrates	[107]
2.	Titanium dioxide NPs	Intranasal	Mice	Ovalbumin	• Titanium dioxide NPs activated level of transient receptor potential vanilloids (TRPVs) and TRPV P 2 × 7 • Secreted neuromediators that cause airway inflammation and exacerbate asthma	[108]
3.	Silica dioxide NPs	Intranasal	Mice	Ovalbumin	• Enhanced mRNA expression • Attenuated level of thioredoxin-interacting protein, NLRP3 inflammasome, and IL-1β proteins	[109]
4.	Zinc oxide NPs	Intranasal	Mice	Ovalbumin	• Reduced airway inflammation • Enhanced Th2 cytokine	[110]
5.	Zinc oxide NPs	Intranasal	Mice	Ovalbumin	• Reduced IL-4, IL-5, and IL-13	[111]
				NPs		
6.	Carbon black NPs	Intranasal	Mice	Ovalbumin	• Attenuated airway hyperreactivity,	[112]

7.	Conjugated α alumina NPs with vasoactive intestinal peptide (VIP)	Intranasal	Mice	Ovalbumin	• Reduced remodeling • Produced antiinflammation • Inhibited IL4-, IL-6, IL-13, IL-1β and TNF-α • Alleviated level of IL-10 • α-alumina NPs prevented enzymatic denaturation of VIP in the respiratory tract • Attenuated numbers of eosinophils, Th2 cytokines, serum IgE level and hyperresponsiveness • Attenuated pro-inflammatory cytokines such as IL-13, eosinophiles	[113]
8.	DNA NPs mediated thymulin gene therapy	Intranasal	Mice	Ovalbumin	• Mitigated lung remodeling, airway inflammation and leading • Improved pulmonary mechanics • Attenuated collagen deposition	[114]
9.	Bilirubin NPs	Intravenous	Mice	Ovalbumin	• Ameliorated level of Th2-related infection in lungs during allergic inflammation	[115]
			Liposomes			
10.	Salbutamol sulphate	Intraperitoneal	Rats and guinea pig	Ovalbumin	• Enhanced encapsulation efficiency by 70% in rats • Reduced the risk of asthma in guinea pigs	[116]
			Solid lipid NPs			
11.	Curcumin	Intraperitoneal	Rats	Ovalbumin	• Enhanced bioavailability by 26 folds as compared to naïve curcumin • Attenuated airway hyperresponsiveness	[117]

					• Reduced expression of T-helper-2-type cytokines like IL-4, IL-5 and IL-13 • Offers antioxidant effects and helped in decreasing levels of ROS • Attenuated the pro-inflammatory mediator such as IL-6, IL-1β and TNF-α	
12.	Curcumin	Intraperitoneal	Mice	LPS	• Augmented the level of cytokine IL-10 by ELISA • Inhibited the expression of TLR4, TLR2, and TNF-α in lymph node tissues	[118]
13.	CCR3 antagonists (R321 peptide)	Intranasal	Mice	Eotaxin	• Inhibited the level of eosinophiles • Blocked airway hyperresponsiveness	[119]
				Exosomes		
14.	Exosomal miRNA	Intranasal	Human	--	• Attenuated the level of various cytokinin such as IL-13 and TNF-α	[120]
15.	B-cell-derived exosomes	Intraperitoneal	Mice	Ovalbumin	• Enhanced the levels of various cytokinin such as IL-4, IL-5, and IL-13 • Attenuated levels of IFN-γ, and TNF-α	[121]

711 **Table 2 Clinical trials using advanced drug delivery systems to target asthma**

Clinical Trials	Drug and Drug Delivery Method	Outcomes	Clinical Trial Identifier	References
Pre-Phase I	Liposome-entrapped *D. Pteronyssinus* vaccination	Formulation protects mild asthma patients from worsening of symptoms following dust mite exposure	N/A	[103]
Phase I	Salbutamol Sulphate entrapped in niosome-based nanoparticles	Niosome-based formulation resulted in sustained, controlled pulmonary release of salbutamol sulphate compared to classical formulation	NCT03059017	[49]
Phase II	Virus-like nanoparticles loaded with bacterial oligonucleotide CpG-motif G10	Formulation was effective in controlling asthma upon steroid reduction in patients on moderate or high-dose inhalational steroids	NCT00890734	[45]
Phase II	Nebulised Liposomal Amphotericin B (Ambisome®)	Formulation failed to show a reduced risk of severe ABPA clinical exacerbations when used as maintenance treatment. Positive secondary outcomes (reduction of immunoglobulin-E and Aspergillus precipitins) achieved.	NCT02273661	[104]

712

713

714

Author contributions

VKP formatted and compiled the manuscript. VKP, SV, RK, SDS, KRP, GDR, BM, DKC, VSRA, PMH, BGO, RML and KD contributed to manuscript writing and proof reading; VKP, DKC, SV, RK and SKS prepared the figures. All authors approved the content of the manuscript.

Acknowledgements

The authors are thankful to the Graduate School of Health, University of Technology Sydney, Australia. KD is supported by a project grant from the Rebecca L Cooper Medical Research Foundation and the Maridulu Budyari Gumal Sydney Partnership for Health, Education, Research and Enterprise (SPHERE) RSEOH CAG Seed grant, fellowship, and extension grant; Faculty of Health MCR/ECR Mentorship Support Grant and UTS Global Strategic Partnerships Seed Funding Scheme. KD is supported by a project funding from the Uttaranchal University to undertake respiratory research. GDR is supported by the UTS International Research Scholarship and the UTS President's Scholarship. KRP is supported by a fellowship from Prevent Cancer Foundation (PCF) and the International Association for the Study of Lung Cancer (IASLC).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Collaborators GBDCRD. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med. 2020;8(6):585-96.

[2] Australian Bureau of Statistics (2020-21) A, ABS Website, accessed 18 January 2023.

[3] Australian Bureau of Statistics [Internet]. ABS website. 2020-21.

[4] Shastri MD, Chong WC, Dua K, Peterson GM, Patel RP, Mahmood MQ, et al. Emerging concepts and directed therapeutics for the management of asthma: regulating the regulators. Inflammopharmacology. 2021;29(1):15-33.

[5] Australian Bureau of Statistics, Asthma [Internet]. ABS website. 2020-21.

[6] Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet. 2015;386(9998):1075-85.

[7] Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. Lancet. 2018;391(10122):783-800.

[8] Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol. 2015;15(1):57-65.

[9] Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clin Rev Allergy Immunol. 2019;56(2):219-33.

[10] King GG, James A, Harkness L, Wark PAB. Pathophysiology of severe asthma: We've only just started. Respirology. 2018;23(3):262-71.

[11] Bush A. Pathophysiological Mechanisms of Asthma. Front Pediatr. 2019;7:68.

[12] Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. Nat Rev Dis Primers. 2015;1:15025.

[13] Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med. 1989;320(5):271-7.

[14] Holgate ST. Innate and adaptive immune responses in asthma. Nat Med. 2012;18(5):673-83.

[15] Ghaffari J, Rafiei AR, Ajami A, Mahdavi M, Hoshiar B. Serum interleukins 6 and 8 in mild and severe asthmatic patients, is it difference? Caspian J Intern Med. 2011;2(2):226-8.

[16] Yssel H, Groux H. Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. Int Arch Allergy Immunol. 2000;121(1):10-8.

[17] Dworski R. Oxidant stress in asthma. Thorax. 2000;55 Suppl 2:S51-3.

[18] Peebles RS, Jr. Prostaglandins in asthma and allergic diseases. Pharmacol Ther. 2019;193:1-19.

[19] Balan S, Radford KJ, Bhardwaj N. Unexplored horizons of cDC1 in immunity and tolerance. Adv Immunol. 2020;148:49-91.

[20] Klein Wolterink RG, Kleinjan A, van Nimwegen M, Bergen I, de Bruijn M, Levani Y, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. Eur J Immunol. 2012;42(5):1106-16.

[21] Ricardo-Gonzalez RR, Van Dyken SJ, Schneider C, Lee J, Nussbaum JC, Liang HE, et al. Tissue signals imprint ILC2 identity with anticipatory function. Nat Immunol. 2018;19(10):1093-9.

[22] Van Dyken SJ, Nussbaum JC, Lee J, Molofsky AB, Liang HE, Pollack JL, et al. A tissue checkpoint regulates type 2 immunity. Nat Immunol. 2016;17(12):1381-7.

[23] St-Laurent J, Bergeron C, Page N, Couture C, Laviolette M, Boulet LP. Influence of smoking on airway inflammation and remodelling in asthma. Clin Exp Allergy. 2008;38(10):1582-9.

[24] Varricchi G, Ferri S, Pepys J, Poto R, Spadaro G, Nappi E, et al. Biologics and airway remodeling in severe asthma. Allergy. 2022;77(12):3538-52.
[25] Limb SL, Brown KC, Wood RA, Wise RA, Eggleston PA, Tonascia J, et al. Irreversible lung function deficits in young adults with a history of childhood asthma. J Allergy Clin Immunol. 2005;116(6):1213-9.
[26] Bartemes KR, Kita H. Dynamic role of epithelium-derived cytokines in asthma. Clin Immunol. 2012;143(3):222-35.
[27] Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. J Appl Physiol (1985). 2013;114(7):834-43.
[28] Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. Front Microbiol. 2013;4:263.
[29] de Groot JC, ten Brinke A, Bel EHD. Management of the patient with eosinophilic asthma: a new era begins. ERJ Open Research. 2015;1(1):00024-2015.
[30] Papi A, Blasi F, Canonica GW, Morandi L, Richeldi L, Rossi A. Treatment strategies for asthma: reshaping the concept of asthma management. Allergy Asthma Clin Immunol. 2020;16:75.
[31] Barnes PJ. New therapies for asthma: is there any progress? Trends in Pharmacological Sciences. 2010;31(7):335-43.
[32] Sulaiman I, Lim JCW, Soo HL, Stanslas J. Molecularly targeted therapies for asthma: Current development, challenges and potential clinical translation. Pulmonary Pharmacology & Therapeutics. 2016;40:52-68.
[33] Hanania NA. Targeting Airway Inflammation in Asthma: Current and Future Therapies. Chest. 2008;133(4):989-98.
[34] Marandi Y, Farahi N, Hashjin GS. Asthma: beyond corticosteroid treatment. Arch Med Sci. 2013;9(3):521-6.
[35] Cazzola M, Segreti A, Matera MG. Novel bronchodilators in asthma. Current Opinion in Pulmonary Medicine. 2010;16(1).
[36] Sunkara K, Mehta M, Satija S, Dhanjal DS, Sharma P, Shukla SD, et al. An Introduction to Respiratory Diseases and an Emerging Need for Efficient Drug Delivery Systems. In: Chellappan DK, Pabreja K, Faiyazuddin M, editors. Advanced Drug Delivery Strategies for Targeting Chronic Inflammatory Lung Diseases. Singapore: Springer Singapore; 2022. p. 1-24.
[37] Quirce S, Phillips-Angles E, Domínguez-Ortega J, Barranco P. Biologics in the treatment of severe asthma. Allergologia et Immunopathologia. 2017;45:45-9.
[38] Jin HJ. Biological treatments for severe asthma. Yeungnam Univ J Med. 2020;37(4):262-8.
[39] Hansbro PM, Kaiko GE, Foster PS. Cytokine/anti-cytokine therapy - novel treatments for asthma? Br J Pharmacol. 2011;163(1):81-95.
[40] Tan R, Liew MF, Lim HF, Leung BP, Wong WSF. Promises and challenges of biologics for severe asthma. Biochemical Pharmacology. 2020;179:114012.
[41] Allam V, Chellappan DK, Jha NK, Shastri MD, Gupta G, Shukla SD, et al. Treatment of chronic airway diseases using nutraceuticals: Mechanistic insight. Crit Rev Food Sci Nutr. 2021:1-15.
[42] Allam V, Paudel KR, Gupta G, Singh SK, Vishwas S, Gulati M, et al. Nutraceuticals and mitochondrial oxidative stress: bridging the gap in the management of bronchial asthma. Environ Sci Pollut Res Int. 2022.
[43] Ahmad A. Pharmacological Strategies and Recent Advancement in Nano-Drug Delivery for Targeting Asthma. Life (Basel). 2022;12(4).
[44] Alvarez MJ, Echechipia S, Garcia B, Tabar AI, Martin S, Rico P, et al. Liposome-entrapped D. pteronyssinus vaccination in mild asthma patients: effect of 1-year double-blind,

placebo-controlled trial on inflammation, bronchial hyperresponsiveness and immediate and late bronchial responses to the allergen. Clin Exp Allergy. 2002;32(11):1574-82.
[45] Beeh KM, Kanniess F, Wagner F, Schilder C, Naudts I, Hammann-Haenni A, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. J Allergy Clin Immunol. 2013;131(3):866-74.
[46] Agarwal R, Muthu V, Sehgal IS, Dhooria S, Prasad KT, Aggarwal AN. Allergic Bronchopulmonary Aspergillosis. Clin Chest Med. 2022;43(1):99-125.
[47] Godet C, Meurice JC, Roblot F, Kauffmann-Lacroix C, Verdaguer M, Frat JP, et al. Efficacy of nebulised liposomal amphotericin B in the attack and maintenance treatment of ABPA. Eur Respir J. 2012;39(5):1261-3.
[48] Godet C, Couturaud F, Marchand-Adam S, Pison C, Gagnadoux F, Blanchard E, et al. Nebulised liposomal amphotericin-B as maintenance therapy in allergic bronchopulmonary aspergillosis: a randomised, multicentre trial. Eur Respir J. 2022;59(6).
[49] Arafa MG, Ayoub BM. Bioavailability Study of Niosomal Salbutamol Sulfate in Metered Dose Inhaler: Controlled Pulmonary Drug Delivery. J Aerosol Med Pulm Drug Deliv. 2018;31(2):114-5.
[50] Serra MF, Cotias AC, Pimentel AS, Arantes ACS, Pires ALA, Lanzetti M, et al. Gold Nanoparticles Inhibit Steroid-Insensitive Asthma in Mice Preserving Histone Deacetylase 2 and NRF2 Pathways. Antioxidants (Basel). 2022;11(9).
[51] Barua S, Mitragotri S. Challenges associated with Penetration of Nanoparticles across Cell and Tissue Barriers: A Review of Current Status and Future Prospects. Nano Today. 2014;9(2):223-43.
[52] Gupta R, Rai B. Effect of Size and Surface Charge of Gold Nanoparticles on their Skin Permeability: A Molecular Dynamics Study. Sci Rep. 2017;7:45292.
[53] Barreto E, Serra MF, Dos Santos RV, Dos Santos CE, Hickmann J, Cotias AC, et al. Local Administration of Gold Nanoparticles Prevents Pivotal Pathological Changes in Murine Models of Atopic Asthma. J Biomed Nanotechnol. 2015;11(6):1038-50.
[54] Park HS, Kim KH, Jang S, Park JW, Cha HR, Lee JE, et al. Attenuation of allergic airway inflammation and hyperresponsiveness in a murine model of asthma by silver nanoparticles. Int J Nanomedicine. 2010;5:505-15.
[55] Jang S, Park JW, Cha HR, Jung SY, Lee JE, Jung SS, et al. Silver nanoparticles modify VEGF signaling pathway and mucus hypersecretion in allergic airway inflammation. Int J Nanomedicine. 2012;7:1329-43.
[56] Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001;70(1-2):1-20.
[57] Vishwas S, Singh SK, Gulati M, Awasthi A, Khursheed R, Corrie L, et al. Harnessing the therapeutic potential of fisetin and its nanoparticles: Journey so far and road ahead. Chem Biol Interact. 2022;356:109869.
[58] Zielinska A, Carreiro F, Oliveira AM, Neves A, Pires B, Venkatesh DN, et al. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. Molecules. 2020;25(16).
[59] Ullah F, Shah KU, Shah SU, Nawaz A, Nawaz T, Khan KA, et al. Synthesis, Characterization and In Vitro Evaluation of Chitosan Nanoparticles Physically Admixed with Lactose Microspheres for Pulmonary Delivery of Montelukast. Polymers (Basel). 2022;14(17).
[60] Jin H, Li J, Zhang M, Luo R, Lu P, Zhang W, et al. Berberine-Loaded Biomimetic Nanoparticles Attenuate Inflammation of Experimental Allergic Asthma via Enhancing IL-12 Expression. Front Pharmacol. 2021;12:724525.
[61] Matsuo Y, Ishihara T, Ishizaki J, Miyamoto K, Higaki M, Yamashita N. Effect of betamethasone phosphate loaded polymeric nanoparticles on a murine asthma model. Cell Immunol. 2009;260(1):33-8.

[62] Pangestuti R, Kim SK. Neuroprotective properties of chitosan and its derivatives. Mar Drugs. 2010;8(7):2117-28.
[63] Garg U, Chauhan S, Nagaich U, Jain N. Current Advances in Chitosan Nanoparticles Based Drug Delivery and Targeting. Adv Pharm Bull. 2019;9(2):195-204.
[64] Wang D, Mehrabi Nasab E, Athari SS. Study effect of Baicalein encapsulated/loaded Chitosan-nanoparticle on allergic Asthma pathology in mouse model. Saudi J Biol Sci. 2021;28(8):4311-7.
[65] Dhayanandamoorthy Y, Antoniraj MG, Kandregula CAB, Kandasamy R. Aerosolized hyaluronic acid decorated, ferulic acid loaded chitosan nanoparticle: A promising asthma control strategy. Int J Pharm. 2020;591:119958.
[66] Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, Joo SW, et al. Dendrimers: synthesis, applications, and properties. Nanoscale Res Lett. 2014;9(1):247.
[67] Shao N, Su Y, Hu J, Zhang J, Zhang H, Cheng Y. Comparison of generation 3 polyamidoamine dendrimer and generation 4 polypropylenimine dendrimer on drug loading, complex structure, release behavior, and cytotoxicity. Int J Nanomedicine. 2011;6:3361-72.
[68] Nasr M, Najlah M, D'Emanuele A, Elhissi A. PAMAM dendrimers as aerosol drug nanocarriers for pulmonary delivery via nebulization. Int J Pharm. 2014;461(1-2):242-50.
[69] Su Y, Zhang B, Sun R, Liu W, Zhu Q, Zhang X, et al. PLGA-based biodegradable microspheres in drug delivery: recent advances in research and application. Drug Deliv. 2021;28(1):1397-418.
[70] SreeHarsha N, Venugopala KN, Nair AB, Roopashree TS, Attimarad M, Hiremath JG, et al. An Efficient, Lung-Targeted, Drug-Delivery System To Treat Asthma Via Microparticles. Drug Des Devel Ther. 2019;13:4389-403.
[71] Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules. 2022;27(4).
[72] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8(1):102.
[73] Ng ZY, Wong JY, Panneerselvam J, Madheswaran T, Kumar P, Pillay V, et al. Assessing the potential of liposomes loaded with curcumin as a therapeutic intervention in asthma. Colloids Surf B Biointerfaces. 2018;172:51-9.
[74] Lin H, Wang P, Zhang W, Yan H, Yu H, Yan L, et al. Novel Combined Preparation and Investigation of Bergenin-Loaded Albumin Nanoparticles for the Treatment of Acute Lung Injury: In Vitro and In Vivo Evaluations. Inflammation. 2022;45(1):428-44.
[75] Shi X, Xu M, Luo K, Huang W, Yu H, Zhou T. Anticancer activity of bergenin against cervical cancer cells involves apoptosis, cell cycle arrest, inhibition of cell migration and the STAT3 signalling pathway. Exp Ther Med. 2021;21(6):653.
[76] Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantu L, et al. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. J Control Release. 2021;332:312-36.
[77] Khursheed R, Paudel KR, Gulati M, Vishwas S, Jha NK, Hansbro PM, et al. Expanding the arsenal against pulmonary diseases using surface-functionalized polymeric micelles: breakthroughs and bottlenecks. Nanomedicine (Lond). 2022;17(12):881-911.
[78] Peng X, Wang J, Li X, Lin L, Xie G, Cui Z, et al. Targeting Mast Cells and Basophils with Anti-FcepsilonRIalpha Fab-Conjugated Celastrol-Loaded Micelles Suppresses Allergic Inflammation. J Biomed Nanotechnol. 2015;11(12):2286-99.
[79] Yoo D, Guk K, Kim H, Khang G, Wu D, Lee D. Antioxidant polymeric nanoparticles as novel therapeutics for airway inflammatory diseases. Int J Pharm. 2013;450(1-2):87-94.

[80] Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem. 1987;262(19):9412-20.
[81] Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019;88:487-514.
[82] Ye Y, Zhang X, Xie F, Xu B, Xie P, Yang T, et al. An engineered exosome for delivering sgRNA:Cas9 ribonucleoprotein complex and genome editing in recipient cells. Biomater Sci. 2020;8(10):2966-76.
[83] Vishwas S, Kumar R, Khursheed R, Ramanunny AK, Kumar R, Awasthi A, et al. Expanding Arsenal against Neurodegenerative Diseases using Quercetin based Nanoformulations: Breakthroughs and Bottlenecks. Curr Neuropharmacol. 2022.
[84] Li C, Deng C, Zhou T, Hu J, Dai B, Yi F, et al. MicroRNA-370 carried by M2 macrophage-derived exosomes alleviates asthma progression through inhibiting the FGF1/MAPK/STAT1 axis. Int J Biol Sci. 2021;17(7):1795-807.
[85] Shang Y, Sun Y, Xu J, Ge X, Hu Z, Xiao J, et al. Exosomes from mmu_circ_0001359-Modified ADSCs Attenuate Airway Remodeling by Enhancing FoxO1 Signaling-Mediated M2-like Macrophage Activation. Mol Ther Nucleic Acids. 2020;19:951-60.
[86] Guo SJ, Ma CG, Hu YY, Bai G, Song ZJ, Cao XQ. Solid lipid nanoparticles for phytosterols delivery: The acyl chain number of the glyceride matrix affects the arrangement, stability, and release. Food Chem. 2022;394:133412.
[87] B. Mukherjee SC, L. Mondal, B.S. Satapathy, S. Sengupta, L. Dutta, A. Choudhury, D. Mandal. Multifunctional drug nanocarriers facilitate more specific entry of therapeutic payload into tumors and control multiple drug resistance in cancer2016.
[88] Lv C, Li H, Cui H, Bi Q, Wang M. Solid lipid nanoparticle delivery of rhynchophylline enhanced the efficiency of allergic asthma treatment via the upregulation of suppressor of cytokine signaling 1 by repressing the p38 signaling pathway. Bioengineered. 2021;12(1):8635-49.
[89] Li N, Li X, Cheng P, Yang P, Shi P, Kong L, et al. Preparation of Curcumin Solid Lipid Nanoparticles Loaded with Flower-Shaped Lactose for Lung Inhalation and Preliminary Evaluation of Cytotoxicity In Vitro. Evid Based Complement Alternat Med. 2021;2021:4828169.
[90] Patil-Gadhe A, Pokharkar V. Montelukast-loaded nanostructured lipid carriers: part I oral bioavailability improvement. Eur J Pharm Biopharm. 2014;88(1):160-8.
[91] Al-Kouba J, Wilkinson AN, Starkey MR, Rudraraju R, Werder RB, Liu X, et al. Allergen-encoding bone marrow transfer inactivates allergic T cell responses, alleviating airway inflammation. JCI Insight. 2017;2(11).
[92] De Stefano D, Ungaro F, Giovino C, Polimeno A, Quaglia F, Carnuccio R. Sustained inhibition of IL-6 and IL-8 expression by decoy ODN to NF-kappaB delivered through respirable large porous particles in LPS-stimulated cystic fibrosis bronchial cells. J Gene Med. 2011;13(4):200-8.
[93] Bezzerri V, Borgatti M, Nicolis E, Lampronti I, Dechecchi MC, Mancini I, et al. Transcription factor oligodeoxynucleotides to NF-kappaB inhibit transcription of IL-8 in bronchial cells. Am J Respir Cell Mol Biol. 2008;39(1):86-96.
[94] Luhrmann A, Tschernig T, von der Leyen H, Hecker M, Pabst R, Wagner AH. Decoy oligodeoxynucleotide against STAT transcription factors decreases allergic inflammation in a rat asthma model. Exp Lung Res. 2010;36(2):85-93.
[95] Mehta M, Paudel KR, Shukla SD, Allam V, Kannaujiya VK, Panth N, et al. Recent trends of NFkappaB decoy oligodeoxynucleotide-based nanotherapeutics in lung diseases. J Control Release. 2021;337:629-44.

[96] Ungaro F, De Stefano D, Giovino C, Masuccio A, Miro A, Sorrentino R, et al. PEI-engineered respirable particles delivering a decoy oligonucleotide to NF-kappaB: inhibiting MUC2 expression in LPS-stimulated airway epithelial cells. PLoS One. 2012;7(10):e46457.
[97] da Silva AL, de Oliveira GP, Kim N, Cruz FF, Kitoko JZ, Blanco NG, et al. Nanoparticle-based thymulin gene therapy therapeutically reverses key pathology of experimental allergic asthma. Sci Adv. 2020;6(24):eaay7973.
[98] Gavitt TD, Hartmann AK, Sawant SS, Mara AB, Szczepanek SM, Rouge JL. A GATA3 Targeting Nucleic Acid Nanocapsule for In Vivo Gene Regulation in Asthma. ACS Nano. 2021;15(7):11192-201.
[99] Kumar M, Kong X, Behera AK, Hellermann GR, Lockey RF, Mohapatra SS. Chitosan IFN-gamma-pDNA Nanoparticle (CIN) Therapy for Allergic Asthma. Genet Vaccines Ther. 2003;1(1):3.
[100] Kong X, Hellermann GR, Zhang W, Jena P, Kumar M, Behera A, et al. Chitosan Interferon-gamma Nanogene Therapy for Lung Disease: Modulation of T-Cell and Dendritic Cell Immune Responses. Allergy Asthma Clin Immunol. 2008;4(3):95-105.
[101] Krishnamoorthy N, Oriss TB, Paglia M, Fei M, Yarlagadda M, Vanhaesebroeck B, et al. Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. Nat Med. 2008;14(5):565-73.
[102] Wu W, Chen H, Li YM, Wang SY, Diao X, Liu KG. Intranasal sirna targeting c-kit reduces airway inflammation in experimental allergic asthma. Int J Clin Exp Pathol. 2014;7(9):5505-14.
[103] Alvarez MJ, Echechipía S, García B, Tabar AI, Martín S, Rico P, et al. Liposome-entrapped D. pteronyssinus vaccination in mild asthma patients: effect of 1-year double-blind, placebo-controlled trial on inflammation, bronchial hyperresponsiveness and immediate and late bronchial responses to the allergen. Clin Exp Allergy. 2002;32(11):1574-82.
[104] Godet C, Couturaud F, Marchand-Adam S, Pison C, Gagnadoux F, Blanchard E, et al. Nebulised liposomal amphotericin-B as maintenance therapy in allergic bronchopulmonary aspergillosis: a randomised, multicentre trial. Eur Respir J. 2022;59(6).
[105] Arafa MG, Ayoub BM. NANO-VESICLES OF SALBUTAMOL SULPHATE IN METERED DOSE INHALERS: FORMULATION, CHARACTERIZATION AND IN VITRO EVALUATION. International Journal of Applied Pharmaceutics. 2017;9(6):100-5.
[106] Vrijens B. A Six Sigma framework to successfully manage medication adherence. Br J Clin Pharmacol. 2019;85(8):1661-3.
[107] Omlor AJ, Le DD, Schlicker J, Hannig M, Ewen R, Heck S, et al. Local Effects on Airway Inflammation and Systemic Uptake of 5 nm PEGylated and Citrated Gold Nanoparticles in Asthmatic Mice. Small. 2017;13(10).
[108] Kim BG, Park MK, Lee PH, Lee SH, Hong J, Aung MMM, et al. Effects of nanoparticles on neuroinflammation in a mouse model of asthma. Respir Physiol Neurobiol. 2020;271:103292.
[109] Ko JW, Shin NR, Je-Oh L, Jung TY, Moon C, Kim TW, et al. Silica dioxide nanoparticles aggravate airway inflammation in an asthmatic mouse model via NLRP3 inflammasome activation. Regul Toxicol Pharmacol. 2020;112:104618.
[110] Huang KL, Chang HL, Tsai FM, Lee YH, Wang CH, Cheng TJ. The effect of the inhalation of and topical exposure to zinc oxide nanoparticles on airway inflammation in mice. Toxicol Appl Pharmacol. 2019;384:114787.
[111] Huang KL, Lee YH, Chen HI, Liao HS, Chiang BL, Cheng TJ. Zinc oxide nanoparticles induce eosinophilic airway inflammation in mice. J Hazard Mater. 2015;297:304-12.
[112] Deng R, Ma P, Li B, Wu Y, Yang X. Development of allergic asthma and changes of intestinal microbiota in mice under high humidity and/or carbon black nanoparticles. Ecotoxicol Environ Saf. 2022;241:113786.

[113] Athari SS, Pourpak Z, Folkerts G, Garssen J, Moin M, Adcock IM, et al. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol. 2016;791:811-20.
[114] da Silva AL, Martini SV, Abreu SC, Samary Cdos S, Diaz BL, Fernezlian S, et al. DNA nanoparticle-mediated thymulin gene therapy prevents airway remodeling in experimental allergic asthma. J Control Release. 2014;180:125-33.
[115] Kim DE, Lee Y, Kim M, Lee S, Jon S, Lee SH. Bilirubin nanoparticles ameliorate allergic lung inflammation in a mouse model of asthma. Biomaterials. 2017;140:37-44.
[116] Chen X, Huang W, Wong BC, Yin L, Wong YF, Xu M, et al. Liposomes prolong the therapeutic effect of anti-asthmatic medication via pulmonary delivery. Int J Nanomedicine. 2012;7:1139-48.
[117] Wang W, Zhu R, Xie Q, Li A, Xiao Y, Li K, et al. Enhanced bioavailability and efficiency of curcumin for the treatment of asthma by its formulation in solid lipid nanoparticles. Int J Nanomedicine. 2012;7:3667-77.
[118] Wang J, Wang H, Zhu R, Liu Q, Fei J, Wang S. Anti-inflammatory activity of curcumin-loaded solid lipid nanoparticles in IL-1beta transgenic mice subjected to the lipopolysaccharide-induced sepsis. Biomaterials. 2015;53:475-83.
[119] Grozdanovic M, Laffey KG, Abdelkarim H, Hitchinson B, Harijith A, Moon HG, et al. Novel peptide nanoparticle-biased antagonist of CCR3 blocks eosinophil recruitment and airway hyperresponsiveness. J Allergy Clin Immunol. 2019;143(2):669-80 e12.
[120] Levanen B, Bhakta NR, Torregrosa Paredes P, Barbeau R, Hiltbrunner S, Pollack JL, et al. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. J Allergy Clin Immunol. 2013;131(3):894-903.
[121] Almqvist N, Lonnqvist A, Hultkrantz S, Rask C, Telemo E. Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. Immunology. 2008;125(1):21-7.

Figure Caption

Figure 1 Schematic representation of asthma pathophysiology

Figure 1 Caption

Upon exposure to various allergens several inflammatory cells, cytokines and mediators are recruited or activated, producing acute effects on the airway epithelium –such as bronchoconstriction, mucus secretion, plasma leakage, together with airway remodeling leading to fibrosis of sub-epithelium, angiogenesis and narrowing airway smooth muscles. Abbreviations: TLR – Toll like receptor; DC – dendritic cell; IL-interleukin; GMCSF – Granulocyte macrophage colony-stimulating factor; TNFAIP3 – Tumour Necrosis Factor Alpha Induced Protein 3; NFκB - Nuclear factor kappa B; ROS- reactive oxygen species; Th2 – T helper cell type 2; TGF-β - Transforming growth factor beta; PAR2 - Protease activated receptor 2; AAM – alternative activated macrophages; ILC2 - type 2 innate lymphoid cells.

Figure 2 Nanoformulations and their delivery to lung

Figure 2 Caption

(A) Various nanoformulations explored to treat asthma. Delivery of drug in section (B) the central lung and (C) peripheral lung nanoparticles (NPs) circumvent the pulmonary barriers (such as mucociliary clearance and macrophage clearance) and get absorbed as well as internalised into the cells. After being absorbed, they enter the blood.

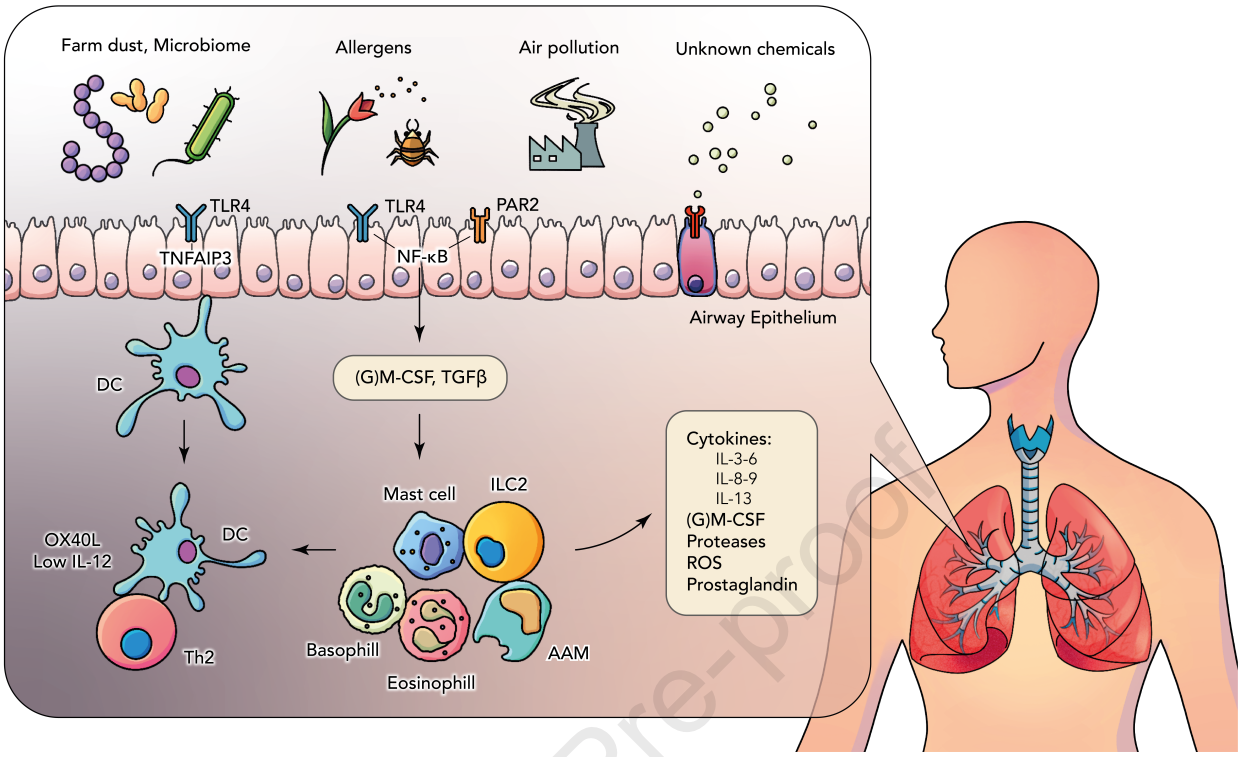
Figure 3 Effects of gold nanoparticles (NPs) against asthma

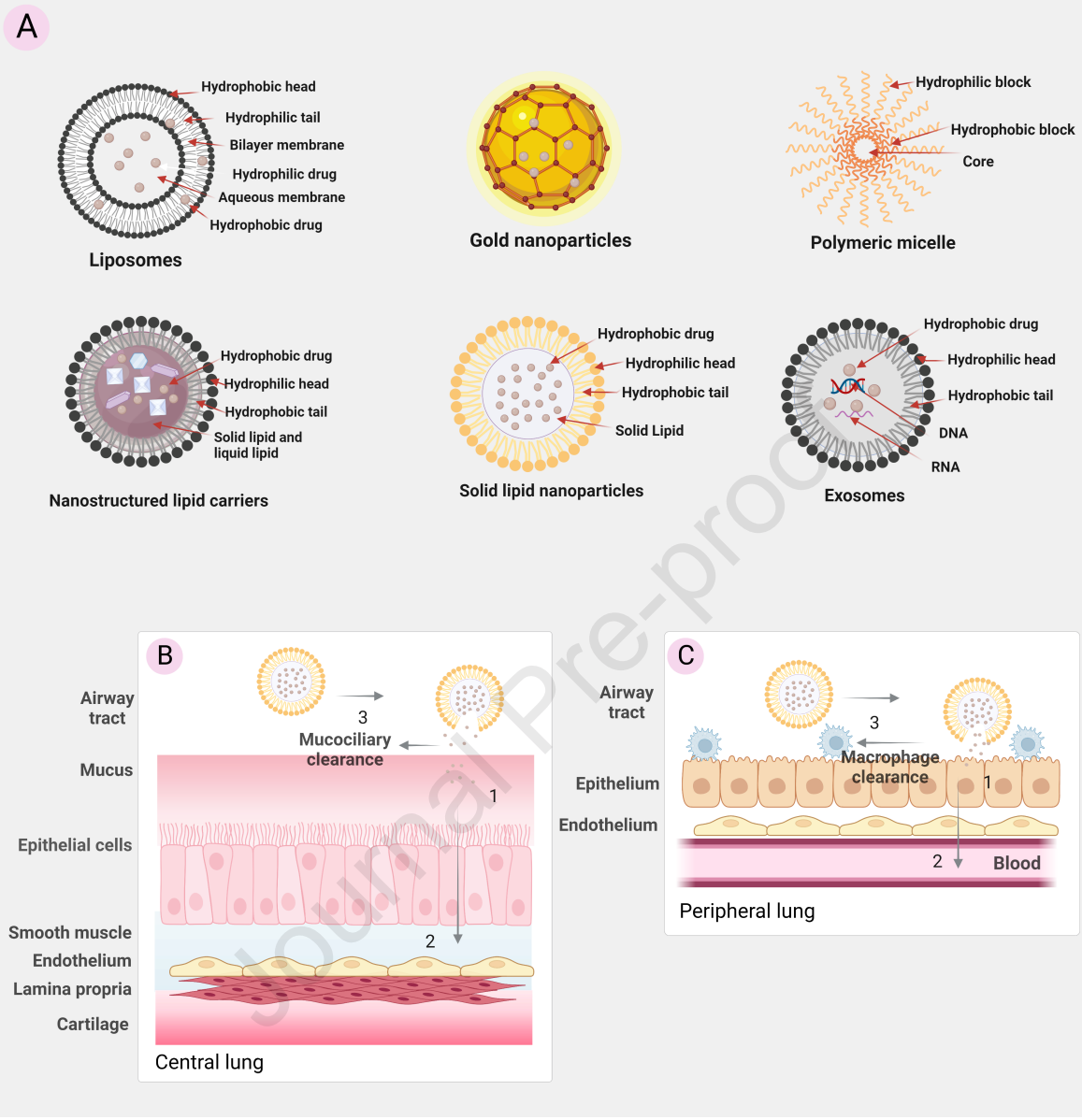
Figure 3 Caption

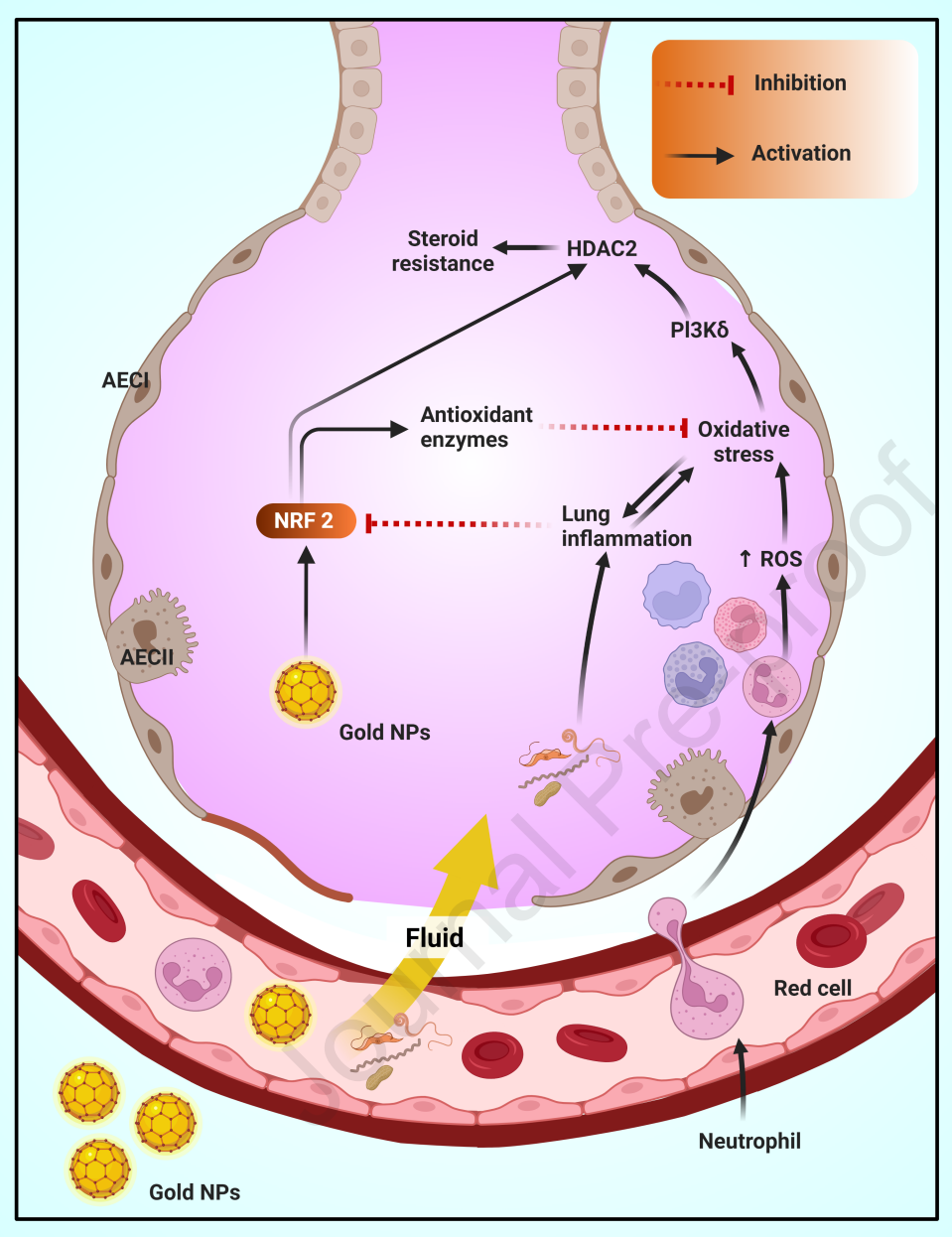
Gold NPs decreased the expression of NRF2, HDAC2, and PI3K. The results indicated that the gold NPs lowered the levels of oxidative stress and inflammation in asthmatic biomarkers.

1102 Abbreviations: NRF2 - nuclear factor erythroid 2–related factor 2; HDAC2 - Histone
1103 deacetylase 2; PI3K - Phosphoinositide 3-kinases.

1104




A
Hydrophobic head
Hydrophilic tail
Bilayer membrane
Hydrophilic drug
Aqueous membrane
Hydrophobic drug
Liposomes
Gold nanoparticles
Hydrophilic block
Hydrophobic block
Core
Polymeric micelle
Hydrophobic drug
Hydrophilic head
Hydrophobic tail
Solid lipid and liquid lipid
Nanostructured lipid carriers
Hydrophobic drug
Hydrophilic head
Hydrophobic tail
Solid Lipid
Solid lipid nanoparticles
Hydrophobic drug
Hydrophilic head
Hydrophobic tail
DNA
RNA
Exosomes
B
Airway tract
Mucus
Epithelial cells
Smooth muscle
Endothelium
Lamina propria
Cartilage
3
Mucociliary clearance
1
2
Central lung
C
Airway tract
Epithelium
Endothelium
3
Macrophage clearance
1
2
Blood
Peripheral lung



Declaration of interests – None