DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF CISPLATIN LOADED CUBOSOMES FOR HUMAN LUNG CARCINOMA

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DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF CISPLATIN LOADED CUBOSOMES FOR HUMAN LUNG CARCINOMA

by

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LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
ABZ	Albendazole
ALT	Alanine transaminase
AP	Activator protein
AST	Aminotransferase
BQ	Bedaquiline
CDDP	Cis-dichlorodiamineplatinum
CRC	Colorectal cancer
Cs	Cubosomes
Ctr	Calcitonin receptor
DACHPt	1,2-diaminocyclohexane-platinum
DLS	Dynamic light scattering
DOX	Doxorubicin
dUTP	Deoxy uridine phosphate
ECM	Extracellular matrix
EE	Encapsulation efficiency
EPR	Enhanced permeability and retention
ER	Endoplasmic reticulum
ETP	Etoposide
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GMO	Glyceryl monooleate
GSH	Glutathione

HNSCC	Head and neck squamous cell carcinoma
HPGs	Hyperbranched polyglycerols
ICBs	Immune checkpoint blockers
IND	Investigational new drug
JNK	C-jun N-terminal kinases
LAG-3	Lymphocyte activation gene-3
LC	Lung Cancer
LDH	Lactate dehydrogenase
LUAD	Lung adenocarcinomas
MDA	Malondialdehyde
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MNs	Microneedles
NADPH	Nicotinamide adenine dinucleotide phosphate
NPs	Nanoparticles
NSCLC	Non-small cell lung cancer
OCT2	Octamer-binding protein 2
OCTs	Organic cationic transporters
P407	Poloxamer 407
PAsp	Poly (aspartic acid)
PCL	Polycaprolactone
PD-1	Programmed death-1
PDI	Polydispersity index
PD-L1	Programmed death ligand-1
PEG	Polyethylene glycol

PEO	Polyethylene oxide
PGlu	Poly (glutamic acid)
PHYT	Phytantriol
PMAA	Polymath acrylic acid
PPO	Polypropylene
PTX	Paclitaxel
PVA	Polyvinyl alcohol
ROS	Reactive oxygen species
SCLCs	Small cell lung cancers
SMVT	Sodium-dependent multivitamin transporter
SWCNTs	Single walled carbon nanotubes
TCI	Trans-cutaneous immunization
TMB	Tumor mutation burden
TME	Tumor microenvironment

PEMBANGUNAN, PENGOPTIMUMAN DAN PENCIRIAN KUBOSOM DIMUATKAN CISPLATIN UNTUK KARSINOMA PARU-PARU MANUSIA

ABSTRAK

Kanser paru-paru kekal sebagai cabaran kesihatan global utama disebabkan oleh keterbatasan terapi konvensional. Kemoterapi berasaskan platinum, seperti cisplatin, digunakan secara meluas tetapi terhalang oleh ketoksikan sistemik dan penyingkiran pantas. Bagi menangani isu ini, kami telah menghasilkan platform kubosom berasaskan gliseril monooleat (GMO) yang baharu untuk penghantaran cisplatin berterusan, memanfaatkan struktur lipid dua fasa berterusan yang unik bagi mengatasi masalah pengkapsulan dan pelepasan pembawa nano sedia ada seperti liposom dan nanopartikel polimer. Kubosom disintesis menggunakan pendekatan "top-down" dengan menggabungkan GMO cair dan poloxamer 407 dengan air terdeionisasi suam yang dimuatkan cisplatin. Reka bentuk faktorial penuh dua peringkat secara rawak digunakan untuk mengoptimumkan formulasi kosong, menghasilkan kubosom berisi cisplatin dengan saiz partikel 168.25 ± 5.73 nm, cas permukaan -9.56 ± 1.33 mV, dan kecekapan pengkapsulan 60.64 ± 0.11%, mengatasi kecekapan yang dilaporkan untuk liposom konvensional (biasanya <50%). Kajian pelepasan in vitro menunjukkan pelepasan cisplatin yang berterusan (94.5% dalam 30 jam), berbeza ketara dengan larutan cisplatin bebas (99% dibebaskan dalam 1.5 jam). Profil pelepasan yang berpanjangan ini, digabungkan dengan kestabilan serum pada 4–8°C, menonjolkan kelebihan kritikal berbanding sistem misel dan liposom, yang sering menunjukkan pelepasan mendadak yang lebih pantas. Ujian sitotoksisiti pada sel NCI-H226 mengesahkan keberkesanan kubosom bermuatan cisplatin, manakala kubosom kosong menunjukkan ketoksikan yang boleh diabaikan. Kajian kestabilan

pecutan mengesahkan pengekalan sifat fizikokimia pada 4–8°C, manakala bagi formulasi yang disimpan pada 40°C/75%, kelembapan relatif menunjukkan pengagregatan partikel. Dengan menggabungkan kapasiti pemuatan ubat yang tinggi, pembebasan terkawal, dan kestabilan, kajian ini dapat mendorong penghantaran cisplatin melebihi platform sedia ada. Reka bentuk struktur kubosom membolehkan sasaran tumor yang cekap dan mengurangkan kesan luar sasaran, secara langsung menangani masalah ketoksikan dan rintangan dalam kanser paru-paru. Keputusan praklinikal ini menyokong pembangunan lanjut kubosom untuk aplikasi klinikal dalam terapi kanser paru-paru bukan sel kecil (NSCLC), dengan menawarkan strategi yang menjanjikan untuk meningkatkan hasil terapeutik.

DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF CISPLATIN LOADED CUBOSOMES FOR HUMAN LUNG CARCINOMA

ABSTRACT

Lung cancer remains a major global health challenge due to the limitations of conventional therapies. Platinum-based chemotherapy, such as cisplatin, is widely used but hindered by systemic toxicity and rapid clearance. To address these issues, we developed a novel glyceryl monooleate (GMO)-based cubosomes platform for sustained cisplatin delivery, leveraging its unique bicontinuous lipid structure to overcome encapsulation and release limitations of existing nanocarriers like liposomes and polymeric nanoparticles. Cubosomes were synthesized using a top-down approach, combining molten GMO and poloxamer 407 with cisplatin-loaded warm deionized water. A randomized two-level full factorial design optimized blank formulations, yielding cisplatin-loaded cubosomes with a particle size of 168.25 ± 5.73 nm, surface charge of -9.56 ± 1.33 mV, and encapsulation efficiency of 60.64 ± 0.11%, surpassing reported efficiencies for conventional liposomes (typically <50%). In vitro release studies demonstrated sustained cisplatin release (94.5% over 30 hours), contrasting sharply with free cisplatin solution (99% release within 1.5 hours). This prolonged release profile, combined with serum stability at 4–8°C, highlights a critical advantage over micellar and liposomal systems, which often exhibit faster burst release. Cytotoxicity assays on NCI-H226 cells confirmed the efficacy of cisplatin-loaded cubosomes, while blank cubosomes showed negligible toxicity. Accelerated stability studies confirmed retained physicochemical properties at 4–8°C, whereas formulations stored at 40°C/75% relative humidity showed particle aggregation. By integrating high drug-loading capacity, controlled release, and stability, this work

advances cisplatin delivery beyond existing platforms. The cubosomes' structural design enables efficient tumor targeting and reduced off-target effects, directly addressing toxicity and resistance challenges in lung cancer. These preclinical results support further development of cubosomes for clinical translation in non-small cell lung cancer (NSCLC) therapy, offering a promising strategy to improve therapeutic outcomes.

CHAPTER 1

INTRODUCTION

1.1 Background

Lung cancer (LC) ranks as the second most prevalent global cancer and the primary cause of cancer-related fatalities. In 2020, LC contributed to 1.8 million deaths, comprising 18% of all cancer mortalities (Sung et al., 2021). The year also saw 2.2 million new LC cases worldwide, with a disproportionate impact on males (14.3%) and females (8.4%) (Y. Zhang, Luo, Etxeberria, & Hao, 2021). According to the latest GLOBOCAN data from 2022, lung cancer is one of the most common and deadliest cancer globally as it accounts for nearly two and half million new cases and over one and half million deaths worldwide, making up around 12.4% of all cancer diagnoses and 18.7% of all cancer deaths (Figure 1.1).

Survival rates for lung cancer remain quite low, typically below 20% even in the most developed countries (Allemani et al., 2018) (Soerjomataram et al., 2023). The survival outcomes observed are likely influenced by a variety of factors, including the healthcare system, access to treatment, and the presence of other medical conditions (Araghi et al., 2022). Because most lung cancers are not detected until they have reached an advanced stage where curative treatment is no longer feasible, there has been a continued focus on screening high-risk groups, such as current and former smokers, through randomized controlled studies (Frille, Hardavella, & Lee, 2020) (de Koning et al., 2020).

LC, characterized by aggressive growth and metastasis in lung tissue, primarily manifests as non-small cell lung cancer (NSCLC), constituting over 85% of reported

cases, proved to be major cause of global cancer mortalities (Bade & Cruz, 2020). The current treatment strategies are based on tumor type, stage of tumor and mainly patient health. The conventional approaches are chemotherapy, surgery and radiation therapy but innovative treatment techniques like targeted therapy and immunotherapy are also employed (Kunda, 2020). Despite research advances and therapeutic developments, overall 5-year survival remains at approximately 23%, emphasizing the urgent need for treatment strategies with minimal adverse effects, toxicity, and drug resistance in novel drug candidates and delivery systems (Schabath & Cote, 2019).

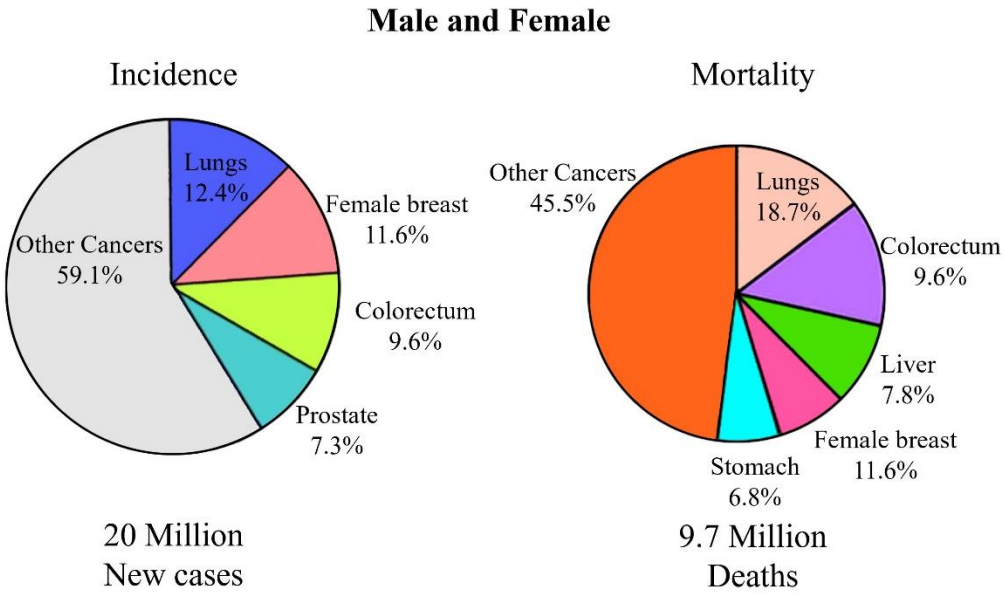

Figure 1.1 Distribution of incidence and mortality for the top cancers among 185 countries in 2022. Adapted from (Bray et al., 2024)

The identification and development of new drug moieties is a costly process, typically requiring 10 to 15 years before they reach the market (Pushpakom et al., 2019). The slow progress, substantial development costs, and high failure rates in new drug discovery have led to the increased focus on redefining existing drugs. (Parvathaneni, Kulkarni, Muth, & Gupta, 2019). Drug remodeling involves repurposing approved medications for therapeutic uses beyond their originally

approved indications or exploring new applications for investigational drugs outside their initial study parameters. Cisplatin is an alkylating drug with a square planar geometry that cause DNA damage and obstruct DNA repair (Alderden, Hall, & Hambley, 2006). Clinical studies have demonstrated that cisplatin is effective in treating various tumors such as sarcomas i.e. tumors of bones, soft tissues, skin and nerves (Rajeswaran, Trojan, Burnand, & Giannelli, 2008).

Lately, tumor-targeted lipid nanoparticles have been developed to deliver cisplatin and PD-1 to cancer sites (Lan et al., 2020). Yet another study reported that the chitosan nanoparticles having magnetic core with cisplatin entrapped were able to enter tumor cells and release the drug precisely (Siavashy et al., 2021). A recent study concluded that falnidamol and cisplatin combinational treatment effectively restrained non- NSCLC progression, and thus can be used as a promising therapeutic strategy (Cui, Li, Zhao, & Chen, 2022). Cisplatin comes with the challenges like drug resistance mainly pre-target, post-target and on- target resistances due to alterations in tumor environment that leads to reduction in the amount of cisplatin in the cytoplasm and molecular damage caused by cisplatin (Galluzzi et al., 2014). So, the combination of cisplatin and carboplatin along with third generation antitumor drugs enhanced survival and response rate in advanced NSCL patients (Vasconcellos et al., 2020). Current developments in the area of nanoparticulate systems have allowed researchers to effectively treat LC while potentially circumventing the drawbacks of traditional therapy approaches. In fact, suboptimal drug concentrations usually show modest antitumor action and may result in drug resistance. To effectively eradicate tumors, anti-cancer drugs have to penetrate cancer tissues to achieve the optimal concentration at site (Yaghmur & Glatter, 2009).

The bi-continuous liquid crystalline nano-structures known as cubosomes which possess superior drug loading properties because of viscosity, biocompatibility and bio-adhesivity and high surface area (Pan et al., 2013; Peng et al., 2010a). Glyceryl monooleate (GMO), or monoolein, is the predominant amphiphilic lipid utilized in cubosomes formation (Umar, Wahab, Gazzali, Tahir, & Ahmad, 2022). It is evident from literature that drug with various affinities like hydrophobic and hydrophilic can be loaded to cubosomes (Nasr, Ghorab, & Abdelazem, 2015) as recently, various antitumor drugs have been incorporated successfully into cubosomes like 5-fluorouracil, etodolac, capsaicin and resveratrol (Abdel-Bar & Abd el Basset Sanad, 2017; Y. Tian et al., 2017). For the treatment of breast cancer, etoposide was incorporated into folate modified cubosomes which depicted sustained release (Y. Tian et al., 2017). For antitumor activity cubosomes was loaded with doxorubicin which proved to be a pH sensitive carrier (Zhen et al., 2012). A pH-responsive lipid nano carriers of GMO containing sparingly water soluble anticancer drug 2-hydroxyoleic acid (2OHOA) specifically target the cancer tissues having acidic extracellular pH environment (Prajapati, Gontsarik, Yaghmur, & Salentinig, 2019).

1.2 Problem Statement

More people die from LC than from any other disease worldwide and it is particularly widespread in developing countries. According to latest American Cancer Society report on global cancer statistics, Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death overall and in men worldwide, with almost 2.5 million cases (1 in 8 cancers) and 1.8 million deaths (1 in 5 deaths). In females, it is the second most prevalent and deadly tumor worldwide (Rebecca L. Siegel, Giaquinto, & Jemal, 2024). Surgery is the primary curative treatment for early-

stage lung cancer. Nonetheless, the majority of patients have advanced NSCLC that is incurable at stage IIIB or stage IV, or they relapse after surgery intended to be curative, indicating the severity of the disease and the bleak prognosis (Bryan & Donington, 2019).

Cisplatin is a widely used chemotherapeutic agent for various cancers; however, its clinical efficacy is often limited by severe systemic toxicity, poor solubility, and rapid clearance from the body. Conventional delivery methods lead to dose-dependent side effects such as nephrotoxicity, neurotoxicity, and drug resistance, reducing its therapeutic potential. There is an urgent need for an advanced drug delivery system that can enhance cisplatin's bioavailability, enhance solubility, prolong circulation time, and allow sustain drug release, thereby, reducing the dose related toxicity. Cubosomes, self-assembled lipid-based nanocarriers with a bicontinuous cubic phase structure, offer a promising approach due to their high drug-loading capacity, biocompatibility, and controlled release properties. Cubosomes can be particularly advantageous for cisplatin delivery due to their ability to enhance solubility, reduce systemic toxicity, and allow sustained drug release. Hence, this study investigates the potential of glyceryl monooleate based cubosomes for delivery of cisplatin for human lung carcinoma. It is anticipated that this research will offer the possibility of encapsulating the cisplatin while remain stable under specified conditions and releasing cisplatin at a controlled, sustained rate to treat human lung carcinoma.

1.3 Study Objectives

The primary objective of this study was to develop optimized glyceryl monooleate based cubosomes for delivery of cisplatin against human lung carcinoma

and then perform the physical and chemical characterization of nano-formulations (Figure 1.2). The specific objectives were as follows:

a) To develop a factorial design for optimizing blank cubosomal formulations by evaluating the effects of lipid composition, surfactant ratio, and processing parameters on particle size, zeta potential, and PDI.

b) To develop and validate a reverse-phase HPLC (RP-HPLC) method with UV detection for accurate quantification of cisplatin in cubosomal formulations.

c) To assess the encapsulation efficiency of cubosomes by loading cisplatin at various concentrations using dialysis membrane method.

d) To characterize the cisplatin loaded cubosomes physically and chemically using various techniques like zeta potential and particle size analysis, Fourier Transform Infrared Spectroscopy (FT-IR), thermal analysis (DSC) and phase identification of cisplatin in cubosomes.

e) To evaluate the in vitro release kinetics of cisplatin from cubosomes in phosphate-buffered saline (pH 7.4) under sink conditions at predetermined time intervals.

f) To assess the in vitro cytotoxicity of blank cubosomes, cisplatin solution, and cisplatin-loaded cubosomes using the MTT assay against the NCI-H226 human lung carcinoma cell line.

g) To evaluate the accelerated stability of cisplatin-loaded cubosomes under controlled conditions (40°C ± 2°C, 75% ± 5% RH) and refrigerated temperature (4-8ºC) over three months, assessing changes in particle size, zeta potential, and encapsulation efficiency.

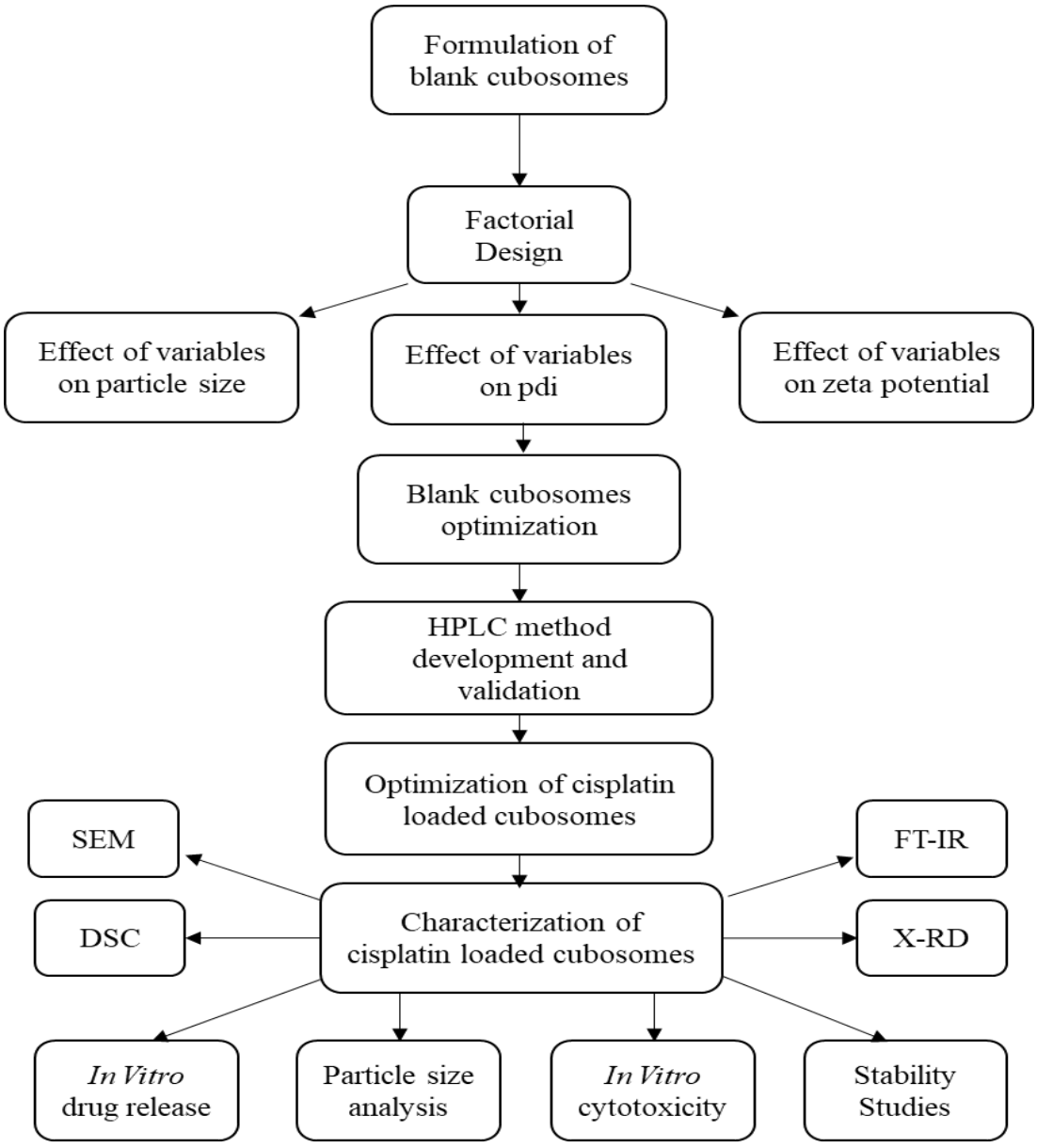

Figure 1.2 Flow chart of the research study

CHAPTER 2

LITERATURE REVIEW

Compared to the combined death rates of the other three most common cancers (colon, breast, and pancreatic cancer), lung cancer continues to be one of the deadliest types of cancer for both men and women. A staggering proportion of individuals diagnosed with lung cancer face a dire prognosis, with over half succumbing within the first year post-diagnosis, and a mere 17.8% surviving beyond five years (Zappa & Mousa, 2016). Lung cancer is one of the most diagnosed cancer types, accounting for approximately 12.5% of all cancer cases globally in 2023. Moreover, it is the leading cause of cancer-related deaths worldwide, responsible for around 21% of all cancer fatalities in the same year (Malvezzi et al., 2023). Lung cancer ranks as the second most common cancer among women and the leading cancer diagnosis among men worldwide (Rebecca L Siegel, Miller, Wagle, & Jemal, 2023).

The disease manifests primarily in two principal subtypes: small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), with NSCLC constituting the majority at 85% (Sher, Dy, & Adjei, 2008). NSCLC comprised of adenocarcinoma and squamous-cell carcinoma (SCC) and the later one contributes to about 25–30% of cases of lung cancer. The SCC develops from primitive squamous cells in the airway epithelial cells of the bronchial tubes, which are found in the center of the lungs. However, there is a strong association between squamous-cell carcinoma and cigarette smoking (Kenfield, Wei, Stampfer, Rosner, & Colditz, 2008).

Adenocarcinoma, the most common type of lung cancer, accounts for about 40% of the disease. Usually appearing in the outer regions of the lungs, adenocarcinoma is derived from the mucus-producing type II alveolar cells and tiny

airway epithelial cells (W. Travis & Travis, 1995). This peripheral localization may be attributed to the inclusion of filters in cigarettes, which block larger particles from entering the lungs, prompting deeper inhalation of cigarette smoke and resulting in lesions in the lung's outer regions. Adenocarcinoma often grows more slowly than other forms of lung cancer and is more likely to be diagnosed before it spreads outside of the lungs (Perez-Moreno, Brambilla, Thomas, & Soria, 2012).

A comprehensive, multidisciplinary strategy is imperative to advance global efforts toward eradicating tobacco-related morbidity and mortality, a critical public health objective in the fight against cancer. Central to this endeavor is the prevention of nicotine addiction through evidence-based interventions. Harm reduction approaches, such as nicotine replacement therapies (NRTs) and electronic cigarettes (e-cigarettes), aim to decouple nicotine consumption from exposure to carcinogenic combustion byproducts found in conventional cigarettes (Shahab et al., 2017). However, the efficacy of e-cigarettes as smoking cessation tools remains contested; while some studies suggest short-term benefits (Hajek et al., 2019), concerns persist regarding their long-term safety and potential to renormalize smoking behaviors among youth (Berry et al., 2019). Socio-structural measures such as tobacco taxation, advertising bans, and plain packaging laws, have demonstrated population-level efficacy in curbing smoking initiation. Notably, the U.S. Food and Drug Administration's (FDA) 2023 proposal to mandate non-addictive nicotine levels in combustible cigarettes represents a groundbreaking regulatory shift, though its long-term impact requires rigorous evaluation (Aaron, Wallace, & Sinha, 2023). Despite these advances, the debate surrounding harm reduction persists, underscoring the need for longitudinal research to resolve uncertainties about e-cigarettes' role in cessation

and their potential to inadvertently perpetuate nicotine dependence or act as gateways to combustible tobacco use (Brandon et al., 2015).

2.1 Lung cancer microenvironment

Since lung cancer has a diverse molecular makeup, improving therapy options requires a grasp of the biology underlying the illness. The management of lung cancer has transitioned from the empirical use of non-specific cytotoxic therapies based on physician preference, to an approach more aligned with personalized medicine (Herbst, Morgensztern, & Boshoff, 2018). Specific subsets of lung cancer patients are now treated based on the genetic alterations present in their tumor, as well as the status of programmed death ligand-1 (PD-L1). These biomarkers are useful in predicting which patients will respond best to immune checkpoint blockers or targeted treatments. This shift towards personalized, biomarker-guided treatment represents a hallmark of the progress made in lung cancer care (Darvin, Toor, Sasidharan Nair, Elkord, & medicine, 2018).

The tumor microenvironment (TME) is shaped by the same genetic events that propel tumor initiation and evolution. As a result, the genetic architecture of a tumor controls both the TME's makeup and the fitness of the cancer cells. One characteristic that sets NSCLC apart is its high somatic tumor mutation burden (TMB) which refers to quantity of nonsynonymous coding mutations per megabase (Mb). This is especially true in smokers, who represent the majority of NSCLC patients. Importantly, the total number of mutations is significantly higher in metastatic lesions compared to the primary lung tumors. This suggests that the genetic complexity and evolution of lung cancers increases as the disease progresses (Robinson et al., 2017).

The author interprets further the relationship between tumor neoantigens, the tumor microenvironment (TME), and immune responses, specifically how these factors influence the effectiveness of immune checkpoint blockade (ICB) therapy. Cytotoxic T cells infiltrating tumors can recognize neoantigens generated by specific mutations. In lung adenocarcinoma (LUAD), a tumor microenvironment (TME) characterized by inflammation, abundant antigens, active effector T cells, and inhibitory immune checkpoints, such as programmed death-1 (PD-1) and lymphocyte activation gene-3 (LAG-3), is associated with a high load of clonal neoantigens. This trait can make a patient more sensitive to ICB treatment. The hallmark of cancer with a high TMB is the microsatellite instability phenotype caused by loss of mismatch-repair capability. These tumors exhibit T cell infiltration and noticeable responses to ICBs, regardless of the tissue of origin (Le et al., 2015).

Genetic alterations can influence the tumor microenvironment (TME) in various ways. One example is the inactivation of the tumor suppressor gene serine/threonine kinase 11 (STK11, also known as LKB1), which occurs in about one-third of KRAS-mutated lung adenocarcinomas (LUAD). Due to STK11 inactivation, the TME is skewed toward immunosuppressive neutrophil increase and decreased PD-L1 expression. Importantly, this genetic event is also associated with fewer tumor-infiltrating lymphocytes in the TME (Koyama et al., 2016).

2.2 Global lung cancer pattern

In 2022, LC was responsible for around 2.5 million new cases and 1.8 million deaths worldwide, making it the primary cause of cancer-related morbidity and mortality worldwide. This amounts to about 18.7% of all cancer-related fatalities and 12.4% of all new cancer diagnoses (Table 2.1).

Table 2.1 New cases and deaths for top 10 cancers and all cancers combined in 2022.

Cancer site	**Incidence**			**Mortality**		
	Rank	**New cases**	**% of all sites**	**Rank**	**New cases**	**% of all sites**
Lung	1	2,480,301	12.4	1	1,817,172	18.7
Female breast	2	2,308,897	11.6	4	665,684	6.9
Colorectum	3	1,926,118	9.6	2	903,859	9.3
Prostate	4	1,466,680	7.3	8	396,792	4.1
Stomach	5	968,350	4.9	5	659,853	6.8
Liver	6	865,269	4.3	3	757,948	7.8
Thyroid	7	821,173	4.1	24	47,485	0.5
Cervix uteri	8	661,021	3.3	9	348,189	3.6
Bladder	9	613,791	3.1	13	220,349	2.3
Non-Hodgkin lymphoma	10	553,010	2.8	11	250,475	2.6

Lung cancer had an incidence and fatality ratio of about 2:1 across males and females, making it the most prevalent cancer in men and the second most common in women. Geographically speaking, the gender gap varied considerably; rates in North America and Northern Europe were equal, whereas rates in Northern Africa and Eastern Europe were four to five times higher (Kratzer et al., 2024)(Figure 2.1).

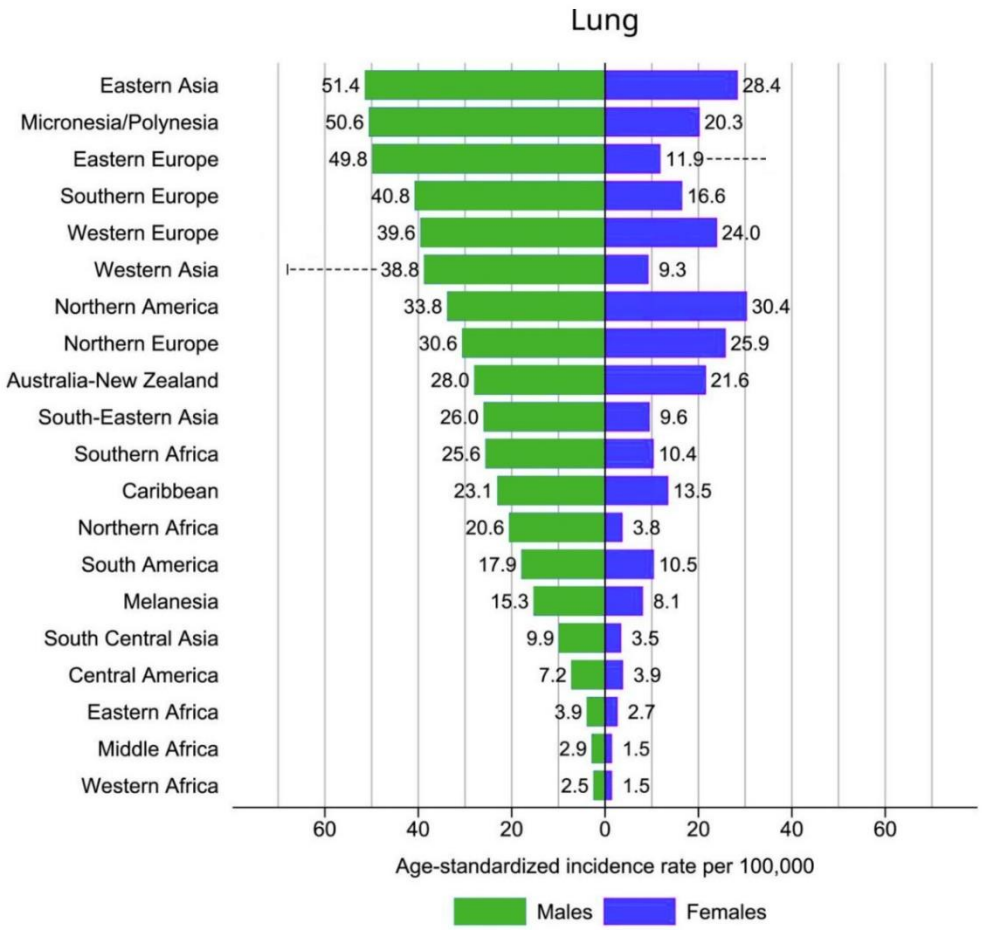

Figure 2.1 Bar chart of the region-specific incidence age-standardized rate by sex for lung cancer among 2022. Adapted from (Cao, Qin, Li, & Chen, 2024)

Figure 2.2 represents that LC was the most common diagnosed cancer in men in East Asian and South Asian countries while it was the main cause of cancer-related fatalities in 89 nations across the globe i.e. Asia, America and Australia (Figure 2.3).

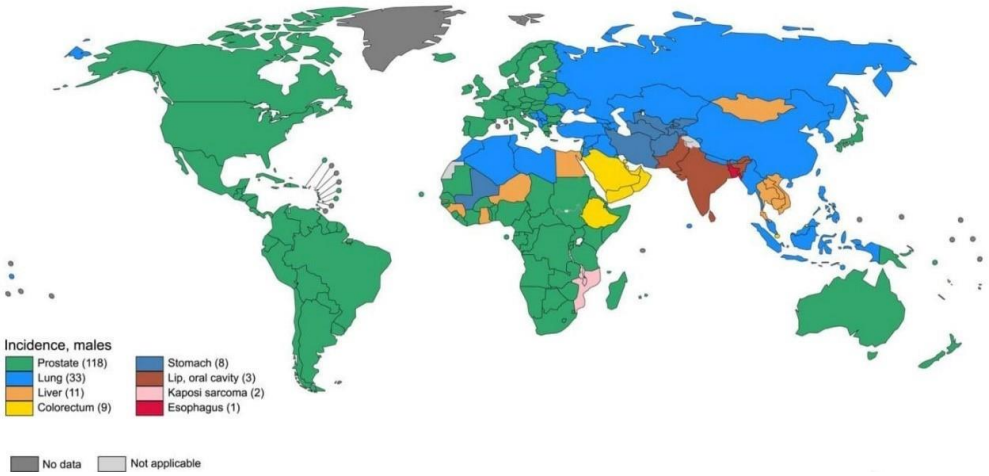

Figure 2.2 Global maps present the most common type of cancer incidence in 2022 in each country among men according to GLOBOCAN 2022. Adapted from (Bray et al., 2024)

Eastern Asia, Micronesia/Polynesia, and Eastern Europe had the highest incidence rates of lung cancer in men; Turkey has the highest national rate in the world for men. In 23 nations, including China and the US, lung cancer was the most common cause of cancer-related fatalities among women (Figure 2.4). North America, Eastern Asia, and Northern Europe had higher incidence rates of lung cancer in women.

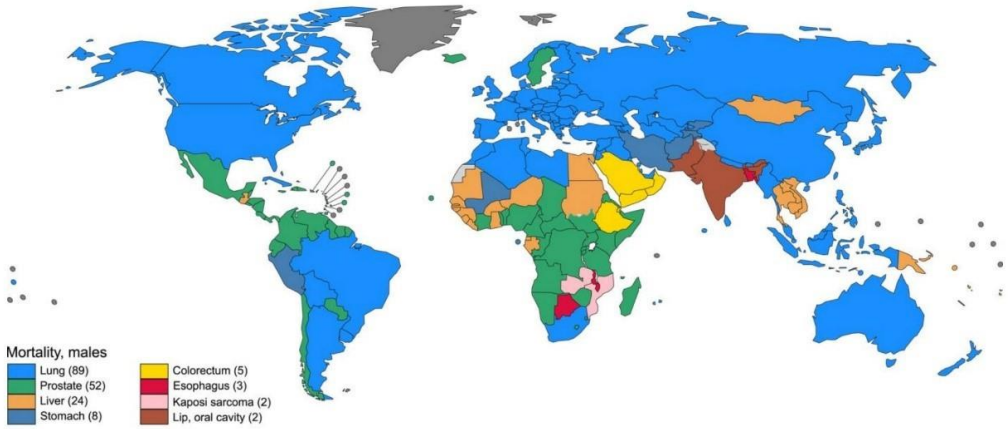

Figure 2.3 Global maps present the most common type of cancer mortality by country in 2022 among men according to GLOBOCAN 2022. Adapted from (Bray et al., 2024)

Lung cancer incidence and death show clear geographic and chronological trends along with poor survival rates, which are mostly due to the different stages of the tobacco epidemic in different nations. This depends on a number of variables, including the amount of smoke inhaled, the kind of cigarettes smoked, and the intensity and duration of smoking (Wéber et al., 2023). Among men, several high-income countries that were early adopters of smoking have seen a decline in smoking exuberance, followed by peak and then subsequent decline in lung cancer rates in the same generations, with a 20-25 year lag between the smoking and cancer trends (Alonso et al., 2018).

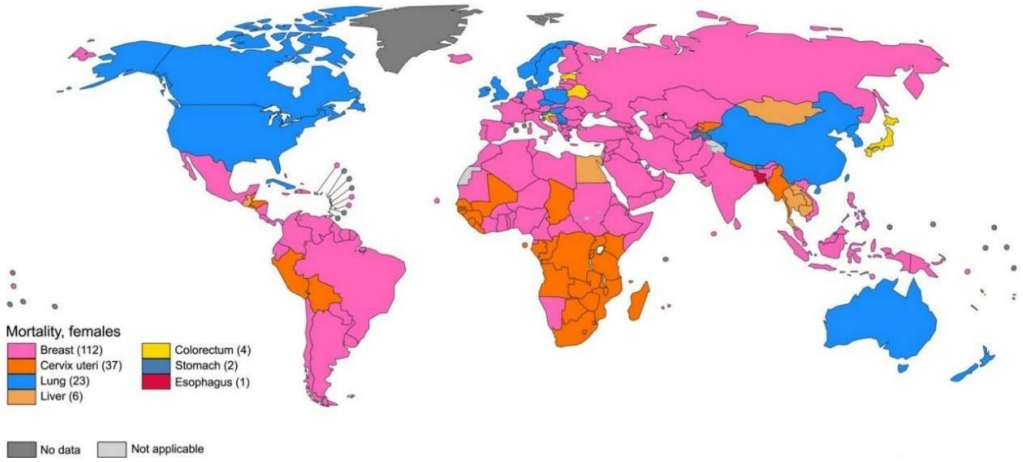

Figure 2.4 Global maps present the most common type of cancer mortality by country in 2022 among women according to GLOBOCAN 2022. Adapted from (Bray et al., 2024)

Compared to men, the global tobacco epidemic among women is still in its early stages. The degree to which smoking tendencies among women are similar to those of men, varies greatly by geographic area (Miranda-Filho, Piñeros, & Bray, 2019). With just a few notable exceptions, such as the United States, women's lung cancer rates are still rising in the majority of developed nations (Thun, Linet, Cerhan, Haiman, & Schottenfeld, 2017). Because of this, women's lung cancer incidence rates

are getting close to or even higher than those of men, especially in younger and middle-aged countries in North America and Europe. This points to a rising incidence of lung cancer in women in general over the next several decades (Jemal et al., 2023).

The prevalence of male smoking is increasing in developing nations where the tobacco outbreak is still in its initial phases. Thus, unless appropriate tobacco control initiatives are put in place to hasten smoking cessation or lower initiation, the rates of lung cancer in these individuals are probably going to keep growing for the foreseeable future. It is especially alarming since there may be a sharp increase in lung cancer mortality worldwide, as some of the world's most populous countries, such as Indonesia (54.4% male smoking prevalence) and China (41.5% male smoking prevalence), have among the highest daily smoking rates among men globally. This represents a pressing public health challenge that will require concerted tobacco mitigation efforts in the coming years (Organization, 2023).

Smoking rates among women in transitional nations vary widely, in contrast to the high prevalence of smoking among men in these same countries. For instance, in nations like Indonesia, China, and the majority of Africa, the proportion of women who smoke every day is believed to be quite low (<5%). Around 25% of lung cancer cases worldwide can be traced back to factors other than tobacco usage (Fidler-Benaoudia, Torre, Bray, Ferlay, & Jemal, 2020). Yet, in some populations like women in Eastern Asia, where smoking is less common and lung cancer among non-smokers represents a large disease burden, this number could potentially be greater. Some theories suggest that elevated exposure to external factors, such as outside air pollution and the use of solid fuels for cooking and heating in homes, may contribute to the high lung cancer rates among women in some regions, such as China, instead of only tobacco usage (Leiter, Veluswamy, & Wisnivesky, 2023).

2.3 Pathophysiology of Non–Small Cell Lung Cancer

The pathogenesis of NSCLC is multifactorial and evolves over many years as a result of chronic exposure to environmental carcinogens (e.g., cigarette smoke, radon, asbestos) and the accumulation of both genetic and epigenetic alterations. These alterations disrupt normal cellular signaling, promote unchecked proliferation, and enable escape from apoptosis and immune surveillance (Gridelli et al., 2015).

2.3.1 Genetic and Epigenetic Alterations in NSCLC

2.3.1(a) DNA Damage and Mutation Accumulation

Tobacco smoke, which contains numerous carcinogens, induces DNA adduct formation and double-strand breaks. In individuals with prolonged exposure, the balance between DNA damage and repair is disrupted. Mutations accumulate in key genes—such as *TP53, KRAS, EGFR,* and *STK11*—leading to a loss of cell cycle control and an increased potential for malignant transformation (W. D. Travis et al., 2013).

2.3.1(b) Driver Mutations and Targetable Alterations:

NSCLC frequently harbors activating mutations in the epidermal growth factor receptor (EGFR) gene. These mutations result in constitutive receptor activation, stimulating downstream signaling pathways such as PI3K/AKT/mTOR and RAS/RAF/MEK/ERK. For example, activating EGFR mutations are pivotal in the oncogenesis of lung adenocarcinomas, which form the basis for targeted therapies using tyrosine kinase inhibitors (TKIs) (Mok et al., 2009; Shaw et al., 2014).

2.3.1(c) DNA Methylation

Aberrant promoter hyper methylation is a common mechanism of tumor suppressor gene silencing in NSCLC. Genes such as *p16* (CDKN2A) and DNA repair genes like *MGMT* are frequently silenced by DNA methylation, contributing to genomic instability and further promoting oncogenesis (Sandoval et al., 2013).

2.3.1(d) Histone Modifications

Alterations in histone acetylation and methylation contribute to the repressive chromatin state observed in NSCLC. Decreased acetylation and aberrant methylation patterns (e.g., reduced H3K4me3 and increased H3K27me3) have been implicated in the down regulation of critical regulatory pathways governing cell cycle and apoptosis. These epigenetic aberrations not only silence tumor suppressors but can also affect the responsiveness to both conventional chemotherapy and emerging immunotherapeutic approaches (Cohen, Poręba, Kamieniarz, Schneider, & cancer, 2011).

2.3.2 Disrupted Signal Transduction in NSCLC

2.3.2(a) EGFR Signaling Pathway

Activating mutations and amplifications of the EGFR gene result in ligand-independent receptor dimerization and autophosphorylation. This aberrant signaling continuously drives cell proliferation, survival, and angiogenesis through downstream pathways such as PI3K/AKT and RAS/RAF/MEK/ERK. The clinical relevance of this pathway is underscored by the success of EGFR inhibitors (e.g., erlotinib, gefitinib, Osimertinib) in subsets of patients with NSCLC harboring EGFR mutations (T. J. Lynch et al., 2004).

2.3.2(b) RAS/RAF/MEK/ERK and Alternative Pathways

Mutations in *KRAS* are another frequent event in NSCLC, particularly in smoking-associated adenocarcinomas. KRAS mutations lead to constitutive activation of the MAPK pathway, contributing to increased proliferation and resistance to apoptosis. Although direct targeting of RAS has been challenging, the downstream effectors of this pathway represent promising therapeutic targets (Pao & Girard, 2011)

2.4 Non-small cell lung cancer treatment approaches

Surgery, radiation, chemotherapy, immunotherapy, and molecularly targeted therapy are often used as treatment options for NSCLC. Based on the stage of disease, its histology, genetic abnormalities, and patient's health, these therapies are applied either alone or in combination. Surgical resection to cure the condition is advised for individuals with early NSCLC (Stage I, Stage II, and Stage IIIA) and who are otherwise healthy. Although patients with illnesses in stages II-IIIA are advised to get adjuvant platinum-based chemotherapy, which has a 5.4% absolute mortality risk reduction at five years, the relapse and toxicity rates are high (Douillard et al., 2010). Multidisciplinary consultation is advised prior to beginning treatment, particularly for stage IIB and stage IIIA illnesses. The overall survival of early-stage patients has not yet been demonstrated to be improved by molecularly targeted therapies. Nearly 30% of NSCLC patients will experience localized disease progression (T3-T4, N2-N3, stage IIIA-C). The majority of stage III NSCLC patients are non-surgical candidates, and a combination of chemotherapy and radiation therapy is currently the backbone of management, followed by immunotherapy (Brierley, Gospodarowicz, & Wittekind, 2017).

2.5 Cisplatin first-line agent for the treatment of NSCLC

Cisplatin (Figure 2.5a) belongs to the class of chemotherapeutic drugs known as alkylating agents comprised of platinum metal, which permanently alters the DNA of dividing cells. This results in the death of rapidly dividing cells, including cancer cells, by stopping or slowing their proliferation (Alderden et al., 2006). The FDA approved the medication in 1978 after finding that hydration could reduce the nephrotoxicity brought on by cisplatin. Cisplatin is commonly used in conjunction with third-generation cytotoxic medicines in the majority of current regimens (Rajeswaran et al., 2008). Cisplatin remains a cornerstone in the first-line treatment of lung cancer, particularly for both non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) (Riely et al., 2024). In NSCLC early stage (I-IIIA), for patients with resect able tumors, surgery is the primary treatment. Adjuvant chemotherapy, often involving cisplatin-based regimens, is recommended to eliminate residual cancer cells and reduce recurrence risk (Calvo et al., 2024). While in advanced-stage (IIIB-IV) where surgery isn't feasible, cisplatin is combined with other chemotherapeutic agents, such as pemetrexed or gemcitabine, as part of the first-line treatment. The choice of combination depends on factors like tumor histology and patient health status (Qiang Chen, Ying, Qin, & Zhang, 2024).

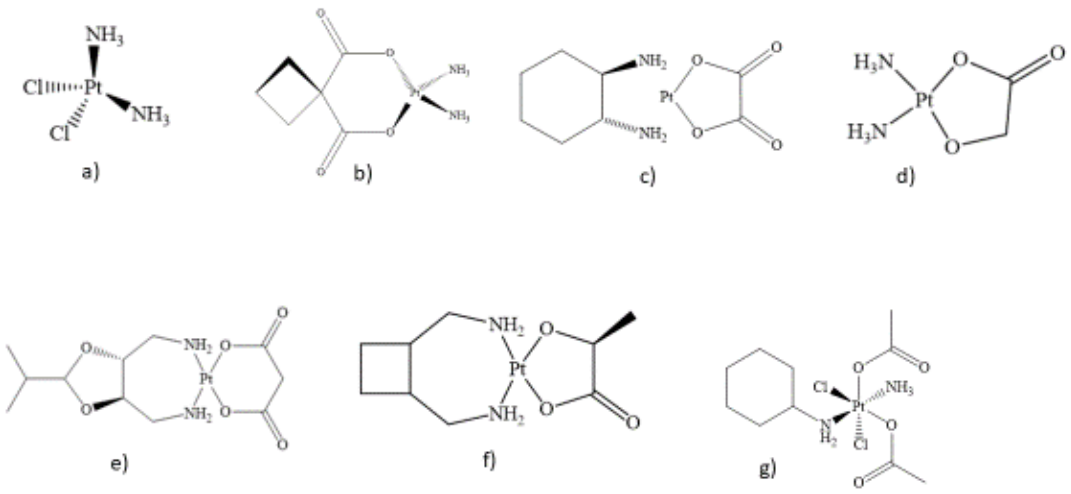

Figure 2.5 Structures of cisplatin and its derivatives

Over time, a concerted effort has been made to define and enhance the usage of cisplatin in the management of NSCLC. A meta-analysis of randomized trials first identified Cisplatin's benefit in patients with advanced NSCLC. Cisplatin-based chemotherapy improved 1-year survival by 10%, median survival by 1.5 months, and mortality risk by 27% compared to supportive care. The necessity for more extensive randomized controlled trials comparing cisplatin-based chemotherapy with supportive care was demonstrated because this meta-analysis only included 416 patients who had received treatment using a wide range of regimens (Group, 1995). Later randomized trials that compared chemotherapy to supportive care with combination therapy found that chemotherapy was once more associated with noticeably better survival. The MIC trial made it clear for the first time that advanced NSCLC patients' quality of life was improved by cisplatin-based therapy. In this trial, cisplatin was administered every three weeks at 50 mg/m² (Cullen et al., 1999).

2.6 Derivatives of cisplatin

2.6.1 Carboplatin

Carboplatin (Figure 2.5b) is the second-generation platinum compound with the best clinical outcomes. The medicine has only ever been recommended for use in the management of testicular and ovarian carcinomas when there are abnormalities in renal function, and the FDA licensed it to treat advanced ovarian cancer. For cancers of the brain, head and neck, lung, breast, esophagus, bladder, cervical, salivary glands, and kidneys as well as retinoblastoma, neuroblastoma, and nephroblastoma is very effective at sensitizing tumor cells to radiation therapy (Ho, Woodward, & Coward, 2016; Johnstone, Suntharalingam, & Lippard, 2016).

Combinations of carboplatin and other medications increase the antitumor effect on several cancers (Bailly, Thuru, & Quesnel, 2020). Due to the slower hydrolysis of the remaining bidentate dicarboxylate ligands compared to Cisplatin's labile chloride ligands, Carboplatin's adverse effects like neuropathy, nephrotoxicity, ototoxicity, and gastrointestinal toxicity, are less severe and easier to manage than those of Cisplatin. Myelosuppression, which manifests clinically as severe thrombocytopenia, neutropenia, and leukopenia, is a compound-limiting side effect that calls for blood parameter monitoring or dose reduction. Although the minimal nephrotoxicity of carboplatin is beneficial, particularly for people with kidney illness, cross-resistance prevents it from serving as a substitute for cisplatin in resistant tumors (Johnstone et al., 2016).

2.6.2 Oxaliplatin

In 1970, scientists at Nagoya University in Japan created oxaliplatin (Figure 2.5c). In 2002, the FDA in the US approved the medication. Because the substance also inhibits protein synthesis, which prevents cell proliferation, it is more effective than Cisplatin. In addition to malignant melanoma, glioblastoma, ovarian and breast cancer, non-small-cell lung cancer, head and neck cancer, and neuroendocrine tumors have all been treated with oxaliplatin (Perego & Robert, 2016).

The medication exhibits more activity and a better therapeutic index in colorectal cancers when compared to carboplatin and cisplatin. The mechanism underlying this uniqueness has to do with variations in absorption. The main determinants of anticancer action are human organic cationic transporters (OCTs), which dramatically increase oxaliplatin accumulation and cytotoxicity in cell lines. OCTs are overexpressed in tumor cells and contribute to antitumor specificity. In Europe, Asia, Latin America, and eventually the United States, the medication has been given the go-ahead for the treatment of metastatic colorectal cancer.

Significant neurotoxicity and tubular necrosis are the dose-limiting side effects of oxaliplatin, but neutropenia and other more severe side effects are more noticeable with cisplatin [103]. The two types of neurotoxicity associated with the drug are paresthesia, dysesthesias in the limbs, clenching of the jaw muscles, and chronic neuropathy. Additional toxicities include significant sensory loss, sensory ataxia, and impairment of function caused by changing the outer surface membrane's potential (Gebremedhn, Shortland, & Mahns, 2018).

2.6.3 Nedaplatin

In preclinical investigations, nedaplatin (Figure 2.5d) outperforms cisplatin, and head, neck, esophageal, non-small cell lung, cervical, testicular, and prostate cancer are the official indications for use in Japan. The drug did not outperform Cisplatin and Carboplatin, but it does exhibit much higher anticancer activity in resected gynecologic carcinoma when compared to Cisplatin. It has been demonstrated that radiation therapy and medication combinations together have a synergistic effect on cancer. Nedaplatin had less leukopenia, nephrotoxicity, neurotoxicity, and gastrointestinal toxicity. Myelosuppression, including thrombocytopenia, neutropenia, and anemia, is a dose-limiting hazard (Shimada, Itamochi, & Kigawa, 2013; Zhu et al., 2019).

2.6.4 Heptaplatin

Heptaplatin (Figure 2.5e) was introduced into clinical trials in 1990 to treat gastric cancer. The drug is more effective against cisplatin-resistant human gastric cell lines than small cell lung cancer. Adducts created by heptaplatin cause cell death by impairing DNA transcription and replication. Heptaplatin's registration is based on its profile of less frequent thrombocytopenia, neuro, hepatic, embryotoxicity, and less nephrotoxicity, even though it is less efficacious than cisplatin (Tsvetkova & Ivanova, 2022; H.-Y. Zhang et al., 2014).

2.6.5 Lobaplatin

For the treatment of small cell carcinoma (Zhou et al., 2018), hypopharyngeal carcinoma, esophageal squamous cell carcinoma, and chronic myelocytic leukemia, lobaplatin (Figure 2.5f) has received approval in China. The drug exhibits action in