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# Nanotechnology in the diagnosis and treatment of lung cancer

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ABSTRACT

Lung cancer is an umbrella term for a subset of heterogeneous diseases that are collectively responsible for the most cancer-related deaths worldwide. Despite the tremendous progress made in understanding lung tumour biology, advances in early diagnosis, multimodal therapy and deciphering molecular mechanisms of drug resistance, overall curative outcomes remain low, especially in metastatic disease. Nanotechnology, in particular nanoparticles (NPs), continue to progressively impact the way by which tumours are diagnosed and treated. The unique physicochemical properties of materials at the nanoscale grant access to a diverse molecular toolkit that can be manipulated for use in respiratory oncology. This realisation has resulted in several clinically approved NP formulations and many more in clinical trials. However, NPs are not a panacea and have yet to be utilised to maximal effect in lung cancer, and medicine in a wider context. This review serves to: describe the complexity of lung cancer, the current diagnostic and therapeutic environment, and highlight the recent advancements of nanotechnology based approaches in diagnosis and treatment of respiratory malignancies. Finally, a brief outlook on the future directions of nanomedicine is provided; presently the full potential of the field is yet to be realised. By gleaning lessons and integrating advancements from neighbouring disciplines, nanomedicine can be elevated to a position where the current barriers that stymie full clinical impact are lifted.



**Contents**



*Abbreviations:* ACUPA, S,S-2-[3-[5-amino-1-carboxypentyl]-ureido]-pentanedioic acid; ADC, adenocarcinoma; BAP1, breast cancer type 1 susceptibility protein (BRCA1)-associated protein; BD, biodistribution; CEA, carcinoembryonic antigen; Ce6, chlorin e6; CT, computerised tomography; CTCs, circulating tumour cells; CR, complete response; DOTAP:Chol, 1,2-dioleoyl-3-trimethylammoniumpropane:Cholesterol; DOX, doxorubicin; EGFR, epidermal growth factor receptor; EPR, enhanced permeability and retention; FDA, Food and Drug Administration; HA, hyaluronic acid; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemical; KRAS, *Kirsten rat sarcoma viral* oncogene homolog; LCC, large cell carcinoma; MELs, macrophage membrane coated emtansine liposomes; MMP, matrix metalloproteinase; MPM, malignant pleural mesothelioma; MPS, mononuclear phagocyte system; MRI, magnetic resonance imaging; MTD, maximum tolerated dose; NGs, nanoghosts; NIR, near-infrared; NPs, nanoparticles; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PEG, polyethylene glycol; PET, positron emission tomography; PFS, progression free survival; PK, pharmacokinetics; PPX, paclitaxel poliglumex; PR, partial response; ROS, reactive oxygen species; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; SD, stable disease; SiBiGdNPs, silica-based bismuth gadolinium nanoparticles; siLuc, siRNA against luciferase; siUBB, siRNA against ubiquitin B; SLNs, solid lipid nanoparticles; SPIONs, superparamagentic iron oxide nanoparticles; TCNPs, targeted chitosan nanoparticles; UCNPs, upconversion nanoparticles..

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## 1. Introduction

Cancer still stands as one of the world's most insidious diseases, with over 18 million new cases and 9.6 million deaths occurring annually (Bray et al., 2018). Disquietingly, the global burden of cancer is set to increase; the International Agency for Research on Cancer estimates that by 2030 there will be 22.2 million new cases and 13.2 million deaths (Bray, Jemal, Grey, Ferlay, & Forman, 2012). Of the subtypes of cancer, lung cancer is the most prevalent cause of cancer mortality, comprising 19% of cancer deaths and 3% of all deaths globally. Moreover, lung cancer has a dismal 5 year survival rate of approximately 19%, second only to pancreatic cancer, as the cancer with the poorest prognosis (Siegel, Miller, & Jemal, 2019), demonstrating a serious unmet need for curative therapies. Currently, there is a lack of reciprocity between the outstanding progress made in deciphering cancer at a molecular level and clinically relevant translational advances. Therefore, it seems our understanding of the disease does not match our ability to treat it. This is principally due to two main reasons; the majority of lung cancers are diagnosed at an advanced stage, and the inability to effectively deliver therapeutic regimens to the tumour at sufficient concentrations without subsequent collateral damage to healthy tissue. One would envision an ideal therapeutic scenario as one where highly selective delivery of a curative remedy to the burden of tumour cells in the very earliest stages of their malignant metamorphosis could be achieved. This "magic bullet" paradigm has been around since the time of Paul Ehrlich's immunological work (Strebhardt & Ullrich, 2008), from which the concept was originally derived. This proposal now has the potential to go from premonition to reality with the advent of nanotechnology.

Nanotechnology can be broadly defined as fabrication and application of man-made materials, devices and systems that fall within the size range of 1-100 nm in at least one dimension, however this size range is often not strictly adhered to in the literature, and is not of critical importance when addressing unmet medical needs. The innate physicochemical properties these materials possess, by virtue of their size and composition, allow them to be engineered towards use in a biological and medical context. One aspect within the remit of nanomedicine that has been an area of intense investigation, particularly over the past two decades, is nanoparticle (NP) research. "Nanoparticle" is an umbrella term for the many different shapes and sizes of nanovector structures (Fig. 1), and these NPs have the potential to revolutionise the way that diseases, such as cancer, are currently diagnosed and treated. NPs are afforded such potential due to their high surface area to volume ratio compared with their macromolecular counterparts, tuneable thermal, magnetic, optical and electrical properties and the diversity of shapes and sizes that can be synthesised, either hollow or solid, with desirable chemical composition and surface chemistry that can be manipulated with exogenous and endogenous stimuli. Due to their multifaceted characteristics, NPs have the potential to overcome the biological and chemical barriers within the human body allowing for augmented therapeutic and diagnostic localisation and efficacy with lower invasiveness and higher biocompatibility, with the aim to improve patient quality of life. However, despite promising pre-clinical results, the full translational potential of NPs is yet to be realised. This is primarily due to the challenges associated with reproducibility, large-scale manufacturing, and potential toxicological and safety hazards that may be inadequately assessed, overlooked or underestimated. For example, elongated and biopersistent NPs, used pre-clinically for pulmonary drug delivery, have been shown to induce fibrosis (Wang et al., 2013) and asbestos-like scarring of the lung (Poland et al., 2008) exemplifying the importance of long-term safety assessments of nanomaterials.

Given the vast realm of nanotechnology, even in an oncological context, this review will focus on the diagnostic and therapeutic application of nanotechnology in lung cancer. However, the concepts addressed here can be applied to a broad spectrum of other cancers and other diseases. We will review the current diagnostic and therapeutic arsenal of lung cancer and, using examples, how nanotechnology seeks to complement and enhance them. We also look to discuss future advances for nanotechnology applications in lung cancer but also on a broader scale, in relation to other pernicious diseases.

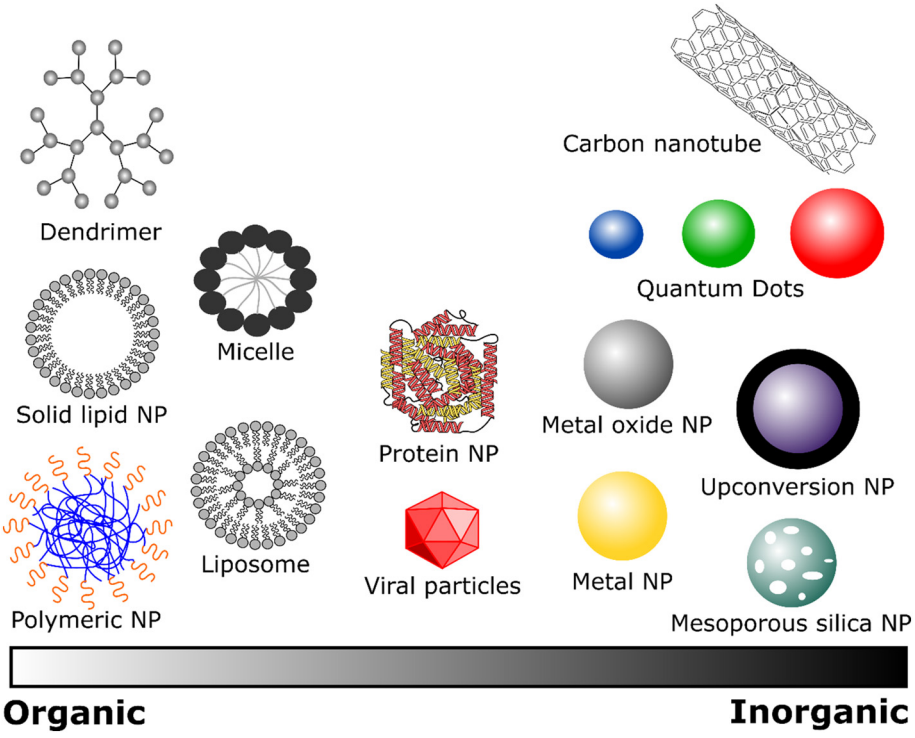


**Fig. 1.** Spectrum of nanoparticles used in diagnosis and treatment of cancer. Nanoparticle composition can generically be defined as organic (polymers, lipids, dendrimers, proteins) or inorganic (metals, rare earth elements, carbonaceous). Although spheres are primarily depicted (and used) nanoparticles can take the form of a variety of shapes such as rods, wires and other geometric structures (triangles, diamonds etc.). The physicochemical properties of nanoparticles dictates their *in vitro* and *in vivo* behaviour meaning they can be engineered to overcome the barriers of the body to effectively target the disease site.

## 2. Classification and biology of lung cancer

Lung cancer is a disease of multiple aetiologies that arises as a result of neoplastic metamorphosis of epithelial cells in the lung. A plethora of epigenetic, genetic and molecular aberrations underlie the progression of the disease and also influence disease heterogeneity, and ultimately the diagnostic, therapeutic and prognostic outcomes (Herbst, Heymach, & Lippman, 2008). In order to be able to devise a comprehensive, personalised treatment strategy for lung cancer, one must consider not only genetic and molecular information, but also histopathological and clinical characteristics. As such, three main categories of respiratory malignancy have been described based on the above criteria; these are non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM).

### 2.1. Non-small cell lung cancer

NSCLC is the predominant form of lung cancer with approximately 85% of total lung cancer cases falling into the diagnostic remit of NSCLC. Of these cases, approximately 65% present with either metastatic or locally advanced disease (Reck, Heigener, Mok, Soria, & Rabe, 2013). Cigarette smoking is the most common risk factor, but other risk factors include second-hand cigarette smoke, certain foods (cured and barbequed meats, deep-fried consumables), alcohol, a sedentary lifestyle, air pollutants, and genetic susceptibility (Molina, Yang, Cassivi, Schild, & Adjei, 2008), most notably activating mutations in the proto-oncogene epidermal growth factor receptor (EGFR) (Rudin et al., 2009). The term NSCLC is a clinical umbrella term used to designate a wide variety of malignancies, including squamous-cell carcinoma (SCC), adenocarcinoma (ADC), large-cell carcinoma (LCC) and other less differentiated variants (Goldstraw et al., 2011) that each possess different cellular, genetic and epigenetic heterogeneity that gives rise to unique tumour microenvironments in patients, significantly contributing to the difficulty of treating NSCLC (Chen, Fillmore, Hammerman, Kim, & Wong, 2014; Gridelli et al., 2015). NSCLC is currently diagnosed using pathological characteristics consisting of histological alterations, immunohistochemical (IHC) staining, mutational and molecular genetics analysis, and imaging techniques in order to ascertain the subtype of NSCLC, and saliently, the stage of disease progression (Ettinger et al., 2012; Travis et al., 2015).

#### 2.1.1. Adenocarcinoma

Of the different histological variants of NSCLC, ADC is the most prevalent worldwide (Tang, Schreiner, & Pua, 2014) and the incidence rate of ADC is increasing fastest in women (Sereno et al., 2012). Due to the expansive heterogeneity that exists within ADC, the disease is notoriously difficult to accurately classify. Recently, the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society created a multidisciplinary consortium in order establish a more precise classification of ADC (Travis et al., 2011). The majority of ADC cases can be distinguished from SCC by absence of SCC-specific morphological characteristics and concomitant positive expression of mucin, thyroid transcription factor-1 and cytokeratin-7 (Rossi et al., 2004; Tang et al., 2014). Mutations in genes such as *EGFR, ELM4-ALK, BRAF* and *KRAS* are almost exclusive to ADC (Bittner, Ostoros, & Géczi, 2014; Yousem, 2012) which may complement histological analysis in order to accurately grade the disease progression. These molecular aberrations combined with reformed ADC classification guidelines facilitate more specific ADC assignment and improved efficacious treatment regimens.

#### 2.1.2. Squamous-cell carcinoma

SCC is the second most common subtype of NSCLC histologically, accounting for approximately 20-30% of all NSCLC cases, and is the most prevalent NSCLC lineage in men (Drilon, Rekhtman, Ladanyi, & Paik, 2012). SCC is most strongly associated with cigarette smoking; other specific risk factors include a familial predisposition and polymorphisms in certain genes involved in DNA repair, such as *MLH1*, and detoxification such as *CYP2A6* and *CYP1A2* (Sereno et al., 2012). Archetypically, SCC was designated as arising from the central airway, however the frequency of diagnosis of more peripheral tumours is increasing and even rivalling cases presenting as a central airway tumour. Histologically, a well differentiated SCC presents with keratinsation and pearl formation with a dense cytoplasm, and irregular hyperchromatic nuclei (Drilon et al., 2012; Stinchcombe, 2014). Although there are no SCC specific markers, positive expression of p63, cytokeratin-5, desmocollin-3 and ΔNp63 have been used to differentiate SCC from other variants of NSCLC, in combination with miRNA profiles (Barbareschi et al., 2011). In addition, genetic analysis is utilised to identify oncogenic driver mutations (typically involved in highly salient cell signalling pathways) and to potentially distinguish between otherwise indiscriminate or undifferentiated subtypes of NSCLC. The Cancer Genome Atlas Research Network characterised the genetic and epigenetic architecture of 178 SCC cases (The Cancer Genome Atlas Research, et al., 2012) and found statistically recurrent mutations in 11 genes, including *TP53*, in almost all of the samples profiled, and a proportion had significantly altered pathways such as the *KEAP2* and *PI3K*, *RB1* and *CDKN2A* pathways. The study also revealed differences from that of ADC, such as altered mutational landscape of *EGFR*, *KRAS* and *FGFR*, that may act as a diagnostic fingerprint when morphological differentiation is unclear

#### 2.1.3. Large-cell carcinoma

LCC is the third most common NSCLC subtype and accounts for approximately 3–9% of NSCLC cases. LCC is the collective term for undifferentiated respiratory malignancies that do not fall into the domain of SCC or ADC, are not of neuroendocrine origin and have no other specific clinical traits. This designation is assigned solely to tumours from surgical resection that, when biopsied or examined cytologically, have a lack of differentiating morphology under light microscopy, however this may not be the case for the actual tumour itself (Sholl, 2014). With the diagnostic hurdles associated with LCC, it is pertinent to differentiate tumours as accurately as possible using IHC analysis for the biomarkers mentioned above and/or genomic profiling of canonical driver mutations (Rekhtman et al., 2013). Traditional pathological and histological methods reveal inconclusive results in up to 70% of cases due to misrepresentative sampling of the tumour (Pelosi et al., 2015), highlighting the need to elucidate a more targetable phenotype in relation to which therapeutic avenues to pursue.

### 2.2. Small cell lung cancer

SCLC encompasses 14% of all lung cancer cases, with approximately 180,000 new cases diagnosed globally each year. SCLC is traditionally thought of as either a geriatric or a smoker's disease, with over 90% of patients falling into at least one of these categories. Indeed, the risk of developing SCLC is increased with duration and intensity of smoking (van Meerbeeck, Fennell, & De Ruysscher, 2012). The World Health Organisation currently classes SCLC as a neuroendocrine malignancy with histological features including nuclear moulding, high number of mitoses, necrosis and crush artefacts. Staining for biomarkers such as CD56 and thyroid transcription factor-1 can aid confirmation of SCLC, whereas chromogranin and synaptophysin staining is more useful as a diagnostic exclusion tool as up to two-thirds of SCLC are negative for these markers. Additionally, as SCLC falls under the remit of neuroendocrine malignancies, it stains strongly for neuroendocrine markers, however these should be used in favour of excluding a carcinoid diagnosis as opposed to confirming SCLC (Travis, 2012). Genetic studies of SCLC have not propagated therapeutic advances in the same fashion as NSCLC, perhaps due to the paucity of putative targets specific to SCLC. *TP53* is lost in the vast majority of cases, as well as *RB1*, the 3p locus and amplification of *MYC* family members (Byers & Rudin, 2015).

### 2.3. Malignant pleural mesothelioma

MPM is a rare thoracic malignancy, with an incidence of approximately 2000-3000 new cases per year. However, the occurrence of MPM has increased over the past five decades and continues to increase. This is widely attributable to the primary aetiological agent associated with MPM, asbestos, although ionising radiation and simian virus 40 infection have also been associated with MPM (Raja, Murthy, & Mason, 2011). Owing to a latency period of between 20 and 60 years, asbestos was not immediately recognised as a carcinogen or to have aetiological pertinence to MPM until a definitive epidemiological study was published in 1960, linking asbestos to mesothelioma (Wagner, Sleggs, & Marchand, 1960). Indeed, this latency period between exposure and diagnosis is the reason we are now seeing an increase in MPM cases worldwide.

Clinically, MPM typically presents with dyspnoea due to pleural effusion, and chest pain due to invasion of the thoracic wall, with the average onset of symptoms occurring 2-3 months prior to diagnosis. Characteristic symptoms of late stage disease include malaise, weight loss, hyperhidrosis and fatigue. The median survival time of untreated cases is in the range of 6-9 months and <5% of all cases survive longer than 5 years (Raja et al., 2011; van Meerbeeck, Scherpereel, Surmont, & Baas, 2011). Diagnosis usually entails chest X-ray and chest computerised tomography (CT) scans. Position emission tomography (PET) scans can be useful to gauge the extent of metastatic disease. Tissue biopsy for cytological, histological and IHC analysis is procured most commonly by thoracoscopy in order to help fully differentiate and assign the correct subtype of MPM, either epithelioid, biphasic or sacromatoid, which then aids in dictating the most suitable treatment course (van Zandwijk et al., 2013).

Unfortunately, due to the intrinsic heterogeneity of MPM, a ubiquitous genetic fingerprint is very difficult to obtain. Surprisingly, mutations in the canonical oncogenes associated with cancer such as *TP53* and *RB1* occur very rarely in MPM (Papp et al., 2001) but in cases of MPM with mutations, loss of heterozygosity at 22q12 is found in almost 100% of cases. At this locus is the *NF2* gene, which encodes for the tumour suppressor merlin that may act as a putative gatekeeper in MPM pathogenesis. Moreover, complete allelic loss of 22q12 can result in the aberration of the *CDKN2A* locus at 9p21 which encodes for two separate tumour suppressors, $p16^{INK4A}$ and $p14^{ARF}$ that have regulatory roles pertaining to the cell cycle and p53 respectively, therefore exacerbating and expediting the malignant transformation (Robinson, Musk, & Lake, 2005; Zucali et al., 2011). Additionally, the *BAP1* gene, located at 3p21, has also been identified as a driver gene in MPM (Bott et al., 2011). BRCA1-associated protein (BAP1) has regulatory functions in highly salient cellular processes such as apoptosis, the cell cycle checkpoint and transcription (Carbone et al., 2013). Mutations in the *BAP1* gene have not only been found in up to 60% of MPM tumours, but may even predispose an individual to MPM, as germline *BAP1* mutations have been reported in families with an extremely high incidence of MPM (Testa et al., 2011). These three chromosomal mutations are the most common in MPM, however there have been reports of frequent deletions within the chromosome arms 1p, 6q and 15q (Murthy & Testa, 1999). There are several proto-oncogenes implicated in MPM pathogenesis, such as the activating protein-1 family member *fra-1*, as well as *c-fos, c-jun, c-Met, c-myc,* and *β-catenin* that all have roles in major cell signalling pathways such as the mitogen activated protein kinase and Wnt pathways, that have a large influence in determining cellular proliferation and growth (Gordon et al., 2005; Weiner & Neragi-Miandoab, 2009). Therefore it stands to reason that dysregulated signalling of these influential pathways can drive cellular proliferation far beyond physiological restrictions. MPM development has also been linked with epigenetic alterations. Promoter hypermethylation has been observed at several loci in MPM (Vandermeers et al., 2013) and there is preclinical data are showing the impact of inhibition of histone deacetylases (HDACs) on MPM (Shao et al., 2007; Symanowski et al., 2009). By inhibiting HDAC activity, the chromatin of, in this case, tumour suppressors remains unfolded and accessible for transcription, preventing loss of heterozygosity and subsequent oncongenic consequences. Based on the role of HDACs in tumourigenesis, there are several HDAC inhibitors in MPM clinical trials, with preclinical data supporting the notion that these class of drugs should be used in combination with chemotherapy for greatest therapeutic effect (Marchion & Münster, 2007; Vandermeers et al., 2009).

## 3. Current state of diagnosis and treatment in lung cancer

### 3.1. Diagnosis

Traditionally, diagnosis of lung cancer was based on histological examination of resected tumours, which was sufficient for decisions on appropriate therapeutic intervention. However, with the advances made in molecular biology and therapeutic options, the diagnostic arsenal has had to expand in order to facilitate accurate differentiation of the subtypes of lung cancer. As most patients are diagnosed with advanced stage disease, it becomes all the more important to be able to identify the different lung cancer variants based not only on histology, but also with profiled molecular aberrations, in order to develop personalised treatment strategies. The discovery of activating mutations in genes such as *EGFR* and *ALK* paved the way for personalised medicine to become clinical reality, and although the therapies targeted towards these mutations (e.g. tyrosine kinase inhibitors) are not curative, they demonstrate encouraging efficacy in patients with sensitising mutations (Wood, Pernemalm, Crosbie, & Whetton, 2015). New activating mutations are also being discovered which will pave the way for further patient stratification and therapeutic innovation, and for this reason, screens for molecular aberrations in *EGFR* and *ALK* are recommended particularly in lung cancers with an adenocarcinoma component (Lindeman et al., 2013). There are a number of diagnostic methods that can aid IHC to discern molecular markers, such as fluorescence (or chromogenic) *in situ* hybridisation, or more sensitive techniques such as Sanger sequencing, restriction length fragment polymorphism, PCR based methods or mass spectrometry based genotyping (Salgia, 2015). Indeed, advances in translational genomics has paved the way for the development of mulitplex diagnostic methods such as a SNaPSHot which is a PCR based multiplexed genotyping technique (Sequist et al., 2011), or Sequenom MassARRAY which is an array based platform for screening of mutational hotspots (Bar et al., 2014). Next generation sequencing based technologies have not yet reached clinical approval but are capable of high throughput omics screening (Hagemann et al., 2015). These technologies could be used to sequence the whole genome or exome for mutations, or the transcriptome for quantification of gene expression, as well as other applications such as miRNA profiling and identifying epigenetic modifications of DNA. This could reveal valuable diagnostic information and aid in creating a more personalised treatment strategy. Sample acquisition is a pertinent diagnostic consideration as insufficient quantity and quality of sample precludes accurate and rapid diagnosis. Multiple biopsy samples, despite their invasive nature, are the best method of obtaining detailed information. By screening for modalities in the respiratory tract (putative airway epithelial cell biomarkers, sputum microRNAs/DNA methylation, microbiome and metabolome) or peripheral circulation (serum auto antibodies, DNA methylation patterns of leucocytes, microRNAs, proteomic signatures, circulating cell free DNA and circulating tumour cells [CTCs]) early detection and diagnosis of respiratory malignancies may be expedited (Abbosh et al., 2017; Beane, Campbell, Lel, Vick, & Spira, 2017; Hasan, Kumar, & Kavuru, 2014; Nagrath et al., 2007) A point of contention with regards to lung cancer screening is the use of CT scans. Although chest X-rays are routinely used, there are currently no lung cancer screening programs in the UK. However the SUMMIT study, which will use low dose CT scans as part of the largest lung cancer screening study done in the UK, will provide information on the potential of a national screening programme. The United States Preventive Services Task Force recently revised their recommendations and now advocate the

use of low dose CT for annual screening of lung cancer in adults aged 55-80 years who have a 30 pack-year smoking history and who currently smoke or have quit within the last 15 years (Moyer, 2014).

### 3.2. Treatment

There are four main categories that encompass currently available lung cancer treatment options: surgical resection, radiotherapy, chemotherapy and biological therapy. Surgical resection of a lung tumour is the most effective curative modality. However, due to the advanced stage or metastatic spread of lung cancer at the time of diagnosis, the resectability of a tumour is greatly diminished, resulting in inoperable cancers. In an attempt to improve resectability, neoadjuvant chemotherapy, radiotherapy or a combination of the two (chemoradiotherapy) are often administered. Indeed, these modalities are not only used as induction therapies but also as a definitive treatment course or postoperatively depending on the status of the patient and the course of disease. Stereotactic ablative radiotherapy involves administration of high dose radiation specifically to the tumour, delineated by advanced techniques such as four dimensional-CT, PET/CT or image guided radiotherapy. Stereotactic ablative radiotherapy is recommended for patients that are medically inoperable, refuse surgery or are ineligible (elderly, poor lung function etc.) and has been shown to achieve control rates of primary tumours comparable to that of lobectomy (removal of an entire lobe containing the tumour) (Onishi et al., 2011; Timmerman et al., 2010).

Chemotherapy (Table 1) comprises an essential part of the treatment regimen for respiratory malignancies, either as neoadjuvant or adjuvant therapy, the primary course, in combination with radiotherapy, or for palliation. Histological information, as well as disease stage and patient status, highly influences the prescribed therapy. This information is of particular relevance in consideration of biological therapy. The identification of oncogenic driver mutations (in genes such as *EGFR, ALK, ROS1, VEGFR*) in lung cancer, led to the development of small molecules that target the products of these mutated genes with varying degrees of clinical responsiveness. With the help of clinical trial data (Inoue et al., 2009; Mok et al., 2009; Sandler et al., 2006; Shaw et al., 2011; Zhou et al., 2011) several of these targeted therapies are now routinely used in the clinic either as first line therapies in their own right or concomitantly with chemotherapy (Table 1). Indeed, extensive clinical trial data has provided information that has facilitated the creation of guidelines as to the most appropriate scenario in which specific therapies (chemo/radio/biological) are to be administered, taking into account patient quality of life and disease status. Furthermore, an area that shows promise is that of immunotherapy. Early clinical trial data demonstrated that co-administration of ipilimumab, an anti-cytotoxic T-cell lymphocyte-4 monoclonal antibody, with paclitaxel and carboplatin resulted in both an improved progression free survival (PFS) and immune-related PFS in patients with stage IIIB/IV lung cancer (Lynch et al., 2012). Recently, nivolumab, a fully humanised IgG4 anti-programmed cell death protein 1 (PD-1) monoclonal antibody, outperformed docetaxel in a clinical trial involving patients with advanced NSCLC, regardless of PD-1 ligand status (Brahmer, et al., 2015). Another monoclonal anti-PD-1 antibody, pembrolizumab, has shown antitumour activity with an acceptable side effect profile in patients with advanced NSCLC (Garon et al., 2015). As a result, both nivolumab and pembrolizumab have been approved by the Food and Drug Administration (FDA) for treatment of patients with metastatic NSCLC with disease progression on or after platinum chemotherapy. As our understanding of lung cancer biology and its mutational landscape increases, more targeted and immunological therapies are being used in the clinic (Herbst, Morgensztern, & Boshoff, 2018).

### 3.3. Defining the problem – why is lung cancer so hard to treat?

Several fundamental factors lie at the centre of the lung cancer conundrum: difficulty in diagnosis, the complex, stochastic and dynamic intra and inter tumour heterogeneity unique to each patient, the gaps in our present understanding of tumour biology and genomic architecture, and the ineffectiveness of the current therapeutic arsenal to achieve a curative clinical response. Perhaps the most important aspect with regards to improving overall survival rates, is that the majority (approximately 85%) of patients have either advanced or metastatic disease at the time of diagnosis and as such, surgical resection is not always a viable treatment avenue. Another pertinent factor is the prevalence of risk factors related to lung cancer, the most prominent of which is tobacco smoking but also include air pollution, second-hand smoke and other occupational carcinogens. On a cellular level, a well-known phenomenon that occurs in lung cancer is its intrinsic heterogeneity. The initial level of heterogeneity arises on an individual basis as evidenced by the diversity in genetic and epigenetic profiles of lung cancers (Kandoth et al., 2013; The Cancer Genome Atlas Research, et al., 2012) and the microenvironments they establish (Quail & Joyce, 2013). The tumour microenvironment is a critical determinant of tumourigenesis and consists of a plethora of components such as infiltrating immune cells and mesenchymal cells as well as close association with the extracellular matrix and vasculature. Each individual will have a different microenvironment based on the genetic background of the tumour, of their

**Table 1**
Currently approved chemotherapy/immunotherapy for treatment of lung cancer.

| Agent | Trade Name | Mechanism of Action |
|---|---|---|
| Cisplatin | Platinol | DNA crosslinking |
| Carboplatin | Paraplatin | DNA crosslinking |
| Mitomycin-C | Mitozytrex | DNA crosslinking |
| Paclitaxel | Taxol | Stabilises microtubules |
| Nab-paclitaxel | Abraxane | Stabilises microtubules |
| Docetaxel | Taxotere | Stabilises microtubules |
| Vincristine | Oncovin | Inhibits microtubule formation |
| Vinblastine | Velban | Inhibits microtubule formation |
| Vinorelbine | Navelbine | Inhibits microtubule formation |
| Topotecan | Hycamtin | Topoisomerase I inhibitor |
| Irinotecan | Camptosar | Topoisomerase I inhibitor |
| Etoposide | Etopophos | Topoisomerase II inhibitor |
| Doxorubicin | Adriamycin | Topoisomerase II inhibitor |
| Ifosfamide | Ifex | DNA alkylating agent |
| Cyclophosphamide | Cytoxan | DNA alkylating agent |
| Temozolomide | Temodar | DNA alkylating agent |
| Mechlorethamine | Mustargen | DNA alkylating agent |
| Pemetrexed | Alimta | Folate antimetabolite |
| Methotrexate | Trexall | Folate antimetabolite |
| Gemcitabine | Gemzar | Nucleoside analogue |
| Erlotinib | Tarceva | EGFR inhibitor |
| Gefitinib | Iressa | EGFR inhibitor |
| Afatinib | Gilotrif | EGFR/HER2 inhibitor |
| Cetuximab | Erbitux | EGFR inhibitor |
| Osimertinib | Tagrisso | EGFR inhibitor |
| Necitumumab | Portrazza | EGFR inhibitor |
| Dacomitinib | Vizimpro | EGFR inhibitor |
| Crizotinib | Xalkori | ALK/ROS1 inhibitor |
| Ceritinib | Zykadia | ALK inhibitor |
| Alectinib | Alecensa | ALK inhibitor |
| Brigatinib | Alunbrig | ALK inhibitor |
| Lorlatinib | Lorbrena | ALK/ROS1 inhibitor |
| Bevacizumab | Avastin | VEGF-A inhibitor |
| Ramucirumab | Cyramza | VEGFR-2 inhibitor |
| Nintedanib | Vargatef | VEGFR/FGFR/PDGFR inhibitor |
| Everolimus | Afinitor | mTOR inhibitor |
| Dabrafenib | Taflinlar | BRAF inhibitor |
| Trametinib | Mekinist | MEK inhibitor |
| Nivolumab | Opdivo | PD-1 inhibitor |
| Pembrolizumab | Keytruda | PD-1 inhibitor |
| Atezolizumab | Tecentriq | PD-L1 inhibitor |
| Durvalumab | Imfinzi | PD-L1 inhibitor |

ALK, anaplastic lymphoma kinase; BRAF, serine/threonine protein kinase B-raf; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; HER2, human epidermal growth factor receptor 2; MEK, mitogen activated protein kinase kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet derived growth factor receptor; PD-(L)1, programmed death receptor-(ligand)1; ROS1, ROS1 proto-oncogene receptor tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

somatic cells, and the immune architecture (Chen, et al., 2014). Furthermore, there is the issue of therapeutic resistance, which is one of the main reasons advanced NSCLC remains incurable, and can also be associated with the microenvironment, amongst other factors (Rotow & Bivona, 2017). Therapies also often have very poor biodistribution (BD) and this non-specific distribution leads to crippling side effects and dose limiting toxicities that further limit the efficacy of treatment. As such, there is an exceptionally high attrition rate of new chemical entities, with many failing in later stage clinical trials principally due to lack of safety and efficacy.

## 4. The use of nanotechnology in lung cancer

Given the difficulties associated with effectively treating lung cancer, nanotechnology can provide an alternate avenue with which to augment traditional therapeutic approaches. As briefly touched on in the introduction, the application of nanotechnology to address medical questions is known as nanomedicine (Kim, Rutka, & Chan, 2010; Thorley & Tetley, 2013), a field that affords great potential in the amelioration of lung cancer and other diseases. The primary innovation in nanomedicine has largely centred on the development of NPs as the delivery vehicle for a plethora of therapeutic molecules including small molecules, nucleic acids, proteins, peptides and hormones (Allen & Cullis, 2004; Chow & Ho, 2013; Davis, Chen, & Shin, 2008; Doane & Burda, 2012; Peer et al., 2007; Thakor & Gambhir, 2013; Wang, Langer, & Farokhzad, 2012). NPs are a versatile technology that can be engineered to overcome the numerous pharmacological, biological and physical barriers of the body which often hinder successful treatment (Jiang et al., 2017; Petros & DeSimone, 2010). This is due to several reasons; there is a multiplicity of different nanomaterials used to create NP based drug delivery systems including lipids (solid liposomes, micelles and lipid NPs) (Allen & Cullis, 2013), polymers (dendrimers, hydrogels, polymeric NPs) (Kamaly, Yameen, Wu, & Farokhzad, 2016), carbon structures (nanotubes, fullerenes, nanodiamonds, graphene) (Chen, Dougherty, Zhu, & Hong, 2015; Yang, Feng, & Liu, 2016) , proteins (Molino & Wang, 2014) and inorganic matter such as metals (gold, silver, iron) (Dreaden, Alkilany, Huang, Murphy, & El-Sayed, 2012; Ge et al., 2014; Mahmoudi, Sant, Wang, Laurent, & Sen, 2011), silica (Yang, Gai, & Lin, 2012), rare-earth elements (Chen, Qiu, Prasad, & Chen, 2014), quantum dots (Zhang, Yee, & Wang, 2008), viral components (Yildiz, Shukla, & Steinmetz, 2011) and others (Gultepe, Nagesha, Sridhar, & Amiji, 2010). Due to the diverse portfolio of materials that can be used to create NPs, their physicochemical properties such as size, shape, surface charge, surface roughness and deformability can be tuned and adjusted, which are critical determinants of their *in vitro* and *in vivo* behaviour (Albanese, Tang, & Chan, 2012; Ernsting, Murakami, Roy, & Li, 2013; Nel et al., 2009). Therapeutic payloads can be incorporated into a nanoformulation either by encapsulation within or conjugation to the NP, allowing potentially multiple types of therapeutics with differing physicochemical properties to be delivered simultaneously. However, as with all new medicines, thorough understanding of potential adverse effects, pharmacokinetics (PK) and pharmacodynamics is required. Due to the unique properties nanomaterials exhibit over their bulk counterparts, special consideration and detailed toxicological studies are essential to understand how nanomedicines interact with various compartments within the body; the challenges and issues surrounding the use of nanotechnology in the medical field are elegantly discussed elsewhere (Soares, Sousa, Pais, & Vitorino, 2018). Within the field of lung cancer nanomedicine, many of the drugs being developed are formulated for administration by injection or inhalation. Both of these routes of administration rapidly expose nanomedicines to a complex mixture of proteins and lipids before they are able to interact with their desired target. The increased surface reactivity of NPs causes a corona to form on the surface, altering the biological identity of the NP which may alter how cells/organs recognise, process, and ultimately clear the particle (Tenzer et al., 2013). Indeed, we and others have previously demonstrated that nanoparticles adsorb proteins from lung lining liquid (Theodorou et al., 2016; Thorley, Ruenraroengsak, Potter, & Tetley, 2014; Whitwell et al., 2016) and blood plasma (Lundqvist et al., 2008; Lundqvist et al., 2017) and that the protein corona formed is dependent on the physicochemical properties of the particle such as size, shape and charge and surface modification (Bertrand et al., 2017). As a result of this corona, particles may be rapidly cleared before eliciting their biological effect. A major clearance mechanism of opsonised particles is the mononuclear phagocyte system (MPS) which exists in all major organs; proteins adsorbed to the surface of particles may facilitate interaction with the MPS and promote clearance (Gustafson, Holt-Casper, Grainger, & Ghandehari, 2015).

NPs can be modified to have a prolonged circulatory half-life and reduced immunogenicity by avoiding opsonisation and sequestration by the MPS. This can be achieved by coating the NPs with hydrophilic polyethylene glycol (PEG) based polymers (D'souza & Shegokar, 2016; Suk, Xu, Kim, Hanes, & Ensign, 2016). However, it should be noted that repeating dosing with PEGylated drugs can lead to the generation of antibodies against PEG, which can diminish circulation times (Grenier, Viana, Lima, & Bertrand, 2018) and potentially lead to type I hypersensitivity reactions (Stone, et al., 2018). The use of native molecules to coat the surface of nanoparticles may overcome the problems associated with PEGylated NPs. Decoration with “self-recognition” peptides (Rodriguez et al., 2013) or biomimicry strategies such as coating with membranes from red blood cells (Hu et al., 2011) or leucocytes (Parodi et al., 2013). Comparatively fewer studies have been conducted to investigate the use of native pulmonary lipids and surfactant proteins to improve chemotherapy delivery to the lung. Preliminary *in vitro* studies have demonstrated that paclitaxel can be effectively encapsulated in NPs consisting of the native pulmonary phospholipids dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol and that these NPs confer improved tumour cell killing compared to paclitaxel alone (Meenach, Anderson, Hilt, McGarry, & Mansour, 2014). The hydrophobicity of surfactant protein D has been taken advantage of as a novel way to conjugate drugs to nanoparticles. A recent study fused a surfactant protein D-derived peptide sequence to the C terminus of a Fab antibody; the peptide sequence readily inserted itself in the membrane of liposomes, thereby circumventing the need for potentially harmful chemical crosslinkers (Ohradanova-Repic et al., 2018) Similarly, insertion of surfactant protein B into a phospholipid nanogel significantly improved delivery of siRNA to pulmonary cells and increased the gene silencing ability of siRNA (Merckx et al., 2018).

Alternatively, therapeutic molecules can be co-delivered with imaging agents to allow for a theranostic approach (Lammers, Aime, Hennink, Storm, & Kiessling, 2011; Xie, Lee, & Chen, 2010) or to monitor response to therapy in real time (Kulkarni et al., 2016; Miller et al., 2015). NPs can also be used to elicit an immunostimulatory response to generate antitumour immunity (Shao et al., 2015), or to concomitantly deliver a drug with molecules that modulate the vasculature (Lee et al., 2011). Furthermore, incorporation of stimulus responsive elements means that release of therapeutic payload can be triggered by either exogenous stimuli such as heat (Kneidl, Peller, Winter, Lindner, & Hossann, 2014), light (Yue, Zhang, & Dai, 2017), ultrasound (Zhang, Yu, Bomba, Zhu, & Gu, 2016) or a magnetic field (Hoare et al., 2011) as well as endogenous stimuli including pH (Du, Du, Mao, & Wang, 2011), redox (Ryu et al., 2010) or components of the physiological microenvironment (Huang et al., 2013), amongst other triggers (Karimi et al., 2016; Torchilin, 2014). Indeed, NP formulations not only benefit from favourable PK but also the cargo is protected from rapid metabolism and degradation *in vivo* along with increased persistence at the disease site. The origin of this enhanced spatial localisation is centred on a passive targeting phenomenon that has been documented in solid tumours known as the enhanced permeability and retention (EPR) effect (Matsumura & Maeda, 1986) (see Fang, Nakamura, & Maeda, 2011; Maeda, Nakamura, & Fang, 2013 for detailed reviews). The preferential passive accumulation of NPs in tumours sites occurs due to their

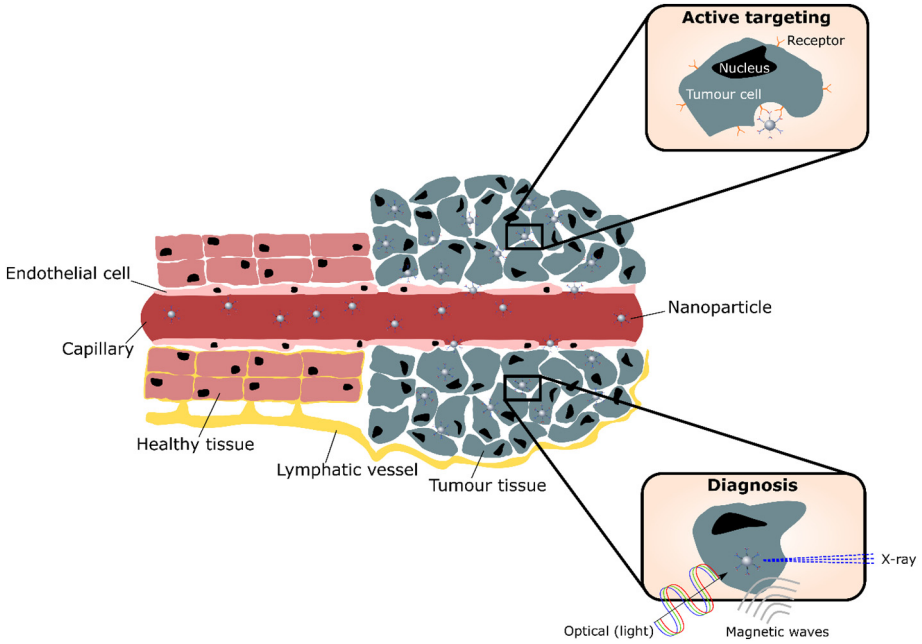


**Fig. 2.** The enhanced permeability and retention effect is a consequence of the rapid development of new and irregular blood vessels (angiogenesis) that present with a disordered and discontinuous endothelium. The resulting fenestrations are approximately 200–2000 nm in size and permit the extravasation of nanoparticles from the circulation into the tumour and not the surrounding healthy tissue which maintains ordered vasculature and tight cell-cell junctions. Tumours also have diminished lymphatic drainage which prevents clearance of nanoparticles, leading to their accumulation in the tumour interstitum. This passive targeting mechanism can be complimented by 'active targeting' whereby the nanoparticle surface is functionalised with affinity ligands that can enhance the cellular uptake of nanoparticles once in the tumour. This selective localisation allows nanoparticles to be used in a therapeutic and diagnostic context.

physicochemical properties and the EPR effect, however this can be increased by the addition of active targeting moieties into the NP formulations (Bertrand, Wu, Xu, Kamaly, & Farokhzad, 2014) (Fig. 2). These surface modifications can include antibody fragments, peptides, proteins, aptamers, nucleic acids or small molecules with specificity towards a component of the tumour or the surrounding microenvironment such as an overexpressed protein (Byrne, Betancourt, & Brannon-Peppas, 2008). In theory, the culmination of these factors results in favourable BD with potentially higher intratumoural drug concentration. This could also lead to a reduction in the amount of drug needed to elicit a therapeutic response contributing to less side effects and dose limiting toxicities, thus improving the therapeutic index compared to conventional (chemo)therapy. However, the reality is that human lung tumours are highly complex and heterogeneous, therefore the EPR effect is also prone to varying levels of physiological prominence and its significance in humans is debated (Danhier, 2016; Maeda, 2015; Nichols & Bae, 2014). The observation that, on average, <1% of an injected dose of NPs reaches the tumour in preclinical models, regardless of NP composition or targeting functionalisation (Wilhelm et al., 2016) reinforces that the EPR effect alone is not sufficient for effective clinical translation of nanomedicines and may instead be used as a biomarker to stratify patients. Insights into the intratumoural fate of passive and actively targeted NPs revealed that of those that reach the tumour microenvironment (<1% of injected dose), a vast majority are taken up by tumour-associated macrophages and not cancer cells themselves (Dai et al., 2018). Moreover, the formation of the protein corona directly affects NP properties and surface functionalisation, significantly hampering their delivery efficacy (Mahmoudi, Bertrand, Zope, & Farokhzad, 2016). Indeed, the challenges and opportunities of overcoming physiological barriers in NP drug delivery related to the EPR effect and in a wider scope have been elegantly outlined elsewhere (Blanco, Shen, & Ferrari, 2015; Dewhirst & Secomb, 2017). The following sections outline how nanomedicines are being used in lung cancer. Going forward, it is important to bear in mind both the advantages and caveats of nanomedicines outlined here.

## 5. Nanotechnology in lung cancer diagnosis

Accurate diagnosis is hugely important as the information gleaned influences the course of treatment. For some modalities such as radiotherapy, the precise tumour location and extent of spread must be known in order to ensure the therapy is as effective as possible. Within the remit of cancer diagnostics there are two main branches, imaging of the tumour body itself and detection of moieties associated with the tumour. Working with materials at the nanoscale provides a means by which to improve the conventional diagnostic methods within each of these categories. These enhancements stem from the unique material properties that are inherent at the nanoscale. For the patient, this translates to higher image resolution and signal strength as well as less toxicity from administered agents due to more specific delivery (Toy, Bauer, Hoimes, Ghaghada, & Karathanasis, 2014). Furthermore, more increased sensitivity and specificity of nano-based sensors means that cancer biomarkers such as specific metabolites or CTCs can be detected earlier, helping to define a malignancy in its infancy which assists in the decision of an appropriate treatment modality (Perfezou, Turner, & Merkoci, 2012).

Imaging agents used for CT scans in current clinical practice are based on iodine molecules however due to their small size they are rapidly cleared from the circulation by the kidneys. In order to overcome this iodine can be encapsulated within a nanoparticle which acts to prolong the circulation time of the agent and also reduce the dose required (de Vries et al., 2010; Kweon et al., 2010). Metal nanoparticles have received heightened interest as contrast agents for CT scans, namely gold, bismuth and tantalum, due to their high atomic number, tuneable size and versatile surface chemistry that allows conjugation or attachment of biomolecules such as aptamers, antibodies or peptides to facilitate targeting to the tumour site (Shilo, Reuveni, Motiei, & Popovtzer, 2012). Gold nanoparticles are the most attractive candidates due to their unique optical properties, excellent biocompatibility and ease of tuning physicochemical properties and surface chemistry (Cole, Ross, Tilley, Vargo-Gogola, & Roeder, 2015). Their potential as enhanced contrast agents has been demonstrated *in vivo* for imaging of lung cancer and other cancers (Chanda et al., 2010). For example, a proof of concept

study using acetylated dendrimer-entrapped gold nanoparticles successfully obtained CT images of a xenograft SPC-A1 lung adenocarcinoma in BALB/c mice (Wang et al., 2011; Wang et al., 2013), lending credence to the notion of the usefulness of specific targeting moieties. Furthermore, gold nanoparticles can be utilised for other types of cancer imaging such as dark field imaging (Nripen, Shukla, Katti, & Kannan, 2009) optical coherence tomography (Oldenburg, Hansen, Ralston, Wei, & Boppart, 2009), two photon luminescence (He, Wang, Hartmann, Cheng, & Low, 2007) and photoacoustic imaging (Galanzha et al., 2009).

Magnetic resonance imaging (MRI) scans are not routinely used for lung cancer imaging due to a low proton density, interference from respiratory and cardiac movement and a heterogeneous magnetic field in the lungs (Yoon et al., 2014) . However, technical and chemical advances have overcome some of these issues and to this end, Koyama *et al.* reported that MRI was as efficacious at detecting malignant pulmonary nodules as multi-detector CT (Koyama et al., 2008). Gadolinium is a commonly used contrast agent and nanoparticle formulations have been investigated in order to favourably alter PK and BD of gadolinium as well as improve contrast, and reduce dosage and toxicity. Such formulations include semiconductor polymer nanoparticles (Hashim et al., 2014), nanoporous silica particles (Ananta et al., 2010) gadolinium nanodiamond conjugates (Manus et al., 2010) or ultra-small rigid platforms that are constructed of a polysiloxane matrix surrounded by gadolinium chelates that are grafted to the matrix. These platforms were shown to either display enhanced signal strength at lower doses compared to a commercially available gadolinium agent, as well as good colocalisation with the tumour observed by MRI (Bianchi, et al., 2014). Superparamagentic iron oxide nanoparticles (SPIONs) have also been investigated for use in MRI as they provide a higher contrast than gadolinium and are biocompatible, and are safely cleared in the liver (Laurent et al., 2008). The FDA approved iron oxide nanoparticle formulation Fermuoxytol (Feraheme®) (Ross et al., 2009), along with other SPIONs, are currently in clinical trials as contrast agents for MRI (Kandasamy & Maity, 2015). Use of targeted SPIONs *in vivo* has also been explored; Hong *et al.* fabricated folate-attached SPION micelles to target xenograft BEL-7402 tumours that overexpress the folate receptor alpha (Hong, Zhou, & Yuan, 2012), a cell surface receptor that is also overexpressed in lung cancer (Cagle, Zhai, Murphy, & Low, 2012). The progress of agents such as Ferumoxytol to the clinic is encouraging, however the SPIO agents Feridex®, Ferumoxsil and Ferumoxtran which were once approved by regulatory bodies, have now been withdrawn from the market. Similarly with Resovist®, which was once approved in the USA and Europe, is now only available in a limited set of countries due to lack of efficacy or safety concerns (Ulbrich et al., 2016; Wáng & Idée, 2017).

Advances have also been made in PET imaging; 2-[$^{18}$F]fluoro-2-deoxy-D-glucose, a commonly used PET tracer, has limited diagnostic value for several types of lung cancers (Miele et al., 2008) therefore longer lived PET isotopes are being investigated with a nanoscale approach in order to increase accumulation of PET tracers within tumours. For example, encapsulation of $^{64}$Cu and $^{177}$Lu into PEGylated liposomes altered radionuclide PK and BD, and demonstrated clinically significant intratumoural accumulation *in vivo* (Petersen et al., 2015). Recently, ultrasmall $^{64}$Cu nanoclusters with a luteinising hormone releasing hormone targeting peptide were synthesised by using bovine serum albumin to act as a scaffold. These targeted nanoclusters exhibited a fourfold increase in tumour uptake when compared with nontargeted (no peptide) nanoclusters in a murine orthotopic A549 lung cancer model. Furthermore, when contrasted with near-infrared (NIR) fluorescence imaging *in vivo*, PET imaging showed enhanced visualisation of tumours (Gao et al., 2015), demonstrating the potential of nanoscale constructions in PET imaging.

Quantum dots are semiconductor nanocrystals used as NIR fluorophores whose light emission wavelength and colour can be tuned based on their size. Utilising this property, Tang *et al.* synthesised silver sulphide quantum dots decorated with cyclic arginine-glycine-aspartic acid-($_D$)phenylalanine-lysine peptides to target the $\alpha_v\beta_3$ integrin receptor. These quantum dots demonstrated preferential tumour uptake when compared with uptake into the liver, spleen or other organs *in vivo* (Tang et al., 2015). In a similar vein, aptamer functionalised zinc doped cadmium:tellurium quantum dots targeted towards mucin 1, which is overexpressed in many cancers including lung adenocarcinoma, were developed and exhibited successful targeting and imaging of A549 cells both *in vitro* and *in vivo* (Zhang et al., 2013).

Another approach centred on using polystyrene nanobeads conjugated with either human CD47 or a synthetic "self" peptide. This resulted in diminished macrophage uptake and reduced splenic clearance leading to enhanced tumour uptake and consequently increased tumour NIR fluorescence intensity in mice with human lung xenografts compared with nontargeted nanobeads (Rodriguez et al., 2013) Multimodal imaging techniques are increasingly being employed in order to obtain more information than is possible with one standalone technique. For example, combined PET-CT has been shown to improve accurate staging of NSCLC compared with PET alone (Yankeelov, Abramson, & Quarles, 2014). At the nanoscale, PEGylated holmium based nanoparticles demonstrated considerable *in vivo* potential as contrast agents for use in CT scans in tandem with MRI (Ni et al., 2016). Sun *et al.* conjugated glycol chitosan polymers to gold nanoparticles which were then functionalised with a peptide that acts as a substrate for matrix metalloproteinase (MMP) 2. A NIR fluorescent dye (Cy5.5) and black hole quencher were also coupled to the peptides. Therefore, in MMP positive tumours, the peptide is cleaved resulting in selective fluorescence facilitating dual modality NIR and CT imaging of tumours (Sun et al., 2011). A trimodal nanoformulation consisting of a SPIO core and a cross linked, aminated dextran coating labelled with a NIR fluorochrome was functionalised with the radionuclide fluorine-18 ($^{18}$F-CLIO). The authors demonstrated that $^{18}$F-CLIO could be detected by MRI and PET/CT and reasoned that detection using NIR imaging could also be performed by virtue of the NIR fluorochrome. The detection threshold of $^{18}$F-CLIO by PET/CT was 200 times lower than by MRI and *in vivo* PET/CT imaging showed a very high signal to noise ratio as well as good tolerability and biocompatibility (Devaraj, Keliher, Thurber, Nahrendorf, & Weissleder, 2009).

Biosensors working at the nanoscale have the potential to improve current diagnostic capability by enhancing detection of cancer biomarkers. Using nanomaterials in this context has lowered the limit of detection to the single molecule level as well as improving molecular specificity. For instance, utilising the unique properties of carbon nanotubes, Heller *et al.* achieved detection of single molecules of reactive oxygen species such as hydrogen peroxide as well as measuring alkylating chemotherapeutic agent concentrations in real time in living mammalian cells (Heller et al., 2009). Gold nanoparticles have been explored in a sensory context as well as in imaging. Oligonucleotide probes with a complimentary sequence to that of mutations in exons 19 and 21 in EGFR were conjugated to gold nanoparticles. Selective aggregation of gold nanoparticles was measured and facilitated colorimetric detection of EGFR mutations in three NSCLC cell lines and in DNA samples isolated from patients with NSCLC (Lee et al., 2010). Differentially functionalised 5 nm gold nanoparticles were assembled to create chemiresistor sensors which were then used to create a cross-reactive array. This array was then subjected to healthy and lung cancer breath samples which were subsequently analysed by gas chromatography-mass spectrometry in order to detect previously identified biomarkers in the form of volatile organic compounds. The array had low parts per billion limit of detection, demonstrated good reproducibility and successfully distinguished between healthy and lung cancer breath samples (Peng et al., 2009)

Nanopore sensors have been employed to detect molecular signatures pertinent to cancer and other diseases. These sensors are interspersed with nano-sized pores that can capture single molecules based on conformation. This causes a change in conductance leading to electrical elucidation and quantification of the molecule. Utilising this technology, Wang *et al.* detected and quantified levels of circulating miR-155, a microRNA, along with over one hundred others, known to be dysregulated in lung cancer. Although both quantitative reverse transcription PCR and the nanopore sensor showed a significant increase of miR-155

levels in lung cancer patients compared with healthy controls, the nanopore sensor was more accurate and showed less intra-assay variability (Wang, Zheng, Tan, Wang, & Gu, 2011). A quantum dot based microarray has also been investigated for detection of circulating microRNAs from serum as a potential method to identify putative biomarkers or signatures in patients with resectable NSCLC (Fan et al., 2015). Furthermore, quantum dots have been employed as dual detection and imaging vectors. The single domain anti-human epidermal growth factor receptor 2 (HER2) antibodies was conjugated to PEGylated 3.3 nm cadmium:zinc quantum dots. This resulted in significantly improved detection and enhanced fluorescence of HER2 in lung cancer cells that weakly express HER2 (A549 and H596) both in monoculture and co-culture with primary human macrophages when compared with conventional monoclonal antibodies against HER2 conjugated with a fluorophore (Rakovich et al., 2014). Quantum dots composed of cadmium:tellurium were attached to silica coated SPIONs which were subsequently decorated with monoclonal antibodies against carcinoembryonic antigen (CEA) to create magnetic and fluorescent bifunctional nanocomposites. The nanocomposites were able to separate (enrich) lung adenocarcinoma SPCA-1 cells from suspension by virtue of their magnetic properties and could also be visualised by fluorescence indicative of CEA binding. Furthermore, this platform was able to detect cancer cells in pleural effusions of lung cancer patients (Lee et al., 2010).

Microfluidic devices are emerging technologies that have the potential to vastly improve cancer diagnostics by detecting cancer at an early stage in a minimally invasive fashion. Although still in their infancy, these technologies have proven to be highly effective for detecting cancer associated biomarkers. CTCs are one such biomarker and using a microfluidic platform based silicon chip with an array of microposts coated with anti-epithelial-cell-adhesion molecule, which is upregulated in lung as well as other cancers, CTCs were able to be captured from millilitres of whole blood in 115 of 116 samples and were in appropriate condition for subsequent molecular analysis (Nagrath et al., 2007). Building on this work, CTCs were captured from the blood of 27 NSCLC patients which were then subjected to *EGFR* mutational analysis. Mutations in *EGFR* were found in 19 out of 20 samples, with 11 out of 20 samples harbouring the substitution mutation T790M which is implicated in resistance to tyrosine kinase inhibitors. Furthermore, rare secondary activating mutations were found in a small subset of patients whose CTC quantity and genotype was monitored over time and in response to therapy (Maheswaran et al., 2008). This information is usually gleaned from invasive biopsies and has a pivotal role in diagnosis, prognosis and therapeutic options. These technologies have also incorporated nanoscale components: semiconductor quantum dots were utilised as detection elements in an immunoassay microfluidics platform that detected cancer biomarkers including CEA and HER2 (Jokerst et al., 2009). Aqueous-phase-synthesised quantum dots were fabricated as signal amplifiers in a microfluidic protein chip framework that allowed multiplexed detection of cancer biomarkers with femtomolar sensitivity (Hu et al., 2010), and a label free system, incorporating a silicon nanoribbon detector, was devised to capture and quantify cancer biomarkers from whole blood in less than 20 minutes. (Stern et al., 2010). Recently, a microfluidic based volumetric bar-chart chip was fabricated using a nanoporous glass membrane as an ELISA substrate in order to immobilise and quantify three serum tumour biomarkers, squamous cell carcinoma antigen, cytokeratin 19 fragment and CEA in lung cancer patients in a rapid and highly sensitive platform (Li et al., 2016). Indeed, these studies are just some of the examples of the potential of nanotechnology to transform the diagnostic landscape, not only in respiratory oncology but human disease on a grander scale.

## 6. Nanotechnology in lung cancer treatment

Although there are in excess of 50 clinically approved nanomedicines or nanoformulations (Farjadian et al., 2018), there are currently only two nanoparticle-based therapies clinically available for the treatment of lung cancer. Abraxane is the first and only to receive FDA approval and is indicated as a first line therapy for locally advanced or metastatic NSCLC in combination with platinum therapy in patients who are not eligible for surgery or radiotherapy. Abraxane, or nanoparticle albumin bound (nab)-paclitaxel is a formulation of paclitaxel that is bound to albumin nanoparticles approximately 130 nm in size, that does not require the toxic and immunogenic polyethoxylated solvent Cremaphor EL and shows improved PK and reduced side effects compared with that of the parent drug (Green et al., 2006). The other therapy, Genexol-PM (Cynviloq) is a polymeric micelle nanoparticle formulation of paclitaxel that is approved in South Korea for treatment of NSCLC (Kim et al., 2007). As with Abraxane, this formulation does not require the adjuvant Cremaphor EL and displayed superior antitumour efficacy and BD in preclinical models (Kim et al., 2001). In a phase I PK study in 24 patients with solid tumours (7 of whom had lung cancer, 11 who had prior taxane-based chemotherapy) Genexol-PM was well tolerated to a maximum tolerated dose (MTD) of 180 mg/m$^2$ (Lim et al., 2010).

### 6.1. Therapies in clinical trials

In stark contrast to the number of clinically available nanomedicines, there are significantly more currently in clinical trials and pre-clinical development. Paclitaxel poliglumex (PPX) is a macromolecular compound that is comprised of paclitaxel conjugated with the biodegradable polymer poly-L-glutamic acid. The prolonged circulatory half-life bestowed by the polymer component allows PPX to take advantage of the EPR effect where it is then internalised by endocytosis and transported to the lysosomes. Due to the nature of the conjugation, PPX is biologically inactive and is only activated by the lysosomal proteases (Chipman, Oldham, Pezzoni, & Singer, 2006). PPX has reached phase III clinical trials which revealed that chemo-naïve patients receiving PPX has similar overall survival compared with either gemcitabine or vinorelbine (STELLAR 3) (Ross et al., 2006). Another phase III clinical trial compared PPX with docetaxel as a second line therapy in 849 patients who were previously treated with a platinum based chemotherapy regimen. PPX and docetaxel were found to produce similar survival results but exhibited different toxicity profiles with PPX being the more favourable (Paz-Ares et al., 2008). Lipoplatin is a PEGylated liposomal formulation of cisplatin (Boulikas, 2009). A phase I dose escalation study was performed using lipoplatin at 100 mg/m$^2$, increasing dosage incrementally by 10 mg/m$^2$ on day 1 and day 8 to determine the MTD. A total of 13 patients with recurrent or refractory NSCLC were evaluated and the MTD was found to be 120 mg/m$^2$ (Froudarakis et al., 2008). A phase II study looked to compare the efficacy of lipoplatin and gemcitabine versus conventional cisplatin and gemcitabine in 88 patients with inoperable (stage IIIB/IV) NSCLC. Lipoplatin (120 mg/m$^2$) was given on day 1, 8 and 15 whereas cisplatin (100 mg/m$^2$) was given on day 1. Gemcitabine (1000 mg/m$^2$) was given on day 1 and day 8 in both treatment arms and patients were given up to 6 cycles. Responses were the average across all histological subtypes, however in the adenocarcinoma subtype, only 3 out of 18 patients (17%) in the lipoplatin/gemcitabine arm experienced disease progression compared with 11 out of 24 patients (46%) that received cisplatin/gemcitabine. Notably, patients receiving lipoplatin had significantly less adverse effects (Mylonakis et al., 2010). Two phase III trials have been conducted involving lipoplatin. One trial focused primarily on the side effect profile of lipoplatin and paclitaxel (arm A) versus cisplatin and paclitaxel (arm B) in 236 chemotherapy naïve patients with unresectable NSCLC. Patients in arm A had significantly lower nephrotoxicity (6% in arm A versus 40% in arm B) and neutropenia (33% in arm A versus 45% in arm B) of grades I-IV (Stathopoulos et al., 2011). The other trial sought to compare the efficacy of the arms described above in 202 patients with inoperable, advanced (stage IIIB/IV) non-squamous NSCLC. There were significantly more partial responses (PRs) in arm A than arm B (Stathopoulos et al., 2011).

Doxil (Caelyx) is a PEGylated liposomal formulation of doxorubicin (DOX) which was the first FDA approved nanoformulation and is currently used to treat several malignancies (Barenholz, 2012). Doxil has been investigated both as a monotherapy and in combination with other agents for treatment of NSCLC and SCLC. In a phase I clinical trial, Doxil demonstrated efficacy as a single agent in locally advanced or metastatic NSCLC that had been pre-treated with platinum based chemotherapy (Numico et al., 2002). In another study, 20 chemotherapy naïve patients with advanced NSCLC were treated with a triplet chemotherapy regimen of docetaxel (50 mg/m$^2$), gemcitabine (1,000 mg/m$^2$) and Doxil (20 mg/m$^2$) (Patlakas, Bouros, Tsantekidou-Pozova, & Koukourakis, 2005). In a phase II study, Doxil (35 mg/m$^2$) was used in combination with cyclophosphamide (750 mg/m$^2$) and vincristine (1.2 mg/m$^2$) for up to six cycles at 21 day intervals as a second line treatment for SCLC. Confirmed PR was seen in 3 patients (10%) and seen but not confirmed by scanning in a further 2 patients (Leighl et al., 2006). Another phase II study investigated Doxil as a single agent therapy for treatment of mesothelioma. Doxil was administered to 15 patients initially at 55 mg/m$^2$ with dose modification based on dose limiting toxicities. Of the 14 evaluable patients, there was 1 PR (7%) and 3 stable disease (SD) (21%) one of whom had previously received and failed chemotherapy (Skubitz, 2002). Another nanoparticle formulation that has reached phase II clinical trials utilises a more targeted approach that can improve tumour specific drug accumulation over conventional EPR-mediated delivery. BIND-014 is a polymeric nanoparticle composed of encapsulated docetaxel and a S,S-2-[3-[5-amino-1- carboxypentyl]-ureido]-pentanedioic acid (ACUPA) moiety on the surface. ACUPA acts as a prostate-specific membrane antigen substrate analogue inhibitor, targeting BIND-014 towards the extracellular domain of prostate-specific membrane antigen, a transmembrane glycoprotein, which has been shown to be overexpressed not only on prostate cancer cells but also the endothelial cells of tumour-associated neovasculature (Chang et al., 1999). It has also been detected in NSCLC and SCLC tumour vasculature (Wang et al., 2015). Preclinical evaluation of BIND-014 in mouse xenograft models of NSCLC as well as breast and prostate cancer showed greater reductions in mean tumour weight when compared with solvent based docetaxel and non-targeted (lacking ACUPA) nanoparticles loaded with docetaxel. Additionally, BIND-014 displayed improved PK properties than that of solvent based docetaxel in tumour bearing mice, Sprague Dawley rats and cynomolgus monkeys (Hrkach et al., 2012). A phase I safety and dose-finding study of BIND-014 in patients with advanced or metastatic solid tumours was performed. Upon study conclusion the MTD was found to be 60 mg/m$^2$ and the PK of BIND-014 was dose proportional and were retained in the plasma of patients affording prolonged release of docetaxel (Von Hoff et al., 2016). A phase II study investigated the efficacy of BIND-014 as a second line therapy in patients with stage III/IV NSCLC previously treated with a platinum containing regimen and with known genomic status (*EGFR*, *ALK* and *KRAS*). BIND-014 was administered at a dose of 60 mg/m$^2$ and amongst the 8 patients with *Kirsten rat sarcoma viral* oncogene homolog (KRAS) mutations there were 2 PR (25%) and 3 SD (38%) (Natale et al., 2014). There are currently two phase II trials investigating BIND-014 as a second line therapy in patients who have failed one prior platinum containing regimen. One study looks to evaluate the safety and efficacy of BIND-014 in patients with stage III/IV NSCLC (NCT01792479) and the other study seeks to assess BIND-014 in KRAS positive or squamous cell NSCLC (NCT02283320). A further example of nanomedicines in phase II clinical trials is CRLX101. CRLX101 is composed of the topoisomerase I inhibitor camptothecin conjugated to a linear copolymer consisting of alternating units of β-cyclodextrin and PEG that subsequently self-assemble into nanoparticles ranging from 20–60 nm (Svenson, Wolfgang, Hwang, Ryan, & Eliasof, 2011). Promising data from mouse xenograft models of multiple cancers, including NSCLC and SCLC (Schluep et al., 2006), prompted a phase I/IIa clinical trial of CRLX101 as a treatment for patients with solid malignancies. The phase I portion of the study sought to determine the MTD of CRLX101 which was found to be 15 mg/m$^2$ given bi-weekly, and from this a phase IIa expansion cohort (6 patients in phase I, 38 in phase IIa, 22 with NSCLC, 44 total) was added. Of the 22 patients with NSCLC, 19 were evaluable and there were 16 SD (73%, 8 confirmed, 8 unconfirmed) (Weiss et al., 2013). The encouraging results in the NSCLC cohort prompted further investigation and as a result a phase II clinical trial of CRLX101 in advanced NSCLC was initiated and has now been completed (NCT01380769). A total of 157 patients with NSCLC were enrolled who had failed 1-2 previous chemotherapy regimens and were treated with either CRLX101 (15 mg/m$^2$ every two weeks) or best supportive care. Despite failing to meet its primary efficacy endpoint of overall survival benefit, CRLX101 demonstrated a favourable safety profile and is currently being investigated in another phase I/II clinical trial. This trial investigates CRLX101 and olaparib as a therapy for relapsed/refractory SCLC (NCT02769962). Etirinotecan pegol (NKTR-102) is a polymer conjugate of irinotecan, another topoisomerase I inhibitor. When administered, the progressive hydrolysis of the polymer linker results in slow and sustained release of irinotecan which is metabolised into the more potent active compound 7-ethyl-10-hydroxy-camptothecin. Etirinotecan pegol demonstrated superior efficacy in mouse xenograft models of several cancers, including lung cancer, compared with that of irinotecan and displayed favourable PK properties such as longer plasma half-life, in Sprague-Dawley rats and Beagle dogs as well as tumour bearing mice (Hoch, Staschen, Johnson, & Eldon, 2014). A phase I study sought to establish the MTD and safety profile of etirinotecan pegol in patients with refractory solid tumours. A total of 76 patients were recruited, 18 of which had lung cancer (3 SCLC, 15 NSCLC). The MTD was found to vary based on the frequency of administration, with higher doses tolerated if given less frequently (up to 145 mg/m$^2$). One third of patients had some form of antitumour response, including 8 confirmed PR (11%, 2 of which were patients with lung cancer) (Jameson et al., 2013). Phase II clinical trials are currently investigating etirinotecan pegol as a treatment for patients with metastatic or recurrent NSCLC who have failed second line therapy (Aggarwal et al., 2018), patients with refractory brain metastases and advanced NSCLC, SCLC or breast cancer (NCT02312622) and patients with relapsed SCLC (NCT01876446).

A polymeric micelle formulation of cisplatin has also seen clinical progression. NC-6004 consists of a PEG outer shell and the coordination complex of the sodium salt of poly(glutamic acid) and cisplatin which comprises the inner core of the micelle, creating a block co-polymer-metal complex. These micelles measure approximately 30 nm in diameter and are highly stable when dispersed in solution (Matsumura, 2008). Encouraging data from preclinical studies (Nishiyama et al., 2003; Uchino et al., 2005) led to an open label, dose escalating phase I trial of NC-6004 in patients with advanced solid tumours. The MTD was found to be 120 mg/m$^2$ and the recommended dose was 90 mg/m$^2$. NC-6004 was reasonably well tolerated, with no significant cases of neurotoxicity or ototoxicity observed at any dose level. PK analysis displayed evidence of longer blood circulatory half-life of NC-6004 compared to cisplatin as well as delayed and sustained release of Pt containing species (Plummer et al., 2011). A phase Ib/II trial is currently recruiting patients with stage IV NSCLC as well as other advanced solid tumours to examine the efficacy of NC-6004 in combination with gemcitabine with MTD of NC-6004 as the primary outcome measure (NCT02240238).

Even though the majority of therapeutics in the clinic or in clinical trials are chemotherapy drugs or other small molecules, nucleic acids are equally potent tools that can be used to ameliorate disease. Nano-delivery systems of such molecules have proven efficacious in preclinical models of not only lung cancer, but other malignancies. For example, cationic liposomes have been developed to deliver the tumour suppressor genes *p53* and *FHIT* to human H1299 (*p53*$^{null}$/*FHIT*-) and human A549 (*p53*$^+$/*FHIT*-) mouse xenograft primary tumour and disseminated metastatic tumour models of lung cancer. Liposomes composed of 1,2-dioleoyl-3-trimethylammoniumpropane: Cholesterol (DOTAP: Chol)-*LacZ* (where the plasmid containing the *LacZ* gene encoding β-galactosidase was encapsulated) were found to enable delivery and

successful transfection of plasmid in 25% of primary tumour cells (H1299 cells) and 10% of A549 lung metastatic tumour cells 48 h after a single intratumoural or tail vein injection respectively. Building on this, delivery of DOTAP:Chol-*p53* or DOTAP:Chol-*FHIT* complexes to subcutaneous human H1299 or A549 derived primary murine tumours significantly inhibited tumour growth compared to control groups (Ramesh et al., 2001). This concept has also been used for the complexation of plasmids containing the tumour suppressor gene *FUS1*, which has been found to be part of a 120kb sequence that is missing in 80% of primary lung cancers. DOTAP:Chol-*FUS1* liposomes (*FUS*-1 NPs) were found to significantly inhibit tumour growth in subcutaneous xenograft lung-cell derived (H1299 and A549) mouse models and reduce the number of metastatic tumour nodules (Ito et al., 2004). A phase I clinical trial found the MTD of *FUS*-1 NPs was 0.06 mg/kg in patients with recurrent or metastatic NSCLC/SCLC previously treated with platinum chemotherapy. Biopsies of tumours taken 24 hours post-treatment revealed the presence *FUS-1* mRNA levels in 7/8 patients and protein expression in all 3 patients tested (Lu et al., 2012). A phase I/II clinical trial is currently underway investigating *FUS-1* NPs in combination with erlotinib in Stage IV NSCLC (NCT01455389).

Bacterial minicells are anucleate nanoparticles that arise as a result of deletion of genes that control normal bacterial cell division. Minicells, 400 nm in diameter, could be loaded with hydrophobic (cisplatin), hydrophilic (irinotecan) or amphiphatic (vinblastine) cargo and were functionalised with bispecific antibodies recognising the O-antigen component of lipopolysaccharide and a cell surface receptor of the mammalian cell to be targeted (e.g. EGFR). The minicells demonstrated good *in vitro* and *in vivo* efficacy; in an A549 xenograft model, EGFR targeted minicells loaded with paclitaxel were highly effective at inhibiting tumour growth, significantly more so than the 11 other treatments, and efficacy was demonstrated in other tumour models as well with no evidence of toxicity or adverse side effects (MacDiarmid et al., 2007). Minicells have also been used to successfully encapsulate and deliver siRNA, restoring drug sensitivity to previously resistant tumours *in vivo* (MacDiarmid et al., 2009). Building on this, a study showed miR-16 expression to be 22-fold lower in pleural tumours compared to normal pleura. This system was then tested *in vivo*; using EGFR targeted minicells, a miR-16 mimic was administered to MSTO-211H tumour bearing mice via intravenous injection once, twice or four times a week for 3 weeks. Treatment with minicells induced a dose dependant inhibition of tumour growth compared to controls, and increasing the dose caused stagnation of tumour growth (Reid et al., 2013). A recently completed phase I trial investigated EGFR targeted, EnGeneIC Delivery Vehicle packaged miR-16 mimic (TargomiRs) (Reid et al., 2016) as a second or third line treatment for patients with recurrent MPM. Acceptable safety profiles and initial efficacy (one patient had a PR documented in detail (Kao et al., 2015)) in a notoriously difficult disease to treat set foundations for further trials in combination with immunotherapy (van Zandwijk et al., 2017).

Whilst it is encouraging to see NP therapies in clinical trials, progression from this stage is not guaranteed. MRX34 is a liposomal miRNA therapy that reached clinical trials, however was terminated during phase I assessment due to a series of adverse events in different patients. There are further examples of nanomedicines prematurely terminated at the clinical trial stage after promising preclinical results. CALAA-001 is a siRNA-NP formulation that did not progress through clinical trials due to unacceptable inflammatory cytokine release (NCT00689065). DCR-MYC (NCT02314052) and BiKDD (NCT00968604) are NP formulations that succumbed to a similar fate due to safety and stability issues respectively. Some clinical trials investigating the approved nanotherapy Abraxane in combination with other therapies also fall short of their primary endpoints. These examples serve as a testament to the importance of rigorous safety testing of nanomedicines and as a reminder that more physiologically relevant preclinical models may aid to circumvent future clinical trial failures.

### 6.2. Preclinical therapies

Solid lipid nanoparticles (SLNs) have been a feature of drug delivery since the early 1990s, demonstrating their utility and applicability in different diseases. In the context of lung cancer, docetaxel loaded SLNs were grafted with tetraiodothyroacetic acid (tetrac) and hyaluronic acid (HA) with the intention of targeting the $\alpha_v\beta_3$ integrin and CD44 respectively. In a B16F10 mouse model of lung metastasis dual functionalised SLNs (HA/Tet NPs) were found to accumulate in the lung at higher levels compared with other formulations tested. Mice treated with HA/Tet NPs also had fewer metastatic nodules, a lower lung weight and more TUNEL positive cells (Shi et al., 2016). Peng *et al.* synthesised polymersomes (a type of vesicle) via self-assembly of two amphiphilic graft copolymers, poly(mPEG ethyl-*p*-aminobenzoate phosphazene) and poly(mPEG *N*, *N*-diisopropylethylamine phosphazene) in order to encapsulate miR-200c (NanoED-200c), the absence of which has been observed in several tumours and is known to play a key role in cell proliferation, differentiation and apoptosis. NanoED-200c were found to be the most cytotoxic to A549 cells and produced the largest fold increased in miR-200c expression. *In vivo*, A549 xenografted mice treated with NanoED-200c were observed to have the greatest inhibition of tumour growth with the highest levels of miR-200c observed in these tumours compared with other treatments, including those treated with commercially available lipofectamine (Peng, Zhu, & Qiu, 2016). Another study combined elements from both the above in an attempt to overcome cisplatin resistance. Chitosan nanoparticles were synthesised using a 1:1 ratio of PEGylated chitosan and chitosan functionalised with a peptide to target EGFR (targeted chitosan NPs - TCNPs). TCNPs were used as the delivery vehicle for siRNA against *Mad2*; knockdown of *Mad2* which leads to premature exit from mitosis and extensive cell death as the protein is an essential component of the mitotic checkpoint complex. The chitosan formulations were tested *in vivo* using A549 wild type and cisplatin resistant xenograft mouse models. The combination of TCNP-*Mad2* siRNA and cisplatin elicited the most effective growth inhibition in both tumour models, more so in resistant than wild type, followed closely by non-targeted TCNP-*Mad2* siRNA and cisplatin. Knockdown of Mad2 was confirmed at both the mRNA and protein level upon treatment with chitosan NPs (Nascimento et al., 2017). NPs created from modified polyesters were manufactured in order to selectively deliver siRNA targeting ubiquitin B (siUBB) to tumour cells based on the inherent composition of the NP. A library of 840 functional polyesters was created that varied in molecular weight and the ratio of alkyl thiol to amino thiol modifications. NPs formed from the polyester PE8K-A17-0.2C6 (nomenclature is starting molecular weight of the polyester, the amino thiol then alkyl thiol feed ratio) were found to preferentially deliver siUBB to tumour cells, 14 times more so than to normal cells. In an HCC4017 xenograft model, targeted NPs containing Cy5.5 labelled siRNA were retained in tumours for over 7 days whereas non-targeted NPs containing Cy5.5-siRNA and free Cy5.5-siRNA were rapidly cleared from the circulation. Targeted NPs with no siRNA or with encapsulated DOX were also observed to reside within tumours for over 5 days indicating the intrinsic physical chemistry of the NPs were responsible for their retention. In a long-term growth inhibition study targeted NPs incorporating siUBB induced the most tumour shrinkage compared to other treatments controlling for siRNA and NP. Moreover, orthotopic HCC1299-Luciferase tumours established in order to test if delivery via the inhalation route was a viable option. Upon treatment with targeted NPs containing siRNA against luciferase (siLuc), observations confirmed that luciferase expression was significantly decreased 48 hours post-delivery, thus extended the translational impact of this study (Yan et al., 2016). A follow up study synthesised another library of 540 functional polyesters modified in the same fashion and developed NPs from these polyesters. NPs made from PE4K-A13-0.33C6/C10 were found to elicit the highest luciferase knockdown and were therefore taken forward for further studies. *In vivo* studies in an A549-Luc xenograft

mouse model found Cy5.5-siRNA loaded NPs injected via the tail vein primarily accumulated in the liver and tumour with minimal signal in other organs. A single intravenous injection of siLuc NPs at a dose of 1 mg/kg could reduce the luciferase signal in tumours by 50%, however for orthotopic tumours that are more clinically relevant, intravenous delivery may not be able to recapitulate these results. Therefore, an orthotopic A549-Luc tumour model was established and exposed to aerosolised Cy5.5siRNA NPs or free Cy5.5siRNA via inhalation, with *ex vivo* analysis revealing specific accumulation in the lung and not in any other organs. Inhalation of siLuc-NPs caused significant luciferase knockdown (approximately 65%) compared to free siLuc and untreated mice at 48 hours post NP exposure (Yan et al., 2017). A layer-by-layer platform was used to construct NPs with a cisplatin loaded core decorated with alternating layers of RNA films (siRNA against KRAS and miR-34a) and poly L-arginine with a final coating of HA. This triple-therapy formulation demonstrated efficacy in studies performed with lung cells derived from a human autochthonous "KP" mouse model (oncogenic KRAS mutation G12D and deletion of p53 (DuPage, Dooley, & Jacks, 2009). These cells were then used to create orthotopic tumours in the lungs of mice for subsequent *in vivo* studies which revealed NPs successfully accumulated in the lungs of tumour bearing mice due to overexpression of CD44, the receptor for HA. Administration of the combo-NPs resulted in prolonged survival of tumour-bearing mice and IHC staining provided evidence for a mechanism; knockdown of the KRAS oncogene (by siRNA) and restoration of p53 regulated pathways (by miR-34a) sensitised tumours to the chemotherapeutic effect of cisplatin, resulting in a reduction of tumour burden in a physiologically relevant model (Gu, Deng, Roy, & Hammond, 2017).

The concept of biomimicry, although not new, is gaining increasing traction in the drug delivery field. For example, Cao *et al*. designed a macrophage membrane coated emtansine liposome (MEL) to target lung metastases of breast cancer. The liposome was composed of DSPE-PEG and 1,2-dioleoyl-sn-glcero-3-phosphoethanolamine with the tubulin inhibitor emtansine encapsulated within. The liposome is also decorated with the membrane of RAW264.7 macrophages with intact $\alpha_4\beta_1$ integrin in order to target vascular cell adhesion molecule-1. *In vitro* activity studies demonstrated MELs were taken up avidly by RAW 264.7 macrophages and were better induces of apoptosis in cancer cells compared with emtansine alone. In a 4T1 Luc lung metastasis model, multimodal *in vivo* imaging demonstrated that fluorescently labelled MELs co-localised with lung metastases. Furthermore, mice treated with MELs had far fewer metastatic nodules than in other treatment groups (87.1% inhibition of metastasis when compared with untreated control), observed by whole lung imaging and H&E staining, confirming the anti-metastatic potential of MELs (Cao et al., 2016). Another study utilises a type of cell membrane derived vesicular delivery system originating from mesenchymal stem cells known as nanoghosts (NGs). These NGs lack the cytoplasmic machinery of mesenchymal stem cells but possess their targeting capabilities, immune evasiveness and tropism towards inflammation. NGs have been found to accumulate both in the cytoplasm and nucleus of tumour cells and as such have been investigated as non-viral gene delivery vehicles. NGs were produced, PEGylated and loaded with plasmid DNA encoding the C terminal of human MMP-2, also known as hemopexin-like domain via electroporation. *In vivo* safety studies revealed no changes in body weight 7 days post-administration and there were no significant changes in blood counts or blood chemistry in mice given empty NGs twice, 7 days apart, as well as no aberrant immune response when compared to PBS treated mice. An orthotopic metastatic A549 model was established and it was found that fluorescently labelled NGs accumulate in the lungs of tumour bearing animals. Furthermore, systemic administration of three weekly doses of NGs, resulted in a 50% inhibition of tumour growth compared to untreated mice with significantly less and smaller neoplastic nodules reinforcing NGs as a viable and promising biomimetic candidate for non-viral gene delivery to pulmonary tumours (Kaneti et al., 2016).

## 7. Theranostic strategies in lung cancer

The versatility of nanotechnology has meant that it is possible to more effectively combine therapeutic and diagnostic components into one single agent. This concept, known as theranostics, is not new, however, the advent of nanotechnology has allowed an extension from traditional theranostics to dedicated co-delivery of both diagnostic and therapeutic platforms, allowing imaging to take place not only before and after treatment but also during. This is facilitated by the common prerequisite that sufficient accumulation of the agents at the disease site is required for efficacy (Xie et al., 2010). Despite being at an early stage of translational development there is an increasing number of promising preclinical studies emerging. Hou *et al*. developed NPs using *cis*-aconitic anhydride modified DOX bonded with the FDA approved vitamin E derivative D-α-tocopherol polyethylene glycol 1000 succinate. This amphiphilic construct self assembles in water to form NPs with DOX in the core and the vitamin E derivative provides the hydrophilic shell. Chlorin e6 (Ce6), an FDA approved photosensitising molecule, was also encapsulated in the NP in order to allow photodynamic therapy via production of ROS upon photoexcitation as well as NIR fluorescence imaging (TCAD-Ce6 NPs). This pH sensitive construct possesses an amide linker that under low pH, such as those present in tumours or lysosomes, will degrade therefore releasing DOX and relieving the self-quenching constraints of Ce6. In an A549 xenograft model, BD fluorescence imaging revealed that TCAD-Ce6 NPs accumulated in the tumour to a greater extent than free Ce6 with peak fluorescence observed at 12 hours. At 24 hours, TCAD-Ce6 NPs were primarily detected in the tumour, liver and lungs, more so than free Ce6. Treatment of tumours with TCAD-Ce6 NPs and laser irradiation essentially abrogated their growth, whereas other treatments comparatively ineffective (Hou et al., 2016). Another study investigated the use of upconversion NPs (UCNPs) in a theranostic context. UCNPs, typically lanthanide doped nanocrystals, were loaded with mPEG-COOH, Ce6 and ROS cleavable thioketal conjugated camptothecin (Ce6-CPT-UCNPs). UCNPs can convert NIR 980 nm light to light of wavelengths 645-675 nm which can then activate Ce6 via fluorescence resonance energy transfer. Active Ce6 produces ROS that not only forms the basis of photodynamic therapy but can also cleave the thioketal linker in order to release camptothecin. Moreover, Ce6 has a fluorescence emission between 650-750 nm which can be utilised for imaging. The Ce6-CPT-UCNPs were extensively characterised prior to *in vivo* studies in a H460 orthotopic mouse model that showed that Ce6-CPT-UCNPs accumulated in the liver and lung as well as the tumour, determined by fluorescence, whereas free Ce6 showed low levels of accumulation. Ce6-CPT-UCNPs could be detected in the tumour for at least 24 hours but free Ce6 was absent at this time point. Laser irradiated Ce6-CPT-UCNPs completely arrested tumour growth whereas other treatments were not significantly different to the PBS/laser group (most tumour growth) (Yue et al., 2016). Detappe *et al*. developed silica-based bismuth gadolinium nanoparticles (SiBiGdNPs) to act as a radiosensitising agent and provide enhanced contrast for MR and CT imaging. A polysiloxane core- (2,2′,2″-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid) (DOTA derivative)-Gd complex was initially formed followed by further addition of chelating DOTA groups to the surface and finally complexation of bismuth ions. After characterising the physical and functional properties of SiBiGdNPs the *in vitro* radiosensitising ability was assessed; it was observed that SiBiGdNPs induced more DNA double strand breaks upon irradiation than just the beam alone due to production of secondary photoelectrons and Auger electrons. A clinical workflow was then devised and carried out *in vivo* in an A549 xenograft mouse model. Results demonstrated that SiBiGdNPs could be used to image the tumour both with MRI and CT modalities. Accumulation was observed primarily in the kidneys, liver spleen and also in the tumour with rapid clearance within 24 h. Dosimetry studies were also performed to calculate the radiation dose distribution after organs were segmented by MRI and

registered by CT which revealed that the tumour received substantially more of the radiation dose than the rest of the body. Long term (80 day) therapeutic efficacy studies were performed with mice in the SiBiGdNP group given treatment intravenously 30 min prior to irradiation (10 Gy) as this was when NP uptake in the tumour was at a maximum. Tumours of mice given SiBiGdNPs and irradiation showed no increase in tumour burden 80 days later whereas untreated mice and mice given just SiBiGdNPs experienced rapid tumour growth. *Ex vivo* analysis of radiation induced DNA damage showed the tumours of mice given the combination of SiBiGdNPs and radiation had the highest levels of γ-H2AX positive cells. Levels of γ-H2AX positive cells were not significantly different in healthy tissue such as the kidney, lung, liver and spleen demonstrating the utility of a dual modality NP formulation that can act as both an image contrast and radiotherapy enhancer (Detappe et al., 2017).

## 8. Conclusions and outlook

Even with the considerable body of research and variety of studies, the full potential of nanomedicine has yet to be realised. This is echoed by the paucity of clinically approved therapies despite the bustling activity of the field. The oscillations in confidence of this maturing field are clear, however nanomedicine is currently at an inflection point as more and more researchers discard old ideas in favour of new, emerging concepts. This paradigm shift acts in concert with the ever increasing knowledge gained from neighbouring fields such as molecular biology and immunology. However, deeper understanding of the heterogeneity of tumours, the utility of the EPR effect, transport of NPs to tumours, nano-bio interactions, the immunological crosstalk between host and tumour, the tumour microenvironment and prevention of metastasis will greatly increase the efficacy of nanomedicines. Furthermore, the advent of more sophisticated drug delivery systems, triggered by extrinsic mechanisms or intrinsic biological stimuli, will enable greater spatiotemporal release of therapy as well as adroit targeting to the site of disease (Wang & Kohane, 2017). Machine learning-assisted predictions of effective nanomedicines and their consequences are beginning to emerge (Shamay et al., 2018) and will inevitably play a sizeable role in the future of drug delivery. Moreover, there is a drive for more physiologically relevant preclinical models as well as large-scale, reproducible synthesis of NPs with rigorous characterisation that adheres to good manufacturing practices. The role of the microbiome (Alexander et al., 2017) and metabolomic inferences (Wishart, Mandal, Stanislaus, & Ramirez-Gaona, 2016) may also provide important insights into the design of nanomedicines. This becomes even more important when considering the next and future generation of molecular entities (small molecules, RNAs, CRISPR components, immunotherapy). The challenges facing nanomedicine both in a diagnostic and therapeutic context have been extensively outlined (Anchordoquy et al., 2017; Hare et al., 2017; Sengupta, 2017; J. Shi, Kantoff, Wooster, & Farokhzad, 2017; Venditto & Szoka, 2013); encouragingly, however, the prevailing view is that nanotechnology has the potential to revolutionise not only the oncological landscape but medicine in a wider context. A recent review outlined, amongst other concepts, a "collaborate not conquer" approach whereby efforts should be focused on leveraging the intrinsic properties of nanomedicines to work with biology (e.g. prevention of a metastatic niche by virtue of preferential sequestration of NPs in the liver) as opposed to overcoming these barriers (Björnmalm, Thurecht, Michael, Scott, & Caruso, 2017). As such, nanomedicine is now poised to surmount the challenges presented and exert a larger influence on the diagnosis of disease and therapies available in the clinic. Over the coming decades, advances in knowledge and nanotechnological innovations will elevate nanomedicine, specifically NP based drug delivery, from a field of potential to a powerhouse in the transformative treatment of lung cancer and other diseases.

## Conflicts of interest

The authors declare no conflict of interest.

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