Chapter 2

Literature Review

2 Literature Review

2.1 Lung Cancer biology and its classification

Lung cancer develops due to neoplastic metamorphosis in the epithelial cells of the lung arising as a result of genetic, epigenetic, and molecular alterations. Development of malignancy is a multi-step process and results in invasiveness and colonal expansion of cancer [38]. Accumulation of genetic and epigenetic factors influence the invasiveness and response to cancer therapy including development of resistance [39]. Identification and development of biomarkers can help in early detection of cancer. As already mentioned, the two most prevalent type of lung malignancies are: non-small cell lung cancer and small cell lung cancer which are further classified as shown in **Figure 2.1**.

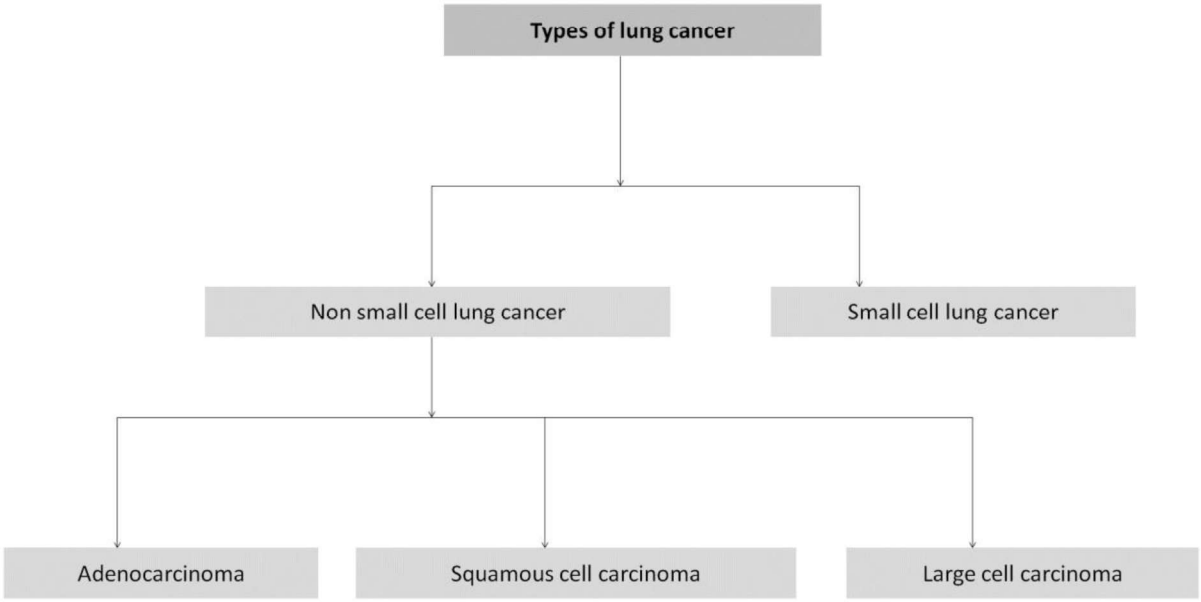


Figure 2.1 Classification of lung cancer [40].

The survival and proliferation of cancer cells is promoted by genomic alterations, which consist of rearrangement (transversions and transitions), manipulation of somatic genetic copy number, and point mutations (missense, nonsense mutations, slicing site, and frameshift alterations). These alterations activate tumour precursor cells, and lineage survival pathways, which are required for development of cancer cells. In molecular alterations, (1) activation of mutation of proto-oncogenes like BRAF, KRAS, PI3K, MEK, EGFR, and HER2; (2) amplification of proto-oncogenes like DDR2 and FGFR1 in squamous cell lung carcinomas, MET in adenocarcinomas; (3) overexpression of

oncogenic gene by microRNAs (miRNAs); (4) inactivation of tumour suppressor genes and (5) enhancement of telomerase activity occurs [40,41].

Non-small cell lung cancer is a clinical condition which includes a variety of malignancies like squamous-cell carcinoma (SCC), adenocarcinoma (ADC), large-cell carcinoma (LCC) and other less differentiated types that possess unique and characteristic cellular, genetic and epigenetic heterogeneity which make the treatment of NSCLC complex [42]. NSCLC mostly occurs due to overexpression of proto-oncogene epidermal growth factor receptor (EGFR) [43]. The prime factor which triggers NSCLC is cigarette smoking, followed by excessive consumption of processed food like deep-fried, cured and barbecued meat, alcohol, air pollution, sluggish lifestyle, and genetic factors [44].

Adenocarcinomas mainly occur on the outer edge of the lungs and are the most common subtype of lung cancer with a 40% frequency of occurrence and, higher rate of incidence in women [45]. The International Association for the Study of lung cancer (IASLC), European Respiratory Society (ERS), and American Thoracic Society (ATS) have classified adenocarcinomas into non-mucinous and mucinous type. Non-mucinous type has been further classified based on growth pattern of cells, i.e., acinar, lepidic, papillary, micropapillary, or solid. According to the Cancer Genome Atlas (TCGA), the molecular alternation in 18 different types of genes is responsible for lung adenocarcinoma. These molecular alterations, in combination with adenocarcinoma classification guidelines, can be used for formulation of efficacious treatment therapy[46].

Squamous-cell carcinoma is a subtype of NSCLC, which accounts for 20-30% of all NSCLC cases and is mostly prevalent in men. SCC occurs as a result of changes in the morphology of chronically inflamed bronchial epithelium that leads to squamous metaplasia (SM), basal cell hyperplasia (BCH), dysplasia I–III. SCC is mainly caused by smoking; besides, other factors like exposure to heavy metals, polycyclic aromatic hydrocarbons, asbestos, radioactive substances, infectious agents, inflammation (CD3, CD4, CD8 T lymphocytes, dendritic cells, T helper cells, T regulatory cells, macrophages, and neutrophils) and gene polymorphism may also lead to the progression of SCC

[47,48]. Genetic, epigenetic, oncogenic abnormalities, such as the amplification of chromosome and significant change in transcriptional regulators p63 and SOX2, also cause SCC[49].

Large cell carcinoma, accounts for 3–9% of all NSCLC cases and is of least occurring type of NSCLC. World Health Organization (WHO) in 2004 defined LCC as an "undifferentiated non-small cell carcinoma that lacks the cytological and architectural feature of small cell carcinoma and glandular or squamous differentiation''[50]. The definition was modified in 2015 by WHO based on lineage-specific immunomarkers, and it was defined as solid predominant adenocarcinoma or non-keratinizing squamous cell carcinoma, showing positive staining for markers such as TTF-1 or p40[51]. Due to presence of undifferentiated morphology of the cells, inconclusive results are obtained in upto 70% of the cases, requiring a need for targeted diagnostic and therapeutic approach.

Small cell lung cancer occurs primarily because of a geriatric or a smoker's disease and accounts for 14% of total lung cancer cases. Exposure to smoking proportionally determines the propensity of developing SCLC [52]. WHO classifies SCLC as a neuroendocrine malignancy associated with neurologic syndromes (sensory neuropathy, encephalomyelitis, and Lambert-Eaton syndrome) and endocrine paraneoplastic syndrome (Cushing syndrome and syndrome of abnormal secretion of antidiuretic hormone). The molecular aberrations in SCLC are due to variation in retinoblastoma 1 (RB1) and tumor protein p53 (TP53) genes [53,54].

2.2 Therapeutic options for treatment and diagnosis of lung cancer

The diagnostic techniques used for lung cancer have evolved from histo-pathological examination to profiling of molecular aberrations for identification of lung cancer variants. Chest radiography, computerized tomography, bronchoscopy, endoscopic ultrasound, and endobronchial ultrasound are the techniques which have been used for diagnosis of lung cancer [39]. Screening and identification of specifically expressed genetic biomarkers like those for EGFR, ALK, etc. is being used for the prognosis and diagnosis of lung cancer type and also for formulation of personalized therapeutic intervention [40]. Immuno-histochemical analysis such as fluorescence (or chromogenic) in situ hybridisation, Sanger sequencing, restriction length fragment polymorphism, PCR based methods or

mass spectrometry based genotyping are used for the identification of these molecular markers [41]. Newer techniques like ambient mass spectrometry are also being used for real-time profiling of molecular information with minimal sample treatment [42]. Once the level of malignancy is determined, using the screening techniques described in **Table 2.1** then treatment modalities are determined and adopted for the treatment of tumour.

Table 2.1 Description of different diagnosis methods for lung cancer.

Techniques	Description	References
Chest radiography	The diagnosis of bronchogenic carcinoma is often suggested by a chest radiograph, If anomalies are discovered on chest radiography, a computed tomography scan is performed.	[55]
Computerized tomography (CT)	CT scans are more likely than standard chest x-rays to reveal lung tumours. It may also demonstrate the shape, size and location of any lung tumours. Because of its high resolution and clear contrast, it is the most common and appropriate modality for examining lung tissues.	[56]
Positron emission tomography (PET)	Non-invasive technique that detects NSCLC stages I, II, and III and provides high sensitivity and biochemical data.	[57]
Bronchoscopy	Bronchoscopy is a comparatively non-invasive technique used to diagnose lung cancer in patients.	[58]
Fluorescence bronchoscopy	Invasive procedure used to spot pre-invasive lesions and newly developing tumours in patients that have already undergone NSCLC surgery.	[59]
Endoscopic ultrasound	An invasive technique used to diagnose early forms of lung cancer.	[60]
Magnetic resonance imaging (MRI)	Detecting SCLC, less sensitive for lesions <3mm. It can be combined with superparamagnetic iron oxide nanoparticles (SPIONs) to provide high spatial resolution, as well as anatomical and functional details.	[61,62]
Sputum cytology	Detects lung cancer in its early stages. This method is easy to use, non-invasive, and patient-friendly.	[63]
Biopsy	Involves excision of a small portion of the infected tissue for microscopic examination to assess the type of lung cancer.	[64]
Biosensors and biomarkers	Advanced methodology, high sensitivity, fast reaction time, and reliability for cancer markers. Used to diagnose lung cancer at an early stage.	[65]

Chemotherapy, surgery, biological and radiation therapy are the four main techniques being used in the treatment of lung cancer. Chemotherapy helps in killing of cancerous cells, and prevents further proliferation, invasion, and metastasis of cancerous cells [66]. The chemotherapy based on platinum-based compounds is the first-line choice for treatment of metastatic NSCLC in patients who lack targetable gene mutations [67]. Platinum-based drugs (e.g., cisplatin, carboplatin, oxaliplatin) can be used as monotherapy or in combination with paclitaxel, gemcitabine, etoposide, or vinblastine. There are dose-limiting side effects like nephrotoxicity, intestinal injury, cardiotoxicity, peripheral neuropathy, anemia, uneasiness, fatigue, and nausea associated with the use of chemotherapeutic agents [68]. Paclitaxel being poorly water-soluble is administered intravenously. The water-soluble drug doxorubicin used for treatment of lung metastasis induces myelosuppression, anemia, neutropenia, and thrombocytopenia. Overall, poor physico-chemical properties of chemotherapeutic agents is responsible for non-specific distribution in the body, low efficacy and toxicity to healthy cells, and multidrug resistance [69].

Surgical resection is considered as an effective remedial option for early-stage NSCLC [70]. Atypical pulmonary resection is the commonly used surgical technique and the tumours are removed by thoracotomy, lobectomy and segmentectomy or pneumonectomy [71,72]. The metastatic spread of the cancerous cells during advanced stages poses limitations to surgical resectability in addition to chances of organ loss, and the possible reoccurrence of cancer after surgery [73]. Co-administration of chemo/biological therapy or chemo-radiotherapy can help improve the outcome of surgical resection.

Biological therapy is being developed to target many of the pathways involved in tumour development and differentiation. These therapies target specific tumour cell receptors or signalling events that are critical to tumour progression and reduce toxicity to normal cells. Furthermore, this therapy involves administration of small molecules that target the mutated genes responsible for occurrence of lung cancer and have been found to improve the clinical responsiveness of the patients to therapy. Genes like VEGFR, EGFR, ROS1, ALK, BRAF, MET, RET, NTRK1 and HER2 are being targeted by genotype-directed therapies [43]. Alongside, immune-based therapies like antibody- targeted therapies, vaccines, checkpoint inhibitors / immune control inhibitors designed to

boost the immune system are proving to be a useful alternative [74]. Inhibition of anti-tumor T-cell immunity occurs due to up-regulation of surface proteins like programmed death (PD)-1 and cytotoxic T-lymphocyte-associated 4 (CTLA4) etc. [44]. Administration of agonistic monoclonal antibodies can inhibit PD-1/PD-ligand 1 interaction and activate T-cells. Substantial clinical evidence has been obtained for nivolumab and pembrolizumab (anti PD-1 monoclonal antibodies) and atezolizumab, durvalumab and avelumab (anti-PD-L1 monoclonal antibodies). Food and Drug Administration (FDA) has approved the use of nivolumab and pembrolizumab for treatment of metastatic NSCLC either alone or with platinum chemotherapy. Various growth factors (GF) and growth factor receptors (GFR) which are generally over-expressed in lung cancer are relevant targets for therapeutic intervention. EGFR is a tyrosine kinase glycoprotein which is abnormally expressed by amplification of genes or overexpression of a normal or mutated receptor gene product [75]. NSCLC patients with EGFR tyrosine kinase mutations, when treated with EGFR tyrosine kinase inhibitor have a response rate of more than 70% and greater progression-free survival as compared to conventional chemotherapy [76]. Continuous assessment of biologic agents targeting angiogenesis, sonic hedgehog pathways, Bcl-2 proteins, DNA repair pathways, and immune checkpoint modulators are expected to improve SCLC outcomes. The clinical outcomes obtained for NSCLC with targeted therapy approach have provided a positive lead for the use of same in SCLC facilitated by improved biological understanding of SCLC [77]. Despite the promising clinical outcomes of biological therapeutic agents, the idea of "pathway-specific" targeted therapies still has substantial limitations. The drawback associated with biological therapy is that these agents are successful only in tumour types that rely on the inhibited pathways. It is easily evident that most solid tumours are the result of multiple genetic mutations, so substantial therapeutic activity may not result from inhibiting a single cellular pathway. The design of agents that target a variety of pathways can increase the therapeutic impact, but will also increase the risk of toxicity associated with treatment [78].

Radiotherapy is generally opted by patients who are reluctant to undergo surgical resection. Stereotactic ablative radiotherapy (SABR) is generally used during stage I NSCLC treatment. The advanced treatment modalities, including image-guided radiotherapy (IGRT), four-dimensional

computed tomography (4DCT), positron emission tomography (PET), intensity-modulated radiotherapy and volumetric modulated arc therapy facilitate targeted delivery of lethal doses of radiation to the malignant cells and reduce damage to surrounding healthy tissues[79,80]. Exposure to radiation causes pneumonitis and toxicity and damages bronchial tree and surrounding cells with loss of lung functions [81,82]. Biological heterogeneity exists between the primary tumour and the metastatic tissue, which leads to diverse response to therapy, so, strategic therapeutic combination of biological, chemotherapy, radiation, and surgery can help plan effective therapy and improve treatment outcomes.

2.3 Nanotherapeutic interventions in the treatment of lung cancer

Nanotechnology can potentially change the course of lung cancer treatment by overcoming the limitations of conventional therapy, diagnostic and treatment techniques [83]. The nanoparticulate carriers can be used to provide a range of therapeutic and diagnostic i.e. theranostic benefits like imaging, targeted delivery of encapsulated anticancer drugs, sustained drug release, therapeutic dose regulation, and subsidized dose-related side effects, etc. These carriers can be customized and tailor-made to suit the requirement of therapy in comparison to other drug carriers [84]. Besides size, surface properties of these carriers can be engineered with targeting moieties for receptors which are over-expressed on the tumorous cells**,** for example, monoclonal antibodies, peptides, antibody growth fragments, and growth factors, etc. [69]. The lungs provide a vast surface area for drug absorption, and nanocarriers of size >200 nm are well trapped in lung capillaries [85]. Nanocarriers can deliver anticancer drugs to the tumorous cells via passive tissue drug transport as well as active cellular transport mechanisms. Increased permeability of tumour vasculature due to angiogenesis, results in enhanced permeability and retention effect (EPR effect), which helps in passive delivery of drugs**.** Nanocarriers have two main pathways for selectively destroying lung cancer cells: passive and active targeting as shown in **Figure 1.2**. The diagram depicts the different mechanisms through which nanocarriers can obtain access to a lung cancer patient's affected areas.

Nanocarriers focus on passive lung tumour targeting through a process known as the "enhanced permeability and retention effect" (EPR effect). It involves the targeted transfer of medications or genes to cancer-affected regions based on specific pathophysiological properties of the cancerous cells. Tumour microvasculature is usually leaky, with irregular branching and widened inter-endothelial openings, breakdown of close junctions between endothelial cells, and a disrupted basement membrane. The extravasation of nanocarriers loaded with therapeutic and/or diagnostic moieties from surrounding vessels into the tumour is facilitated by these large inter-endothelial openings, resulting in increased accumulation and concentration of drug/imaging agent in the targeted pulmonary tissue while reducing circulation and toxicity in healthy tissues [86]. However, nanocarrier-mediated delivery of drug/imaging agent through passive targeting is not an appropriate treatment choice for lung cancer and causes toxicity to underlying healthy tissues.

Active binding of a therapeutic and/or imaging agent to cell surface receptors and cellular absorption are critical for accurate and selective delivery of therapeutic and/or diagnostic agents to lung tissue. In order to enable entry of therapeutic/imaging agents inside the tumour interstitium, this involves alteration or process simulation. Active targeting and internalization of anticancer drug-loaded nanocarriers to lung tumour cells is possible through receptor-mediated endocytosis. Endocytosis is a vesicular-mediated mechanism in which cells engulf materials or compounds, causing them to enter the cells. To ensure successful targeting of drugs to the infected sites, ligands that directly bind to tumor cell surface receptors are coupled to the surface of long circulating nanocarriers [87]. High affinity ligands, antibodies, nucleic acids, peptides, proteins, small molecules, and aptamers are examples of targeting moieties that can be conjugated to nanocarriers via various mechanisms [86]. Monoclonal antibodies (mAbs) and antibody fragments, as well as non-antibody ligands, may be used as targeting ligands (peptidic or nonpeptidic). Bevacizumab (Avastin®), for example, is an FDA-approved treatment for non-squamous NSCLC that targets vascular endothelial growth factor (VEGF) [88].

2.4 Influence of the properties of the nanocarriers on lung cancer therapy

The lungs are definitely a well-fit body organ for systemic or local administration of nanoparticulates via inhalation, as nano-sized particles have a large surface area which interact with thin epithelial membrane barrier, resulting in high bioavailability, fast absorption, restricted proteolytic action, and prevents first-pass metabolism [89]. Moreover, charge present on nanoparticles play a vital role in their deposition and clearance from the lungs, as shown in **Figure 2.2** [90,91].

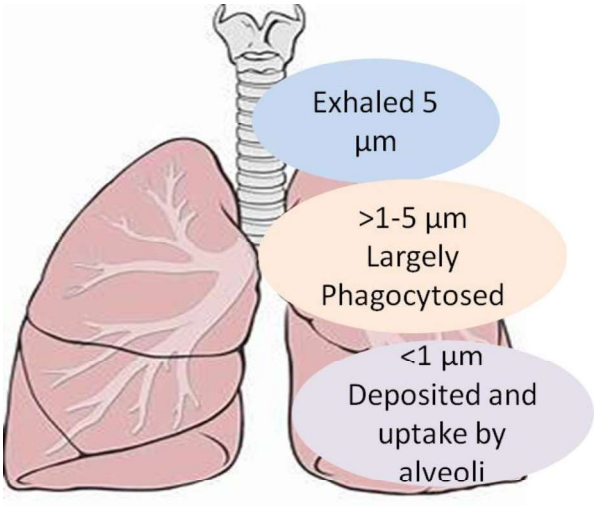


Figure 2.2 Schematic diagram showing the deposition of particles of different size in lungs.

Physicochemical factors play a critical role in determining the type of interaction between the particles inside the biological environment, aggregation property, protein absorption on the surface of nanoparticle, and intracellular trafficking. A significant alteration in any of these parameters leads to poor delivery of drug moieties, decrease in therapeutic potential, and/or increased toxicity. Thus, balance between efficacy and toxicity of nanoparticles mostly relies on their physicochemical parameters. Particle size greater than 500 nm are not suitable for intravenous administration as these particulates quickly exterminate from the blood circulation. The ideal shape and size of nanoparticles is spherical with less than 200 nm for delivering therapeutic dosages to solid tumors to ease the transport into tumorous vasculature and cells. Above-mentioned physical properties are probably beneficial for the nanoparticles in order to attain the enhanced permeation and retention (EPR) potential associated with solid tumorous cells. This exceptional microphysiology of tumorous cells is utilized by various FDA-approved nano drug delivery systems such as Abraxane and Doxil. In various

solid tumors, including lung tumor, the EPR effect plays a vital role in determining efficacy of nano drug delivery carriers [92]. Furthermore, the various characteristics of nanoparticulates including shape, size, and surface properties are significantly very important for passive targeting of drugs to solid tumors. The EPR effect generally occurs for particles having size less than 200 nm. However, particles size less than 50 nm often undergo extravasations from the tumor site via the fenestrations and are less likely to be retained in the tumor environment for longer time [93].

2.5 Nanotechnology in the diagnosis of lung cancer

In lung cancer systemic or local symptoms such as cough, heavy breathing, wheezing, sputum, weight loss, and hard swallowing occur. Radiography is subsequently used as the next step in the diagnosis of the disease, which also helps in determining the type of lung cancer (SCLC, NSCLC), primary tumor location, tumor size, metastasis, and clinical rating [94]. Accurate diagnosis is also necessary since it helps in selection of course and type of therapy, in order to ensure maximum recovery. A two pronged approach is adopted in the diagnosis of cancer, one is the imaging of the tumor body itself and the other is identification of tumor related groups [40].

Nanoparticles are being used in the treatment and diagnosis of cancer due to the multifarious advantages offered by these carrier systems. Targeted nanoprobes conjugated with ligands and chemotherapeutic drugs can be used to mark tumors with high efficiency and can be beneficial in early detection and identification of tumor cells. Gold and iron oxide nanoparticles can help in colorimetric diagnosis of cancer, and; quantum dots and upconversion nanoparticles can be used for *in vivo* imaging based on their fluorescence property [77,78].

Magnetic resonance imaging (MRI) is performed using superparamagnetic iron oxide nanoparticles (SPIONs) as contrast agents. Immune SPIONs coated with oleic acid and carboxymethyl dextran, and conjugated to mouse anti-cluster of differentiation CD44v6 monoclonal antibody have been developed to identify metastases in lung cancer. Nebulized magnetic nanoparticles (MNPs) have been investigated for pulmonary imaging by using Magnetic particle imaging (MPI) to determine mucociliary clearance [45,95]. Tomographic imaging technology based on magnetic powder imaging

has been done in animals using nebulized MNPs tagged with EGFR, a highly expressed protein in NSCLC and has shown high resolution in cancer tissues. Furthermore, positron emission tomography (PET) conducted *in vitro* using self-assembled dendritic amphiphilic molecules showed organization of these dendritic molecules into homogenous supramolecular nanoparticles with abundant surface PET reporting units. Multivalency and enhanced penetration and retention effect of these dendritic molecules resulted in sensitive and accurate imaging of tumor cells [96].

Metallic nanoparticles i.e. gold, tantalum and bismuth have gained importance as contrast agents for Computed tomography, facilitated by high atomic number, tuneable size and flexible surface chemical properties, which enables conjugation or connection of bioactive compounds, such as aptamers, antibodies or proteins, to promote targeting of cancer cells. They have been used as contrast agents for imaging of lung cancer also [40].

Targeted computed tomography (CT) imaging of SPC-A1 cells (human lung adenocarcinoma) and xenograft tumor model has been performed using gold nanoprobes entrapped with folic acid-modified dendrimers (Au DENPs-FA). Transmission electron microscopy data showed that Au DENPs-FA was efficiently taken up by the lysosomes. Au DENPs-FA are highly biocompatible and their cell morphology and viability was not influenced by MTT cell viability assay [97]. Galactose core-shell nanospheres grafted on magnetic silica when used for intranuclear imaging of A549 LC cells, showed uptake by cytoplasm and nucleus of lung cancer cells via carbohydrate-lectin recognition effects [98].

A contrast-enhanced ultrasound paired with a nanoscale microbubble contrast agent loaded with liposome-encapsulated epidermal growth factor receptor administered by nebulisation, facilitated diagnosis and detection of the early-stage lung cancer, in addition to understanding of the pathophysiology of NSCLC. Contrast-enhanced ultrasound technique is a non-invasive imaging modality which produces high-resolution images and has been found to be safer with wider applications in comparison to conventional imaging examinations, including X-ray and bronchoscopy [99].

Nanopore sensors are also used as non-invasive biomarkers for early diagnosis of lung cancer. These sensors have scattered nano-size pores tagged with selective binding sites for the biomarkers/bio-elements, and upon binding generate signals, which are processed by various methods that eventually lead to the elucidation and evaluation of the bio-elements. Using this technique, Wang et al. described and evaluated circulating miR-155 levels, microRNA reported to be abnormally regulated in lung cancer. The reverse transcription polymerase chain reaction (RTPCR) and nanopore sensor reported presence of substantially higher amounts of miR-155 in patients with lung cancer relative to control subjects. Further, the nanopore sensor proved to be reliable and displayed small variation in the intra-assay results [100].

2.6 Use of nanocarriers for the treatment of lung cancer

The most effective way of drug targeting at tumor site is to nanoencapsulate the anticancer moieties. These nanocarrier systems have attracted great attention in cancer treatment because of their nano size (1 to 100 nm) which is advantageous to adsorb and carry the hydrophobic and hydrophilic drugs to the targeted site in a controlled manner [101]. The main benefit of nanoformulations over conventional carrier systems is targeted delivery of chemotherapeutic drugs with improved pharmacokinetic properties, which leads to increased anti-cancer potential and minimized drug toxicity as shown in the **Figure 2.3**. Various nanoparticle preparations have been developed recently to effectively administer chemotherapeutic agents and nucleic acids such as DNA and siRNA to carcinoma lung cells, and they may be candidates for next-generation lung cancer therapy [102].

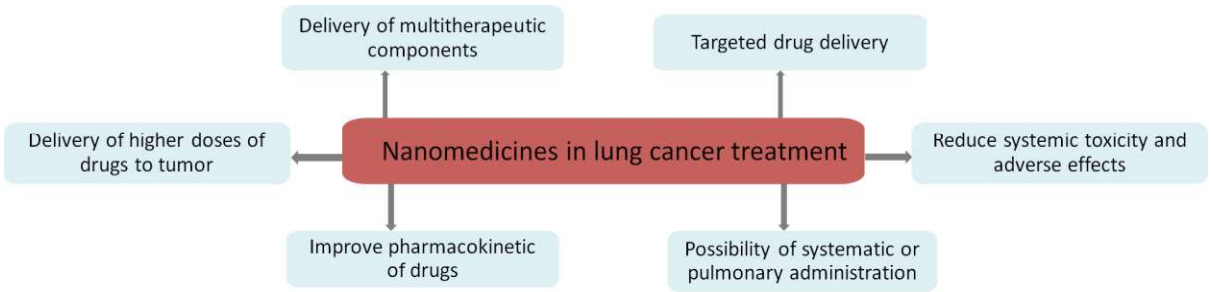


Figure 2.3 Advantages of nanomedicines as delivery vehicles of chemotherapeutic moieties for lung cancer treatment.

The drugs can be loaded on a plethora of nanoplatforms with specific molecular structures depending on the nature of polymer or lipid used in its preparation. Accordingly, they can be categorized as lipid-based carriers (liposomes, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs)), polymer-based carriers (polymeric micelles, dendrimers, and polymeric nanoparticles), metal based and magnetic nanoparticles for diagnosis and treatment of lung cancer as shown in the **Figure 2.4.** Nanocarriers used must be biodegradable; otherwise, they may deposit in the airways and cause irritation of the respiratory track and lungs [103].

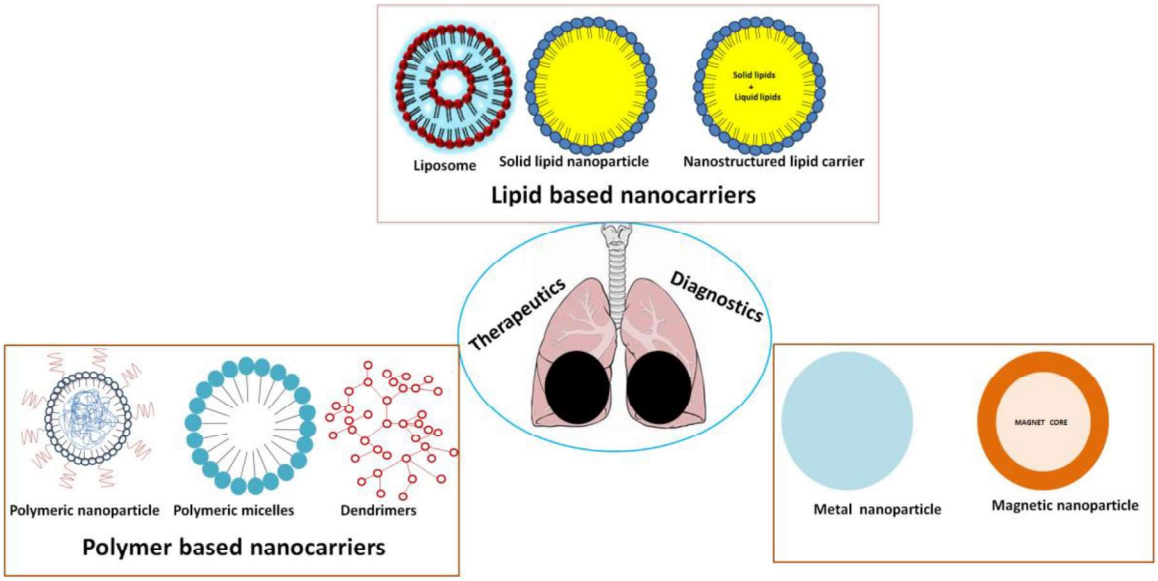


Figure 2.4 Schematic illustration of nanoplatforms used for lung cancer therapy and diagnosis.

2.6.1 Lipid-based nanocarriers

Liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) are lipid- based nanocarriers, which can be used for encapsulation of both small molecules and macromolecules. Liposomes are vesicular structures having one or more concentric phospholipidic bilayers with an aqueous core [104]. Phosphatidylcholine and phosphatidylethanolamine, along with cholesterol, act as building blocks of liposomes. Based on the number of lipid bilayers present in liposomes, they can be categorized as unilamellar vesicles (ULVs) or multilamellar vesicles (MLVs). Thin-film hydration technique is commonly used in the preparation of liposomes. Initially, multilamellar vesicles are formed, which later undergo mechanical extrusion to form unilamellar vesicles. The bilayer and aqueous core can be used for the loading of lipophilic and hydrophilic drugs,

respectively [105]. Modification of surface of liposomes with polyethylene glycol (PEG) prolongs the elimination half-life of drugs by reducing uptake by alveolar macrophages site [106].

Liposomes can suitably be used as carriers for drugs and genes. Cisplatin is used as a drug of choice for the treatment of NSCLC but is associated with nephrotoxicity. When incorporated into liposomes, cisplatin produces lower nephrotoxicity and higher efficacy in comparison to plain cisplatin [107]. Other drugs like, etoposide and docetaxel are also used in the treatment of NSCLC, but presence of inactive or mutant p53 gene limits their therapeutic efficacy due to development of multidrug resistance or loss of apoptotic function. Encapsulation of these drugs in liposomes, resulted in enhanced therapeutic efficacy against lung cancer cell lines, that too at a lower dose [108]. Phase I clinical trial of liposomal paclitaxel conducted in 2010 in NSCLC patients having malignant effusions, showed significantly higher therapeutic efficacy and reduced drug resistance in comparison to the plain drug [109].

Immunoliposomes have strategically been used for targeted delivery of anti-cancer drugs. Sterically stabilized immunoliposomes tagged with tumor-specific antigens showed effectiveness against murine squamous lung cancer cells (both *in vitro* and *in vivo*) and; murine fibrosarcoma cells *in vitro* [110].

Stealth liposomes loaded with doxorubicin showed therapeutic activity in nude Balb/c xenografts mice model injected subcutaneously with human SCLC H69 cells. Mice were administered both non-targeted stealth liposomes (SL) and antagonist G surface coupled stealth liposomes (SLG). SLG showed a 30-44fold higher binding efficiency to harvested H69 cells as compared to SL [111]. Similar results were reported by Moreira et al., who studied antagonist G-targeted *s*terically stabilized liposomes in H82 cell lines, a variant of human small cell lung cancer (SCLC), and suggested potential of their use in this highly heterogeneous disease [112]. Biodistribution studies of liposomal encapsulated 9-nitrocamptothecin (9-NC) administered via pulmonary route showed 3.4 times higher mean residence time in lungs as compared to 9-NC solution. With sustained-release characteristics, approximately 2.2-fold higher total area under the curve (AUC_{0-t}) in all tissues in mice was reported for liposomes as compared to 9-NC solution [113]. Liposomal-based vectors have also shown potent

tumor suppressor activity against human lung cancer *in vivo*. Gene delivery vector 1,2-dioleyol-3-trimethyl ammonium propane (DOTAP)/cholesterol (Chol.) cationic liposome delivered tumor suppressor genes p53, FHIT and FUS1 intravenously to lungs and limited the propagation of lung metastasis [114].

Solid lipid nanoparticles, also called lipospheres or solid lipid nanospheres, have a diameter in the range of 50-1000nm and are prepared using solid lipids in the range of 0.1-30 % (w/w) [115]. The lipids generally used in the preparation of SLNs are monoglycerides, diglycerides and triglycerides, fatty acids, and waxes. SLNs provide stability and safety to vulnerable drugs and are biodegradable and biocompatible. SLNs have shown promising results during preclinical studies in overcoming multidrug resistance to anti-cancer drugs [116,117]. Also, SLNs act as promising transporters for targeted delivery of drugs to lungs, especially of lipophilic drugs, thereby reducing the systemic side effects [118]. SLNs of mannosylated-DSPE (Distearoyl-phosphatidyl-ethanolamine) loaded with paclitaxel (PTX), was studied against A549 lung's epithelial cancer cells. Cytotoxicity assay and biodistribution study of SLNs showed presence of higher concentration of PTX in alveolar area [119]. In various malignant carcinomas, including NSCLC, transferrin receptors are up-regulated. Solid lipid nanoparticles of etoposide targeted against transferrin receptors expressed on A549 human NSCLC cells showed significant antitumor activity, which was confirmed by the anti-proliferation assay, morphological changes and apoptosis of A549 cells [120]. Paclitaxel-SLNs coated with chitosan-derivative targeted against over-expressed folate receptors (FRs) were designed to increase the residence time of the drug in the respiratory tract [121]. SLNs of 3β-[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol hydrochloride (DC-Chol) were formulated using 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and Tween 80, which were then complexed with p53-EGFP plasmid DNA for gene transfection in H1299 NSCLC cell line [122]. Lipoplexes of DNA (pp53-EGFP) and SLNs formulated using tricaprin, DC-Chol, DOPE, and Tween 80, when transfected into human NSCLC cells (H1299 cells) showed higher transfection efficiency as compared to commercially available Lipofectin [123].

A highly ordered matrix created using solid lipids, cause leaching of drugs during storage. In order to overcome this shortcoming, liquid lipids are incorporated to induce imperfections in the matrix, which decrease drug leaching during storage and also improve loading capacity of active compound [124]. These nanoplatforms, which consist of both liquid and solid lipids, are described as nanostructured lipid carriers (NLCs). With efficient drug loading capacity, NLCs offer advantages such as increased drug solubility, improved storage ability, enhanced permeability and bioavailability, and targeted delivery [125]. NLCs are potentially good colloidal carriers for the delivery of various therapeutic substances like siRNA and anticancer drugs into the lungs. These systems present a prospective approach for selective silencing of oncogenes and multidrug resistance related genes [46,126]. NLCs can be modified with multifunctional targeting moieties to form PEGylated NLCs, and ligand-targeted NLCs for targeted delivery to lungs [127]. PEGylated liposomes such as Doxil®, DaunoXome®, DepoCyt®, and ONCO-TCS® have reached phase II trials [18]. NLCs loaded with paclitaxel and doxorubicin (NLC-PD) showed significantly higher cytotoxic effects *in vitro* as compared to both unencapsulated drugs as well as NLCs loaded with a single drug. When delivered *in vivo*, NLC-PD exhibited high tumor-targeting capacity and strong anti-cancer activity [128]. Shao et al. studied transferrin (Tf) tagged NLCs co-encapsulated with paclitaxel and DNA (Tf-PTX-DNA-NLCs) for treatment of LC. Tf-PTX-DNA-NLCs showed strong anti-tumor effect *in vitro* and *in vivo* respectively on NCL-H460 cell lines and in mice infected with NCL-H460 cells [129].

2.6.2 Metallic nanocarriers

Metallic nanoparticles come in different sizes and shapes, and sizes between 10-100 nm are usually used for drug delivery. The metallic nanoparticles are made up of metals such as gold, nickel, silver, zinc oxide, titanium dioxide, iron oxide, and gadolinium [73]. Metallic nanoparticles have been investigated as nontoxic carriers for drug and gene delivery applications. The metal nanoparticles have exclusive characteristics such as ease of synthesis, high surface to volume ratio, proficient surface chemistry, and broad optical properties that make these nanoparticles useful in the clinical field for cancer therapeutics [130].

Inorganic nanoparticles were prepared by Ramalingam and coworkers wherein doxorubicin was conjugated to gold NPs with polyvinylpyrrolidone. These gold nanoparticles limited the proliferation of A549 cells, improved production of cellular ROS (reactive oxygen species), and induced cell apoptosis. Similarly, Kalaiarasi and colleagues formulated copper oxide nanoparticles that could decrease the expression of specific oncogene i.e. histone deacetylase in A549 cancer cells, which promotes apoptosis. Although platinum based anticancer agents have been extensively studied in LC cell lines, their clinical results are mainly limited by multidrug resistance. Tsai and research team fabricated self-assembled diaminocyclohexane-platinum-(II) (DACH-Pt-II) loaded nanoparticles that could be effectively internalized by platinum (Pt) resistant LC cell lines, prompting higher tumor toxicity. Hence, these DACHP loaded nanoparticles can be potentially used as a nanocarrier system for combating multidrug-resistant LC [131].

Peng et al. developed gold nanoparticles for use as biosensors for diagnosis of lung cancer that differentiate the breadth of lungs of cancer patients from that of healthy ones. The sensor was also capable to discriminate between SCLC, and NSCLC [132,133]. Gold nanoparticles conjugated with methotrexate showed high tumor retention and increased therapeutic effect in a Lewis lung carcinoma mouse model as compared to free methotrexate [134]. Other studies have shown that nanoclusters of gold/iron oxide combined with fluorescently labelled antibodies can proficiently target EGFR expressed on epidermoid carcinoma cells [135]. Silver nanoparticles also have anti-proliferative effects in the cancerous cells [136]. But, *in vitro* exposure of silver nanoparticles to human lung cancer cells resulted in reactive oxygen species (ROS) induced genotoxicity that raised the concern of adverse risk to benefit ratio [137,138]. He et al. showed that biosynthesized AgNPs have antitumor activity against lung cancer in H1299 lung cancer cells *in vitro* and an *in vivo* xenograft immunodeficient (SCID) mouse model. The cytotoxicity of AgNPs was shown using trypan blue and MTT assay. AgNPs induced apoptosis (increased caspase-3 and decreased bcl-2) in lung cancer cells, which was linked to inhibition of NF-κB activation shown via mechanistic experiments [139].

Amongst other rare earth, silica and nanodiamonds nanoparticles have been used as theranostics in lung cancer. Wu et al. used silica-based nanocomposites for p53 gene therapy and near infrared tumor

imaging of lung cancer both *in vitro* and *in vivo*. In a study in a lung cancer cell model in immunodeficiency mice, using nanodiamond (ND) loaded with paclitaxel, potential regression of tumor was observed. Mechanistic experiments showed that ND induced cell death by causing mitotic arrest and apoptosis. In another study, Zhang et al. used silica dependent imaging device to detect an miRNA that can be used as a biosensor in the treatment of lung cancer[140–142].

2.6.3 Magnetic nanocarriers

Magnetic nanoparticles are ultra-small nanoplatforms with versatile characteristics like biocompatibility, flexible surface chemistry, and find multi-dimensional use in biomedical applications such as biosensing, bioseparation, drug delivery, imaging, and diagnosis. With such unique properties, magnetic nanoparticles are sumptuously being used in the evaluation of different types of cancers. Iron oxide magnetic nanoparticles have been investigated in human clinical trials and have shown excellent safety profile and serum bioavailability. Polyethylene glycol or Pluronic polymers are used as prime stabilizers in magnetic nanoparticles because they enhance circulation, reduce uptake by RES, and improve tumor targeting due to EPR effect. Various combinations of iron oxide magnetic nanoparticles with PLGA and PCL have extensively been used for drug delivery [143]. Magnetic nanoparticles (MNPs) are favoured as chemotherapy substitutes as they transmit drugs selectively to the desired region. It only harms cancer cells because it has unique physical properties and the ability to behave at the cellular and molecular levels of biological interactions. The magnetic properties of these nano-sized materials are being used as contrast agents in magnetic resonance imaging (MRI). The different MNPs, including surface coated MNPs, Polymer based MNPs, Super Paramagnetic Iron Oxides nanoparticles (SPIONPS), Magnetic Hyperthermia, MNP conjugated with DNA and RNA have been studied for targeting of lung cancer. The advances in MNP technologies have helped to solve many diagnostic and treatment issues related to lung cancer [144]. Magnetic hyperthermia is a non-invasive technique for tumor ablation. Application of an alternating magnetic field along with superparamagnetic iron oxide (SPIO) nanoparticles, sub lethal temperature change is produced which then damage the local tissue. Significant inhibition of *in vivo* lung tumor growth in a mouse orthotopic model of NSCLC was observed using this technique [145]. Hybrid

plasmonic magnetic NPs combined with anti-EGFR antibody (Clone 225) resulted in autophagy, apoptosis, and cytotoxicity of lung cancer cells of NSCLC type as compared to free clone 225 antibody [146]. Wang et al. detected micro-metastasis in lung cancer using magnetic nanoparticles. Conjugation of magnetic nanoparticles with pan-cytokeratin epithelial tumor cell markers could effectively isolate circulating tumors cells in individuals diagnosed with lung cancer. Furthermore, the metastatic cells were detected using quantum dots coupled with lung-specific X protein (LUNX) and surfactant protein-A (SP-A) antibody NSCLC micro-metastasis markers. This was the first study which described the identification of micro-metastasis in peripheral blood in patients with lung cancer [147].

MNPs are also being studied to combat drug resistance. In a study, Cisplatin infused MNPs were used to chemosensitize a cisplatin-resistant A549 lung cancer xenograft representation. The tumors were treated with iron oxide-magnetic-nanoparticle-cisplatin (Fe3O4-MNP-DDP), which lead to reduction in resistant proteins and improvement in cisplatin cytotoxicity. The cytotoxicity findings showed that DDP completely suppressed A549 cell growth and DDP-resistant A549 cells in a dose-dependent and time-dependent fashion, which was amplified by Fe3O4-MNP, with no significant impact of Fe3O4-MNP alone on DDP-resistant A549 cells. The study showed that Fe3O4-MNP improves DDP's anticancer efficacy without causing any toxicity in both *in vitro* and *in vivo* trials. As a result, Fe3O4-MNP-DDP exhibited the ability to overcome DDP resistance in lung tumor cells [148].

Aerosolized MNPs have also shown significant clinical efficiency in the treatment of lung cancer. Verma et al. used nebulized Fe_3O_4 MNPs coated with poly(lactic-co-glycolic acid (PGLA) combined with quercetin, (poorly water-soluble flavonoid) and tested the same after nebulization on human cultivated tumor cells A549 and found to be sustained in both cell culture and in animal culture (mice). High content screening (HCS) found that quercetin formulation significantly reduced the number of viable A549 cells [95,149].The roles of various nanocarriers in lung cancer therapy have been summarized in **table 2.1.**

2.6.4 Polymer-based nanocarriers

During formulation of polymeric nanoparticles, drugs either can be loaded physically or bound covalently to the polymeric matrix. These drug carriers could be capsules like polymeric nanoparticles, hyperbranched macromolecules (dendrimers), and amphiphilic core/shell (polymeric micelles) [150].

Polymeric nanoparticles can be composed using synthetic polymers (poly caprolactone (PCL), poly lactic-co-glycolic acid (PLGA), polyethylene glycol (PEG), or natural polymers like chitosan and polysaccharides or amphiphilic diblock copolymers. PLGA can be used to encapsulate both hydrophilic and lipophilic drugs. PEGylation (addition of PEG on the surface) forms a stealth layer and reduces nonspecific cellular uptake of nanoparticles. As compared to lipid carriers, polymeric nanoparticles offer higher stability, tunable drug release patterns, sharper size distribution, and higher drug loading [151].

Polymer based nanoparticles have proved to be effective carrier system for the treatment and management of cancers, as their morphology and composition can be altered as per the need. The polymers commonly used for the preparation of nanoparticles are alginic acid, gelatin, chitosan, polycaprolactone, polylactic acid and polylactide-co-glycolide. However, when these nanoparticles conjugate with sulfide bond, they control the release of the drugs. The biocompatible and biodegradable characteristics of these polymers cause less toxicity and lead to improvement in bioavailability. In contrast, cationic polymers cause cell toxicity and higher aggregation in the capillaries of the lungs because they have poor compatibility and are non-degradable. Therefore, regular toxicity monitoring is necessary in case of use of cationic polymeric nanoparticles, as they can cause breathlessness on interaction with a biosurfactant [152].

Polymeric nanoparticles have been extensively used for the delivery of chemotherapeutic agents. Polymeric nanoparticles help to potentiate the chemotherapeutic and radio-therapeutic effects of anti-cancer drugs. Encapsulation of taxanes in polymeric nanoparticles enhanced the efficacy of chemotherapy and radiation therapy *in vivo* in A549 lung tumor xenograft model [153]. Gelatin based

polymeric nanoparticles decorated with biotinylated epithelial growth factor (BEGF) were used to target EGFR overexpressed in lung cancer. Enhanced cellular uptake of these nanocarriers was observed in A549 lung adenocarcinoma cells *in vitro* and upon administration as aerosol, higher accumulation was found in lung Cancer cell [154,155]. Recently, polymeric nanoparticles coated with hyaluronan/polyethyleneimine have been developed for targeted delivery of Docetaxel to target the CD4 receptor of the lung cancer cells. Another study, evaluated the effectiveness of polylactide-tocopheryl polyethylene glycol (1000) succinate based nanocarriers for targeted delivery of crizotinib to the lung tumor cells. Further, small interfering RNA (siRNA) based drug delivery has always remained a big challenge. Advancement in field of nanotechnology has helped in fabricating novel cationic polymers that can suppress siRNA genes in lung tumor. A research showed that *in vivo* study of these cationic polymeric nanoparticles silence 50% of expression of targeted genes. Another study emphasized on the potential of polymeric nanoparticles in suppressing the expression of genes coding for endothelial cells [152]. Further, clinical trials, and preclinical evaluation of polymeric nanoparticles containing small interfering RNAs (siRNAs), microRNA (miRNA) has been undertaken [156].

Polymeric micelles are generally prepared using amphiphilic block copolymers, which assemble to form a layered structure with an inner lipophilic core and an outer hydrophilic shell. The core is used to entrap drugs with low water-solubility while the shell supports the core against the aqueous environment and helps to protect the micelles from RES recognition *in vivo* [150]. Polymeric micelles, like other nanocarriers, offer certain advantages like biodegradability, prolonged circulation, smaller particle size, high drug loading efficiency, and accumulation at the target site [157]. PEG-polyglutamic acid block copolymer micelles of cisplatin showed approximately 20 times higher accumulation in solid tumors of Lewis lung carcinoma cells in comparison to free cisplatin. Also, they exhibited prolonged circulation *in vivo* [158]. Worm-like polymeric micelles of amphiphilic block copolymer poly (2-oxazoline) co-loaded with etoposide and alkylated cisplatin prodrug showed slow release of both drugs, improved pharmacokinetic profile, enhanced tumor distribution and cytotoxicity in comparison to single drug micelles in various animal models of SCLC and NSCLC [159]. Genexol-

PM (PEG-poly (D, L-lactide)-paclitaxel) is the first polymeric micelle of paclitaxel [150]. Polymeric micelles of various anticancer drugs are under different phases of preclinical trials for evaluation of their efficacy and targeted delivery to the tumors. A phase II clinical trial of Genexol-PM with cisplatin showed significant antitumor activity against advanced NSCLC, and was found to be safe and efficacious. Similar results for phase II trials of Genexol-PM - gemcitabine combination were obtained [160].

Dendrimers are globular structures made up of multiple branches of polymers with a central atomic core and repeating units of polymeric branches with inner shell cavities and outer shell with a multivalent functional group. The functional groups combine with the charged polar molecules, and the inner shell entraps the uncharged non-polar molecules. The functional groups are responsible for the controlled release of drugs at a certain pH or by the action of specific enzymes. Studies have shown that dendrimers of poly (glutamic acid)-b-poly (phenylalanine) copolymers, get self-assembled into micelles, and deliver the drug at a controlled rate [161]. Dendrimers act as an ideal delivery vehicle and can be designed to achieve desirable pharmaceutical characteristics, for example, i) negatively-charged and neutral dendrimers are biocompatible, while positively-charged dendrimers possess toxicity; ii) stearic properties of dendrimers influence their pharmacokinetics and biodistribution characteristics; iii) PEGylation of dendrimers leads to increase in water solubility, dendrimer size, improved retention and biodistribution characteristics; iv) tagging of dendrimer surface with the targeting moieties can be used to treat cancerous cells with overexpressed receptors. Over the last few years, dendrimers have progressively been used for diagnostic and therapeutic purposes in the treatment of cancer, including use in photothermal therapy, neutron capture therapy, and photodynamic therapy [162].

Novel peptide conjugated dendrimers, when delivered to lung tumor-bearing athymic mice, effectively targeted the tumor cells and showed efficacy as a drug delivery vector for the treatment of lung cancer [163]. Aptamer (Ap) conjugated polyamidoamine (PAMAM) dendrimers encapsulated with microRNA-34a (miR-34a) were used to form the targeted gene delivery nanoparticles (PAM-Ap/MiR-34a NPs) for treatment of the lung cancer. The PAM-Ap/MiR-34a NPs improved cellular

uptake as well as gene transfection efficiency in cultured NSCLC cells, and increased the regulation of targeted genes BCL-2 and p53 *in vitro* and significantly induced apoptosis and inhibited cell growth, migration, and invasion of lung cancer cells as compared to non-targeted nanoparticles [164]. PEGylated dendrimers have promising applications when administered as aerosols. It has been found that smaller sized dendrimer particles enter into the bloodstream when administered via inhalation route while larger sized particles sequestrate into the lungs for an extended period. This method in the future could be used as an alternative to injectable drug delivery system [165]. PEGylated dendrimers conjugated with doxorubicin delivered as inhalation can extend the exposure of chemotherapeutic drugs to lungs with improved antitumor potential [166].

Table 2.2 Potential functions of various nanocarriers for targeting lung cancer.

Nanocarriers	Targeting moiety/Drug	Nanocarrier loaded with drug	Therapeutic functions	References
Liposomes	Vinorelbine (VRB) and Quinacrine (QNC)	PEGylated liposomes entrap VRB and QNC	VRB have anticancer effect and QNC induce apoptosis for the treatment of NSCLC.	[167]
	Etoposide (ETP)and Docetaxel (DCT)	liposomal formulation encapsulated with ETP & DCT	Enhanced cytotoxicity in A549 and H-1299 lung tumor cell lines mediated through p53 tumor suppressor gene.	[168]
	Epirubicin	Epirubicin liposomes	Multifunctional targeted epirubicin liposomes showed higher antitumor efficacy for NSCLC, increased cellular uptake via receptor-mediated endocytosis, blocked the tumor metastasis, induced apoptosis of Lewis lung tumor cells through activating caspase 3.	[169]
Solid-lipid nanoparticles (SLNs)	Paclitaxel and Bcl-2 siRNA	SLN loaded with Bcl-2 siRNA and paclitaxel	Showed effective synergistic combination therapy.	[170]
	Paclitaxel (PTX)	SLNs of glyceril tripalmitate (tripalmitin) loaded with PTX (Tripalm-	Enhanced paclitaxel antitumor activity against breast and lung cancer cells *in vitro*, Superior cytotoxic effect.	[171]

		NPs-PTX)		
Nanostructured lipid carrier (NLCs)	Docetaxel (DCT) and curcumin (CR)	Folate (FA)–appended nanostructured lipid carriers (NLCs) loaded with DCT and CR (FA-DTCR-NLCs)	Combination oncotherapy used in the treatment of NSCLC, higher apoptotic, anti-proliferative, anti-angiogenic, and anti-metastatic potential.	[172]
	Rapamycin (RAP) and Berberinee (BER)	Nanostructured lipid carriers (NLCs) loaded with RAP and BER (RAP-BER-NLCs)	Showed superior anticancer activity in Lung carcinoma A549 cell lines.	[173]
	Paclitaxel (PTX) and doxorubicin (DOX)	Nanostructured lipid carriers (NLCs) loaded with PTX and DOX (PTX-DOX-NLCs)	PTX-DOX NLC achieved highest cytotoxic effect amongst all formulations *in vitro*, as compared to single drug delivery NLC. *In vivo* investigation on NSCLC animal models showed that co-delivery of PTX and DOX possessed high tumor-targeting capacity and strong antitumor activity.	[128]
Polymeric Nanoparticles	Paclitaxel (PTX)	PTX-Polycaprolactone/ Poly(ethylene-glycol)/Polycaprolactone (PCEC) nanoparticles with chronomodulated chemotherapy	Greater anti-tumor activity against A549 cells.	[174]
	Paclitaxel	(Abaraxane) Albumin based NPs carrying Paclitaxel	Effectively used as first line treatment of advanced or metastatic NSCLC in combination with Carboplatin in patients who are not good candidates for surgery or radiation therapy.	[175]
	Lomustine(LMT)	Chitosan-NPs loaded with LMT	Marvellous control over drug release and enhanced *in vitro* cytotoxicity of lomustine (potent antineoplastic agent) against lung cancer cell lines L132.	[176]
	Resveratrol(RVT)	Gelatin-NPs loaded with RVT	Effectively delivered hydrophobic drug (Resveratrol) towards NSCLC	[177]

			cells.	
	EFGR-targeted biotinylated EGF (bEGF)	Gelatin-NPs conjugated with EGF (bEGF)	Showed enhanced cellular uptake in EFGR over-expressing cancer cell lines. It holds a great promise for targeted lung cancer treatment.	[154]
Dendrimers	Paclitaxel (PTX)	GE11 peptide and (PTX) conjugated dendrimer	Successfully targeted overexpressed EGFR receptors in lung cancer, increased receptor-mediated endocytosis, improved therapeutic efficacy.	[178]
	Cis-diamminedichloroplatinum-II (CDDP)	CDDP encapsulated polyethyleneimine based siRNA and folic acid (FA) conjugated dendrimer	Targeted specifically overexpressed receptor in lung cancer.	[179]
Polymeric Micelles	Paclitaxel (PTX) and parthenolide (PRT)	EGF conjugated micelles co-loaded with PTX and PRT	Increased cellular uptake by the internalization of drugs into lung cancer cells.	[180]
	siRNA	cRGD conjugated polyethylene glycol-polyaspartamide (tetraethylenepentamine) block copolymer-based siRNA-loaded micelle	Enhanced accumulation at target site, achieved tremendous tumor growth inhibition, induced cell apoptosis.	[181]
Metallic Nanoperticles	Cetuximab (C225)	C225-AuNPs	Improved efficiency of antibody delivery and promoted cytotoxicity specifically to EGFR positive NSCLC cells.	[182]
	Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)	AuNPs	The AuNPs showed increase in sensitivity of NSCLC cells to TRAIL-induced apoptosis.	[183]
	Afatinib (Afb)	Afb-AuNP conjugates	There was 3.7 times increase in potency of Afb-AuNPs as compared to free drug signifying higher	[184]

			inhibition of cell proliferation of tumor cells in EGFR positive NSCLC.	
Magnetic nanoparticles	Quercetin	Quercetin-MNP coated with PLGA	Targeted lung cancer cells via nebulization and decreased number of viable A549 cells.	[149]
	EGFR targeted SPIO-nanoparticles	Spion coated MNP	Suppressed lung tumor growth.	[145]
	Actein (AT)	AT-MNPs combination	Strong antitumor effect in NSCLC with no toxicity to normal cells.	[185]

2.7 Chitosan based Nano particulate system

The particle systems with a polycation in their composition are known as CSN based particle systems. Our observations stemmed from studies where CSN was employed as a structural element or to modify the surfaces of polymer and lipid-based particles. The main goal here was to assess the probable correlations between previously investigated synthesis variables and particle attributes (Zeta Potential, PDI and particle size).

2.7.1 Chitosan-sodium tripolyphosphate particulate system

Sodium tripolyphosphate (Na-TPP) is often utilised in the pharmaceutical industry as excipient in detergents formulation. In order to produce CSN-based micro and nanoparticles, this non-polymeric polyanion is used in the ionic gelation process. Na-TPP contains 5 pKa values from 1 to 8.5, and a molecular mass of 367.86 g/mol. The polyanionic Na-TPP has the capacity to attain a maximum of 3 negative charges within the pH range of 4 to 7, and up to 5 negative charges at pH 10 during its dissociation in an aqueous medium.

Na-TPP acts as a crosslinking agent for CSN through its electrostatic connection with the protonated amino groups. This phenomena may take place inside the same polymer chain or between dissimilar polymer chains.

2.7.2 Chitosan polyelectrolyte complexes

The primary technique for producing polyelectrolyte complexes is complexation. This method is based on the polycation-polyanion interaction which occurs when polyelectrolytes with opposing charges react to each other. Drug delivery systems for a range of pharmaceuticals and biomolecules such as proteins are frequently produced using this technique. The primary cause of polyelectrolyte complexation, which yields colloidal particles, is supramolecular interactions including dipole-dipole, hydrophobic, electrostatic and hydrogen bond interactions.

2.7.3 Surface modification of Chitosan particulate systems

One of the best-known polymer for nanoparticle formulations is poly (lactic-co-glycolic acid) (PLGA), which is approved for parenteral delivery by regulatory bodies due to its biocompatibility. The different molecular weights and copolymer concentrations of commercially available PLGA have a direct impact on the physical and chemical attributes of these polymers. The monomer ratio used to identify the polymer, such as PLGA 15:85, shows that it is made up of 15% lactic acid and 85% glycolic acid. Several volatile organic solvents, such as dichloromethane, ethyl acetate, acetone, and chloroform, are miscible with this polymer, making it possible to create nanoparticles by a range of techniques. CSN may adhere to the surface of PLGA by chemical binding or physical adsorption.

Positive Zeta potential is produced by CSN-coated nanoparticles. The positive charge of CSN and the negative charge of the PLGA surface interact electrostatically to coat the PLGA particle surface with CSN. Important characteristics of the PLGA nanoparticles including improved physicochemical stability and affinity to biological membranes, are provided by this coating [186].

2.8 Techniques for preparation of chitosan nanoparticles (CSN-NPs)

CSN-NPs have been extensively documented in the scientific literature as a carrier system for various drug delivery applications. A diverse range of approaches has been employed in the development of CSN-NPs.

2.8.1 Emulsion cross-linking method

In this procedure, an aqueous CSN solution is first used to create a water-in-oil (W/O) emulsion in the oil phase, and subsequently, the aqueous droplets are rendered stable through the utilization of a suitable surfactant. The most adaptable cross-linking agent, glutaraldehyde, is then used to cross-link the stable emulsion. Here, CSN amino groups and glutaraldehyde aldehydes undergo cross-linking process that precipitates into particles [187].

2.8.2 Spray-drying

A nanospray drier is used in this approach to prepare CSN-NPs. The technique uses heated air to dry atomized droplets. The first step is to make a CSN solution by dissolving pure CSN powder in an acetic acid solution and storing it overnight. Atomisation produces little droplets. The solvent evaporates immediately from tiny droplets, resulting in the production of NPs [188].

2.8.3 Emulsion droplet coalescence method

First, two stable emulsions are formed, a stable emulsion that contains an aqueous CSN solution and drugs is created in liquid paraffin oil. Then, in liquid paraffin oil, another stable emulsion containing CSN aqueous solution with NaOH is prepared. Using high-speed stirring, the two emulsions are then combined. When these two emulsions are combined, the emulsion particles randomly collide and coalesce, precipitating CSN droplets to create small size particles [189].

2.8.4 Nanoprecipitation

First, CSN is dissolved in a suitable solvent to generate the diffusing phase in this approach. Under magnetic stirring, this phase is then introduced to the dispersion phase, i.e. methanol. At a rate of 0.86 ml/min, the diffusing phase is introduced to the dispersion phase using a peristaltic pump and a needle positioned 2 cm above the surface. Then, irrespective of the non-solvent to solvent volume ratio, a very tiny amount of polysorbate-80 is added to the non-solvent phase resulting in formation of smaller NPs [190,191].

2.8.5 Reverse micellization method

W/O droplets are referred to as reverse micelles in the reverse micellisation procedure. To make a W/O emulsion, a lipophilic surfactant, such as sodium bis(ethyl hexyl) sulfosuccinate or cetyltrimethyl ammonium bromide, is dissolved in an appropriate organic solvent, such as n-hexane. The organic phase is then added to and continuously stirred to prevent turbidity during the addition of an aqueous CSN solution, the medication and glutaraldehyde. The NPs formed by removing excess of surfactant by precipitation with $CaCl_2$ followed by dialysis to remove unreacted components and freeze drying [192].

2.8.6 Desolvation/simple coacervation/phase separation

In this procedure, a CSN solution is first made using an appropriate solvent. The solvent-competing agent with higher hydrophilicity is then added to the CSN solution (for example, sodium sulphate). Due to the higher affinity of water for salt, when salt comes into contact with the aqueous environment of the CSN solution, a progressive elimination of the solvating water surrounding CSN takes place. Precipitation results from this step due to insolubilization of polymer. In this procedure, the precipitating agents are sodium sulphate and acetone. In order to stabilise the suspension of nanoparticles, polysorbate 80 is utilised as a stabilising agent in the preparation medium. To harden the NPs, glutaraldehyde is then added to the mixture [191].

2.8.7 Emulsion solvent diffusion

In this procedure, an aqueous solution comprising CSN and a stabilising agent (such as poloxamer and lecithin) is first mixed with the organic phase (such as methylene chloride and acetone) containing the hydrophobic medication. This creates an oil-in-water (O/W) emulsion. The created O/W emulsion is then homogenised under high pressure and the methylene chloride is removed at room temperature while under reduced pressure. At this stage, CSN solubility declines as acetone diffuses into the aqueous phase and resulting NPs are precipitated by polymerization. In order to allow for full acetone diffusion, additional water is typically supplied. Finally, NPs are separated by centrifugation [191].

2.8.8 Ionic gelation

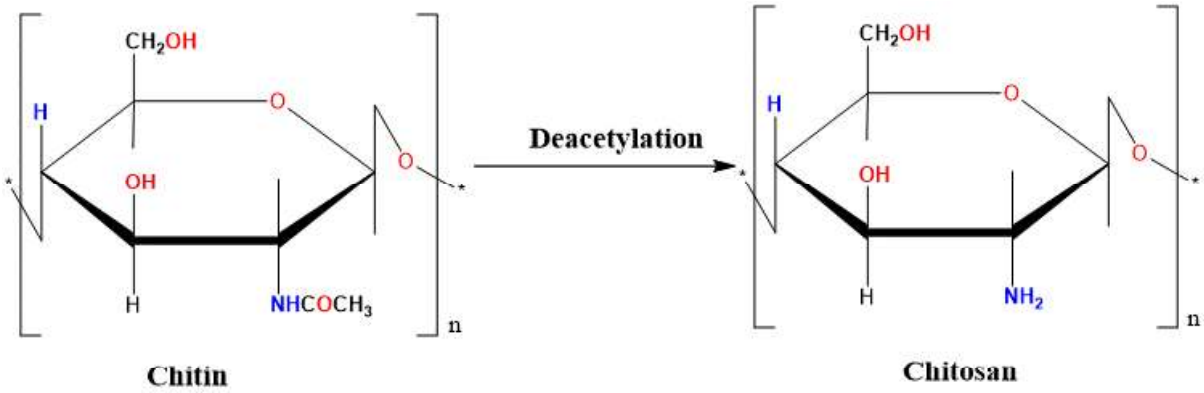
In this process, CSN is first dissolved in an aqueous acidic solution to get the cationic CSN. This solution is then added dropwise to an anionic tripolyphosphate (TPP) solution with constant magnetic stirring. Ionic gelation occurs as a result of TPP and cationic CSN electrostatic complex, and CSN precipitates to form spherical particles [193]. Ionic gelation can be chosen as a preferred method for creating chitosan nanoparticles because of its gentle conditions, lack of harmful materials, and ease of preparation.

2.9 Excipients profile

2.9.1 Chitosan

2.9.1.1 Synonyms: Chitosan, Poliglusam, Chicol, Deacetylchitin

2.9.1.2 Chemical structure:



Chitin Chitosan

2.9.1.3 IUPAC name:

Methyl*N*-[(2*S*,3*R*,4*R*,5*S*,6*R*)-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6(hydroxymethyl)oxan-2-yl]oxy-2-[(2*R*,3*S*,4*R*,5*R*,6*S*)-5-amino-6-[(2*R*,3*S*,4*R*,5*R*,6*R*)-5-amino-4,6-dihydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-3-yl]carbamate.

2.9.1.4 Molecular formula: $C_{56}H_{103}N_9O_{39}$

2.9.1.5 Molecular weight: 1526.5 Daltons

2.9.1.6 Storage: 8 to 25°C (Cool & dry area)

Chitosan is a linear polysaccharide comprised of randomised β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) group with an amino (NH_2) and two hydroxyl (OH) groups in each repeating glycosidic unit [194]. It is produced by treating the chitin shells of crustaceans such as crabs and shrimps, as well as the cell walls of fungus with an alkaline chemical such as sodium hydroxide [195]. NMR spectroscopy can be used to determine the degree of deacetylation (%DD), and %DD in commercial chitosans ranges from 60 to 100%. The molecular weight of commercially manufactured chitosan ranges between 3800 to 20,000 daltons [196]. The amino group in chitosan has a pKb value of 6.5 [197], resulting in considerable protonation in neutral solution, which increases with increased acidity (lower pH) and %DD value [198]. This renders chitosan to be water soluble and a bioadhesive capable of binding to negatively charged surfaces [199]. Chitosan's free amine groups can form crosslinked polymeric networks with dicarboxylic acids to increase its mechanical properties [200].

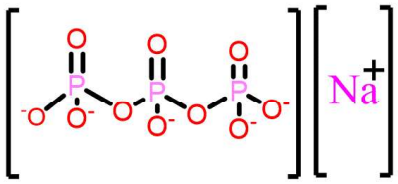
Chitosan is a popular biopolymer because of its active amino groups, which enable the attachment of different functional groups under gentle reaction conditions. Furthermore, the cationic nature of chitosan and its water solubility at low pH levels are attributed to these amino groups. Chitosan exhibits remarkable biocompatibility, remarkable biodegradability, minimal immunogenicity and antibacterial activity making it a suitable and useful biomaterial. Additionally, muco-adhesion, regulated drug distribution, and in situ gelation are all made possible by the primary amine functional groups of chitosan. Given its versatility, chitosan finds significant application in drug delivery methods. In addition, chitosan exhibits several important properties including bacteriostatic, fungistatic, anti-carcinogenic and haemostatic effects. Depending on the intended uses and preparation techniques, chitosan is employed in a variety of ways to form conjugates, hydrogels, NPs, microspheres and capsules etc. [201].

Chitosan NPs can be modified to target certain tissues; hence, cell selective targeting of chitosan NPs appears to be a viable strategy to avoid non-specific interactions, boost local drug concentration and reduce systemic toxicities and adverse events. Modifying NPs with peptides, antibodies, aptamers, or small molecules allows for targeted drug delivery [201]. These techniques for targeting NPs, not only reduce the required dosage of medications, but also facilitate their delivery to the receptors.

2.9.2 Sodium Tripoly-phosphate

2.9.2.1 Synonyms: Pentasodium triphosphate, Sodium tripolyphosphate anhydrous

2.9.2.2 Chemical structure:



2.9.2.3 Molecular formula: $Na_5O_{10}P_3$

2.9.2.4 Molecular weight: 367.86 g/mol

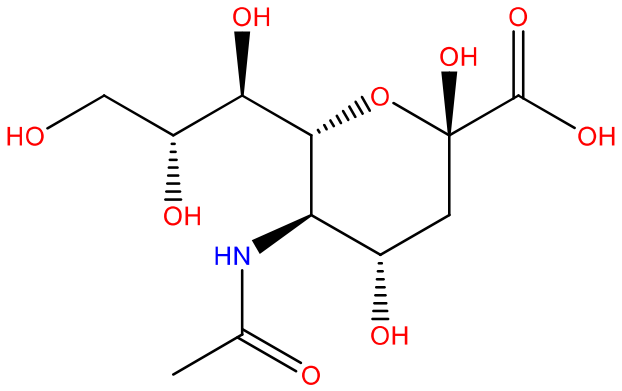
Sodium tripoly phosphate (Na-TPP) is a cross linker that is frequently used to electrostatically crosslink polycationic polymers such as chitosan by ionic gelation. In contrast to other chemical cross-linkers such as glyoxal and glutaraldehyde, Na-TPP does not pose a physiological risk. Its aqueous solubility and one-step ionic gelation reaction are other advantages. During ionic gelation, the high charge density of Na-TPP (six ionic groups) guarantees a high cross linking density with CSN amine groups. The molar ratio of CSN to TPP is known to have a significant impact on the mean diameter and drug release characteristics of the resultant nanoparticles. Drugs were encapsulated using CSN-TPP as a nanocarrier [202]. Na-TPP was chosen for the formulation of chitosan nanoparticles because of its efficacy of nanocarrier formation.

2.10 Targeting Ligands Profile

2.10.1 Sialic acid

2.10.1.1 Synonyms: 5-N-acetyl-neuraminic acid, NANA, Neu5Ac

2.10.1.2 Chemical structure:



Sialic acid

2.10.1.3 IUPAC name: 5-acetamido-2,4-dihydroxy-6-(1,2,3-trihydroxypropyl)oxane-2-carboxylic acid

2.10.1.4 Molecular formula: $C_{11}H_{19}NO_9$

2.10.1.5 Molecular weight: 309.27

2.10.1.6 Storage: 0-4°C

Salic acid (SA) is a nine-carbon carbohydrate that has α-keto acids sugars. Of the more than 50 sialic acid derivatives that have been found so far, 5-N-acetyl-neuraminic acid (Neu5Ac) and N-glycolyl-neuraminic acid (Neu5Gc) are the most prevalent types. The only form of sialic acid that is present in healthy humans is called Neu5Ac. Neu5Gc, on the other hand, is thought to be a non-human sialic acid and is present in many animal species as well as human cancer cells [203,204]. It's interesting to note that SA modulates cell-cell interactions between leukocytes, platelets, cancer cells, and endothelial cells. As a result, it is a well-known ligand for selectins, the adhesion molecule that spreads tumor metastasis and inflammation. When applied as ligands or targeting moieties, sialic acids have the ability to identify and bind particular molecules, such as proteins, on the surface of the chosen target. In addition, because SA moieties are found on the surface of viruses and cancer cells, SAs can be recognised and bound using nanocarriers that have been designed to bind WGA or

specific antibodies against SA. Over the past three decades, several hydrophilic compounds, such as carbohydrates, have been added to the nanosystem surface to inhibit opsonization and escape immune system identification. Among these, sialic acid (SA), the most prevalent carbohydrate on the membrane of the erythrocyte, is crucial for the RES escape of these cells, enabling them to enter the circulation unabated by immune system absorption [205].

This discovery led to the surface engineering of nanocarriers containing SA to target tumor cells, as described in multiple publications. The sialic acid-blocking glycomimetic P-3Fax-Neu5Ac was rationally designed to effectively prevent cancer spread. P-3Fax-Neu5Ac was formulated into poly(lactic-co-glycolic acid nanoparticles coated with antityrosinase-related protein-1 antibodies), which allowed for gradual release, long-term sialic acid blockage, and targeted delivery of P-3Fax-Neu5Ac into melanoma cells. Most notably, in a mouse lung metastasis model, intravenous infusions of melanoma-targeting P-3Fax-Neu5Ac nanoparticles inhibited the generation of metastases. These results highlight the role that sialoglycans play in the spread of cancer and support the idea that cancer metastases can be effectively prevented by sialic acid blockage utilising glycomimetics that are specifically designed to target cancer cells. This targeted approach to disrupt sialic acid-dependent pathways is widely applicable to infections and inflammations in addition to many cancer types [26].

The most significant derivative utilised in a study coating liposomes with SA to target tumor cells is sialyl Lewis X (SLEx). A significant reduction in side effects and toxicity was seen when compared to the free medication following *in vivo* administration of modified LPs loaded with various antitcancer agents in mice. Furthermore, a notable rise in drug accumulation within the tumor region resulted in the suppression of tumor expansion [206,207]. Two more recent studies have been described using several SA derivatives to target tumors. Specifically, Zheng et al. synthesised modified selenium nanoparticles (NPs) with SA to investigate their impact on human cervical cancer cells (HeLa): SA-modified NPs showed improved cytotoxicity and absorption by cancer cells. Conversely, it was found that incubating SA-modified selenium NPs on non-cancer cells resulted in a decreased level of toxicity [208].

In order to target elevated cancer cells, sialic acid (Neu5Ac) was employed as a targeting moiety or ligand in the formation of chitosan nanoparticles intended to target lung cancer.

2.10.2 Cetuximab

2.10.2.1 Brand name: Erbitux

2.10.2.2 Biological Type: Chimeric (mouse/human) Monoclonal antibody (mAb)

2.10.2.3 Molecular Formula: $C_{6484}H_{10042}N_{1732}O_{2023}S_{36}$

2.10.2.4 Molecular Weight: 145781.6 Da

2.10.2.5 Therapeutic Target: EGFR receptor

Cxmab is a ligand for the endothelial growth factor receptor. It is a recombinant chimeric (human/mouse) IgG1 mAb that competes with EGFR for binding and competitively inhibits EGF. EGFR belongs to the ErbB family of tyrosine kinase receptors, which has been identified in both healthy and cancerous cells. It controls the homeostasis and growth of epithelial tissues. EGFR seems to be associated with many distinct forms of cancer because it is frequently overexpressed in cancerous cells [209].

EGFR frequently undergoes mutations in cancer and acts as a promoter of carcinogenesis. Under normal circumstances, EGFR controls alveolar and bronchial epithelial cell development, differentiation, and migration; nevertheless, when overactive in cancer, EGFR promotes lung cancer cell proliferation, metastasis, and invasion. With little involvement in SCLC, EGFR is one of the onco-receptors that are strongly expressed in 85% of NSCLC. To treat lung cancer, several mAbs (panitumumab, cetuximab) and tyrosine kinase inhibitors (erlotinib, gefitinib, lapatinib) target EGFR. Cxmab has been demonstrated to produce anti-tumor effects *in vitro* in a variety of cancer cell lines and human tumor xenografts [210].

Cxmab was approved by the FDA in February 2004 and available in market under the brand name ERBITUX. It has been researched for advanced colon cancer, head and neck cancer, NSCLC with EGFR expression, and squamous cell skin cancer treatment. Cxmab is given intravenous infusion and

can be used alone or in conjunction with other chemotherapy agents such as platinum drugs, leucovorin, fluorouracil, and irinotecan and with radiation treatment [211].

The previously reported study postulated that targeting EGFR on surfaces of cancerous cells with an anti-EGFR mAb coupled to drug-loaded PLGA-NPs could be a viable strategy for reversing drug resistance and improving drug absorption in EGFR overexpressing tumor cells. This strategy would involve targeting EGFR with an anti-EGFR mAb [212].

The delivery of nanoparticles to various antigens on the surface of cancer cells is now being accomplished in a significant way by the use of monoclonal antibodies. Drug-targeting therapy can benefit from the greater expression of this antigen in cancerous cells that separates them from healthy cells. By coupling monoclonal antibodies to the nanoparticles, the therapeutic treatment's systemic toxicity is decreased due to improved drug targeting [213]. Generally, many receptor expressions were discovered in cancer cells.

Targets of monoclonal antibodies include receptors that are highly expressed in lung cancer, including the EGFR, EPCAM and NSE receptor and other receptors. However, it has a significant disadvantage of quick clearance from circulation, leading to a short half-life and rendering the dose inactive. Therefore, mAbs continue to be conjugated with nanoparticles to enhance their targeting capabilities and mitigate their drawbacks [214].

Recently, it was discovered that ligand-anchored NPs selectively attach to EGFR extracellular zones, releasing medicaments intracellularly to block the signalling pathway in tumors. In this particular methodology, gelatin nanocarriers that are coupled to biotinylated-EGF ligands have been employed to transport higher quantities of cisplatin to lung cancer cells via inhalation route, and demonstrated a substantial reduction in tumor volume [155]. In the same line, the targeted delivery of erlotinib to lung cancer cells through the use of chitosan-liposome complexes coupled with DNA aptamers has been demonstrated, precisely targeting the epidermal growth factor receptor (EGFR) [215]. In addition, polymeric nanoparticles that are coupled to monoclonal antibodies have demonstrated good efficacy against acquired EGFR-kinase resistance in cancerous cell lines and may be used to decrease

EGFR-resistant pathways in lung tumors. In addition to improving treatment efficacy with prolonged drug release rate, ligand-bound nanocarriers promote the transport of monoclonal antibodies or site-specific tyrosine kinase inhibitors to lung cancer cells. They also minimise off-site toxicities and endosomes clearance [216].

Lung cancer treatment uses a monoclonal antibody (cetuximab) conjugated with chitosan nanoparticles as a therapeutic strategy to target the overexpressed EGFR receptor.

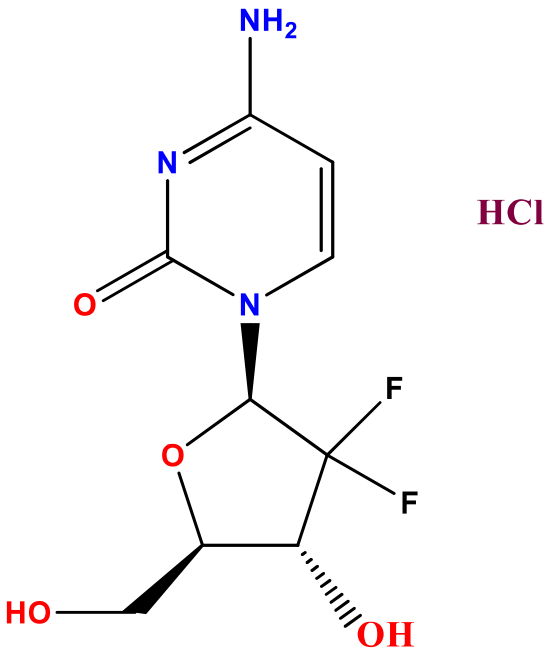
2.11 Drug Profile

2.11.1 Gemcitabine

2.11.1.1 Synonyms: Gemzar

2.11.1.2 Brand Name(s): Gemzar, Infugem

2.11.1.3 Chemical structure:



2.11.1.4 IUPAC name: 4-amino-1-[(2*R*,4*R*,5*R*)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one;hydrochloride

2.11.1.5 Molecular formula: $C_9H_{11}F_2N_3O_4.HCl$

2.11.1.6 Molecular weight: 263.20 + 36.46

2.11.1.7 Storage: Desiccate at 4°C

2.11.1.8 Physical state & appearance: White to off white solid powder

2.11.1.9 pKa: 3.6

2.11.1.10 Partition coefficient: log (P) value of -1.4.

2.11.1.11 Melting point: 168.64˚C.

2.11.1.12 BCS class: III

2.11.1.13 Solubility: Soluble in water, slightly soluble in methanol and practically insoluble in ethanol and polar organic solvents.

2.11.1.14 Formulation: IV Injection: 200 mg vial powder and 1 g vial powder.

2.11.1.15 Pharmacokinetics:

2.11.1.15.1 Absorption: IV infusion of 30 minutes: Peak plasma concentrations of GMC 10 to 40 mg/L.

2.11.1.15.2 Plasma Protein Binding: Less than 10%.

2.11.1.15.3 Volume of distribution: IV infusion < 70 min: 50 L/m^2; IV infusion 70-285 min: 370 L/m^2

2.11.1.15.4 Metabolism: Metabolized intracellularly by nucleoside kinases to active metabolites GMC diphosphate (dFdCDP) and GMC triphosphate (dFdCTP), also metabolized intracellularly and extracellularly by cytidine deaminase to inactive metabolite difluorodeoxyuridine (dFdU).

2.11.1.15.5 Clearance: IV infusion < 70 min: 41 to 92 L/h/m^2.

2.11.1.15.6 Excretion: Renal excretion.

2.11.1.15.7 Biological Half-life: IV infusion < 70 min: 0.7 to 1.6 hours; IV infusion 70-285 min: 4.1 to 10.6 hours.

2.11.1.16 Indications and uses:

GMC marketed under the brand name Gemzar and is administered intravenously. The Food and Drug Administration has approved it for the treatment of pancreatic cancer as monotherapy, advanced ovarian cancer when combined with carboplatin, metastatic breast cancer when combined with paclitaxel, non-small cell lung cancer when combined with cisplatin.

2.11.1.17 Adverse effects:

Myelosuppression like anaemia, leukopenia, neutropenia, and thrombocytopenia. Also raises alanine transaminase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase levels.

2.11.1.18 Gemcitabine loaded Nano-particulate systems

Wang et al., developed folic acid-grafted CSN-NPs for the targeted and controlled administration of GMC in the treatment of lung cancer. The research findings show that the formulation has improved targeting, localised delivery, increased bioavailability, and higher retention potential in tumor cells. Also, the abundance of folic acid receptors made it easier to target tumors. Newly developed nanoparticles (NPs) ensure that the medicine reaches and remains in tumor tissues. In comparison to the free drug, this accumulation is significantly greater in ligand-conjugated NPs. Further, the addition of folic acid to nanoparticles might dramatically boost their affinity for human lung cancer cells, according to *in vitro* cell uptake studies [217].

Wang et al., specifically targeted NSCLC (A549) cells that overexpress the epidermal growth factor receptor (EGFR) with gemcitabine loaded cetuximab-modified poly (lactic) acid (PLA) nanoparticles. The resulting NPs were extremely homogenous in size (120 nm) and shape (spherical). NPs displayed controlled release pattern at pH 5.0 as compared to pH 7.4. The effectiveness of the therapeutic system's chemotherapeutic regimen can be considerably enhanced by sustained drug release under physiological settings and a quicker release under tumor pH. Cxmab decorated PLA NPs were taken

up more efficiently by A549 cancer cells, demonstrating their ability to actively target epidermal growth factor receptor (EGFR) dependent receptors. For targeted NPs, the fluorescence intensity was almost twice as much of non-targeted NPs in cancerous cells. Confocal microscopy was used to show that the targeted NPs were taken inside the cell by EGFR mediated signalling. Due to the specific delivery of gemcitabine to the EGFR overexpressing cancer cells, the MTT assay demonstrated that the enhanced effect of killing cancerous cells by Cxmab-conjugated NPs. Last but not least, gemcitabine cetuximab attached PLA NPs demonstrated higher levels of cell death (early and late apoptosis 40%) than non-targeted NPs. The outcomes demonstrated that antibody conjugation on the surface of NPs might be a promising therapeutic approach for cancerous cells that overexpress EGFR. The findings imply that nanoparticles (NPs) targeted towards the epidermal growth factor receptor (EGFR) have the ability to be utilized in the therapeutic management of non-small cell lung cancer (NSCLC) [6].

Guo et al. discovered that incorporating a hydrophobic phosphorated GMC prodrug in A6-conjugated micelles (A6-mHPG) mediates targeted treatment of CD44+ lung tumor xenografts in mice. Notably, A6-mHPG exhibits enzymatic degradation resistance, increased absorption by CD44+ lung tumors, reduced side effects, and efficient suppression of lung tumor growth, all of which contribute to a significant rise in overall survival compared to free HPG and mHPG controls. A6-mHPG is small and homogeneous in size, relatively easy to develop. The targeted administration of GMC prodrug via biodegradable micelles investigates to be an interesting technique for enhancing GMC therapy in advanced tumors [17].

Mottaghitalab et al., developed silk fibroin NPs (SFNPs) with gemcitabine loaded for the treatment of lung cancer in animals. The SP552 peptide was coupled to gemcitabine-loaded SFNPs in order to target the tumorigenic lung tissue. The structural and physicochemical characteristics of the SFNPs were characterized using a variety of techniques. The created nanoparticles (NPs) had the perfect size, zeta potential, shape, and structural features for drug delivery. In addition, compared to non-targeted SFNPs and control groups, the targeted gemcitabine-loaded SFNPs showed higher increased cytotoxicity, cellular uptake, and accumulation in the lung tissue. After that, Lewis lung carcinoma

(LL/2) cells were injected intratracheally into the lungs of mice in order to evaluate the effectiveness of treatment of the designed drug delivery system using a mouse model of lung tumor induction. Histopathological evaluations and single-photon emission computed tomography (CT) images demonstrated effective induction of lung tumors. Additionally, the results demonstrated that targeted gemcitabine-loaded SFNPs had a better potential for treating induced lung tumors than the control groups. According to histological and radiological examinations, animals treated with targeted NPs also showed higher survival rates, lower death rates, and no evidence of metastasis. This study illustrated a successful chemotherapeutic drug delivery method for specialised targeting of a produced lung tumor, which may be beneficial in the future for the treatment of lung malignancies [218].

Zhang et al., reported a unique method using the tumor-penetrating peptide iRGD to increase the treatment effectiveness of gemcitabine for NSCLC. In this work, iRGD and gemcitabine were used to treat xenografts of human NSCLC made with the A549 cell line in nude mice. The findings showed that iRGD might efficiently boost gemcitabine to stop tumor cell growth and trigger apoptosis. The therapeutic effectiveness in *in vivo* model was also markedly improved. These findings showed that combination therapy with iRGD would be a potential strategy for enhancing gemcitabine's clinical effectiveness in the management of NSCLC [219].

Hatami et al. claimed that gambogic acid (GA) enhances the therapeutic efficacy of gemcitabine (GMC) in NSCLC. In NSCLC cells, the GA and GMC combo therapy showed synergistic anti-tumor effects using a variety of functional tests. These findings demonstrated that GA can enhance GMC induced cell cycle arrest and apoptosis. By downregulating the expression of MDR1, RRM1, anti-apoptosis protein (Bcl-2) and upregulating the expression of apoptosis proteins (Bax), co-treatment method improved the anti-tumor effects of GMC. Particularly, the *in vivo* assessment conducted in a xenograft tumor mouse model demonstrated an improved efficacy of GMC when administered in conjunction with GA. In summary, the results of this study demonstrate that GA can be used as an adjuvant therapy for NSCLC treatment in addition to GMC. In future, additional research is necessary

in order to validate the efficacy of this combined treatment for non-small cell lung cancer (NSCLC) in animal models on a larger scale [220].

2.12 Nanomedicine system suited for parenteral administration

Typically, injectable parenteral dosage forms can be prepared as solutions, suspensions, or emulsions. However, the emergence and progress of the field of nanotechnology has opened up new avenues for improving efficiency and expanding the applications of these traditional dosage forms. A number of advantages justify the use of nanoparticle platforms for injection-based parenteral drug delivery. These involve improving the solubility of water insoluble drugs, which improves their absorption into the body i.e bioavailability, producing long-term release parenteral depots, allowing for targeted administration to specific cells and tissues and protecting the incorporated drugs from harsh extracellular and intracellular environment [221].

Nanomedicines can be delivered parenterally, but they must be sterile. Nanomedicines can be sterilized using a variety of techniques. Presently, the most common sterilization methods for nanoparticles are sterile filtration, autoclaving, ionizing radiation, and nonionizing radiation; yet additional techniques used include treatment with chemical substances such as gases ethylene oxide, and formaldehyde. But according to the type of nanoparticles, the sterilization method must be very carefully chosen [222].

2.13 Stability of nanomedicine

Stability problems are major issue in nanomedicines. Most of nanomedicnes needs precisely defined storage conditions. Techniques like lyophilization, spray drying, addition of PEG imparts good physical stability and can help in prolonging the shelf-life of the nanomedicine [223].