Chapter 1

Introduction

1 Introduction

According to the report published by International Agency for Research on Cancer, 2020, lung cancer (LC) is the one of the most commonly occurring types of cancer (2.1 million new cases of lung cancer were reported in 2018), in addition to being the leading cause of death worldwide (causing 1.8 million deaths in 2018). A schematic distribution of the cancer related deaths from various types of cancers (i.e., liver, colon, rectal, stomach, breast, and other tumours) has been shown in **Figure 1.1** [1]. Of the deaths occurring due to lung cancer, 63% are attributed to tobacco smoking and the percentage can go as high as 90% in countries where smoking is prevalent in both the sexes. Cigarette smoking is identified as major factor contributing to occurance of lung cancer [2]. A potent carcinogen found in cigarette smoke is benzo(a)pyrene (B[a]P). Its reactive metabolite7,8-diol-9,10-epoxide, can cause mutations and makes DNA adducts that initiate the progression and proliferation of cancer [3]. Besides smoking, genetic and environmental factors, previous occurrence of lung disease, and occupational exposures are also responsible for causing lung cancer. Lung diseases like emphysema, tuberculosis, chronic bronchitis, pneumonia, and other chest related infections increase the probability of occurrence of lung cancer. Further, exposure to crystalline silica, asbestos, radon, heavy metals, polycyclic aromatic hydrocarbon compounds increase the risk of lung cancer [4].

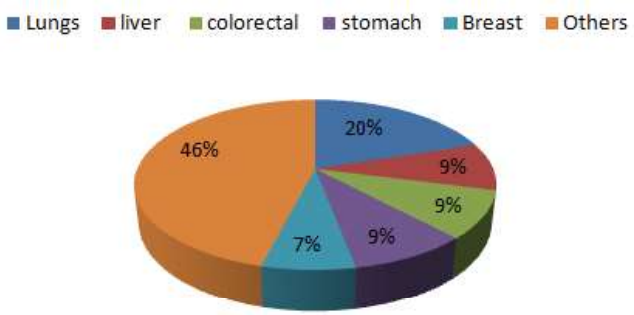

Figure 1.1 Schematic percentage distribution of deaths worldwide due to various types of cancers [1].

Existing remedies for curing lung cancer include chemotherapy, radiotherapy, surgery and biotherapy. These therapies are, however, associated with some limitations as shown in **Table 1.1** that restrict their usefulness. However, chemotherapy still remains the first line of therapeutic approach for treatment. Conventional chemotherapeutic drugs, which have been frequently used to combat LC, are

challenged by high rates of drug resistance and significant toxicity load [5]. Despite the fact that several medications, including gemcitabine, paclitaxel, and docetaxel are being utilised to treat tumours, these medications have shown little success in completely curing the disease. The primary disadvantage and unfavourable effects of conventional chemotherapeutic agents has been caused by the excessive concentration of anticancer drugs in normal tissues and the unavailability of these medicines in malignant tissues. As a result, there is an immediate need to develop novel formulations or therapeutic strategies to enhance LC treatment [6].

Gemcitabine (2,2-difluoro-2-deoxycytidine), is one of the most widely used chemotherapeutic drug authorised by the FDA for NSCLC treatment [7]. GMC is a cell cycle specific nucleotide like antimetabolite that usually entails an active transport method in which it is phosphorylated into active state (50-triphosphategemcitabine), acts by cross linking with DNA to arrest malignant cells in G1/S phase, inhibiting DNA synthesis and tumour growth, and eventually result in cell death [8]. However, the drug is metabolised to its inactive metabolite (2′,2′-di fluorodeoxyuridine) through an enzyme cytidine deaminase (CDA), which is found in plasma, kidney, and liver [9]. Gemcitabine has a low plasma half-life and a limited exposure time to tumours, as around 77% of it is eliminated into the urine within 24 hours of treatment, either unaltered or as an inactive metabolite [10,11]. However repeated delivery of GMC has undesirable cytotoxic impact on healthy cells. It has been found that some individuals experience drug resistance to GMC because of decreased intracellular transport of GMC [12]**.** These are the main factors limiting its effectiveness as a chemotherapeutic agent.

Table 1.1 Overall limitations associated with traditional lung cancer strategies.

Chemotherapy	Surgery	Radiation therapy	Biotherapy
Non-specific distribution of the chemotherapeutic agent in the body	Chance of organ loss	Severe toxicity to bronchial tree	Depends only on inhibition of specific cellular pathways
Multidrug resistance(MDR)	Risk of reoccurrence of cancer	Radiation-induced lung injury and pneumonitis	Targeting of multiple cellular pathways results in toxicity
Toxicity to healthy cells	Not used for all types of lung cancer	Loss of various lung functions	

The nanocarrier based targeted therapeutics has the potential to selectively enhanced drug deposition at the target regions. It can improve therapeutic efficacy with minimization of adverse and toxic

effects of chemotherapeutic agents [13]. Many nanocarrier based strategies have been explored in recent years to improve therapeutic efficiency of GMC in a variety of malignancies and has demonstrated enhanced tumour tissue accumulation and anticancer efficacy of GMC [14]. If nanocarriers exhibit appropriate physicochemical attributes (e.g., size distribution, shape, and surface charge), the EPR effect leads them to concentrate in malignancies. [5]. However, passive tumour targeting based on the EPR effect results in nonspecific tumour targeting, hence cell specific targeting functionalities need to be linked to nanocarriers to improve their tumour specific targeting potential [15]. The coupling of specific ligands (small molecules, peptides, and antibodies) to nanocarriers capable of interacting with receptors abundantly expressed in cancerous cells is an active tumour targeting method and has been extensively applied in association with passive tumour targeting approaches as shown in **Figure 1.2** [16]. GMC nanomedicines were developed for active targeting to improve the treatment of pancreatic and lung tumours by targeting the epidermal growth factor receptor (EGFR), folate receptor, plectin-1, or substance P [17]. So, molecularly targeted strategies might be a viable option for delivering chemotherapeutic medicines into cancer tissues [18]. In lung cancer epidermal growth factor receptor (EGFR) and sialic acid binding receptors are highly expressed and can be used for EGFR and sialic acid binding receptor targeted anticancer therapeutics [19,20].

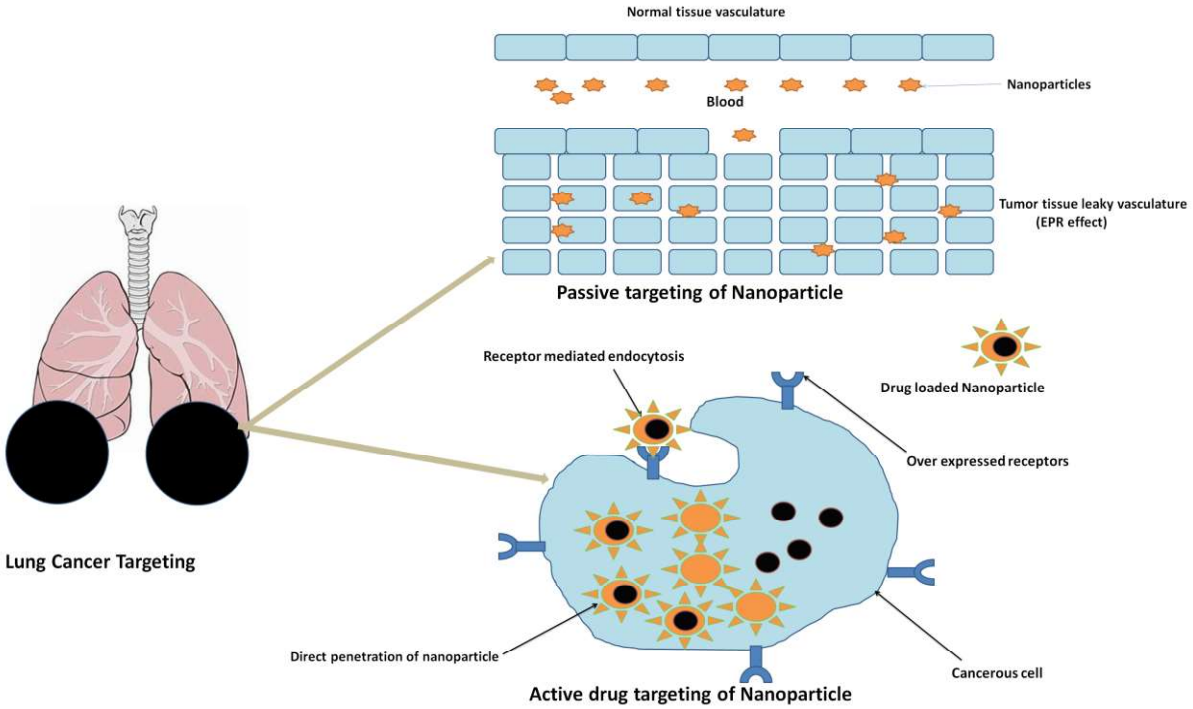

Figure 1.2 Schematic illustration of targeting mechanism in lung cancer cells by nanotechnology. Passive tumour targeting of nanocarriers by enhanced permeability and retention effect and active tumour targeting of nanocarriers via receptor mediated endocytosis.

EGFR, a part of the human EGF category of tyrosine kinase receptors is abundantly expressed in several cancerous cells, particularly LC [21]. It has been demonstrated that NSCLC is accompanied with 85% of EGFR overexpression and EGFR mutations are actively involved in multiple physiological functions such as tumour growth, differentiation, angiogenesis, metastases, and tumour development [22]. As a result, EGFR has emerged as a therapeutic target for nanoparticle-mediated cancer therapy in the management of NSCLC [23]. It has been discovered that anti-EGFR specific target treatments based on specific monoclonal antibodies and inhibitors of tyrosine kinase efficiently suppress the EGFR downstream signalling pathway and offer a promising therapeutic option for LC treatment [24]. Various monoclonal antibodies, including cetuximab, gefitinib, erlotinib, oximertinib, and panitumumab, have been designed to inhibit EGFR overexpression [23]. Cetuximab (Cxmab), an immunoglobulin G monoclonal antibody, has continually been used either as standalone or in conjunction with other drugs for advanced NSCLC. It is noted that Cxmab targets EGFR over-expressing cancerous cells through the use of receptor-mediated EGFR phosphorylation and signal

block down. According to investigations, Cxmab has the capability to be used a targeting ligand for the delivery of chemotherapy medicines [25].

Sialic acids (N-acetylneuraminic acid) are a broad class of sugar compounds that attach to the terminal end of glycans (sialoglycans) on the surface of the cell of glycoproteins and glycolipids in nearly all vertebrate cells [26]. Cancer cells increase sialyltransferase expression as they undergo tumour progression, which causes an influx of sialoglycans to accumulate on their surface [27]. Sialyltransferases precisely bind sialic acids to other sugar molecules in glycans via particular glycosidic linkages [28]. On cancerous cells, hypersialylation (overexpression of glycan chains, primarily N-acetylneuraminic acid), enhances immune evasion and carcinoma cells proliferation, is related to metastasis and resistance to treatment [29]. In general, hypersialylation is more common in tumour tissues than in normal tissues [30]. Interfering with sialic acid expression in cancerous cells might potentially be significant in suppressing tumour metastasis since various phases of the metastatic process could be targeted simultaneously. Exogenously administered sialic acid may detect sialic acid expression on cancer cells and hence can be employed as tumour targeting moiety [31]. Accordingly, desialylation of cancerous cells by synthesized small compounds (glycomimetics) that reduce sialic acid production in cancerous cells may have a significant therapeutic potential and has been shown to limit metastasis development in murine metastasis models [26]. To improve intratumoral transportation of nanocarriers, strategies for sialic acid targeting may be used [16].

Chitosan (CSN) is a cationic, linear and naturally occurring polysaccharide consisting of varying quantities of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units that are produced from partial deacetylation of chitin in an alkaline conditions [32]. It has remarkable qualities for drug delivery, being biodegradable, biocompatible, pH-stimuli sensitive with nontoxicity, and mucoadhesive properties [33]. The aqueous solubility of CSN containing amino groups is greater at acidic condition than those at neutral pH. It has shown to improve the passage of complex macromolecules through mucosal membranes and intracellular compartments [34]. It is anticipated that polymeric NPs, remain stable for an extensive period under normal physiological environments (pH 7.4), indicating the presence of intact nanocarriers in bloodstream, hence significantly lowering

the adverse effects to normal tissues [35]. However, the use of pH sensitive nanoformulations may increase the effectiveness of chemotherapeutic medications in malignancies by delivering drugs at targeted sites [36]. When drug loaded CSN-NPs are taken up by cancerous cells through endocytosis, a rapid release may take place at a lower pH (pH 5.5), area around the tumour site or within the endosomes and lysosomes of tumour cells, and this may help in increasing the therapeutic effectiveness of the chemotherapeutic agents [37].

Accordingly, the goal of the current research was to increase the therapeutic effectiveness of GMC by designing a drug loaded formulation with dual targeted characteristics, (based on pH-stimuli and receptor-targeting). Chitosan (CSN) is a popular pH-responsive drug carrier and was used in the present study to facilitate pH-dependent GMC release. In order to facilitate receptor targeting, conjugated CSN nanoparticles with receptor targeting moieties were developed to improve the site specific delivery of anticancer agents. The addition of targeted ligands is likely to enhance the affinity and specificity of chemotherapeutic agents.

So, we have designed and developed targeted nanocarriers, wherein chitosan nanoparticles functionalized with EGFR antibody cetuximab (Cxmab) and glycan receptor targeting moiety 5-N-acetyl-neuraminic-acid (Neu5Ac) could deliver gemcitabine to tumour tissues in a targeted fashion with improved cellular internalization and therapeutic efficiency, as shown in **Figure 1.3**.

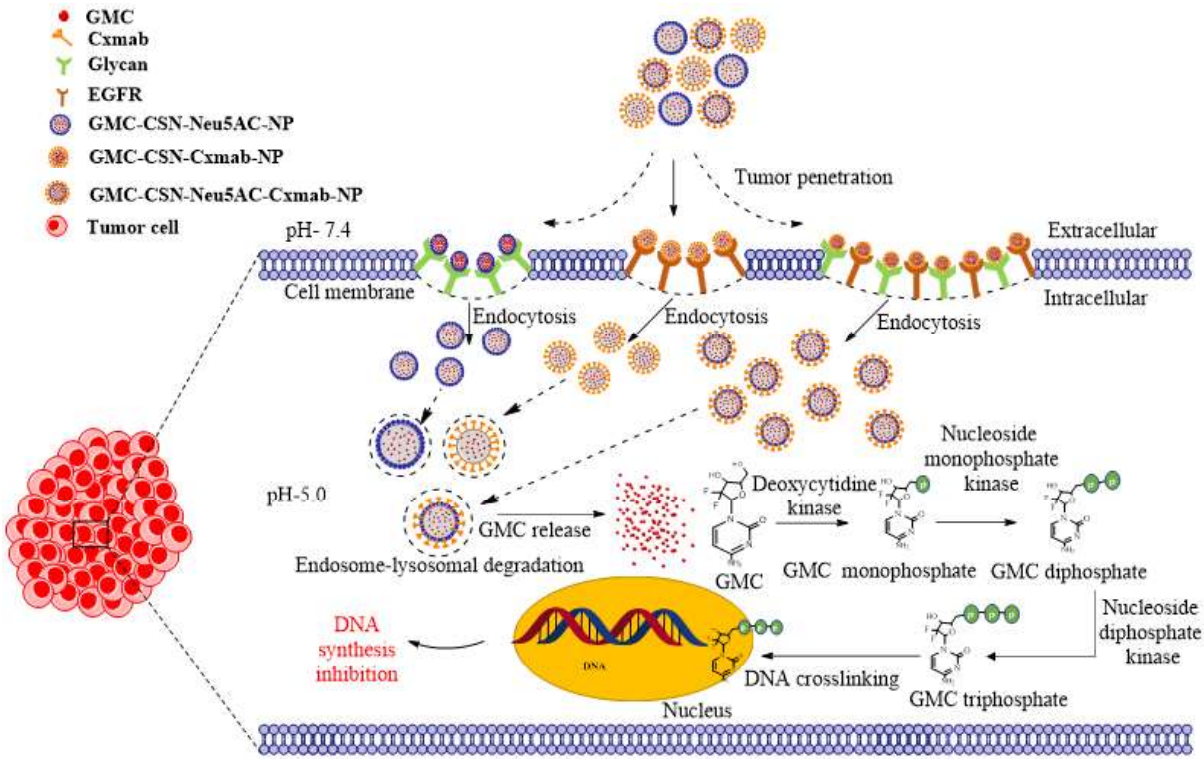

Figure 1.3 A schematic demonstration of mechanism of targeting EGFR and N-acetyl-neuraminic acid receptors (glycans) and proposed mechanism for intracellular pH dependent release, tumour penetration and circulatory route of chitosan nano-formulations functionalized with EGFR antibody and glycan receptor targeting moiety.

As the methods used for the formulation of CSN-NPs are complex and are greatly influenced by the variables involved, optimization of critical parameters by conventional method is not possible. So, advanced techniques like design of experiments (DoE) has been used to reduce the number of trials to large extent. The experiments were designed using design expert software and Box-Behnken design was applied. DoE works based on variables to optimize the results and predict the influence of factors in a suitable range. DoE was comprehensively used in the application of Quality by design (QbD) in the study. In QbD, the product and process knowledge is the vital designer of acceptable quality in the final product. Knowledge is accomplished by creating models linking the inputs with the outputs of the process. The optimization was done to predict the best formulation by response surface methodology based Box Behnken Design (BBD) followed by desirability approach based numerical optimization technique.