Nanotechnology based therapeutics for lung disease

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ABSTRACT

Nanomedicine is a multidisciplinary research field with an integration of traditional sciences such as chemistry, physics, biology and materials science. The application of nanomedicine for lung diseases as a relatively new area of interdisciplinary science has grown rapidly over the last 10 years. Promising research outcomes suggest that nanomedicine will revolutionise the practice of medicine, through the development of new approaches in therapeutic agent delivery, vaccine development and nanotechnology-based medical detections. Nano-based approaches in the diagnosis and treatment of lung diseases will, in the not too distant future, change the way we practise medicine. This review will focus on the current trends and developments in the clinical translation of nanomedicine for lung diseases, such as in the areas of lung cancer, cystic fibrosis, asthma, bacterial infections and COPD.

INTRODUCTION

Lung diseases are one of the leading causes of mortality worldwide, accounting for one in six deaths globally each year,[1] and are projected to account for one in five deaths globally by 2030.[2] As such, the development of new therapeutic strategies, as well as the improvement of current therapies, has been the focus of considerable research efforts to address this pressing clinical need.[3] It is widely believed that the use of nanotechnology for medical applications (termed nanomedicine) will revolutionise the way we practise medicine, through the development of new approaches in therapeutic agent delivery,[4] vaccine development,[5] nanotechnology-based medical detections,[6] diagnosis-therapy (theranostics)[7] and drug formulations in the personalised medicine space.[8] Moreover, nano-drug carriers can be targeted to treat specific disease sites, and drug releases can be also controlled to maximise drug sustained release, leading to prolonged dosage and efficiency. The development of nanomedicine has allowed scientists to achieve these outcomes, without the need to modify existing drugs.[9] This review will focus on the current state of development of nanotechnology as well as future opportunities for nanotechnology, with a focus on the treatment of lung diseases.

APPLICATIONS OF NANOMEDICINE IN LUNG DISEASES

The most advanced areas for current nanotechnology research, particularly as it applies to the lung, relate to drug delivery, vaccination and disease diagnosis (see tables 1 and 2 and figure 1A), whereas nanotechnology research as it pertains to imaging, personalised medicine and theranostics (therapeutic and diagnostic capabilities into one single nanoplatform) is at an earlier stage of development. Currently, a significant body of published work relates to the use of nanoparticles as a drug delivery system (DDS) to the lung.[10 11] As almost 40% of currently marketed drugs and virtually 90% of small molecules being developed as novel drug candidates are poorly water-soluble, a variety of nanodrug delivery systems (nano-DDS) offer an opportunity to overcome these solubility issues and enhance the circulating half-life of the therapeutic agents (see figure 1B).[12 13] Biomacromolecular drugs such as DNA, small interfering RNA (siRNA) and messenger RNA (mRNA) are often degraded in biological fluids, and delivering therapeutic concentrations to the target organ presents challenges.[14 15] Nanoparticles as DDS have shown to improve the stability of biomacromolecular drugs, and prevent premature degradation and rapid clearance in vivo, thereby enabling the delivery of biomacromolecular drugs.[15 16] Moreover, nano-DDS as a non-viral vector are less toxic and immunogenic than the viral vectors which are traditionally used for biomacromolecular agents.[15 17 18] Furthermore, nano-DDS can be administered via different routes, such as intravenous,[19] oral[20] and inhalation[21] (see figure 1C). Employing nanoparticles to delivery drug candidate can enhance bioavailability and overcome natural barriers, such as the mucosa.[20] Anticancer drugs loaded in a nano-DDS tend to preferentially accumulate in tumour tissue compared with normal tissues through the enhanced permeability and retention (EPR) effect, namely ‘passive targeting’. EPR is the result of the leaky tumour vasculature having interendothelial gaps due to poor lymphatic drainage of the tumour,which provides an opportunity for nanotherapeutics to escape the circulation and functionally localise in the tumourtissue.[22]

One of the most exciting concepts in nanomedicine as it pertains to the lung is in the design and development of nanoparticles for targeting specific sites, which is referred to as ‘active targeting’.[23] As a precision medicine, targeted nano-DDS has been designed to enhance the accumulation of drug compounds in specific disease sites while limiting exposure to healthy tissues, thus reducing the dose to be administered to the patient and thereby the risk of side effect.[24–26] Conjugating specific moieties, which include antibodies, small molecules, proteins/peptides and nucleic acids, to the surface of nanoparticles is the most common strategy to design a targeted nanodrug delivery carrier.[27] It has been shown that nanoparticles loaded by antibiotics targeted to the site of infection significantly enhance antibacterial efficacy.[25 28] Additionally, overexpression of surface markers on tumour cells which are absent in normal cells provides an

Table 1 Clinical trials, registered at https://clinicaltrials.gov/, investigating nanomedicine for lung cancer

Therapeutic delivery				
Trade name	**Route of administration**	**Particle type/therapeutic agent**	**Indication**	**Approval/Status**
Abraxane	Intravenous	Polymeric/paclitaxel	NSCLC	FDA-approved (2012)
Genexol-PM	Intravenous	Micellar/paclitaxel	Lung cancer	(phase II) in US*
Genexol-PM	Intravenous	Micellar/paclitaxel in combination with cisplatin	Lung cancer	NCT02667743 (phase III)
Nab-P/C	Intravenous	Polymeric/paclitaxel in combination with free carboplatin	NSCLC	NCT02027428 (phase III)
Onivyde	Intravenous	Liposomal/topotecan	Small cell lung cancer	NCT03088813 (phase II/III)
Abraxane	Intravenous	Polymeric/paclitaxel in combination with pembrolizumab and carboplatin	NSCLC	NCT02775435 (phase III)
Xyotax (CT-2103)	Intravenous	Polymeric/paclitaxel compared with gemcitabine or vinorelbine	Advanced or recurrent NSCLC	NCT00054197 (phase III)
Xyotax (CT-2103)	Intravenous	Polymeric/paclitaxel compared with docetaxel	Progressive NSCLC	NCT00054184 (phase III)
Xyotax (CT-2103)	Intravenous	Polymeric/paclitaxel in combination with carboplatin	NSCLC	NCT00576225 (phase III)
Xyotax (CT-2103)	Intravenous	Polymeric/paclitaxel in combination with carboplatin	Stage III NSCLC	NCT00352690 (phase II)
Genexol-PM	Intravenous	Micellar/paclitaxel in combination with gemcitabine	NSCLC	NCT01770795 (phase II)
Genexol-PM	Intravenous	Micellar/paclitaxel in combination with free cisplatin	NSCLC	NCT01023347 (phase II)
Abraxane	Intravenous	Polymeric/paclitaxel in combination with carboplatin and atezolizumab	NSCLC	NCT02716038 (phase II)
BIND-014	Intravenous	Polymeric/docetaxel	NSCLC	NCT01792479 (phase II)
BIND-014	Intravenous	Polymeric/docetaxel	Squamous cell non-small and cell lung cancer	NCT02283320 (phase II)
Abraxane	Intravenous	Polymeric/gemzar	Lung carcinoma	NCT02449122 (phase I/II)
NC-6004	Intravenous	Micellar/cisplatin in combination with free gemcitabine	Advanced solid tumours, including NSCLC	NCT02240238 (phase Ib/II)
Oncoprex	Oral	Lipid/plasmid DNA encoding FUS1 (TUSC2)	Lung cancer	NCT01455389 (phase I/II)
Oncoprex	Oral	Lipid/plasmid DNA encoding FUS1 (TUSC2)	Lung cancer	NCT00059605 (phase I/II)
CRLX101	Intravenous	Polymeric/olaparib in combination with free camptothecin	Non-small cell and non-small cell lung cancer	NCT02769962 (phase I/II)
Abraxane	Intravenous	Polymeric/paclitaxel in combination with free nintedanib	NSCLC	NCT03361319 (phase I/II)
SGT-53	Intravenous	Liposomal/plasmid DNA encoding wild-type p53	Solid tumours	NCT00470613 (phase I)
TargomiRs	Intravenous	Non-viable minicells/miRNA	Pleural mesothelioma and NSCLC	NCT02369198 (phase I)
Abraxane	Intravenous	Polymeric/paclitaxel	NSCLC	NCT02495896 (phase I)
BIND-014	Intravenous	Polymeric/docetaxel	Advanced or metastatic cancer, including lung cancer	NCT01300533 (phase I)
AuroLase (Nanospectra Biosciences)	Intravenous	Silica-gold nanoshell /AuroLase therapy (hyperthermia cancer therapy)	Metastatic lung tumours	NCT01679470
Doxil/Caelyx	Intravenous	Liposomal/doxorubicin	NSCLC	NCT01051362 (phase II)

Continued

Table 1 Continued

Therapeutic delivery				
Trade name	**Route of administration**	**Particle type/therapeutic agent**	**Indication**	**Approval/Status**
OSI-211	Intravenous	Lurtotecan/topotecan	Small cell lung cancer	NCT00046787 (phase II)
Diagnosis				
Trade name	**Mechanism**	**Nanoparticle type**	**Indication**	**Approval/Status**
Electronic nose	Volatile organic compounds detection	Polymeric nanoparticles	Lung cancer	NCT01386203
Vaccination				
Trade name	**Route of administration**	**Particle type**	**Vaccine antigen/adjuvant**	**Approval/Status**
BLP25 (Stimuvax)	Intravenous	Liposomal	MUC1/MPLA	NCT01015443 (phase III)
BLP25 (Stimuvax)	Intravenous	Liposomal	MUC1/MPLA	NCT00409188 (phase III)

*Approved in Korea (2007)
miRNA, microRNA.FDA, Food and Drug Administration; NSCLC, non-small-cell lung cancer.

opportunity for targeting the nano-DDS to cancer cells.[29] The concept of passive and active targeting and the major advantages of targeted nano-DDS are schematically outlined in figure 2.

Applications of nanomedicine in respiratory conditions are summarised in figure 3 and in tables 1 and 2 as drug carriers and as diagnostic and detection tools for a selection of pulmonary diseases. The advantages of nanocarriers as potential diagnostic platforms include both negligible toxicity and controlled sizing, which offer both highly specific and sensitive early disease.[30 31]

Considerable progress has also been made in vaccine research, where nanocarriers offer the potential to overcome vaccine degradation, non-specific targeting and a lack of antigen-presenting cells to take up of cross-presentation. Moreover, nano-DDS has the potential to mimic features of pathogens including viruses, which means nano-DDS could also present themselves as the real pathogen to the immune system.[32 33] To achieve higher immunity response, they have the capacity to present multiple peptide epitopes in a repetitive pattern, and this strategy can improve the poor immunogenicity in peptide-based subunit vaccines.[34]. All the above-mentioned nanomedicine applications can be achieved by encapsulating/conjugating the therapeutic agents in polymeric, liposomal, micellar or dendrimer nanoparticles, as illustrated schematically in figure 4.

LUNG CANCER

Nanomedicine and anticancer drugs

Lung cancer remains the leading cause of death from cancer worldwide, accounting for nearly 1.6 million deaths in 2012,[35] and efforts are ongoing to develop more effective cancer drugs to address this clinical burden. Nanotherapeutics have been approved for the treatment of cancer for over 20 years, since the approval of Doxil in 1995 for AIDS-related Kaposi's syndrome. This was the first Food and Drug Administration (FDA)-approved nanotherapeutic, and this has continued to motivate the development of novel nanotherapeutics for the treatment of cancer (see table 1).

There are currently two nanotherapeutics approved for the treatment of lung cancer. Both contain the potent chemotherapeutic paclitaxel as the active drug component, which is widely used to treat a number of different solid tumours including ovarian, breast and lung cancer.[36] Due to its poor water solubility, paclitaxel is administrated with surfactants such as Cremophor EL as solubilisers to increase its bioavailability. However, such excipients have been associated with high toxicity and adverse events, such as hypersensitivity and neurotoxicity.[37] When formulated as advanced nano-DDS, the use of such excipients, and their associated toxicities, can be minimised. Genexol-PM is the first nanotherapeutic approved for the treatment of non-small-cell lung cancer (NSCLC); paclitaxel is encapsulated into nanosized polymeric micelles, which allows a significantly higher paclitaxel dose to be administered to patients without an increase in toxicity.[38]

Abraxane is a protein-based nanotherapeutic which gained FDA approval in 2012 as a first-line treatment for advanced NSCLC.[39] Abraxane, which was originally approved in 2005 for the treatment of metastatic breast cancer, comprised albumin-bound paclitaxel nanoparticles, and similar to Genexol-PM also allows the administration of higher doses of paclitaxel, while avoiding the need for additional excipients.[40] In addition to the successful translation of Abraxane into clinical use, several other nanoformulations of clinically approved anticancer drugs have been recently conducted in various stages of clinical trials. As a hopeful candidate for nanodrug delivery to treat lung cancer, irinotecan is a semisynthetic inhibitor of topoisomerase I that causes DNA damage and cell death in cancer cells and is FDA-approved for the treatment of advanced or metastatic pancreatic cancer. Onivyde is a liposomal nano-DDS that carries irinotecan. The liposomal nanodrug carrier provides a slow and sustained drug releases over time, with reduced drug toxicity; however, Onivyde is not approved for use as a single drug without 5-Fluorouracil/Leucovorin (5-FU/LV), two other medicines.[41] Recent clinical studies in patients with lung cancer showed a promising anticancer activity of Onivyde with higher prolonging circulation time compared with free drug.[42 43] Onivyde is currently undergoing clinical investigation as a randomised, open-label, phase III trial in patients with small cell lung cancer progressed on or after platinum-based first-line therapy (NCT03088813).

Lipoplatin is another liposomal nano-DDS which encapsulates cisplatin, an FDA-approved anticancer drug. Lipoplatin has shown promising results in several clinical trials and strong anticancer activity with fewer side effects, compared with traditional cisplatin, which is discussed in detail elsewhere.[44] A new clinical study was carried out using a combination of lipoplatin

Table 2 Clinical trials, registered at https://clinicaltrials.gov/, investigating nanomedicine for lung diseases (cancer excluded)

Therapeutic delivery				
Trade name	**Route of administration**	**Particle type/therapeutic agent**	**Indication**	**Approval/Status**
Arikace	Inhalation	Liposomal/amikacin	Acute exacerbation of non-cystic fibrosis bronchiectasis	NCT02081963 (phase IV)
Arikayce	Inhalation	Liposomal/amikacin	Non-tuberculous mycobacterial lung infection due to *Mycobacterium avium* complex	NCT02344004 (phase III)
Arikayce	Inhalation	Liposomal/amikacin	Chronic *Pseudomonas aeruginosa* infections in patients with cystic fibrosis	NCT01315678 (phase III)
Arikayce	Inhalation	Liposomal/amikacin	*P.aeruginosa* infections in patients with cystic fibrosis	NCT01316276 (phase III)
Pulmaquin (Linhaliq)	Inhalation	Liposomal/ciprofloxacin	Non-cystic fibrosis bronchiectasis	NCT01515007 (phase III)
Pulmaquin (Linhaliq)	Inhalation	Liposomal/ciprofloxacin	Non-cystic fibrosis bronchiectasis	NCT02104245 (phase III)
Arikayce	Inhalation	Liposomal/amikacin	*M. abscessus* lung infection	NCT03038178 (phase II)
Arikayce	Inhalation	Liposomal/amikacin	Patients with cystic fibrosis with *P. aeruginosa* infection	NCT00777296 (phase II)
Arikayce	Inhalation	Liposomal/amikacin	Non-tuberculous mycobacteria infection	NCT01315236 (phase II)
Arikace	Inhalation	Liposomal/amikacin	Patients with bronchiectasis	NCT00775138 (phase I/II)
Arikace	Inhalation	Liposomal/amikacin	Patients with cystic fibrosis with bacterial infection	NCT00558844 (phase I/II)
Nanovesicles of salbutamol sulfate	Inhalation	Vesicular/salbutamol sulfate	Asthma	NCT03059017 (phase I)
Diagnosis				
Trade name	**Mechanism**	**Nanoparticle type**	**Indication**	**Approval/Status**
Electronic nose	Volatile organic compounds detection	Polymeric nanoparticles	Diagnosis of bacterial infection in COPD	NCT01976117
NanoDisk-MS assay		Silica nanoparticles	*M. tuberculosis*	NCT03271567
Exhaled breath olfactory signature	Cross-reactive gold nanoparticles coated with organic ligands	Gold nanoparticles	Pulmonary arterial hypertension	NCT02782026

and gemcitabine to treat advanced NSCLC, which showed a significant prolonged time to tumour progression and survival.[45]

Nanomedicine and lung cancer immunotherapy

Immunotherapy has emerged as a potent additional therapeutic strategy against lung cancer.[46] Programmed death ligand 1 (PD-L1) is an immune regulatory molecule that causes the negative regulation of effector T-cell activation. Immunotherapy studies in patients with advanced NSCLC revealed that drugs targeting programmed cell death 1 (PD-1) and its ligand, PD-L1, are highly effective agents significantly prolonging patient survival.[47 48] A natural extension of these studies is currently under way assessing the efficacy of Abraxane plus carboplatin in combination with cancer immunotherapies: these include a phase III trial with the humanised antibody pembrolizumab (which targets PD-1) in patients with metastatic squamous NSCLC (NCT02775435) and a phase II trial with the humanised monoclonal antibody atezolizumab (which targets PD-L1; NCT02716038).

Active targeting of nanomedicines for lung cancer

Active targeting represents a therapeutic strategy whose aim is to maximise the concentration of the active therapeutic agent at the cancer site with minimum adverse side effects, leading to increased treatment efficiency and patient compliance.[49] This is achieved by adding targeting ligands to the surface of the nanocarriers, which bind to specific receptors on tumour cells which are absent in normal cells.[29] BIND-014 is a polymeric nanodrug

carrier encapsulating docetaxel and targeting prostate-specific membrane antigen. A preclinical development and clinical translation study phase II clinical trial for BIND-014 resulted in a significant reduction in tumour volume in patients with cholangiocarcinoma with multiple lung metastases, as illustrated in figure 5,[50] whereas treatment with free docetaxel showed low activity in cholangiocarcinoma.[51]

Additional novel targeted nanodrug formulations carrying siRNA, microRNA (miRNA) or DNA therapeutic agents have been examined for the treatment of lung cancer. In fact, therapeutic agents such as siRNA, miRNA and DNA have shown great promise for the treatment of lung cancer. Engeneic Delivery Vehicle (EDV) is a bacterially derived minicell nano-DDS that is similar to liposomal nanodrug carriers and can be loaded with therapeutic agents including miRNA and siRNA molecules.[52] TargomiR is a targeted EDV against epidermal growth factor receptor (EGFR)-specific antibody carrying miR-16-based mimic miRNA.[53] TargomiR was designed to offset the loss of the miR-15 and miR-16 7 family of miRNAs, which are linked to unsuppressed tumour growth in preclinical models of malignant pleural mesothelioma. To investigate the safety, optimal dosing and activity of this targeted nano-DDS, an open-label, dose-escalation phase I study was performed in patients with malignant pleural mesothelioma. This clinical study presented the safety profile and early antitumour activity of TargomiR, suggesting that further studies of TargomiR in combination with chemotherapy or immune checkpoint inhibitors are required. An example of radiology from a treated patient is illustrated in figure 6.[54]

Recently, a novel EDV system loaded with paclitaxel and targeted with an anti-EGFR-specific antibody (^{EGFR}minicellsPac) has been evaluated in a phase I study in patients with thoracic cancer, which showed this nanotherapeutic strategy to be safe (ACTRN12609000672257).[55] As a targeted nano-DDS that carries DNA therapeutic agents for gene therapy, SGT-53 is a liposomal drug carrier encapsulating a plasmid encoding normal human tumour suppressor p53 DNA. SGT-53 targets cancer cells by using an antibody against the transferrin glycoprotein receptor, which is highly expressed on tumour cells. This targeting strategy has resulted in a greater internalisation efficiency of the nanotherapeutic, compared with untargeted nanodrug carriers in preclinical studies.[56] A recent phase I clinical study in patients with advanced solid tumours indicated that SGT-53 is a safe treatment with minimal side effects. Importantly, SGT-53 only accumulated in metastatic tumours and no drug was detected in normal skin tissue, concluding that this targeted drug delivery can protect healthy cells from being exposed to anticancer drugs (NCT00470613).[57]

As an untargeted nano-DDS that carries the biomolecule therapeutic agents for gene therapy, Oncoprex is a cholesterol-based nanovesicle encapsulating *TUSC2* gene, which expresses tumour suppressor candidate 2 protein lacking in cancer cells of many different cancer types, including NSCLC.[58] A phase I clinical study with systemically administered Oncoprex was conducted in metastatic lung cancer previously treated with platinum-based chemotherapy (NCT00059605). At the end of the study, five patients achieved stable disease (2.6–10.8 months, including two minor responses), and Oncoprex showed a safe therapeutic treatment for patients with lung cancer.[59] This encouraging outcome has led to ongoing phase I/II trials with patients with stage IV lung cancer (NCT01455389).

One of the most exciting aspects of nanomedicine is to tackle challenging issues in the new era of subunit vaccines made of small molecule, protein/peptides or DNA antigens by solving the common problems in the vaccine industry, including degradation, non-specific targeting and lack of antigen-presenting cells to take up of cross-presentation.[33] A nanovaccine consists of nanoscale-based particles attached or formulated with antigens that are preferentially internalised by antigen-presenting cells.[60] Lung cancer-fighting nanovaccines have shown significant promise, and nanotechnology has been employed in many attempts to develop vaccination and immunisation against lung cancer.[61] In preclinical studies nanoparticles have presented the potential of long-lasting immunity, improvement of antigen stability, various platforms as adjuvant possibility and mimicking features of pathogens such as viruses.[62] To achieve higher clinical efficacy, nanoparticles have the capacity to present multiple peptide epitopes in a repetitive pattern, and this strategy can overcome the poor immunogenicity in peptide-based subunit vaccines.[34]

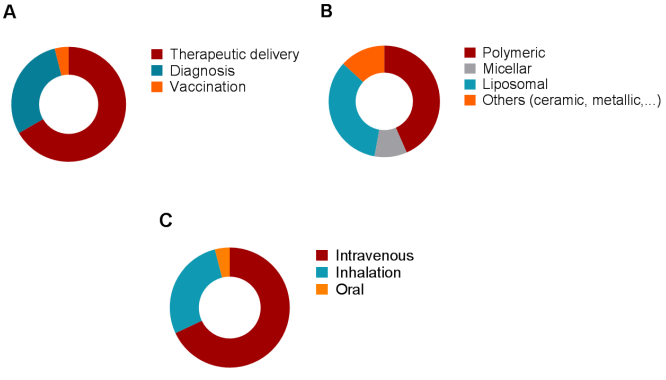

Figure 1 Data collated from ClinicalTrials.gov. (A) Proportion of the most common applications of nanomedicine in lung diseases. (B) The most common nanocarriers used for the treatment of lung diseases. (C) The most common nanoparticle administrations in lung diseases. (Last data of collection 2019.)

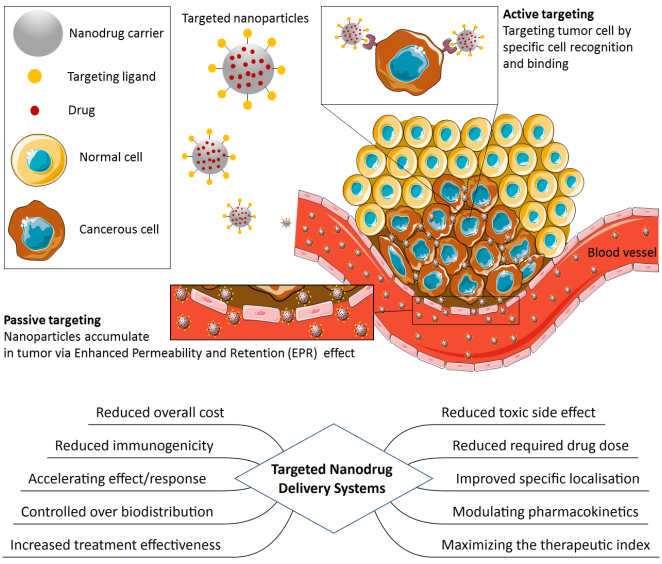

Figure 2 Passive and active targeting of cancerous cells and their advantages. Passive targeting, which is preferential accumulation in the tumour tissue due to enhanced permeability and retention effect, provides an opportunity for localisation of nanotherapeutics within the neoplastic tissues effectively. Active targeting can be achieved by enabling the active cellular uptake of nanotherapeutics by the tumour cells. Advantages of targeted nanodrug delivery systems (nano-DDS) in lung disease.

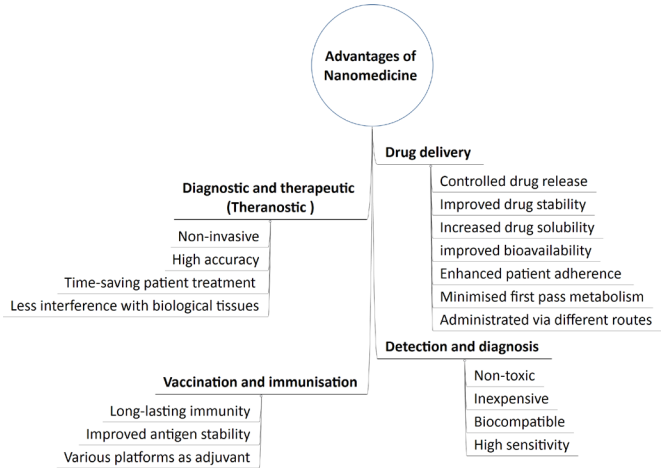

Figure 3 Main nanomedicine applications and their benefits for lung disease treatment.

Most of the currently developing nanovaccines for lung cancer have been employing anti-MUC1 as a peptide vaccine candidate. MUC1 is a cell membrane glycoprotein, an antigen that is widely expressed in lung carcinomas.[63] L-BLP25 (Stimuvax) is a liposome vaccine that targets the MUC1 antigen. In a previous clinical study in patients with advanced stage III NSCLC, L-BLP25 showed a significant survival benefit.[64] In another clinical trial, treatment with L-BLP25 resulted in median survival time of 30.6 months vs 13.3 months in the best-supported-care control arm (NCT01015443).[65] Detailed information of L-BLP25 against NSCLC has been highlighted somewhere else. [66]

CYSTIC FIBROSIS

Cystic fibrosis (CF) is the most common autosomal recessive inherited disorder, and results from mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Since CF is caused by a CFTR gene mutation, gene therapy has been actively employed to correct the mutation at a cellular level. In this approach, correct copies of CFTR gene are placed in the affected epithelial cells in the airways. Generally, two main types of vectors are used to transfer the CFTR gene in the studies, viral vectors and non-viral vectors.[67] In comparison with viral vectors, non-viral vectors, such as nanomaterials, are less challenging and inexpensive to produce, have a much longer shelf-life, and show reduced immunomodulatory responses with better tolerance for repetitive administration.[68] Non-viral vectors are most often designed to be positively charged, to form an electrostatic bond with the negatively charged therapeutic nucleic acid and also improve

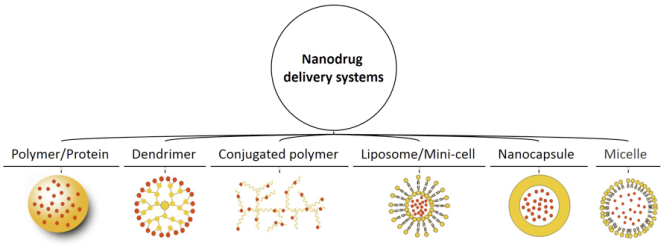

Figure 4 Schematic illustration of the most common designs of the nanodrug delivery systems in current clinical use or undergoing clinical/preclinical development for the treatment of lung diseases. Red circles represent therapeutic agents.

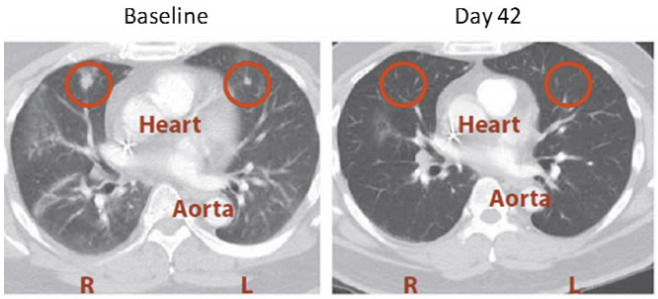

Figure 5 The impact of the nanotherapeutic BIND-014, a polymeric nanodrug carrier, encapsulating docetaxel and targeting prostate-specific membrane antigen on human drug metastases from a primary cholangiocarcinoma. Reproduced with permission from ref [50].

immobilisation of particles in the CF mucus, which possesses high concentrations of negatively charged macromolecules, such as mucins and actin filaments.[69–71] The first clinical study of a nanoparticle-based, non-viral vector consisted of plasmid DNA, carrying the CFTR encoding gene, encapsulated in polyethylene glycol nanoparticles. The safety and gene transfer efficiency evaluation demonstrated nanoformulation to be a successful vector for gene transfer, resulting in a partial nasal potential difference correction with no adverse side effects, suggesting that this approach can be developed for the treatment of patients with CF.[72] Preclinical evaluation of different non-viral vectors showed that the cationic liposomal formulation, GL67A, was the most effective non-viral vector.[73–75] Previous clinical investigation confirmed a sustained level of gene expression for over 2 months after administrating the cationic lipid-mediated transfer of the CFTR gene to the nasal epithelium of patients with CF.[76] A single-dose phase I and IIa study of aerosolised pGM169 plasmid DNA encoding the CFTR gene delivered by a nanoliposome (GL67A) showed it to be a safe strategy for the treatment of patients with CF (EudraCT reference: 2007-004050-85). This study was recently followed by a randomised, double-blind, phase IIb trial to evaluate the clinical benefits of GL67A/pGM169 for patients with CF. The results indicated that the nebulised formulation of GL67A/pGM169 therapy was well tolerated. Moreover, monthly administration of GL67A/pGM169 led to sustained improvement in pulmonary function parameters. Patients with CF succumb to chronic pulmonary bacterial infections leading to progressive lung injury. Therefore, the potential of nanotherapeutics to potentially address this global clinical unmet need is generating increased interest.[77]

BACTERIAL INFECTION

Lower respiratory infection is the fourth leading cause of death, contributing 3.2 million deaths per year, reported by the WHO.[78]

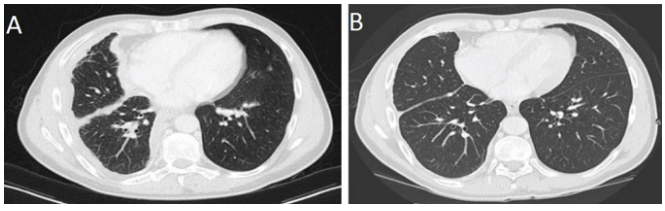

Figure 6 CT scan taken from a patient with a right-sided mesothelioma before (A) and after treatment (B) with TargomiR, an Engeneic Delivery Vehicle targeted against EGFR-specific antibody and located with miR-16-based mimic microRNA.[54]

Strong evidence has been presented linking bacterial infections to increased mortality, frequent exacerbations, accelerated loss of lung function and intensified inflammation in patients with CF and non-CF infection.[79]

Amikacin is a semisynthetic aminoglycoside antibiotic derived from kanamycin A, which is used against a broad spectrum of Gram-negative organisms, to treat severe chronic lung infections with multidrug-resistant (MDR) organisms, including *Pseudomonas aeruginosa* in CF/non-CF patients. Amikacin has been formulated into liposomal nanoparticles in order to improve both its therapeutic half-life and reduce its side effects.[80] Encapsulated amikacin in liposomal nanoparticles also provides sustained release, rapid delivery times and drug protection from charged sputum to compare with free amikacin.[81] The nanopackaged amikacin had been adapted for aerosolised administration, hence its general name liposomal amikacin for inhalation (LAI), under the current trade name Arikayce.[81 82] It is worth highlighting that aerosolised drug delivery therapies are considered a promising alternative strategy to maximising pulmonary drug concentration while reducing the risk of systemic toxicity.[83] Moreover, LAI has shown promising progress in the development of robust antimicrobials to treat lung infections caused by MDR organisms. Its nanoformulation features provide a more prolonged and sustained release of drug with efficient penetration into bacterial biofilms. An in vitro study has recently shown that LAI can effectively penetrate throughout *Mycobacterium avium complex* biofilms (see figure 7), which are the most common *non-tuberculous mycobacteria* (NTM) species isolated from patients with CF. Incorporation of the drug into the liposomes resulted in increasing the uptake of amikacin fourfold in pulmonary macrophages, when compared with free amikacin.[84]

In a phase II trial on patients with CF against *P. aeruginosa*, LAI was examined to evaluate the lung function and CF quality of life questionnaire. No difference in toxicity between experimental arms and placebo arms and no significant shift in minimum inhibitory concentrations were observed. When compared with placebo, patients receiving a drug dose of 560 mg showed a significant improvement in FEV_1 lung function and a reduction in sputum *P. aeruginosa* of more than one log unit (NCT00777296).[85] A phase III efficacy and safety study in patients with CF with chronic infection due to *P. aeruginosa* compared LAI and TOBI, the nebulised formulation of the antibiotic tobramycin, which is the standard of care in patients with CF chronically infected with *P. aeruginosa*. The study revealed comparable improvements in the lung function of patients receiving once-daily administered LAI with those receiving TOBI twice. A more recent clinical study on a small group of patients with *Mycobacterium abscessus* infection in patients with CF showed patients who received a single daily dose of LAI (590 mg) no longer showed the presence of *M. abscessus* in their sputum following either the stabilisation or improvement of their pulmonary function. This study concluded that LAI is active on both *P. aeruginosa* and *M. abscessus*.[86]

Ciprofloxacin is another commonly used antibiotic to treat a number of acute and chronic lung infections.[87] Preclinical studies have shown favourable pharmacokinetic characteristics of aerosolised liposomal ciprofloxacin, a nanostructured liquid vesicle made from lipids that encapsulate ciprofloxacin. In vitro studies on *M. avium* and *M. abscessus* strains have shown that aerosolised nanoliposomal ciprofloxacin had higher efficacy, in comparison with free ciprofloxacin.[88] Moreover, preclinical mouse models revealed that aerosolised nanoliposomal ciprofloxacin had an elimination half-life of up to 10 hours and ciprofloxacin could still be detected in the lung of mice 24 hours after aerosol therapy compared with the half-life of free ciprofloxacin of approximately 2 hours.[89] Liposomally encapsulated ciprofloxacin has been confirmed to have superior antibacterial infection compared with unencapsulated ciprofloxacin.[90] Pulmaquin (Linhaliq) is a dual-release formulation that is a 1:1 mixture of nanoliposomal ciprofloxacin and free ciprofloxacin and has a slow release liposomal formulation.[91] In a phase II study, treatment of non-CF patients with bronchiectasis by Pulmaquin resulted in a reduction in *P. aeruginosa* density in the sputum by 4.2 log colony-forming unit (CFU) from baseline on day 28.[87] This encouraging result led to two parallel phase III studies (NCT01515007 and NCT02104245) to evaluate the efficacy of Pulmaquin in non-CF patients with bronchiectasis infected with *P. aeruginosa*. The results showed that annual exacerbation frequency and sputum *P. aeruginosa* density declined significantly compared with the placebo group, while the treatment was safe and well tolerated.[92]

MYCOBACTERIUM TUBERCULOSIS

TB, which is caused by *M. tuberculosis (Mtb)*, is the world's second leading cause of death from an infectious disease.[93] A considerable challenge in treating the disease is the requirement of daily drug administration for long periods of time (up to 9 months). This often leads to poor adherence by patients, which is not only a risk to the patient's well-being, but due to its highly infectious nature represents a serious risk to public health. The current approach to tackle poor adherence is directly observed treatment, where each day during their treatment patients are observed taking their medication by a healthcare worker, which presents a considerable burden to the healthcare system, both in cost and time. In addition, the drugs currently used for the treatment of TB suffer from serious adverse side effects, such as hepatotoxicity, as well as short plasma life and rapid clearance.[94] Due to the ability to control and sustain the release of drugs from nanoparticle formulations, nanotechnology offers

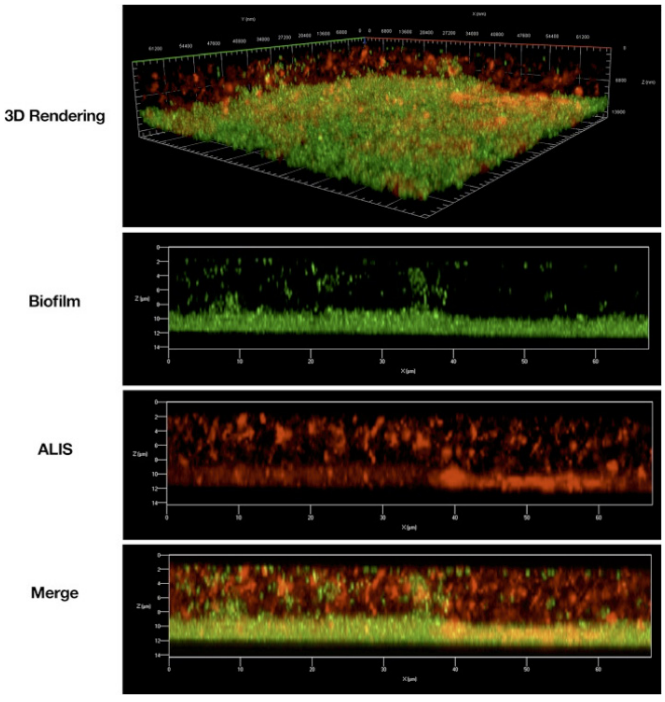

Figure 7 Penetration of amikacin liposome inhalation suspension (ALIS) in *Mycobacterium avium* biofilms via confocal microscopy. 3D, three-dimensional.[84]

the advantage of reducing the dosage frequency associated with poor adherence. A variety of nanocarriers have been evaluated in preclinical studies as potential DDS for TB therapies; however, clinical studies have yet to be undertaken.[95–97] Significant work in the nanotechnology field has been devoted in the diagnosis of TB. Early, rapid and accurate diagnosis of TB infection is crucial at preventing high prevalence of systemic disease and mortality.[98–100]

The gold standard for TB detection is based on microscopy and molecular techniques, which are limited by low sensitivity and high cost. Recently, a high-throughput method, such as Nano-Disk-MS, has been developed by employing nanotechnology to improve *Mtb* diagnosis. Antigenic 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) are two orthologues proteins which play an important role for *Mtb* infection by inducing a transient release of Ca^{2+} from intracellular stores in human immune cells, which protects the bacteria from immune responses and promotes intracellular survival.[101] Thus ESAT-6 and CFP-10 are pivotal to active *Mtb infection*.[102] Published work has identified ESAT-6 and CFP-10 as indicative biomarkers for TB infection due to the strong correlation of detection in patient samples and active TB disease.[103] The NanoDisk-MS assay was designed to permit the sensitive multiplex quantification of serum CFP-10 and ESAT-6 concentrations in suspected TB cases to allow rapid measurement of active *Mtb* with high clinical sensitivity and specificity.[104] The CFP-10 and ESAT-6 antibodies are conjugated to silica nanoparticles, and the two antigens can be detected from the serum samples collected from patients. The NanoDisk increases the target peptide enrichment and effect to enhance matrix-assisted laser desorption/ionisation signal of bound peptides, resulting in the quantification of ESAT-6 and CFP-10 peptides at low concentrations, which provides maximum sensitivity. The advantages of this system include the following: (1) measures severity of active TB infections, allowing monitoring of treatment effects; (2) only minute concentrations of the antigens in the blood are required to detect the bacteria infection; and (3) it is effective in clinical scenarios where standard culture techniques are associated with low specificity, for example, HIV-positive patients with TB and extrathoracic TB. The NanoDisk-MS assay, in contrast, detects TB infections in lung or non-lung tissues with comparable sensitivity by over 90%, regardless of the patient's HIV status. In a clinical study, NanoDisk-MS demonstrated promising specificity in both healthy and high-risk groups in the range of 87.1%–100%. Moreover, it has shown as a reliable technique to differentiate patients with related disease cases such as latent TB, NTM infections and healthy subjects with an accuracy of 87%, 91% and 100%, respectively.[104 105] Published work to date on the NanoDisk-MS shows clinical efficacy and supports its future approval by regulatory authorities.

ASTHMA

Asthma is the most prevalent long-term inflammatory disease of the lungs and is characterised by intermittent reversible airway obstruction, bronchial hyper-responsiveness and chronic airway inflammation.[106] Electronic nose (E-nose) is a novel device made up of nanosensors capable of detecting specific volatile organic compounds (VOCs) in exhaled gas. Generally, the non-invasive diagnosis of various diseases is the key advantage of exhaled breath analysis techniques, like E-nose, over other commonly used methods.[107] Previous clinical study showed that the E-nose method is able to discriminate the exhaled breath of patients with asthma from healthy controls and can make a difference between the degrees of asthma severity; it was the first study using pattern analysis of exhaled VOC mixtures by an E-nose in the field of asthma.[108] E-nose technique is also applied for the detection and discrimination of pulmonary diseases.[109] It has been well described that asthma has four inflammatory phenotypes, including eosinophilic, neutrophilic and paucigranulocytic, which are categorised based on induced sputum inflammatory cell counts. The inflammatory asthma phenotypes have been shown to differ with respect to airway microbiology and even response to corticosteroid treatment; thus, diagnosis of the phenotypes of asthma is important for personalised approaches to asthma therapy.[110] Recently, nanotechnology has been employed to develop a non-invasive assessment method with high accuracy to discriminate patients with eosinophilic, neutrophilic and paucigranulocytic asthma phenotypes. The results of a clinical trial (NCT02026336) that was conducted to assess the ability of this method indicated that different inflammatory asthma phenotypes based on induced sputum analysis can be readily discriminated by their breath-prints using an E-nose device.[111]

Nanomedicine was also employed to deliver therapeutic agents for the treatment of asthma.[112] Salbutamol is a bronchodilator that is used for the symptomatic relief and prevention of bronchoconstriction. However, poor deposition of the inhaled drug in the lung presents a consistent and ongoing challenge for the symptomatic relief and control of asthma.[113] By formulating salbutamol as nanoparticles, Bhavna *et al* demonstrated lower drug in healthy volunteers after dry powder inhalation, as well as a 2.3-fold increase in total lung deposition, when compared with the standard microparticle formulation used for dry powder inhalation.[113]

In another nanoformulation of salbutamol to be clinically investigated, the drug was encapsulated into a nanosized non-ionic surfactant-based vesicles (niosomes). Preclinical studies showed a controlled release of the drug over an 8-hour period. A phase I clinical trial is currently under way to study the relative bioavailability of this nano-salbutamol and sustained release of the drug after inhalation (NCT03059017).[114]

One of these studies examined the use of nanotherapeutics for the treatment of allergic asthma by subcutaneous immunisation of mice with poly (lactic-co-glycolic acid) polymeric nanoparticles containing recombinant birch profilin protein, an allergen in pollen, latex and plant food. In a murine model of allergic asthma, this therapeutic strategy was found to prevent and show therapeutic benefit by regulating the Th1/Th2 equilibrium (see figure 8).[115]

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

COPD, as with most chronic non-communicable diseases, is the third most common cause of death worldwide.[78] It has been pointed out that the diagnosis of COPD at an early stage can alter the rate of disease progression and severity of lung function impairment. Spirometry is the gold standard method for COPD diagnosis and monitoring progression,[116] and needs expert operator and general practitioners to perform and evaluate. Moreover, in the early stage of COPD, breathing symptoms might not be clinically manifest.[117] Previous studies have confirmed the ability of nanosensor-based E-nose method as a simple and easily operated method to diagnose COPD and to discriminate patients with COPD and asthma (accuracy of 96%),[118] and also lung cancer from COPD (accuracy of 85%).[119] In a recent clinical trial (NCT01976117), an E-nose method was used to identify bacterial colonisation in patients with COPD and was compared with the quantitative culture of protected specimen brush, which is the

gold standard but an invasive method for the diagnosis of distal airway infections. This investigation resulted in 88% accuracy of the E-nose method to distinguish the colonised and non-colonised patients with COPD; however, their demographic, functional and similar. This study introduced the E-nose tool as a non-invasive, easy-to-use, feasible and reliable method to detect bacterial colonisation in patients with COPD.[120]

CURRENT CHALLENGES AND FUTURE PERSPECTIVE

Nanomedicine presents an excessive opportunity for the fundamental improvement of current therapies and development of new treatment options for lung diseases previously thought difficult or impossible to treat. However, we are still in the early stages of nanomedicine in respiratory diseases care, and more human data and a lot more investment are required before they can be viewed as a mainstream proposition for vaccination, diagnosis and treatment purposes. At the commercialisation and industrial scale, there are some obvious challenges, which include non-uniform size distribution, lack of reproducibility, sterilisation and storage of the large-scale production.[121 122] Hence, it remains difficult to reproducibly manufacture the nanoparticle batches with identical properties, and these challenges can lead to irregular structure/shape, undefined surface chemistry, and finally increase the risk of unwanted biodistribution, as it has been shown that there is a direct correlation between physicochemical properties (eg, size and surface charge) of nanoparticles and their biodistribution pattern.[123] Developing methods/tools to characterise physicochemical features of nanoparticles for medical purpose can help to achieve a safe and robust nanotherapeutic. Moreover, standards for in vivo models are needed to predict the performance of nanoformulation in the clinical studies to investigate total body clearance. The accumulation of nanotherapeutics in unwanted tissues and organs brings about long-term toxicity concerns. For instance, it has been well demonstrated that almost 75% of liposomal nanotherapeutics, which are administered intravenously, are accumulated in other organs (liver, spleen, kidney and heart) and never can be found in the lung for the treatment of lung diseases.[21] This could cause poor treatment efficiency and high systemic adverse side effects. Therefore, determination of the nanoparticle biodistribution following systemic administration through any route should be considered in preclinical and clinical studies. In the context of cancer, nanoparticles are inclined to accumulate in solid tumour due to EPR effect, which is referred to as passive targeting. However passive targeting has a very low efficiency, and less than 1% of the nanotherapeutics can be found in the tumour .[124] In order to effectively control biodistribution and subsequently reduce adverse effect, nanoparticles can be targeted to specific cells or tissues, the so-called active targeting.[125] As we describe previously, there are a few targeted nanodrug carriers which are undertaken to determine their safety and efficiency in clinical trials (eg, BIND-014, TargomiR and L-BLP25). Also, to reduce systemic toxicity and improve treatment efficiency, nanocarriers can be engineered to be responsive to physical or chemical stimulus (eg, thermal and pH) to release their payload, also known as triggered nanoparticles. These types of nanoparticles release their therapeutic agents under certain conditions, providing maximum treatment efficiency and minimum side effect. Although numerous efforts have been taken to investigate triggered nanoparticles in preclinical studies, to the best of our knowledge there are no ongoing clinical trials based on this strategy for lung diseases. Both targeted nanotherapeutics and triggered nanoparticles can be categorised in the same group as

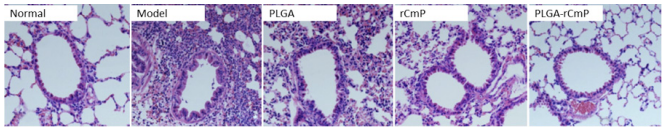

Figure 8 Histology of H&E-stained lung slices in a murine model of allergic asthma, with and without treatment with PLGA-rCmP nanoparticles. Mice in the normal group were treated with saline. The PLGA-rCmP group was subcutaneously injected with nanoparticles, the rCmP absorbed on 2 mg of Alhydrogel or Al(OH)3 and the PLGA group was injected with an equivalent amount of PLGA. Figure reproduced with permission from ref [115]. PLGA, poly (lactic-co-glycolic acid); rCmP, recombinant birch profilin protein.

'smart nanoparticles', and represent a future strategy to overcome the current limitations of a nanotherapeutics lung strategy.[126] It is expected that preclinical and clinical studies evaluating smart nanoparticles will lead to the delivery of safe, efficient, nano-based diagnostic, vaccine and therapeutic platforms.

CONCLUSION AND FUTURE DIRECTION

As Robert Langer, winner of Kabiller Prize in Nanoscience and Nanomedicine for 2017, believes, *"I personally would be shocked if 20 years from now, we're not seeing a lot of nanotechnologies helping a lot of people."*[127] As shown in this review, considerable work has been done in nanoformulating therapeutics and vaccinations; nonetheless, it is expected that more preclinical and clinical studies in nanomedicine will continue to lead towards safe and efficient solutions for lung diseases. Challenges however remain particularly in the investment in both the scale-up and industrialisation processes to produce large-scale compounds for appropriately powered phase III clinical trials that address the safety and efficacy of specific nanotherapies in human patients. Moreover, international standards are required to be defined for in vitro and in vivo models in order to allow valid comparisons between studies and to define the safety and efficacy of proposed treatments. There is now a substantial body of work in this relatively young field to predict that nanotherapeutics as applied to the lung and other organs will change the way we practise medicine in the not too distant future.

Cancer is the archetypical disease that illustrates how the initial concept of nanopackaged treatments given systemically has translated to effective therapies in the oncology field. The next stage in the development of this evolving field will be refinement of nanoparticle delivery systems to be organ-specific and tissue-specific. The lung represents the classical organ for the development of an enhanced capacity in the nanotherapeutics arena.

In the context of chronic respiratory diseases, such a strategy offers the ability to deliver enhanced concentrations of inhaled therapeutic payloads that minimise systemic toxicity. In addition, it offers the ability to deliver controlled, sustained slow-release compounds with specific absorption characteristics that overcome the significant mucus and bacterial biofilm burden characteristics of many chronic respiratory diseases, thus overcoming the problem of poor lung penetration of many of our current antimicrobial agents across this biological barrier. All these will lead to improved clinical care for patients.

Published work has shown that nanopackaged corticosteroids produce significantly more sustained anti-inflammatory benefits compared with orally administered compounds.[128] In the context of bronchial hyper-reactivity and asthma, nanoparticle packaged salbutamol has been shown to have more efficient

airway absorption compared withstandard inhalers, leading to sustained symptomatic relief. [129]

We are now entering a brave new world where nanotherapeutics will change the way we practise respiratory medicine. It offers improved clinical efficacy for our patients, particularly to those individuals who are currently treatment-resistant to conventionally administered medications.

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