Chapter 2

Literature Review

2.1. Treatment of lung cancer

The basic choice of therapy depends upon the size of cancer and its location. Lung cancer can be treated with various methods such as surgery, radiation therapy, photodynamic therapy, and chemotherapy and target specific therapy [69].

2.1.1.Surgery

The surgery is performed basically for stage-I and II type of Non-small cell lung cancers. Three majorly employed techniques for removal of lung cancer include (a) Lobectomy (removal of lung lobe), (b) Wedge resection (removal of mass harmful tissues of the lung), (c). Pneumonectomy (removal of lungs). Generally, 10-35% of lung cancers can be cured with surgery but complete eradication may not be possible. Towards small cell lung cancer, the surgery is not possible because of its presence in more than one specific location which is difficult to operate. Whereas, non-small cell lung cancer can be cured with surgery when it is not spread beyond the lungs [70].

Win et al., in a prospective study of 110 patients undergoing potentially curative lung cancer surgery showed that patients had high levels of functioning and low levels of symptoms after surgery. Overall quality of life had deteriorated significantly 1 month after surgery ($p = 0.001$) but had returned to preoperative levels by 3 months ($p = 0.93$). Symptoms have also returned to baseline levels by 6 months [71].

Myrdal et al., observed that Lung cancer patients who undergo open traditional surgical resection have a quality of life comparable to that of coronary bypass surgery patients. Lung cancer patients have poorer physical function because of reduced pulmonary function, but show no sign of increased anxiety or depression. Those who continued to smoke after surgery had impaired mental health [72].

Handy et al., in a study of lung cancer surgery patients from three hospitals that were administered the Short-Form 36 Health Survey (SF-36) and the Ferrans and Powers' quality-of-life index (QLI) before surgery and 6 months after surgery, observed a significant impairment in preoperative functional health status in patients after lung cancer surgery. A significant number of patients died during the 6 months after surgery. Pain and impairment of functional health status persists for 6 months after lung cancer resection [73].

2.1.2.Radiation Therapy

The radiation therapy is used to shrink the tumor size but complete eradication is difficult. In ration therapy initially the tumor size is reduced using high energy X-Rays/Ionizing Radiation such as Radium (228 Ra), Iridium (192 Ir), Phosphorus (32 P), Cobalt (60 Co) followed by surgery for the removal of remaining harmful cells. The radiation therapy is generally categorized into two types (a). Brachy therapy (radioactive source in pellets is placed close to cancer cells), (b). Tele-therapy (source placed outside the body (irradiate). The major side effects are fatigue, reduction of White Blood Cells (WBC). If the patient is very sensitive to the radiation they may have nausea, vomiting, skin irritation [74].

Kashowitz et al., in a retrospective study of clinical Stage I non-small cell carcinoma patients treated with definitive radiation therapy alone, observed that the actuarial overall survival rate was 19% at 3 years and 6% at 5 years, with a median survival time of 20.9 months. Of the 49 deaths, 35 died of lung cancer; 13 died of intercurrent illness, and one died of pancreatic cancer, which made the actuarial cause-specific survival 33% at 3 years and 13% at 5 years. The results were found to be inferior compared to surgical therapy [75].

Wisnivesky et al., also observed in a study involving patients with histologically confirmed, stage I and II non-small cell lung cancer between 1988 and 2001, who did not undergo surgical resection and had been treated with radiation therapy that the survival of patients was significantly better compared to the untreated patients (stage I cancer, $p = 0.0001$; stage II cancer, $p = 0.001$). The overall survival time of patients with stage I disease who underwent radiotherapy was 21 months compared to 14 months for untreated patients. Also, stage II patients who received and did not receive radiation therapy had median survival times of 14 and 9 months, respectively [76].

Sause et al., in a phase III clinical trial involving patients with surgically unresectable clinical stage II, IIIA, and IIIB non-small cell lung cancer, with a Karnofsky Performance Status of$\geq$ 70 and a weight loss of < 5% for 3 months before study, tried to test whether chemotherapy followed by radiation therapy resulted in superior survival to either hyperfractionated radiation or standard radiation in surgically unresectable non-small cell lung cancer. The results revealed that median survival for standard radiation was found to be 11.4 months and, for chemotherapy and irradiation, it was 13.2 months. In case of hyperfractionated irradiation, median survival was 12 months. The respective 5-year survivals were 5% for standard radiation therapy, 8% for chemotherapy followed by radiation therapy, and 6% for hyperfractionated irradiation [77].

2.1.3.Photodynamic Therapy (PDT)

PDT employs a photosensitizing agent that is activated by light of a specific wavelength to produce reactive singlet oxygen ($^1O_2$) that mediates cellular cytotoxicity [78]. The Porfimer sodium is the first-generation photo sensitizer where it is widely used for the diagnosis of the lung cancer. When the photo sensitizers (Porphyrin, Texaphyrin and

Chlorine) incorporated into the specific site and irradiation with external light of specific wavelength, they move from the ground state to excited singlet later to triplicate state. Oxygen plays a major role in PDT, it stays in the ground triplet state. As singlet oxygens are highly reactive, it reacts with the substrate (S) to generate oxidized products. Alternatively, a Type I process can occur when the triplet state photosensitizer reacts directly with an organic molecule or substrate to produce a radical or radical ion both in the photosensitizer and the substrate. These radicals destroy the cancer cells. This PDT is likely useful in the diagnosis [79].

Furuse et al., conducted a prospective phase-II study to evaluate the activity and toxicity of photodynamic therapy (PDT) with photofrin II in centrally located early-stage lung cancer and to determine the complete response (CR) rate as the primary end point. They observed that PDT with photofrin II has an excellent effect on patients with centrally located early-stage lung cancer who have limited tumor invasion extending over a small area (< or = 1 cm) [80].

Cai et al., investigated the effectiveness of photofrin-photodynamic therapy (PDT) for stage II–IV intractable bronchial lung cancer in a group of thirty patients with lumen obstruction, who failed previous treatment regimens such as surgery, radiotherapy and chemotherapy. PDT was performed with 630 nm laser light (Diomed) delivered through cylinder diffusing tip quartz fibers that was passed through the biopsy channel of a flexible endoscope 48 h after intravenous injection of photofrin (2 mg/kg body weight). 72 h after the first irradiation, the endoscopic procedure was repeated, necrotic tissues were mechanically removed and the deep original lesions and newly exposed cancer lesions were re-treated, and, if necessary, the areas were cleaned repeatedly. The results showed that photofrin-PDT effectively reduced the amount of lumen obstruction and improved the quality of life of patients [81].

2.1.4.Chemotherapy

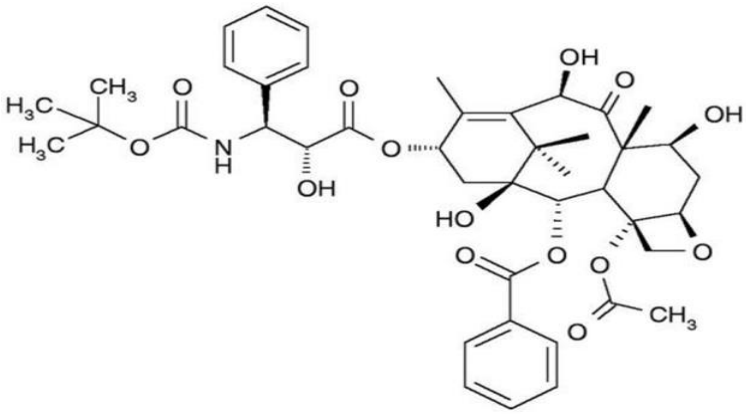
Due to the deficiency in early-stage diagnostics, most lung cancers are only detected at advanced stages, with local tumor invasion or distant metastasis and are not suitable for surgery [82]. Systemic chemotherapy treatment modality that addresses the majority of lung cancers is currently the mainstay of advanced lung cancer treatment regimens, aimed at extending survival and improving quality of life [83]**.** The recommended treatment for patients with advanced NSCLCs involves systemic platinum-based chemotherapy (e.g., cisplatin, oxaliplatin) combined with taxanes (such as paclitaxel or docetaxel) or gemcitabine [84]. Combination drug therapies are being explored, as the synergistic action of multiple drugs could potentially lead to better therapeutic efficacy and reduced possibility of drug resistance development by the cancer cells [85] . Gemcitabine-platinum and cisplatin-gemcitabine-bevacizumab are some of the drug combinations currently in clinical trials for the treatment of advanced NSCLC [86,87].

2.2. Docetaxel

Docetaxel is a second-generation taxane derived from the needles of the European yew tree. It has a wide spectrum of antitumor activity and a number of unique preclinical characteristics compared to other chemotherapeutic agents, including the taxane paclitaxel. For instance, in several murine and human tumor cell lines, docetaxel exhibited 1.3- to 12-fold greater cytotoxicity relative to paclitaxel. A similar pattern emerges from its broad spectrum of activity *in-vivo* with murine tumor models and human tumor xenografts. Furthermore, unlike paclitaxel, docetaxel exhibits linear pharmacokinetics and, due to differences in drug efflux, is retained intracellularly for a longer period. Docetaxel is highly effective as monotherapy and combination therapy across a variety of tumor types, including breast, lung, and ovarian, as well as head and

neck, gastric, and prostate carcinomas. It acts by promoting microtubulin assembly and thus stabilize the polymers against depolymerization, thereby inhibiting microtubule dynamics. This in turn blocks number of vital cellular functions in which microtubules play a critical role such as impairment of mitotic progression leading to cell cycle arrest. This blocks progression of a cell through its natural division cycle and, consequently, inhibits cell proliferation. Docetaxel has also shown to inhibit angiogenesis and its effect is four times stronger than that of paclitaxel [88,89].

2.2.1. Chemical structure:



***2.2.2. IUPAC name*:** 1,7β,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-{(2*R*,3*S*)-3-[(*tert*-butoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoate}

***2.2.3. Solubility*:** In water (0.0127 mg/ml), DMSO (25 mg/ml), and Ethanol (25 mg/ml)

2.2.4. Molecular formula & molecular weight: 807.88 Da, $C_{43}H_{53}NO_{14}$

2.2.5. Pharmacokinetics:

Half-life elimination: 11 h (terminal)

Protein bound : 94-97%

Volume of distribution : 80-90 l/m²

Metabolism : Liver (CYP3A4)

Clearance : 21 l/h/m²

Excretion : Feces

2.2.6. Mechanism of Action:

Docetaxel prevents the depolymerization of cellular microtubules, which results in DNA, RNA, and protein synthesis inhibition. Docetaxel promotes microtubulin assembly and stabilize the polymers against depolymerization, thereby inhibiting microtubule dynamics. This mechanism of action employed by docetaxel is distinct from that of other antimicrotubule agents, which prevent microtubule assembly. As a consequence, number of vital cellular functions will be hampered in which microtubules play a critical role. Mainly, impairment of mitotic progression leading to cell cycle arrest is considered to be a principal component of docetaxel's mechanism of action. This blocks progression of a cell through its natural division cycle and, consequently, inhibits cell proliferation.

2.2.7. Safety:

Docetaxel enhanced progression-free survival and overall response rate compared with vinca alkaloid in numerous clinical trials on 2,080 subjects as first-line treatment ($p \sim 0.05$). Docetaxel led to a lower rate of anemia as first-line treatment ($p \sim 0.05$). Moreover, docetaxel caused less grade 3/4 hematological and non-hematological toxicity compared with vinca alkaloid. Docetaxel showed better effectiveness and safety on patients with advanced non-small-cell lung cancer as first-line therapy and also causes lower toxicity as second-line therapy compared with vinca alkaloid [90-94].

2.2.8. Recent studies:

Maya et al., prepared docetaxel loaded chitosan cross-linked γ-poly(glutamic acid) (γ-PGA) nanoparticles were prepared by ionic gelation method and decorated with cetuximab *via* EDC/NHS chemistry, for targeting EGFR over-expressing non-small-cell-lung-cancer. Prepared targeted nanoparticles exhibited enhanced cellular internalization and therapeutic activity, by actively targeting EGFR on NSCLC cells. The A549 cells treated with targeted formulation underwent a G2/M phase cell cycle arrest followed by reduction in mitochondrial membrane potential of A549 cells, inducing apoptosis and necrosis resulting in enhanced cancer cell death [95].

Gu et al., developed a novel type of reduction-sensitive nanoparticles based on cross-linked lipoic acid (LANPs)for targeted delivery of docetaxel to lung cancer. The results showed that DTX-LANPs had a particle size of 110 nm and a negative zeta potential of −35 mv with excellent colloidal stability. LANPs efficiently encapsulated DTX with a high drug loading of 4.51% ± 0.49% and showed remarkable reduction-sensitive drug release *in-vitro*. Cellular uptake experiments demonstrated that LANPs significantly increased intracellular DTX uptake by about 10 fold as compared with free DTX. The cytotoxicity of DTX-LANPs showed significantly higher potency in inhibiting A549 cell growth than free DTX, while blank LANPs had a good biocompatibility. In addition, *in-vivo* experiments demonstrated that DTX-LANPs could enhance tumour targeting and anti-tumour efficacy with low systemic toxicity [96].

Chi et al., formulated biomimetic platelet membrane (PM)-coated docetaxel (DTX)-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles for satisfactory lung cancer therapy. The resulting core–shell NP exhibited a hydrodynamic diameter of 98.2 nm, quite suitable for the enhanced permeability and retention (EPR) effect and slowed down the release of loaded DTX and effectively suppressed the growth of tumor cells

in-vitro. NP showed long circulation times and effective lung tumor-targeting ability due to PM facilitated immune escape. Prepared biomimetic NP significantly inhibited the tumor growth in A549 cell-bearing nude mice. In addition, the prepared NP effectively reduced the DTX toxicity compared with that of free DTX [97].

Jin et al., formulated docetaxel-loaded PEG-albumin nanoparticles (PEG-DANPs) for the treatment of non-small cell lung cancer (NSCLC) and their efficacy was compared with the commercial product of docetaxel (Aisu®) and docetaxel-albumin nanoparticles (DANPs). PEG-DANPs showed a dose- and time-dependent efficacy in the *in-vitro* cytotoxicity studies; the tumors growth and the metastases in the livers of NSCLC-bearing nude mice *in-vivo* were reduced markedly by PEG-DANPs, and the PEG-DANP-treated mice had a minimum of weight loss; furthermore, the mice which were treated with PEG-DANPs can survive longer than the other groups. PEG-DANPs have the lowest side-effects, and the highest antitumor and metastases activity [98].

Zhu et al., developed novel T7 peptide-modified nanoparticles based on carboxymethyl chitosan, which is capable of targeted binding to the transferrin receptor (TfR) expressed on lung cancer cells and precisely regulating drug-release according to the pH value and reactive oxygen species (ROS) level. The results showed that the drug-loading of docetaxel (DTX) and curcumin (CUR) was approximately 7.82% and 6.48%, respectively. Good biosafety was obtained even when the concentration was as high as 500 μg/ml. More importantly, the DTX and CUR coloaded formulation exhibited better *in-vitro* and *in-vivo* anti-tumor effects than DTX monotherapy and other nanocarriers loaded with DTX and CUR alone. Furthermore, The prepared nanoformulation can ameliorate the immunosuppressive micro-environment to promote the inhibition of tumor growth [99].

2.3. Targeted therapy of lung cancer

The main limitations of current chemotherapy for the treatment of lung cancers are lack of target specificity, recurrence and superficial increase in lifespan of human beings. Further, oral and IV administrations of the anti-cancer drugs have many drawbacks such as degradation of the drug molecule in the stomach pH, alterations in the drug molecule during the process of metabolism in the liver, lack of specificity in the conventional method of treatment and lack of specificity which causes toxicity and side effects [100]. Poor overall survival rates in NSCLC patients may also be attributed to intrinsic radiation resistance due to the increased ability of cancer cells to repair DNA damage after radiation therapy (RT). Therefore it is crucial to develop a system that can overcome the above limitations and provide targeted and controlled releases of these therapeutic reagents for effective chemo-radiation therapy to treat these lung cancer patients [101].

Targeted therapy is a treatment that targets cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival. These treatments are very focused and work differently than chemotherapy. This type of treatment blocks the growth and spread of cancer cells while limiting damage to healthy cells. The most important targeted treatment options in lung cancer currently include the vascular endothelial growth factor receptor (VEGFR) inhibitors, EGFR inhibitors, 4–Anaplastic lymphoma kinase fusion gene (ALK) inhibitors, B-Raf enzyme (BRAF) inhibitor [102].

2.3.1. VEGFR inhibitors

For tumors to grow, they need to form new blood vessels to keep them nourished. This process is called angiogenesis. The vascular endothelial growth factor A (VEGF) is the main mediator of angiogenesis. In addition, VEGF contributes to cancer growth and

metastasis. VEGF overexpression and/or high VEGF serum levels have been reported in lung cancer. Some targeted drugs, called angiogenesis inhibitors, block this new blood vessel growth [103].

2.3.1.1. Bevacizumab (Avastin)

It is used to treat advanced lung cancer. It is a monoclonal antibody that targets vascular endothelial growth factor (VEGF), a protein that helps new blood vessels to form. This drug is often used with chemotherapy for a time. Then if the cancer responds, the chemotherapy may be stopped and the bevacizumab given by itself until the cancer starts growing again [104].

2.3.1.2. Ramucirumab (Cyramza)

It can also be used to treat advanced NSCLC. VEGF has to bind to cell proteins called receptors to act. This drug is a monoclonal antibody that targets a VEGF receptor. This helps stop the formation of new blood vessels. This drug is most often given after another treatment stops working. It is often combined with chemo [105].

Because of the risks of bleeding, these drugs typically aren't used in people who are coughing up blood or who are taking blood thinners. The risk of serious bleeding in the lungs is higher in patients with the squamous cell type of NSCLC, which is why most current guidelines do not recommend using bevacizumab in people with this type of lung cancer [106].

Pang et al., developed hyaluronic acid (HA) decorated, pH sensitive lipid-polymer hybrid nanoparticles (LPH NPs) to co-deliver erlotinib (ERL) and bevacizumab (BEV) for targeted therapy of NSCLC. All LPH NPs samples have particle sizes of about 100−120 nm, polydispersity index values range from 0.12 to 0.15, and negative zeta potentials. HA-ERL/BEV-LPH NPs contained pH sensitive adipic acid dihydrazide

(ADH) showed fast drug release at pH 5.5 than pH 7.4. After 21 days, the tumor volume of the HA-ERL/BEV-LPH NPs group (229.2 ± 13.1 mm^3) was significantly smaller than 0.9 % NaCl control group (1126.3 ± 39.4 mm^3), with a tumor inhibition rate of 79.7 ± 3.2 %. The maximum plasma ERL concentrations, half-life, and area under the curve of HA-ERL/BEV-LPH NPs were 21.6 μg/ml, 7.57 h, and 290.3 mg/l·h). VEGF targeted LPH-NPs showed the highest tumor tissue accumulation concentration (25.3 μg/ml) and low system toxicity [107].

Hao et al., retrospectively evaluated the efficacy and safety of the nab-paclitaxel and bevacizumab combination in patients with advanced non-squamous (NSQ) NSCLC after failure of at least one prior systemic regimen between February 2012 and December 2018 at the Cancer Hospital of the Chinese Academy of Medical Sciences (Beijing, China). Results revealed that combined nab-PTX and bevacizumab might be an effective treatment regimen for patients with advanced NSQ NSCLC after failure of at least one prior systemic regimen with prolonged objective response rate (ORR), progression-free survival (PFS), overall survival (OS), and safety [108].

Garon et al., in a multicenter, double-blind, randomized phase 3 trial, assessed the efficacy and safety of docetaxel plus ramucirumab or placebo as second-line treatment for patients with stage IV non-small-cell-lung cancer (NSCLC) after platinum-based therapy. A total of 1825 patients were screened between Dec 3, 2010, and Jan 24, 2013, out of which 1253 patients were randomly allocated to treatment. Results revealed that ramucirumab plus docetaxel improved survival as second-line treatment of patients with stage IV NSCLC with manageable toxicities, appropriate dose reductions and supportive care [109].

2.3.2. EGFR inhibitors

The epidermal growth factor receptor (EGFR) is a cell-surface receptor belonging to ErbB family of tyrosine kinase and it plays a vital role in the regulation of cell proliferation, survival and differentiation. Indeed, EGFR is overexpressed in a variety of human cancers including lung, head and neck, colon, pancreas, breast, ovary, bladder and kidney, and gliomas. More than 60% of non-small cell lung cancers (NSCLCs) show EGFR overexpression, whereas no overexpression is detected in small cell lung cancer. The overexpression of EGFR is presumably caused by multiple epigenetic mechanisms, gene amplification, and oncogenic viruses. Drugs called *EGFR inhibitors* can block the signal from EGFR that tells the cells to grow. Some of these drugs can be used to treat NSCLC [110].

- Cetuximab(Erbutix)
- Erlotinib (Tarceva)
- Afatinib (Gilotrif)
- Gefitinib (Iressa)
- Osimertinib (Tagrisso)
- Dacomitinib (Vizimpro)

These drugs can be used alone (without chemo) as the first treatment for advanced NSCLCs that have certain mutations in the *EGFR* gene. These mutations are more common in women and people who haven't smoked. Erlotinib can also be used for advanced NSCLC without these mutations if chemotherapy isn't effective [111].

2.3.2.1. Cetuximab (Erbitux™)

It is a class of chimeric (mouse/ human) monoclonal G1 immunoglobulin, is the most advanced mAb developed against EGFR that targets and binds to the exterior domain

of the EGFR. The US FDA approved it in February 2004 for clinical applications in the treatment of various cancers *via* intravenous infusion. The halflife of cetuximab is approximately 7 days in humans [112] which allows once-weekly dosing in combination with standard chemotherapy regimens. After internalization of cetuximab, downregulation from cell surface and consequent reduction of EGFR dependent downstream signaling pathways occurs due degradation of receptor without phosphorylation and/or activation. Cetuximab mediated receptor downregulation induces cytotoxicity associated with the inhibition of the receptor function [36].

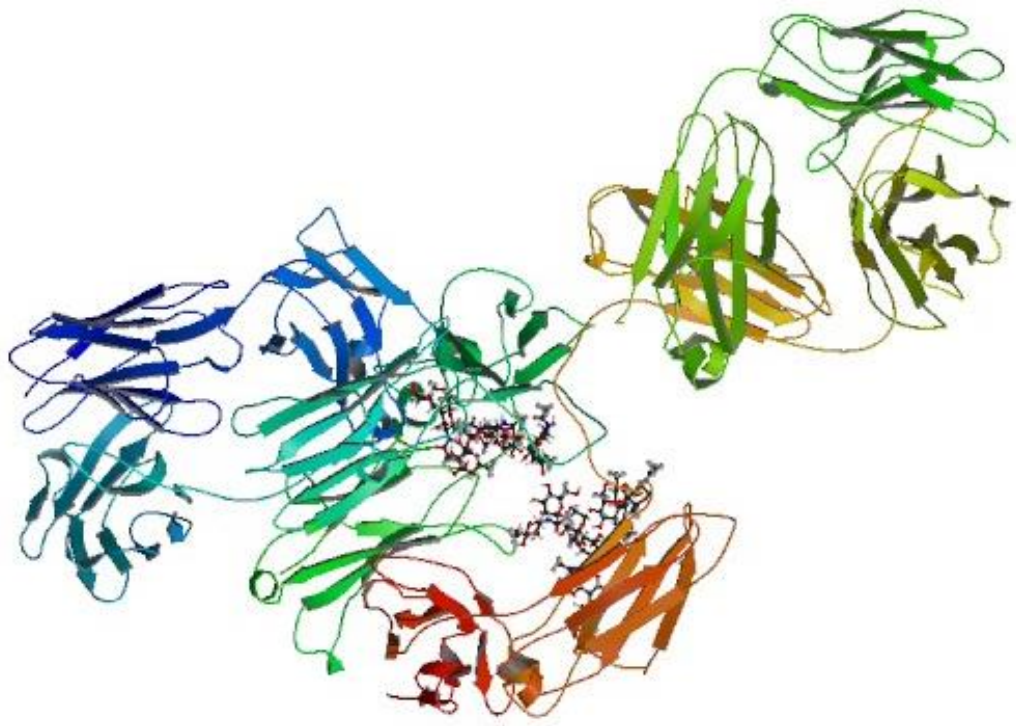


Figure 2.1. 3D structure of cetuximab

- ***Type:*** Whole antibody
- ***Source:*** Chimeric (mouse/human)
- ***Target:*** EGF receptor
- ***Formula:*** $C_{6484}H_{10042}N_{1732}O_{2023}S_{36}$
- ***Molar mass:*** 145781.92 g·mol^{-1}

Pirker et al. evaluated the efficacy of cetuximab plus chemotherapy and chemotherapy alone in a multinational, multicenter, open-label, phase III trial between October, 2004-January, 2006, and the subjects were patients (≥18 years) with advanced EGFR-expressing histologically or cytologically proven stage wet IIIB or stage IV non-small-cell lung cancer. Chemotherapy was cisplatin 80 mg/m^2 intravenous infusion on day 1, and vinorelbine 25 mg/m^2 intravenous infusion on days 1 and 8 of every 3-week cycle) for up to six cycles. Cetuximab at a starting dose of 400 mg/m^2 intravenous infusion over 2 h on day 1, and from day 8 onwards at 250 mg/m^2 over 1 h per week was continued after the end of chemotherapy until disease progression or unacceptable toxicity had occurred. The results established that addition of cetuximab to platinum-based chemotherapy can be an effective treatment option for patients with advanced non-small-cell lung cancer [113].

Patel et al. developed anti-EGFR antibody cetuximab conjugated and docetaxel loaded poly(lactide-co-glycolide) nanoparticles (NP) to target EGFR overexpressed on non-small cell lung cancer cells to improve cytotoxicity and site specificity. I*n-vitro* release study demonstrated sustained release of drug from NP with 25% release at pH 5.5 after 48 h. *In-vitro* cytotoxicity studies demonstrated higher anti-proliferative activity of NPs than unconjugated NP. Cell cycle analysis and apoptosis study were performed to evaluate extent of cell arrest at different phases and apoptotic potential for the formulations, respectively. *In-vivo* efficacy study showed significant reduction in tumor growth and so antibody conjugated NP present a promising active targeting carrier for tumor selective therapeutic treatment [114].

2.3.3. ALK inhibitors

About 5% of NSCLCs have a rearrangement in a gene called *ALK*. This change is most often seen in non-smokers (or light smokers) who have the adenocarcinoma subtype of NSCLC. The *ALK* gene rearrangement produces an abnormal ALK protein that causes the cells to grow and spread. Drugs that target the abnormal ALK protein include:

- Crizotinib (Xalkori)
- Ceritinib (Zykadia)
- Alectinib (Alecensa)
- Brigatinib (Alunbrig)
- Lorlatinib (Lorbrena)

These drugs can often shrink tumors in people whose lung cancers have the *ALK* gene change. Although they can help after chemo has stopped working, they are often used instead of chemotherapy in people whose cancers have the *ALK* gene rearrangement. At least some of these drugs also seem to be useful in treating people whose cancers have changes in the *ROS1* gene [115,116].

2.3.4. Drugs that target cells with BRAF gene changes

In some NSCLCs, the cells have changes in the *BRAF* gene. Cells with these changes make an altered BRAF protein that helps them grow. Some drugs target this and related proteins:

Dabrafenib (Tafinlar) is a type of drug known as a *BRAF inhibitor*, which attacks the BRAF protein directly.

Trametinib (Mekinist) is known as a *MEK inhibitor*, because it attacks the related MEK proteins. These drugs can be used together to treat metastatic NSCLC if it has a certain types of *BRAF* gene changes [117,118].

2.4. Nanomedicine in the treatment of NSCLC

The application of nanotechnology to medicine is the foundation for the development of nanomedicine with the ultimate goal of improving the quality of patient's life [119]. The cancer nanotechnology is the application of nanotechnology for cancer therapy. It is an integrated application of nano platform to draw benefits for the welfare of human beings [120,121]. It is a technology in which the drug loaded nanomedicine of 1-1000 nm, exhibit strong interaction between anticancer drugs and cancer cells or tumor tissues [122-124]. Recent advancements in nanotechnology have contributed to the development of nanomedicine systems that enabled specific delivery of several micro and or macromolecules including anticancer drugs, antibody, protein, targeting ligand and/or imaging agents to the targeted cancer cells [125].

Advanced nanomedicine is multifunctional in nature, capable of delivery of therapy to the diseased cells with the help of targeting ligand and biomarkers. The nanomedicine can work better than other drug delivery systems since they have advanced capabilities in an all-in-one single platform, which include sustained/controlled release, targeted delivery, higher transport efficiency by endocytosis, stimulus responsive agent release (*i.e.,* smart delivery), synergetic performance (e.g., combination therapy, siRNA co-delivery), multimodality diagnosis and/or therapies and quality performances (e.g., oral delivery, escape from multi drug resistance (MDR) protein, autophagy inhibition, etc) [126].

Lung cancer mortality rates are not only due to multi drug resistance, but also attributable to delayed diagnoses. Nanotheranostics may be the solution to NSCLC due to its unique, targeted delivery system that simultaneously allows for diagnosis and treatment assessment as well as improving therapeutic efficacy. Many diagnostic agents have been developed in the recent past for tumour imaging such as [127]

Gold nanoparticles (AuNPs),

Magnetic nanoparticles (MNPs),

Mesoporous silica nanoparticles (MSNPs),

Quantum dots (QDs)

Upconversion nanoparticles (UCNPs)

Carbon nanotubes (CNTs)

Polymeric nanoparticles (PNPs)

Polymeric micelles (PMs)

Solid lipid nanoparticles (SLNs)

Dendrimers

Bio-dots etc.

2.4.1. Advantages of cancer nanomedicine

The anticancer drugs usually suffer from low solubility, rapid *in-vivo* degradation, poor pharmacokinetics and undesirable biodistribution, stimulated *in-vivo* clearance rate and inability to cross biological barrier. These all limitations of anticancer drugs can be overcome by using nanomedicine systems [128]. Nanomedicine has unique properties such as nanoscale size (1-1000 nm), high surface-to-volume ratio, and favorable physicochemical characteristics and also potential to modulate both the pharmacokinetic and the pharmacodynamic profiles of drugs, thereby enhancing their therapeutic index. Furthermore, nanotechnology can help to improve distribution and targeting efficiency to ensure selective deposition of drug in the target site but low concentrations in healthy tissues or organs by virtue of the EPR effect. Hence, nanomedicine may prolong the circulation time of anticancer drug and internalize into cancer cells followed by cellular uptake mechanisms [129,130].

Recently, nanomedicine has also been proved to target multiple cancer markers and deliver multiple agents simultaneously to treat and prevent diseases at cellular and molecular levels. Several thousands of publications suggest that the nanomedicine is effective in several cancer treatments (*i.e.*, lung cancer, brain cancer, and liver cancer etc.) [131]. Some nanomedicine has been developed as multifunctional in nature which was highly capable of the specific delivery of the anticancer drugs to the lung cancer cells with the help of targeting ligands (*i.e.*, hyaluronic acid, folic acid, and transferrin, etc) [132,133].

2.4.2. Polymers used in nanomedicine

The nanomedicine may be constructed from a wide variety of organic and inorganic materials. Owing to their smaller sizes, they can move through microvasculatures and across various biological barriers to preferentially accumulate in tumor tissues due to the EPR effect, because of leaky vasculature and poor lymphatic drainage at cancer site. Previous several studies have shown the advancement in nanotechnology, and material science has resulted in a large number of organic and inorganic nanomedicine platforms. Some important nanomedicines are polymeric nanoparticles, liposomes, silica nanoparticles, polymeric micelles, carbon nanotubes etc. [134].

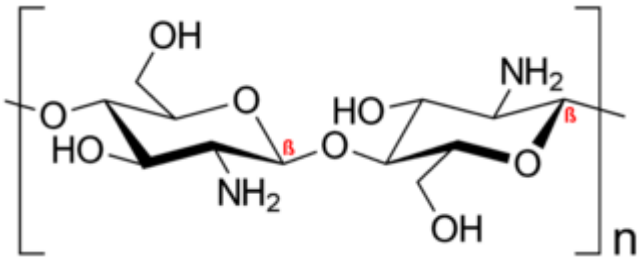
Recently, polymeric nanoparticles based drug delivery systems have been extensively investigated for solid tumor treatment. Tumor targeted nanoparticles have demonstrated significant potential for improved accumulation in the tumor tissue and also reduced side effects of anticancer drugs [135]. Apart from this, these nanomedicines are stable in blood, non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory and do not activate neutrophils, biodegradable and avoid RES uptake [136].

The polymeric nanoparticles offered several advantages for drug delivery such as biocompatibility, storage stability, protection of loaded drug/diagnostic agent and controlled/sustained release on target site [137]. In several previous studies, many biodegradable polymers are used for nanoparticles preparation to provide biological compatibility with less cytotoxicity [138,139]. Chitosan and its derivatives, poly(ethylene glycol)-poly (L-glutamic acid) (PEG-PGA), poly(ethylene-glycol) (PEG), poly(2-hydroxy ethyl methacrylate) (PHEMA), poly(butyl cyanoacrylate), poly(D,L-lactide)–poly (ethylene glycol) (PLA–PEG), poly(D,L-lactide-co-glycolide) acid (PLGA), poly(D,L-lactide-co-glycolide)-D-α-tocopheryl polyethylene glycol 1000 succinate (PLGA-TPGS), poly(ε-caprolactone) (PCL), poly(ε-caprolactone)-D-α-tocopheryl polyethylene glycol 1000 succinate (PCL–TPGS), poly(hydroxy butyrate) (PHB), poly(L-glutamic acid) (PGA) poly-N-(2-hydroxypropyl-methacrylamide) (PHPMA), poly(methyl methacrylate) (PMMA), poly(L-glutamic acid)-poly(ethylene glycol) (PGA-PEG), poly(L-lactide-co-2-methyl-2(2-dicarba-closo dodecarborane) propyl-oxycarbonyl-propyne carbonate), poly(vinyl alcohol) (PVA), polyethylene glycol-poly (D,L-lactide-co-glycolide) acid (PEG-PLGA), poly-lactic acid-d-α-tocopheryl polyethylene glycol 1000 succinate (PLA-TPGS), poly(D,L-lactide-co-glycolide)–poly(ethylene glycol) (PLGA-PEG), poly-lactic acid-D-α-tocopheryl polyethylene glycol 1000 succinate (PLA-TPGS) and poly-lactic acid-poly (ε-caprolactone)-D-α-tocopheryl polyethylene glycol 1000 succinate (PLA-PCL-TPGS), are the highly used biodegradable polymers for the preparation of polymeric nanoparticles [140,141]. PLA and PLGA are easily hydrolyzed into their monomers (*i.e.*, lactic acid or glycolic acid) and eliminated from human systems *via* metabolic pathways [142,143].

2.4.3. Chitosan as polymer material for nanoformulation

Interest in the application of natural polymers as components of drug delivery systems has greatly increased over the past decade. Natural polymers have distinct advantages over their synthetic counterparts; commonly natural polymers are non-toxic, biodegradable and allow for cell-specific targeting. Among the various biopolymers, chitosan is a linear copolymer of β(1→4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose. Chitosan is an example of a basic polysaccharide, the pKa of chitosan amine groups is ~6.5 and therefore solubilized by diluted acids through protonation of the amino group, rendering the corresponding chitosan salt in solution.

2.4.3.1.Chemical structure:



2.4.3.2.Synonyms: Chitosan, Deacetylchitin, Poliglusam, Chicol

2.4.3.3.Molecular formula & molecular weight: $C_{56}H_{103}N_9O_{39}$, 1526.5 Daltons

2.4.3.4.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-5-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-4,5-dihydroxy-6-(hydroxy methyl) oxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-hydroxy-6-(hydroxy methyl)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-(hydroxy

methyl) oxan-3-yl]oxy-4-hydroxy-2-(hydroxymethyl)oxan-3-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-3-yl]carbamate

2.4.3.5. Applications in nanomedicine:

Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it is useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin [144-146].

Chemical modifications have been applied to prepare chitosan derivatives with enhanced biological and physicochemical properties. Chitosan is a nontoxic, semicrystalline, biodegradable and biocompatible linear polysaccharide of randomly distributed N-acetyl glucosamine and glucosamine units. Due to the presence of amine groups in its structure, chitosan is converted to a polyelectrolyte in acidic media. Since many minerals and cells carry negative charges, the positive charge of chitosan interacts strongly with these negatives surfaces. Chitosan can bind to materials such as cholesterol, fats, proteins and tumor cells. It has further shown an affinity towards proteins, such as wheat germ agglutinin and trypsin. Owing to its cationic nature, electrostatic complexes are used for encapsulation of drugs, immobilization of enzymes and as a gene carrier. For instance, fibroblasts which exhibit a more negative charge surface when compared to keratinocytes, exhibit a higher adhesion to chitosan. Interactions of this kind has been applied to build up polymer/polymer ionic complexes involving chitosan and negatively charged polymers. Chitosan can be modified to chitosan nanoparticles by emulsion cross linking, spray-drying, reverse micellar

method, template polymerization, polyelectrolyte complex, precipitation and ionotropic gelation method [147]. Chitosan is biocompatible with living organisms and tissues as it does not cause any allergic or rejection reactions and is biodegradable since it breaks down gradually leaving harmless amino sugars that the body absorbs, as its products. The mucoadhesive and low toxicity properties of chitosan have been well documented and have fueled the continual investigation of chitosan drug delivery systems. Chitosan's mucoadhesive properties increase the residual time at the site of absorption; thus, increasing efficiency of drug delivery. Unlike many hydrophobic and amphiphilic polymers, such as poly (lactide-co-glycolide) (PLGA) and poly(lactic) acid (PLA)–poly(ethylene)glycol (PEG), chitosan can avoid the use of hazardous organic solvents during the nanoparticle production process because it is soluble in aqueous acidic solution [148]. Chitosan's free amine groups and cationic nature allow for ionic crosslinking with multivalent anions for the production of nanoparticles. Chitosan's positive charge also allows for particles to act as permeation enhancer across epithelial cell membranes. It is because of these many benefits and advantageous properties that chitosan is of particular interest in the field of nano-drug delivery. Chitosan as a base component has been explored for use in buccal, intestinal, nasal, periodontal, wound healing and many other drug delivery systems, which have taken the form of beads, microcapsules, microspheres, nanoparticles and tablets [149,150].

2.5. Methods of producing polymeric nanoparticles

The polymeric nanoparticles can be prepared from the dispersion of preformed polymers or by direct polymerization of monomers. On the other hand, polymeric nanoparticles can be directly synthesized by polymerization of monomers using emulsion polymerization method. The choice and the method of preparation depend

on some of the factors such as the type of polymeric system, the area of application, size requirement, etc. Their size depends on the preparation method, ranging from 10-1000 nm. The therapeutic agents are either physically entrapped or chemically conjugated to the hydrophobic core of polymeric nanoparticles. The main advantages of polymeric nanoparticles are high stability and controlled drug release, either through diffusion or degradation of the polymeric matrix [151-153].

2.5.1. Solvent evaporation

In the solvent evaporation, polymer solutions are prepared in volatile solvents and emulsions are formulated with the commonly used volatile solvent such as dichloromethane, chloroform and ethyl acetate. The emulsion is converted into a nanoparticles suspension on evaporation of the solvent from the system, which is allowed to diffuse through the continuous phase of the emulsion [154]. In the conventional methods, two main strategies are being used for the formation of emulsions: the preparation of single-emulsions, *e.g.*, oil-in-water (o/w) or double-emulsions, and water-in-oil-in-water, (w/o/w). These methods utilize high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants and then the product is lyophilized [155].

2.5.2. Dialysis method

The dialysis method is commonly used for the preparation of small and narrow-distributed polymeric nanoparticles. A polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cut-off. Dialysis is

performed against a non-miscible solvent. The displacement of the solvent inside the membrane is followed by the progressive aggregation of the polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles [156,157].

Nah et al. prepared poly(DL-lactide-co-glycolide) (PLGA) nanoparticles by dialysis method and investigated drug loading capacity and drug release. The size of PLGA nanoparticles was found to be 269.9+118.7 nm with spherical shape from the observation of SEM and TEM micrographs. PLGA nanoparticles were monomodal pattern with narrow size distribution in the empty and lower drug loading nanoparticles whereas bi- or trimodal pattern was showed in the higher drug loading ones. Release of clonazepam from higher drug loading PLGA nanoparticles was seemingly slower than that with lower loading [158].

Xie et al. developed self-assembled biodegradable nanoparticles by a direct dialysis method for the delivery of anticancer drug paclitaxel. Paclitaxel-loaded poly(D,L-lactic-co-glycolic acid) (PLGA) and poly(L-lactic acid) (PLA) nanoparticles self-assembly was achieved by direct dialysis. The encapsulation efficiency and *in-vitro* release profile were measured by high-performance liquid chromatography. Particle cellular uptake was studied using confocal microscopy, microplate reader, and flow cytometry. In addition, the cytotoxicity of this drug delivery system was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on C6 glioma cell line to predict the possible dose response of paclitaxel-loaded PLGA and PLA nanoparticles [159].

2.5.3. Coacervation/precipitation

The coacervation/precipitation method is based on the physical and chemical properties of chitosan, which make it insoluble in alkaline pH medium. This insolubility in

alkaline medium means that chitosan precipitates/coacervates out of solution when mixed with an alkaline solution. One method of applying these principles to generate chitosan nanoparticles is to blow chitosan solution into an alkali solution using a compressed air nozzle. Commonly sodium hydroxide, NaOH-methanol or ethanediamine alkali solutions are used. The particles are separated and purified either through filtration, centrifugation or both and are then put through successive washing with hot and cold water. The particle size is controlled through altering the compressed air pressure and/or the spray-nozzle diameter. The release mechanics can be altered through the addition of a crosslinking agent, such as glutaraldehyde, to harden the particles. This method is most commonly used for microparticle production, but can be applied for nanoparticle production. The method that is most frequently used for chitosan nanoparticle production is known as complex coacervation [160].

2.5.4. Emulsion-droplet coalescence

The emulsion-droplet coalescence method employs both precipitation and emulsion cross-linking principles. Unlike the emulsion cross-linking methods applied to produced chitosan microparticles, which cross-links stable droplets, in this method precipitation is induced by allowing the merging (coalescence) of chitosan droplets with NaOH droplets. This method was first utilized by Tokumitsu *et al*. The first step is to produce a stable emulsion containing aqueous chitosan and therapeutic agent solution, which is generated in liquid paraffin oil. The same process is used to produce another stable emulsion containing chitosan aqueous solution in NaOH. Then the emulsions are mixed using high-speed stirring, which causes random collisions and coalescence of droplets from each emulsion; thus, precipitating chitosan droplets and producing nanoparticles. Tokumitsu *et al* encapsulated gadopentetic acid using this method for

gadolinium neutroncapture therapy. They found that particle size depends upon the degree of chitosan deacetylation, which as it decreased the particle size increased, while drug encapsulation efficiency decreased. However, increasing the gadopentetic acid concentration in the chitosan solution did not increase the particle size and did increase the encapsulation efficiency. Their investigation produced nanoparticles with a mean particle size of 452 nm and 45% gadopentetic acid loading using 100% deacetylated chitosan [161].

2.5.5. Reverse micellization

Reverse micelles are mixtures of water, oil and surfactant that are thermodynamically stable and have a dynamic behavior. When viewing up-close at the microscopic scale, the structure of reverse micelles consists of aqueous and oil volumes separated by surfactant films, as the observer zooms out to a more macroscopic scale, reverse micelles appear homogeneous and isotropic. The advantage of reverse micellar formation is that ultrafine polymeric nanoparticles with narrow size distributions are produced; while applying traditional emulsion polymerization methods, larger nanoparticles (>200 nm) are formed with broad size distributions. The reverse micelle aqueous core acts as a nanoreactor in preparation of ultrafine nanoparticles. The minute dimensions and narrow size distribution of nanoparticles, produced through the reverse micellar method, are due the small size of the reverse micellar droplets themselves, which are usually between 1 and 10 nm, and their high degree of monodispersity. When performing the reverse micelle method, surfactant is first dissolved in an organic solvent in order to create the reverse micelles and then the chitosan and drug aqueous solution is added under constant vortexing to avoid any turbidity. The system can be manipulated based on the amount of aqueous phase and the generally aqueous phase is

maintained at a level where the entire mixture is an optically transparent microemulsion. The aqueous phase also dictates the particle size and, with an increased amount of water, larger nanoparticles are obtained. Like any other type of nanoparticle system, the maximum drug loading varies between different therapeutic agents; but in reverse micelles, maximum drug loading can be achieved by gradually adding drug until the clear microemulsion is converted into a translucent solution. The next step is to add a crosslinking agent under constant stirring and mix overnight to allow for complete cross-linking [162,163].

2.5.6. Ionotropic gelation

Ionotropic gelation is a process where a polyelectrolyte is cross-linked with a counter ion forming a hydrogel. The structures of hydrogels are maintained by hydrogen bonding, hydrophobic forces, ionic forces or molecular entanglements. This technique has been applied using a variety of materials, including gellan gums, alginates, carboxymethyl cellulose and chitosan, to create micro and nanoparticles for encapsulation and controlled release of therapeutic agents. Depending on the material used, the strength of the counter ion and the desired particle size, several methods of ionotropic gelation can be applied, including syringe dropping and air atomization for bead formation, and flush mixing for nanoparticles formation [164]. The application of ionotropic gelation, which utilizes chitosan's positive charge to cross-link it with an anion to form micro- and nanoparticles, has garnered much attention because the process is very simple and takes place under mild conditions. The mild conditions refer to the lack of possible toxic reagents and other undesirable effects associated with chemical cross-linking when compared to reversible physical cross-linking by electrostatic interaction. The most well documented chitosan nanoparticles produced

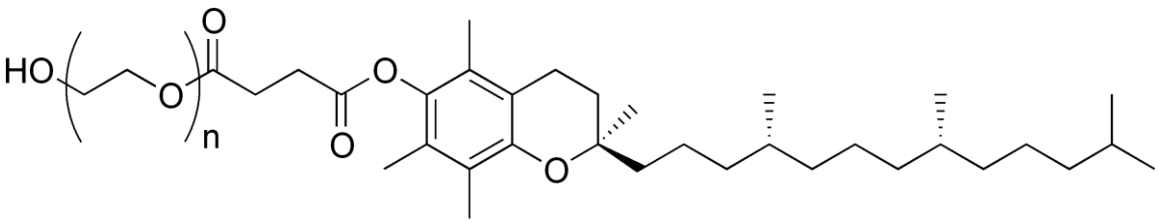
through ionotropic gelation are chitosan-tripolyphosphate nanoparticles, which, many research groups have explored as a potential drug delivery system for various drugs. Potential anions are TPP, sodium alginate, κ-carrageenan and hexadecyl sulphate. When applying the ionotropic gelation process to chitosan, it is first dissolved in aqueous acidic solution, obtained using acetic acid, which generates the chitosan cations [165]. The chitosan solution is added drop wise under constant stirring to polyanionic solution (ie.TPP), which through the ionic interaction between the oppositely charged species, chitosan precipitates to form spherical particles. Fernandez-Urrusuno *et al.* prepared insulin loaded chitosan-TPP nanoparticles by adding insulin to the TPP solution prior to mixing with the chitosan solution. The study used two different molecular weight chitosans and the chitosan to TPP mass ratio used was 6:1. The resulting nanoparticles had a mean particle size between 300–400 nm, a surface charge between +25 to +54 mV and insulin loading as high as 55% [166]. Another group investigated the intestinal absorption of insulin *in-vivo* when delivering using chitosan-TPP nanoparticles. The intention was to determine if the bioadhesive properties of chitosan would further enhance insulin's intestinal absorption. The insulin-loaded chitosan-TPP nanoparticles were prepared through ionotropic gelation producing particles that were positively charged, had a particle size between 250– 400 nm, polydipsersity less than 0.1 and an insulin association of up to 80%. The insulin loaded chitosan-TPP nanoparticles were orally administered to alloxan-induced diabetic rats and through monitoring the plasma glucose levels the intestinal adsorption of insulin was tracked. The chitosan-TPP nanoparticles improved the intestinal absorption of insulin more than aqueous solution of chitosan *in-vivo* and after administration of nanoparticles with 21 I.U/kg of insulin hypoglycemia was prolonged over 15 h. The average pharmacological bioavailability relative to SC injection of

insulin solution improved 14.9%. The *in-vitro* release experiments indicated an initial burst effect, which is pH-sensitive. Another study would go on to the show that insulin encapsulation efficiency is highly dependent on the pH with encapsulation efficiencies ranging between 2 and 85%, with the highest obtained at a pH of 6.1. This study also showed that the release of insulin was pH dependent [167].

2.6. TPGS as surfactant/emulsifier in nanoformulation

D-α- tocopherol glycol succinate 1000 (TPGS) is a surfactant used for pharmaceutical dosage form preparations. It is a water-soluble derivative of natural Vitamin E, which is formed by esterification of vitamin E succinate with PEG.

2.6.1. Chemical structure:



2.6.2. IUPAC name:

α-Hydro-ω-{[4-oxo-4-({(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-yl}oxy)butanoyl]oxy}poly(oxyethylene)

2.6.3. Synonyms:

Tocofersolan; Vitamin E PEG succinate; α-Tocopherol polyethylene glycol succinate (TPGS); Liqui-E

2.6.4. Molecular formula & molecular weight:

$(C_2H_4O)_nC_{33}H_{54}O_5$, 1513 Daltons

2.6.5. Advantages:

Typically, the molecular weight of TPGS with PEG1000 segment is 1513. TPGS has amphiphilic structure of lipophilic alkyl tail and hydrophilic polar head with a hydrophile-lipophile balance (HLB) value of 13.2 and a CMC of 0.02% w/w. The TPGS is widely used emulsifier in pharmaceutical nanotechnology. It is soluble in both water and organic solvent. As such it has advantages of PEG and Vitamin E in application of various drug delivery device, including extending the half-life of the drug in plasma and enhancing the cellular uptake of the drug. The TPGS can be used as an absorption enhancer, emulsifier, solubilizer, additive, permeation enhancer and stabilizer. The TPGS could also suppress multi-drug resistance of cancer cells by inhibiting P-glycoprotein (p-GP) activity, leading to increased cytotoxicity and bioavailability of anticancer drugs. It has already reported that TPGS could effectively inhibit the growth of human lung carcinoma cells *in-vitro* and *in-vivo* [168]. The presence of PEG and vitamin E has been found to improve the half-life of drugs in plasma by preventing opsonization and enhancing cellular uptake of the drug molecules. TPGS coated nanostructures have higher stability *in-vitro* and *in-vivo*. In recent years TPGS has been intensively applied in developing the various drug delivery systems. Feng's group has been focused in the past decade on various applications of TPGS in nanomedicine, including TPGS-based prodrugs, micelles, liposomes, TPGS-emulsified PLGA NPs and NPs of TPGS-based copolymers, which can significantly enhance the solubility, permeability and stability of the formulated drug and realize sustained, controlled and targeted drug delivery [169].

TPGS has been proved to be an efficient emulsifier for synthesis of NPs of biodegradable polymers, resulting in high drug encapsulation efficiency, high cellular uptake *in-vitro* and high therapeutic effects *in-vivo*. For example, TPGS may have more

than 77 times higher emulsification efficiency compared with the traditional emulsifier polyvinyl alcohol (PVA), i.e. to produce the same amount of polymeric NPs by the single emulsion method, the needed TPGS amount can be only 1/77 than that of PVA as the emulsifier used in the process. TPGS-based NPs have been found to increase the cell uptake efficiency on Caco-2, HT-29, MCF-7, C6 glioma cells and thus enhance cancer cell cytotoxicity [170-173].

2.6.6. Recent studies:

In a study, TPGS-DOX-folic acid (FOL) conjugate (TPGS-DOX-FOL) was developed for targeted chemotherapy and compared it with TPGS-DOX conjugate and pristine DOX. Targeting conjugate TPGS-DOX-FOL can be 45.0-fold effective than DOX in cytotoxicity on MCF-7 cells judged by the IC_{50} results, while TPGS-DOX conjugate was only 1.19-fold effective than DOX. The half-life ($t_{1/2}$) of TPGS-DOX and TPGS-DOX-FOL were extended from 2.69 h (DOX) to 10.2 h and 10.5 h, respectively. The AUC values of TPGS-DOX and TPGS-DOX-FOL were 19.2 and 14.5 times than the DOX, respectively. Conjugates also significantly decreased the drug distribution in gastric, intestine, and especially in heart. Indeed, TPGS conjugate, especially TPGS-DOX-FOL can deduce the gastrointestinal side effect of the drug [174].

Kutty et al. developed cetuximab-conjugated micelles of TPGS for targeted delivery of DTX as a model anticancer drug for treatment of the triple negative breast cancer (TNBC), which shows no expression of either one of the hormone progesterone receptor (PR), estrogen receptor (ER) and epidermal growth factor receptor 2 (HER2). The results confirmed that the therapeutic effects of DTX could be greatly enhanced by the formulation of cetuximab conjugated TPGS micelles, which demonstrated 205.6

and 223.8-fold higher efficiency than Taxotere® for the MDA MB 468 and MDA MB 231 cell lines respectively [175].

Here, in this study TPGS conjugates i.e TPGS-CS and TPGS-Tf has been used to prepare bioadhesive micelles and nanoparticles that possess unique bioadhesive and targeting properties via two mechanisms i.e. carrier mediated transcytosis by chitosan and receptor mediated transcytosis by transferrin in brain cancer treatment. Therefore, in this study it was hypothesized that these advanced nanomedicine systems developed for the delivery of DTX will prove more efficacious in brain cancer chemotherapy [175].

2.7. Cancer cell lines for anticancer studies

Multiple cell lines (estimated at 300–400) have been established from human small cell (SCLC) and non-small cell lung cancers (NSCLC). Cancer cell lines offer certain advantages and disadvantages when compared to tumor materials. Cell lines are populations of pure tumor cells without admixed stromal or inflammatory cells. Cell lines are capable of infinite replication, providing a limitless source of materials and permitting their dispersion to laboratories worldwide. Scientists can directly compare their results from identical materials. The NSCLC cell line A549 was established in 1976 and has been very widely studied since then. A549 cells, as found in the lung tissue of their origin, are squamous and responsible for the diffusion of some substances, such as water and electrolytes, across alveoli. If A549 cells are cultured *in-vitro*, they grow as a monolayer; adherent or attaching to the culture flask. The cells are able to synthesize lecithin and contain high levels of unsaturated fatty acids, which are important to maintain membrane phospholipids. A549 cells are widely used as a type II pulmonary epithelial cell model for drug metabolism and as a transfection host. When

grown for sufficient long time in cell culture, A549 cells may begin to differentiate, as signaled by the presence of multilamellar bodies. A549 cells have served as models of alveolar Type II pulmonary epithelium, finding utility in research examining the metabolic processing of lung tissue and possible mechanisms of drug delivery to the tissue. In context of lung cancer drug development, the cells have served as testing grounds for novel drugs such as paclitaxel, docetaxel, and bevacizumab both *in-vitro* and *in-vivo* through cellculture and xenografting, respectively [176-179].

Sun et al., studied the anticancer activity of green synthesized gold nanoparticles (GNP) on A549 lung cancer cells. GNP were synthesized by green synthesis method using Marsdenia tenacissima plant extracts and characterized by UV spectroscopy, AFM, EDS, TEM, and FT-IR. Particle size of GNP was around 50 nm, which is quite suitable nano dimension. *In-vitro* anticancer activity was performed on lung cancer cell lines (A549). MTT assay revealed that AuNPs produce toxicity based on the dose-dependent A549 cells growth inhibition. AuNPs treatment activates caspase expression and down-regulates the anti-apoptotic protein expression in A549 cells. Results pointed out that the AuNPs from M. tenacissima extract are apposite stabilizing agents, which serve as an effective anticancer agent against lung cancer cell lines (A549) [180].

Zhang et al., evaluated the *in-vitro* efficacy of hyaluronic acid (HA)-modified chitosan nanoparticles (CS NPs-HA) loaded with cyanine 3 (Cy3)-labeled siRNA (sCS NPs-HA) on A549 cells. The results showed that noncytotoxic CS NPs-HA of small size (100–200 nm) effectively delivered the Cy3-labeled siRNA to A549 cells via receptor CD44 and inhibited cell proliferation by downregulating the target gene *BCL2*. *In-vivo* experiment results revealed that sCS NPs-HA directly delivered greater amounts of Cy3-labeled siRNA to the tumor sites, resulting in the inhibition of tumor growth by

downregulating *BCL2*, as compared to unmodified NPs loaded with siRNA (sCS NPs) and to naked Cy3-labeled siRNA [181].

2.8. Lung carcinogenesis using chemical carcinogens

It has been well established that tobacco smoke contributes to tenfold increase in risk in long term smokers as compared with nonsmokers. Tobacco smoke contains more than 60 established carcinogens. Among the constituents of tobacco smoke, the polycyclic aromatic hydrocarbons (PAHs) play a major role in lung carcinogenesis. PAHs are one of the prevalent environmental contaminants, which are formed primarily by incomplete combustion of organic matter and also associated with combustion of engines, home heating, biomass burning, and industrial activities. One of the most studied and potent PAH is benzo(a)pyrene (B(a)P), which is often used as a toxicological surrogate or prototype for all carcinogenic PAHs and is ubiquitously present in our environment. B(a)P was the first carcinogen to be detected in cigarette smoke. Since the mechanism of carcinogenesis is similar in animals and humans; based on the experimental studies in animals, B(a)P has been classified as group-1 carcinogen in humans by international agency for research on cancer (IARC). B(a)P, as a carcinogen, has impact on all the three stages of carcinogenesis i.e., initiation, promotion, and progression of disease. Moreover, B(a)P can act as a both genotoxic and non-genotoxic carcinogen by forming DNA adducts and by activating AhR receptors, respectively. One of the well-studied, most potent and remarkably lung specific carcinogen in cigarette smoke is B(a)P, and its ability to induce lung tumors is well documented in animal models. B(a)P generally produces squamous cell tumors of the lung in animal models, including rat, mouse, and hamster and these tumors possess both morphological and molecular similarities to those observed in humans and the

doses of B(a)P administered are c01-06.omparable to the exposure encountered by a smoker during his/her lifetime. Mice spontaneously develop a large number of tumors with age and produce a strong lung tumor response after intoxification with B(a)P in a relatively short time [30]. Consequently, mice have been utilized extensively to study the carcinogenic effects of numerous tobacco smoke carcinogens, including B(a)P and the effects of intervention strategies, most notably chemoprevention [182-184].

Selvendiran et al., evaluated the effects of oral supplementation of the piperine on lung tumour initiation by orally applied B(a)P. Lung cancer bearing mice showed an increase in erythrocyte membrane and tissue ATPase enzymes (Na+/K+-ATPases, Mg2+-ATPases and Ca2+-ATPases) ($p < 0.05$) compared with control groups. These enzyme activities were reversed to near normal control values in animals treated with piperine (50 mg/kg body weight). The beneficial effect of piperine is primarily exerted during initiation phase and post-initiation stage of B(a)P induced lung carcinogenesis [185].

Kamaraj et al., evaluated the chemo-preventive and cancer curative effects of quercetin on B(a)P induced carcinogenesis. Here, the efficacy of quercetin on the level of lipid peroxides, activities of antioxidant enzymes and tumor marker enzymes was assessed in which an increase in lung weight, lipid peroxidation and marker enzymes such as aryl hydrocarbon hydroxylase, gamma glutamyl transpeptidase, 5-nucleotidase, lactate dehydrogenase and adenosine deaminase with subsequent decrease in body weight and antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione-*S*-transferase, glutathione reductase, reduced glutathione, vitamin E and vitamin C were noted in B(a)P induced experimental lung carcinogenesis in Swiss albino mice Quercetin supplementation (25 mg/kg body weight) attenuated all these alterations, which indicates the anticancer effect that was further confirmed by histopathological analysis [186].