Chapter 2

Literature Review

2.1. Treatment of lung cancer

The basic choice of lung cancer therapy depends on its size and location. It can be treated with surgery, chemotherapy, radiation therapy, targeted therapy, or a combination of these treatments [83].

2.1.1. Surgery

Lung cancer surgery may be an alternative for some patients, depending on the kind, location, and stage of the lung cancer and other medical problems. Surgical attempts to treat lung cancer require removing the tumour and some surrounding lung tissue and, frequently, lymph nodes in the tumour's vicinity. Lung cancer surgery is the best treatment when the disease is localized and unlikely to spread. This comprises early-stage non-small cell lung cancer and carcinoid tumours. A thoracotomy is a surgical incision on the side of the chest that follows the curvature of the ribs. It usually entails splitting portions of the chest wall muscles and gently spreading between two ribs using a tool. The muscles are repaired when the incision is closed [84,85].

2.1.2. Radiation Therapy

Lung cancer radiation therapy employs high-energy X-rays to either kill or inhibit the growth of cancer cells [86]. Radiation can enter the body from outside (external) or from radioactive materials inserted directly into the lung cancer tumour (internal/implant). External radiation is the most often utilized method. The radiation is directed towards the lung cancer tumour and destroys cancer cells solely in that location of the lungs. Radiation can be used before or after lung cancer surgery to reduce the tumour or eliminate any cancer cells in the lungs. External radiation is sometimes utilized as the primary form of lung cancer therapy [87].

2.1.3. Photodynamic Therapy (PDT)

In minimal circumstances, PDT may have a limited role in lung cancer treatment. PDT is a treatment that integrates light energy with a light-activated cancer-killing agents. Photodynamic treatment may be an option if lung cancer spreads into the airway, causing difficulty breathing, bleeding, or continuous coughing. It might also be utilized to treat early-stage non-small cell lung cancer in locations easily accessible with the equipment used during therapy. A light-sensitive drug is injected into a vein to commence photodynamic treatment. One to three days later, a specific wavelength is delivered to the tumour from within the body, generally via the mouth into the lungs, using a thin, illuminated tube called a bronchoscope. The light kills the cells that have taken in the light-sensitive drug. Photodynamic treatment is ineffective for malignancies that have migrated beyond the lung or are inaccessible via bronchoscopy. The main side effects of photodynamic treatment include light sensitivity responses. Patients are often advised to avoid exposure to intense light, especially the sun, for several weeks following treatment [88,89].

2.1.4. Chemotherapy

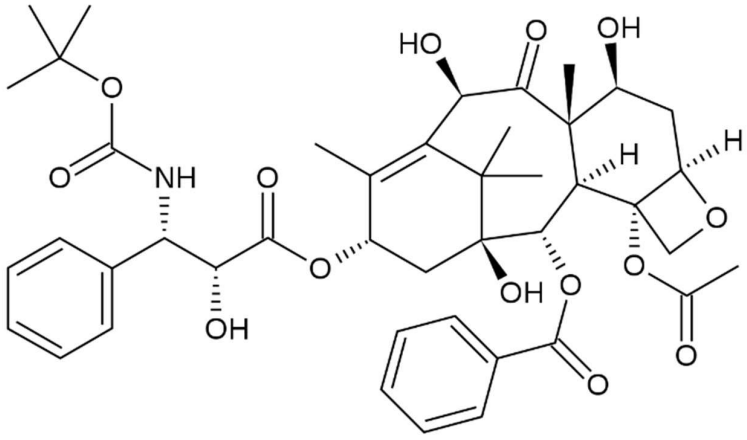
Chemotherapy is frequently used in conjunction with radiation treatment to treat lung cancer. Chemotherapy and radiation treatment may work better together to kill cancer cells. Chemotherapy can help some patients with lung cancer to reduce the tumour size so that radiation can operate more effectively to eradicate them. It may help prevent cancer cells from regrowing following radiation therapy. Chemotherapy following surgery may help persons with NSCLC avoid recurrence, especially if they have stage II or stage IIIa lung cancer. Chemotherapy combined with radiation treatment is suggested for stage III of lung cancer. Chemotherapy is the primary treatment for NSCLC stage IV. Cisplatin and

carboplatin are commonly used with docetaxel (Taxotere), gemcitabine (Gemzar), paclitaxel (Taxol), pemetrexed (Alimta), or vinorelbine (Navelbine) in NSCLC treatment [90, 91].

2.2. Docetaxel (DXL)

Docetaxel is the main ingredient of the DXL injection concentrate and is a semisynthetic diterpene derived from the genus *Taxus baccata* (European yew). It has a broad spectrum of antitumor activities compared with other drugs. For instance, in several murine and human tumour cell lines, DXL exhibited 1.3 to 12-fold greater cytotoxicity than paclitaxel. The drug showed significant and consistent antitumor activities in metastatic, non-small-cell lung and breast cancers. DXL has a major problem with low aqueous solubility when administered as an intravenous (i.v.) injection. To overcome this, DXL concentrate was developed using a mixture of Tween-80 and ethanol [92,93].

2.2.1. Chemical structure of DXL:



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-{(2R,3S)-3-[(tert-butoxycarbonyl)amino]-2-hydroxy-3 phenylpropanoate}

2.2.3. Solubility: The solubility of the DXL in water is 0.0127 mg/ml, in DMSO 25 mg/ml, and in ethanol (25 mg/ml).

2.2.4. Molecular weight: 807.88 Da

2.2.5. Molecular formula: $C_{43}H_{53}NO_{14}$

2.2.6. Pharmacokinetics:

- Half-life elimination : 11 h
- Protein-bound : 94-97%
- Volume of distribution : 80-90 L/m²
- Metabolism : Liver (CYP3A4)
- Clearance : 21 L/h/m²
- Excretion : Feces (76 %), Urine (6%)

2.2.7. Mechanism of Action:

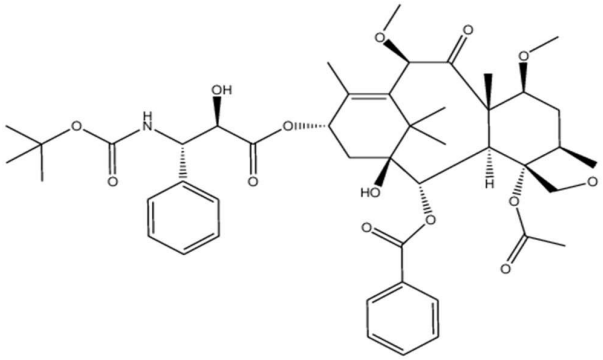
The primary mechanism of action of DXL is to bind beta-tubulin, increasing its proliferation and stabilizing its conformation. This prevents the normal assembly of microtubules into the mitotic spindle, which stops cell cycling during G2/M. DXL also decreases the expression of the BCL2 gene, an anti-apoptotic gene that cancer cells frequently overexpress to increase survival [94,95].

2.3 Cabazitaxel (CZT)

Several semisynthetic taxane analogues, including CZT, have been developed to avoid drug resistance. CZT was chosen for clinical development based on its *in-vivo* efficacy in docetaxel-resistant MDR tumour models. In preclinical trials, this taxane was shown to be as effective as docetaxel in cellular models and even more effective in variations chosen for resistance to taxanes. Cabazitaxel, in combination with prednisone/prednisolone, was

authorized by the FDA in 2010 for the treatment of patients with metastatic hormone-refractory prostate cancer who had previously been treated with docetaxel [96,97].

2.3.1 Chemical structure of CZT:



2.3.2 IUPAC name:

(2α,5β,7β,10β,13α)-4-acetoxy-13-({(2R,3S) 3[(tertbutoxycarbonyl) amino]-2-hydroxy-3-phenylpropanoyl} oxy)-1 hydroxy-7,10-dimethoxy-9oxo-5,20-epoxytax-11-en-2-yl benzoate -propan-2-one.

Solubility: The solubility of cabazitaxel in ethanol is 1.5 mg/ml, 5 mg/ml in DMSO and DMF and 0.00413 mg/mL in water.

2.3.3. **Molecular weight:** 835.93 Da

2.3.4. Molecular formula: $C_{45}H_{57}NO_{14}$

2.3.4. **Pharmacokinetics:**

- Half-life elimination : 2 h
- Protein bound : 89-92 %
- Volume of distribution : 2,643 L/m2
- Metabolism : Liver (CYP3A4/5, CYP2C8)
- Clearance : 26.4 L/h/m2
- Excretion : Feces (76 %), Urine (3.7%)

2.3.5. Mechanism of action

CZT is a novel second-generation semisynthetic microtubule inhibitor (particularly a taxane derivative) that causes cell death by stabilizing microtubules. Microtubules are cytoskeletal polymers made up of alpha-tubulin and beta-tubulin heterodimers that play an essential role in cell shape maintenance, vesicle transport, cell signalling, and cell division. Cabazitaxel binds the beta-tubulin subunit's N-terminal amino acids and increases microtubule polymerization (tubulin dimer elongation). Microtubules grow toward the mitotic spindle, responsible for chromosomal separation and distribution and cell division into daughter cells during mitosis. Cabazitaxel promotes microtubule polymerization while inhibiting microtubule cell division, interrupting the tumour cell cycle and development [98,99].

2.4. Targeted therapy of lung cancer

The main drawbacks of current chemotherapy for lung cancer treatment include a lack of target specificity, recurrence, and a superficial increase in human survival. Furthermore, oral and IV administrations of anticancer drugs have numerous drawbacks, including drug molecule degradation in the stomach pH, drug molecule alterations during the process of metabolism in the liver, and lack of specificity, which causes toxicity and side effects. Poor overall survival rates in NSCLC patients may also be related to inherent radiation resistance due to cancer cells' improved capacity to repair DNA damage after radiation therapy (RT). As a result, it is critical to design a system that can overcome the constraints mentioned earlier and deliver the anticancer drug at the cancer site in the desired concentration. Targeted therapy is a type of cancer treatment that targets specific genes, proteins, or the tissue environment that promotes cancer development and survival. These medicines are highly targeted and operate differently than chemotherapy. This therapy inhibits cancer cell growth and spreads while causing minimal damage to healthy cells. Currently, the most

important targeted treatment strategies in lung cancer are VEGFR inhibitors, EGFR inhibitors, 4-Anaplastic lymphoma kinase fusion gene (ALK) inhibitors, and B-Raf enzyme (BRAF) inhibitors [100,101].

2.4.1. EGFR inhibitors

The epidermal growth factor receptor (EGFR) is a cell-surface receptor that belongs to the ErbB family of tyrosine kinases and regulates cell proliferation, survival, and differentiation. Indeed, EGFR is overexpressed in a wide range of human malignancies, including lung, head and neck, colon, pancreatic, breast, ovary, bladder, and kidney cancers, as well as gliomas. EGFR overexpression is found in more than 60% of non-small cell lung cancers (NSCLCs), but not in small cell lung cancer. EGFR overexpression is thought to be generated by a combination of epigenetic processes, gene amplification, and oncogenic viruses. EGFR inhibitors are drugs that disrupt the EGFR signal that instructs cells to grow. Some of these drugs may be used to treat NSCLC [102,103].

2.4.1.1. Cetuximab (CTXmab)

It is a kind of chimeric (mouse/human) monoclonal G1 immunoglobulin that targets and binds to the EGFR's outer domain. In February 2004, the US FDA authorized it for clinical use in treating different malignancies by i.v. infusion. In humans, the half-life of cetuximab is around seven days, allowing for once-weekly treatment in conjunction with typical chemotherapy regimens. Cetuximab internalization causes receptor degradation without phosphorylation or activation, decreasing EGFR-dependent downstream signalling cascades. Cetuximab-mediated receptor downregulation causes cytotoxicity by inhibiting receptor function [100,104].

2.5. Nanomedicine in the treatment of NSCLC

Currently, nanomedicine-based formulations such as Abraxane and Doxil are available in the market and are being used clinically [75]. The nanotechnology-based formulations such as nanoparticles, liposomes, micelles, solid-lipid nanoparticles, etc. can be used by both such drug delivery strategies as active and passive targeting [76,77]. The extravasation into tumour endothelium is the most prominent pathway for the size-based passive targeting through EPR (enhanced permeability and retention) effect [78]. Moreover, the specific types of receptors overexpressed in lung cancer are the key features for the active targeting through the interaction between corresponding receptors and ligand-decorated nanomedicines, followed by the promoted endocytosis with consequent drug release [79]. A huge number of targeting ligands for active targeting in lung cancer have been identified. The nanomedicines, decorated with such targeting ligands, can specifically target lung cancer and improve the therapeutic efficacy of anticancer drugs. The active targeting of the nanomedicines can be facilitated by various targeting ligands such as folate, transferrin, aptamers, monoclonal antibodies etc. [71,80,81]. Generally, the nanomedicines can be conjugated with targeting ligands either by pre-conjugation or post-conjugation techniques [82]. However, due to the heterogeneity and complexity of lung cancer, the effectiveness of single-receptor targeted nanomedicine is quite limited. The concept of personalized therapy has recently emerged, which can be applied to targeted drug delivery systems for cancer treatment. However, personalized nanomedicine requires extensive clinical research on the human body, from which personalized and targeted nanomedicine can be developed. Under the current circumstances, it is challenging to implement personalized nanomedicine for the treatment of a large number of patients [105]. Therefore, the concept of dual or multiple-receptor targeted nanomedicine, based on the expression levels of receptors in a large

number of patients, is recommended as a promising strategy for lung cancer nanomedicine. Furthermore, to augment their accumulation in cancer cells, targeted nanomedicine may also be decorated with cell-penetrating peptides such as penetratin, TAT, VP22, polyarginine, etc. [106]. The biological response of targeted nanomedicines can vary depending on the density of the targeting ligands so that for the development of multiple- or dual-receptor targeted nanomedicine, it is necessary to optimize the proportions of the targeted ligands based on *in-vitro* and *in-vivo* studies [107]. In this review, we also discussed the common conjugation mechanisms that can be used in the fabrication of dual- or multiple-receptor targeted nanomedicine [108].

2.6. Limitation in single targeted nanomedicines

Targeted drug delivery to cancer is entirely based on the expression level of cancer cell-specific receptors. The lung cancer-specific receptors extensively explored by the researchers in the development of targeted nanomedicines are folate, transferrin, integrin, EGFR, VEGFR, IGF-1, CD44, CXCR-4 and CA-IV. **Table 2.1.** enlists the percent of NSCLC patients showing the overexpression of specific types of receptors from which it is evident that transferrin receptor, folate receptor and EGFR overexpression is seen in 93 %, 87 % and 80 % of total NSCLC patients, respectively. Nanomedicine targeting any of these receptors may not be helpful for all NSCLC patients. Selecting the proper combination of these receptors for active targeting is critical for successful lung cancer therapy. The meticulous combination of ligands for any two or even multiple types of receptors is required to achieve maximum therapeutic benefits in the treatment of NSCLC patients. [109-111].

Moreover, some scientific reports also illustrated that single ligand-targeted nanomedicines showed lesser penetration in solid tumours, including lung cancer [112]. All these factors

may affect the efficiency of single-ligand targeted nanomedicine, leading to diminished effectiveness of anticancer agents. Therefore, dual or multiple-receptor targeted nanomedicines can be developed for the benefit of a large number of patients based on the receptor expression levels in specific cancer types.

Table 2.1. Percentage of NSCLC patients overexpressed with various lung cancer-specific receptors

Targets for lung cancer	Expression in NSCLC patients	Nature of receptors/proteins
Folate receptor-α	66-88% [113,114]	(GPI)-anchored membrane protein
Transferrin receptor	76 -93% [115]	Glycoproteins
Integrin α-1, α-5 and β-3	42 %, 44.4% and 33 % [116]	Glycoproteins
EGFR	40- 80 % [117]	Transmembrane receptor
VEGFR	29 -71 % [118]	Vascular endothelial growth factor
IGF-1	74.3-88.7 % [119]	Transmembrane protein with tyrosine kinase activity
CD44	68.1 % [120]	Transmembrane glycoprotein
CXCR-4	52.2 % [121]	G protein-coupled receptor
(CA-IX)	70.3% [122]	HIF1α-regulated downstream gene

2.7. Dual-receptor targeted nanomedicines for lung cancer

Multiple or dual receptor-targeted nanomedicines for lung cancer therapy are much less explored and reported than single receptor-targeted nanomedicine. Tan and Wang have reported the first dual receptor-targeted nanomedicine for lung cancer treatment, G. They successfully conjugated the ligands, specific for folate and transferrin receptors, to the cisplatin-loaded nanoparticles. For the conjugation of both ligands, they used the pre-conjugation technique. The folic acid-cisplatin conjugate was prepared via cisplatin reaction

with succinic anhydride and cystamine, which was further conjugated with folic acid using EDC/NHS. Whereas transferrin was conjugated with oleic acid-cystamine complex. The chemical structures of both the folic acid and transferrin conjugates were confirmed by NMR spectroscopy, and both the conjugate systems were incorporated into the oleic acid-cystamine nanoparticles during their preparation by the nanoprecipitation method. The physicochemical properties such as particle size, PDI and entrapment efficiency of dual receptor-targeted nanoparticles were compared with that of non-targeted and single receptor-targeted nanoparticles. *In-vitro* cytotoxicity was assessed by MTT assay against A-549 and NCI-H460 cells. The potency of dual-targeted nanoparticles was approximately 25 times and six times higher than non-targeted and single receptor-targeted nanoparticles. *In-vivo* anticancer efficacy of dual-receptor targeted nanoparticles was performed on a lung cancer-induced mice model. The tumour inhibition rate of dual-receptor targeted nanoparticles was about 84 %, while for folate and transferrin, it was about 59 % and 62 %, respectively [123]. Lin et al. have developed dual ligand targeted liposomes loaded with triptolide for pulmonary delivery. They have used an anti-carbonic anhydrase antibody and a cell-penetrating peptide (CPP33 peptide) as ligands. The pre-conjugation technique was used for the conjugation of the CPP33 peptide. The cysteine-modified CPP33 peptide was conjugated with DSPE-PEG2000-MAL using sulfhydryl chemistry and characterized by mass spectrometry. CPP33 targeted liposomes, loaded with triptolide, were prepared by ethanol injection after incorporating DSPE-PEG2000-MAL-CP33 conjugate. On the other hand, DSPE-PEG-MAL micelles were prepared by film hydration method, and the anti-CA-IX antibody was post-conjugated using dithiothreitol (a sulfhydryl-reactive cross-linker). The prepared anti-CA-IX antibody conjugated micelles were incubated with CPP33 conjugated and triptolide loaded liposomes, and the extent of their penetration was

determined by using a confocal laser scanning microscope in a 3-D tumour spheroid model of A-549 cells. The Z-stack images of dual-receptor targeted liposomes showed the highest penetration. Furthermore, an MTT assay was also performed in the 3-D tumour spheroid model of A-549 cells, and the CPP33 and anti-CA-IX antibody conjugated liposomes exhibited the highest cytotoxicity. The *in-vivo* anticancer efficacy was assessed on BALB-c lung cancer-induced mice. The micro-sprayer aerosolizer kit was used for the pulmonary delivery, and the dual-targeted liposomes showed a significant reduction in the tumour volume, which was determined by bioluminescence imaging [124]. **Figure 2. 1.** Illustrated the comparative targeting mechanisms of non-targeted nanomedicine, single-receptor targeted nanomedicine and dual-receptor targeted nanomedicines towards the lung cancer cells.

More recently, Pooja et al. developed the N-acetyl-d-glucosamine (NAG) conjugated poly(amidoamine) dendrimers as dual receptor-targeting agents for efficient delivery of camptothecin to non-small cell lung cancer cells. In contrast to other monosaccharides, NAG offers a unique opportunity, as NAG can target glucose transporter 1 (GLUT1) and lectin receptors. Specifically, NAG-conjugated, camptothecin-loaded poly(amidoamine) dendrimers significantly improved the cytotoxic efficiency through enhanced cellular uptake, decreased cell viability, reactive oxygen species generation, chromatin condensation, apoptosis and inhibition of cellular migration without impacting the mechanism of camptothecin. The *in-vivo* studies confirmed the selective accumulation of the targeted system in the lung tumours and retention of specificity in the biological system. Overall, blocking cellular lectin as one of the two receptors of NAG provides convincing evidence that a dual receptor-targeting strategy offers considerable promise for enhancing the specificity of cancer therapeutics [125].

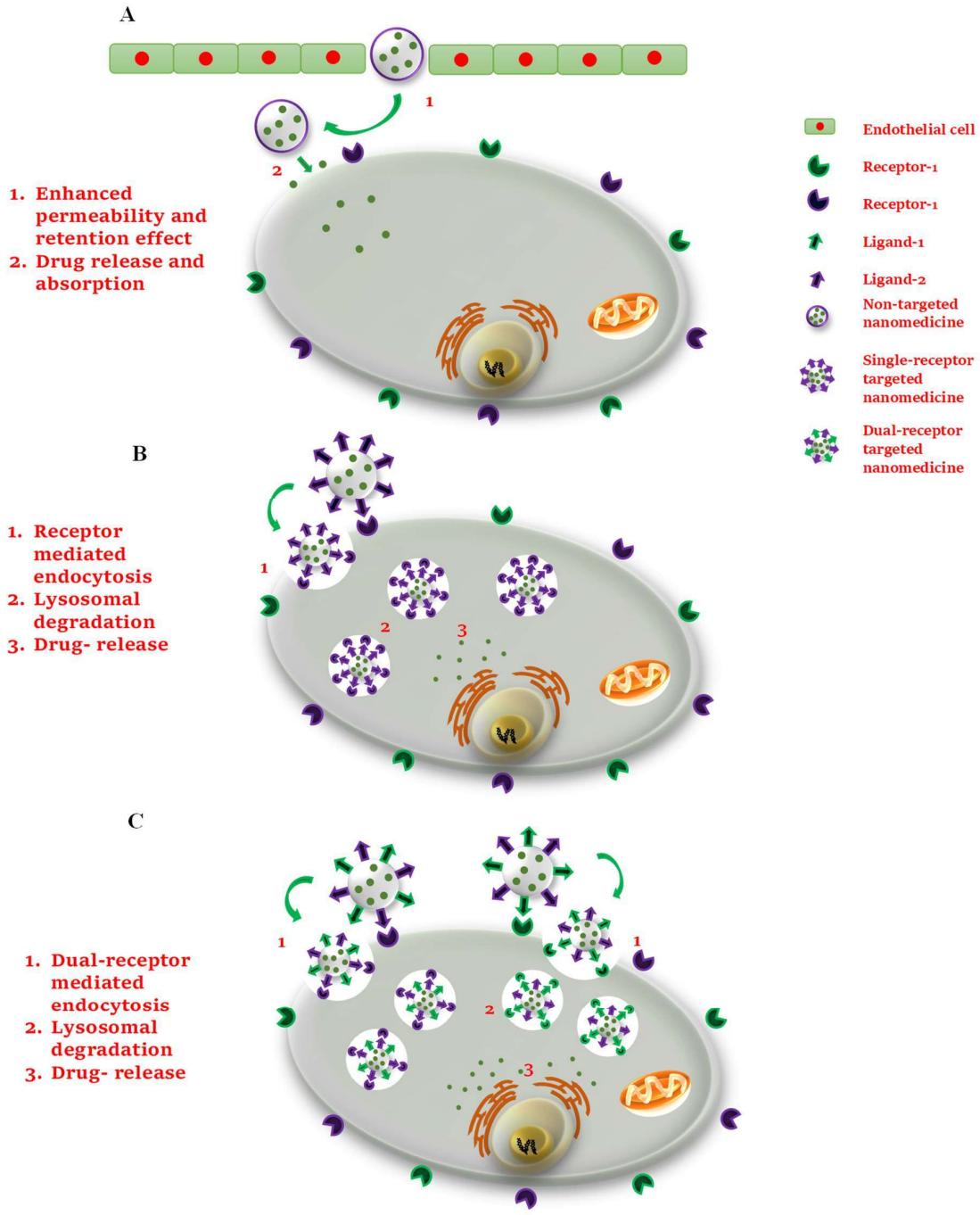
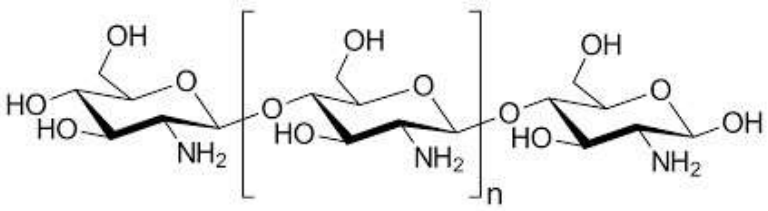


Figure 2.1. The cellular uptake mechanisms of (A) non-targeted, (B) single-receptor targeted and (C) dual-receptor targeted nanomedicines in lung cancer cells

2.8. Chitosan (CS) as polymer material for nanomedicine

CS is a natural, non-toxic, biodegradable polymeric polysaccharide with a positive surface charge. By virtue of its cationic nature can interact with the mucous membranes and thus exhibit excellent mucoadhesion properties. Ionic cross-linking is the most popular method for the preparation of CS nanoparticles by using anionic cross-linkers such as thiamine pyrophosphate and tri-polyphosphate. The solubility of the CS is pH limited; it is only soluble in the acidic medium. To overcome this problem, various chemically modified CS derivatives are also available. CS is also amenable to surface modification by specific chemical conjugation reactions as numerous reactive amino, and hydroxyl groups are present in the ring at the position of carbon no. 2 and 6, respectively [126]. CS exhibits intrinsic anti-neoplastic, antifungal and antibacterial properties, so CS nanoparticles can be manipulated for their synergistic antitumor activities. CS-based targeted drug delivery system is widely used in cancer treatment to deliver antineoplastic agents to the tumour site, such as aptamer (AS1411) decorated chitosan nanoparticles [127], cetuximab decorated chitosan nanoparticles [128], transferrin conjugated chitosan nanoparticles [129] and curcumin loaded chitosan-folate nanoparticles [125,130].

2.8.1. Chemical structure of CS:



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

2.8.3 Molecular weight: 1526.5 Daltons

2.8.4. Chitosan-alginate (CSA) based nanoparticles

Chitosan and alginate are commonly used natural polymers in pharmaceuticals and biomedical applications [131]. Because both of these polymers have excellent biocompatibility, biodegradability, mucosal adhesion, non-toxicity and other characteristics, they have been approved by USFDA [132]. Alginate is a negatively charged, linear co-polymer obtained from brown algae [133]. Contrastingly, chitosan is a positively charged deacetylated derivative of chitin extracted from crustaceans' shells [134]. The main downside of chitosan in the drug delivery system is that it swells and begins to break down in an acidic environment [135]. Since the microenvironment of cancer is inherently acidic, the chitosan nanomedicine can break down before endocytosis [136].

In contrast to chitosan, alginate shrinks in an acidic medium and swells in an alkaline medium [137]. Since both polymers are oppositely charged in nature, they can be used in polyelectrolyte complex reactions to form hybrid-polymeric drug delivery systems [138,139]. The polyelectrolyte complex of the CSA overcomes the shortcomings of CS, including poor mechanical strength and stability at lower pH while maintaining its biocompatible, biodegradable and non-toxic properties [140]. The ionic gelation method is generally used to form the polyelectrolyte complex between the amino group of the CS and the carboxylic group of the alginate [141]. Many researchers have utilized CS and alginate polyelectrolyte complexes in anticancer drug delivery systems loaded with doxorubicin, paclitaxel, and 5-fluorouracil [142-144].

2.9. Methods of preparation of polymeric nanoparticles

Polymeric nanoparticles can be prepared by dispersing existing polymers or by polymerizing monomers. Polymeric nanoparticles, on the other hand, may be directly prepared by the polymerization of monomers through the emulsion polymerization process.

The type of polymeric system, the area of application, the size requirement, and so on all influence the selection and technique of preparation. Their size varies depending on the technique of production, ranging from 10-1000 nm. The pharmaceutical agents are either physically encapsulated or chemically linked to polymeric nanoparticles' hydrophobic core. The primary benefits of polymeric nanoparticles are their great stability and controlled drug release via diffusion or breakdown of the polymeric matrix [145,146].

2.9.1. Solvent evaporation

In this method, the polymer solutions are prepared in volatile solvents, and emulsions are formed using commonly used volatile solvents such as dichloromethane, chloroform, and ethyl acetate. Once the solvent in the system evaporates, the emulsion is changed into a nanoparticle suspension, which is permitted to permeate into the emulsion's continuous phase. These techniques make use of high-speed homogenization or ultrasonication, followed by solvent evaporation by continuous magnetic stirring at room temperature or under reduced pressure. The solidified nanoparticles are then recovered by ultracentrifugation and washed with distilled water to remove additives such as surfactants before being lyophilized [147,148].

2.9.2. Dialysis method

The dialysis method is often used to fabricate small, monodispersed polymeric nanoparticles. A polymer is dissolved in an organic solvent and then deposited in a dialysis tube with the appropriate molecular weight cut-off. Dialysis is carried out in the presence of a non-miscible solvent. The displacement of the solvent into the membrane is followed by increasing polymer aggregation due to loss of solubility and the production of homogenous nanoparticle suspensions [149].

2.9.3. Coacervation/precipitation

The coacervation/precipitation process is based on CS's physical and chemical characteristics, which cause it to be insoluble in alkaline pH media. Because CS is insoluble in alkaline solutions, it precipitates/coacervates out of solution when combined with them. Using a compressed air nozzle, one technique of utilizing these principles to synthesize CS nanoparticles is to spray CS solution into an alkali solution. The particles are separated by filtration, centrifugation, or both, and then washed repeatedly with hot and cold water. Complex coacervation is the most often used process for producing CS nanoparticles [150].

2.9.4. Emulsion-droplet coalescence

The emulsion-droplet coalescence approach makes use of both precipitation and emulsion cross-linking principles. Unlike the emulsion cross-linking techniques used to create CS microparticles, which cross-link stable droplets, precipitation is created in this process by allowing CS droplets to merge (coalescence) with NaOH droplets [151,152].

2.9.5. Reverse micellization

Reverse micelles are thermodynamically stable and dynamic compositions of water, oil, and surfactant. The benefit of reverse micellar formation is the production of ultrafine polymeric nanoparticles with narrow size distributions. When using the reverse micelle approach, the surfactant is first dissolved in an organic solvent to produce the reverse micelles, and then the CS and drug aqueous solution is added while constantly vortexing to avoid turbidity. Maximum drug loading varies amongst drugs, as it does in any other form of nanoparticle system; nevertheless, maximum drug loading in reverse micelles can be reached by progressively adding medication until the clear microemulsion is turned into a translucent solution. The following step is to add a crosslinking agent while constantly stirring and mixing overnight to achieve maximum cross-linking [153,154].

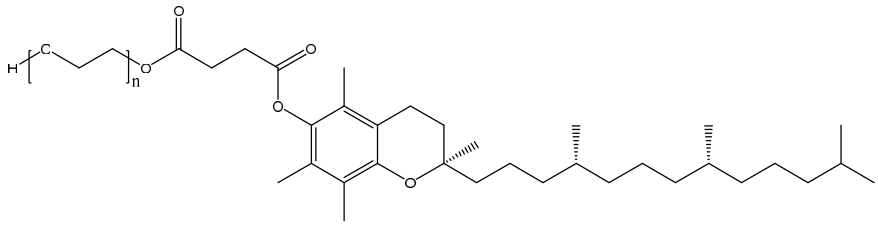
2.9.6. Ionotropic gelation

Ionotropic gelation is the process by which a polyelectrolyte is cross-linked with a counter ion, resulting in the formation of a hydrogel [155]. Hydrogel formations are held together by hydrogen bonding, hydrophobic forces, ionic forces, or molecular interferences. This approach has been used to prepare micro and nanoparticles for encapsulating and controlled release of medicinal compounds utilizing a range of materials such as gellan gums, alginates, carboxymethyl cellulose, and CS [156]. Several techniques of ionotropic gelation can be utilized depending on the material used, the strength of the counter ion, and the desired particle size, such as syringe dropping and air atomization for bead formation and flush mixing for nanoparticle formation [157].

2.10. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)

Recently, some non-ionic surfactants with PEG chains such as TPGS and Tween-80 are commonly employed in the drug delivery systems to enhance their stability and half-life in the blood circulation [158]. TPGS is a vitamin-E derivative and approved by the FDA, so it is often used as an excipient in various pharmaceutical preparations [159,160]. Moreover, TPGS is the inhibitor of the P-glycoprotein efflux pump, making the anticancer drug delivery system more effective [125,161]. In addition, TPGS can also be modified with appropriate chemical groups such as TPGS-COOH, TPGS-NH_2, TPGS-SH, etc., so that necessary functional groups can be used to bind ligands over nanomedicine surface [162-164].

2.10.1. Chemical structure of TPGS:



2.10.2. 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)

2.10.3. Molecular formula: $(C_2H_4O)_{nC33}H_{54}O_5$

2.10.4. Molecular weight: 1513 Daltons

2.11. Chemical induced lung cancer mice model

B(a)P is one of the most well-studied, strong, and impressively lung-specific carcinogens in cigarette smoke, with a well-documented potential to generate lung tumours in animal models. In general, B(a)P induces squamous cell lung tumours in rodents, mice, and hamsters. Mice generate a high number of tumours spontaneously as they age and have a robust lung tumour response following B(a)P toxification in a relatively short period of time. Mice have therefore been frequently utilized to study the carcinogenic consequences of numerous tobacco smoke carcinogens, including B(a)P, as well as the impacts of intervention techniques, most notably chemoprevention. It is frequently used to create lung tumours in mice models since these tumours have genetic and physical similarities to those found in humans. Previous research has shown that the toxicity of B(a)P is caused by its intermediate metabolites and oxidative stress caused by reactive oxygen species (ROS), both of which play an important role in B(a)P-induced lung carcinogenesis. Benzopyrene-induced lung cancer animal models can thus be used to study the anticancer efficacy of anticancer drugs or anticancer nanomedicine [165-167].