Chapter 1

INTRODUCTION

1.1 Introduction to lung cancer and its statistics

Lung cancer represents the most common root cause of deaths related to cancer around the world [1]. According to Cancer Statistics, 2024, published in CA: A Cancer Journal for Clinicians, 2,001,140 new cases of cancer have been reported with 611,720 deaths due to cancer in the United States and nearly 1680 deaths per day [2]. The greatest number of deaths are reported to be due to lung cancer [3] which is reported to be more than breast cancer, prostate cancer, pancreatic cancer, and colorectal cancer [4], [2], [5]. The main etiology reported for the manifestation of lung cancer is tobacco smoking in the United States or other countries where smoking is a common practice. The global fight against lung cancer can be dealt with through proper counselling and eradication programs which include e-cigarettes (these include nicotine without carcinogenic agents present in cigarettes), varenicline which is a partial agonist having affinity for nicotinic acetylcholine receptor, and socio-economic programs encompassing advertisement depicting hazards of tobacco smoking, taxation, and legislative protocols like decreasing the concentration of nicotine used in cigarettes to a concentration which is non-addictive (this is a recently declared policy by FDA against cessation of smoking) [6]. But these preventive measures turned out to be insufficient for a triumph over tobacco smoking and consequently lung cancer.

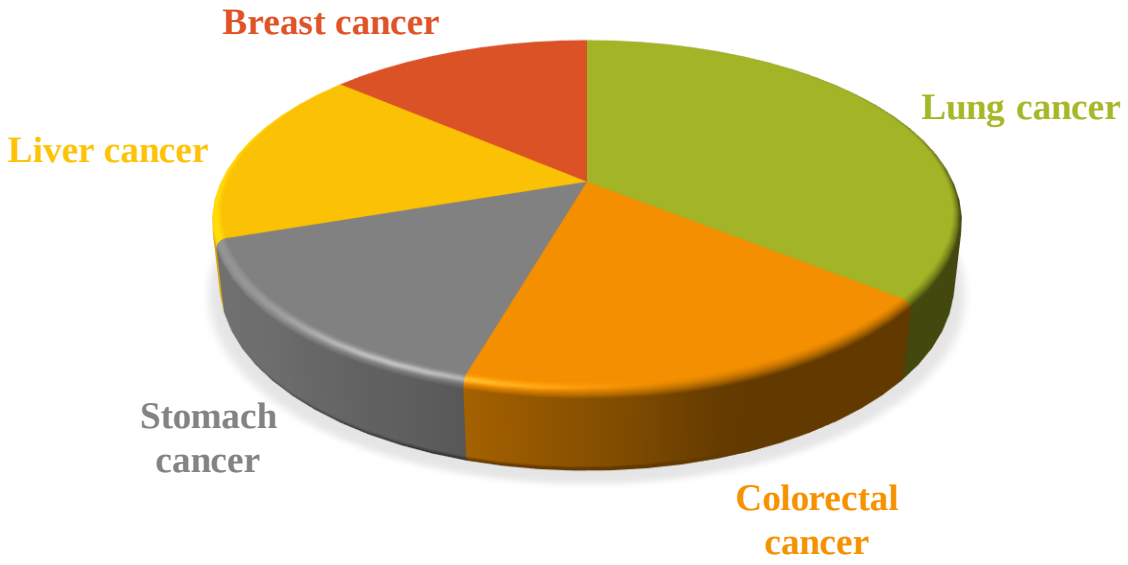

Figure 1: Pie chart depicting the percent of deaths related to different types of cancers as per data documented by the International Agency for Research on Cancer.

1.2 Types of lung cancer

Lung cancer can be categorized as small cell lung cancer (SCLC) (which is surgically resectable) and aggressive and metastatic non-small cell lung cancer (NSCLC), the latter is further divided into subtypes viz., lung adenocarcinoma and lung squamous cell carcinoma [7].

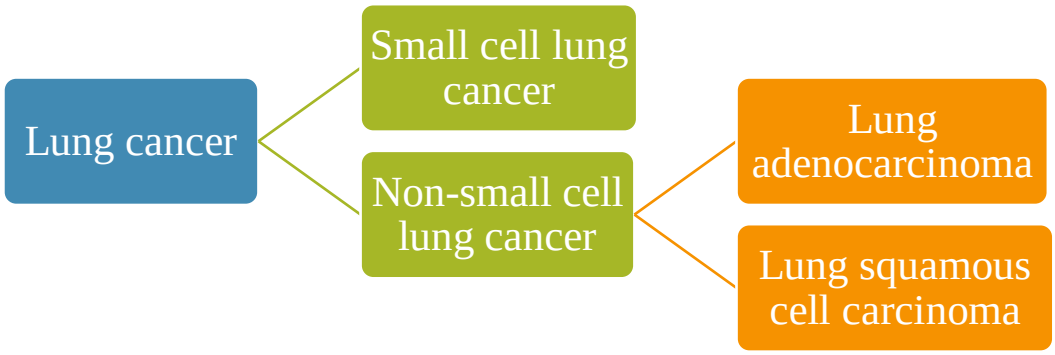

Figure 2: Categorization of lung cancer.

1.3 Biology of lung cancer

For the genesis of effective therapy against any disease, a detailed knowledge about the biology and etiology of the concerned disease is very important.

Lungs represent a sophisticated organ system comprising different cell types with discrete functions responsible for gaseous exchange. It encompasses an airway passage of approximately 2,000 km with thin alveolar membranes corresponding to around 50 m^2 in addition. This airway passage is responsible for the exchange of oxygen and carbon dioxide between the blood and environment along with exposure to toxic gases and contaminants corresponding to fine particulates and infectious agents. Ciliary action of larger airways is involved in the clearance of inhaled large particulates whereas immune cells and phagocytic cells are involved in the eradication of infectious agents [7]. An imbalance in the cell division and cell death of this sophisticated clique of cells results in the assemblage of cell-autonomous and microenvironmental adaptations leading to the progression of cancer. Years of subjection to tobacco smoke leads to structural damage in the lungs which emerges as chronic obstructive pulmonary disease (COPD) and emphysema [8]. Bronchial epithelium represents a concatenation of morphological alterations starting from basal cell hyperplasia to metaplasia followed by severe dysplasia to carcinoma in situ leading to frank carcinoma at a late stage. This concatenation of changes is characteristic of the squamous subtype of NSCLC whereas adenocarcinomas are primarily the dominant subtype in cases of low carcinogen subjection or never smokers with some cases of exceptions of adenocarcinomas in subjects with heavy carcinogen exposure too [9]. Atypical adenomatous hyperplasia is a less well-characterized premalignant lesion associated with adenocarcinomas [7].

1.4 Genetic basis of lung cancer

At the genetic level, the oncogenic mutations involved in the genesis and progression of NSCLC are translocations of the anaplastic lymphoma kinase (ALK) gene and activating

mutations in the epidermal growth factor receptor (EGFR) gene [10]. Lung tumors manifesting in the case of smokers are reported to have the greatest number of somatic alterations, suggesting 10 mutations per megabase with only $1/10^{th}$ the number of somatic mutations as compared with smokers reported in the case of non-smokers. 10-60 % of adenocarcinomas are set forth to have EGFR and ALK mutations. Deletion of exon 19 (E746-A750) and substitutions of exon 18 (G719C, G719S, G719A) and exon 21 (L858R) are the most common mutations reported for EFGR gene [11]. Other possible genetic basis which is deemed as targets for the treatment of NSCLC encompass mutations in MET (which encodes hepatocyte growth factor i.e., HGF receptor), HER2 (ERBB2), and BRAF, translocations of ROS1, RET, and receptor tyrosine kinases, and amplifications of HER2, MET, and fibroblast growth factor receptor 1 i.e., FGFR1. All subtypes of lung cancer promulgate mutations in the tumor suppressor genes viz., TP53 and RBI, and hence these mutations cannot be deemed as effective therapeutic targets for the treatment of specific subtypes of lung cancer. KRAS oncogenic mutations are specific for lung adenocarcinomas and are absent in SCLCs [12]. Other set-forth mutations for the genesis of lung cancer include mutations in tumor suppressor serine/threonine kinase 11 (STK11) which is also known as LKB1, AKT pathway, cyclins, and MAPK pathway which is also known as MEK [7].

Tumor cells secrete certain growth factors to forge an environment conducive to its growth and metastasis, which includes vascular endothelial growth factor (VEGF) for proper blood supply into tumor cells, platelet-derived growth factor, sonic hedgehog (SHH), fibroblast growth factors, and HGF [13], [14]. Bevacizumab is the monoclonal antibody targeting VEGF and sunitinib and sorafenib are the potential tyrosine kinase inhibitors for the treatment of lung cancer [7].

Tumor cells are in fact the intelligent ones that bypass immune recognition by camouflaging neoantigens as a result of structural alterations from cytotoxic T cells viz., deletions or

mutations in genes encoding β2- macroglobulin, class I MHC, and TAP peptide transporter. Other pathways involved in escaping of tumor cells from immune recognition and elimination involved sabotaging of regulatory signals viz., CD80, CD86, and CTLA4 (cytotoxic T lymphocyte-associated antigen 4) axis which is responsible for programmed cell death protein 1 (PD1) and T cell priming, and PDL1 (PD1 ligand 1) axis involved in T cell killing [15].

1.5 Risk factors associated with lung cancer

The most common risk factor associated with lung cancer is the consumption of tobacco or smoking, global data shows that 50 % of women and 80 % of men diagnosed with lung cancer were smokers. Never-smokers are defined as those individuals who have smoked less than 100 cigarettes in their entire lifetime and these never-smokers account for 10-25 % of lung cancer cases. Passive smoking and air pollution are other risk factors included in the list, particulate matter with size corresponding to < 10μm is found to be involved with a greater risk for the development of lung adenocarcinomas [7]. 9 % of death associated with lung cancer arises from subjection to radon and its inhalation. Decay products of radon gas sediments on the epithelium of bronchi followed by emission of α particles and irradiation of bronchial cells. Genetic risk factors include germline mutations of exon 20 T790M of the EGFR gene and mutations in the transmembrane domain of HER2 could be oncogenic and account for hereditary and sporadic lung adenocarcinomas [16], [17].

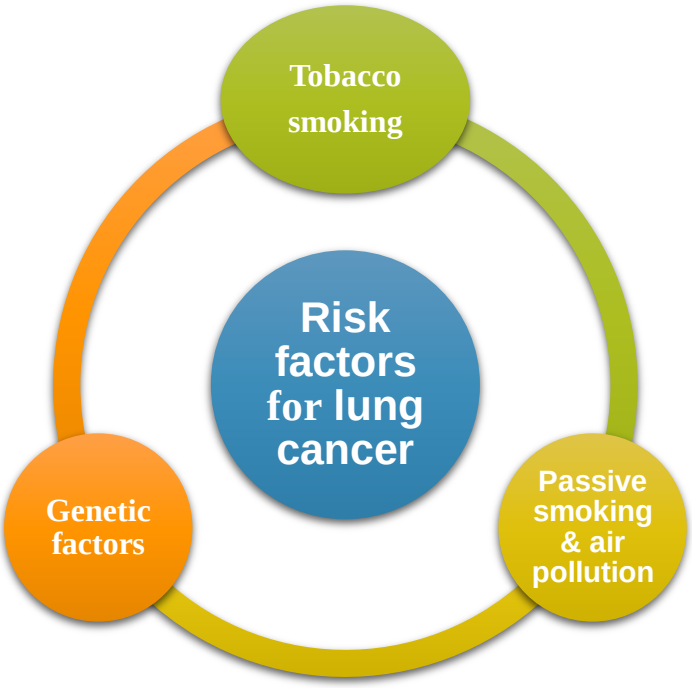

Figure 3: Common risk factors associated with lung cancer.

1.6 Diagnosis and screening of lung cancer

The diagnosis procedure generally commences once the patient reports not-so-good signs and symptoms undergoing in his body. The next step is to locate the lesion with the help of imaging techniques and upon confirmation with the aid of a biopsy sample the following step is to select an appropriate therapeutic strategy for effective treatment [7].

More than 75 % of cases with lung cancer delineate symptoms such as chest pain, cough, hemoptysis, dyspnoea, and weight loss accompanied by other symptoms such as superior vena cava syndrome, which is venous congestion reported in the upper chest, Horner syndrome (infiltration of apical lung tumors into the stellate ganglion and lower branches of brachial plexus), and shoulder pain (an ulnar aspect of the forearm and arm) [18].

Imaging, chest radiography is the first diagnostic test performed when one reports symptoms similar to lung cancer. This includes lateral and posterolateral chest radiography; however, these test results appear to be normal in 4 % of cases with lung cancer [19]. **Contrast-**

enhanced computed tomography (CT) scan of upper abdomen and chest is always advisable for proper gauging of the mediastinum which tends to be pivotal for correct staging of the lesion. CT scan of chest enables to track down the location of the lesions along with their alliance to nearby cellular structures viz., the heart, vessels, esophagus, and chest wall [20], [21].

Magnetic resonance imaging (MRI) seems to be more sensitive than CT scan imaging technique when it comes to lung cancer as lung cancer is reported tometastasize to brain and its detection is very important when one is eligible for the surgery of the lesions in the lung, neurosurgery or brain stereotactic radiotherapy. MRI has potentials to detect even smaller lesions in the brain [22], [23], [24].

Metabolic nature of the tumor and related lesions viz., metastases or lymphonodal invasion can be gauged with the aid of 18-fluorodeoxyglucose positron emission tomography. Assessment of metabolic profile about the tumor aids in deciding whether surgery or radiotherapy will be apt for the patient. Lung tumors located at the centre can be diagnosed by bronchoscopy whereas the tumors located at periphery of the lungs can be efficiently diagnosed with techniques like radiography and ultrasonography. Hilar lymph nodes, enlarged mediastinal, peribronchial mass, and other mediastinal tumors can be scanned by endobronchial ultrasonography transbronchial needle aspiration (EBUS-TBNA) [25].

For peripheral lesions transthoracic needle aspiration biopsy is the scanning procedure of choice. The sensitivity reported for this procedure is 0.9 [7].

1.7 Research gaps, hypothesis and rationale of the study

Lung cancer being a multifactorial disease involve various different pathways in the genesis and progression of tumours, therefore aiming at counteracting different pathways will be a good choice for the treatment of lung cancer. Chemotherapy has emerged as a boon for the

management and treatment of cancer in conjunction with radiation therapy, surgery, and targeted therapy. But conventional synthetic drugs used as chemotherapeutic agents for the treatment of lung cancer have been reported to incite associated nonspecific and systemic cytotoxicity and this leads to a hunt for better biologically compatible compounds of natural origin-based chemotherapy that could be explored as alternative therapy for lung cancer.

Researchers exploring the therapeutic potentials of epigallocatechin-3-gallate (EGCG) have reported its therapeutic effects as antidiabetic, anti-inflammatory, anticancer, and anti-obesity. EGCG not only acts as a chemopreventive agent but also as a chemotherapeutic agent against a wide array of cancer cell lines. The inhibition of tumorigenesis, proliferation, and angiogenesis has been attributed as the anticancer mechanism exhibited by EGCG. EGCG exhibits its anticancer effects by multiple pathways in different types of cancers [26].

The compromised bioavailability and stability of EGCG obstructed its clinical application as a therapeutic agent. Its solubility and pharmacokinetic profile could be amplified by novel drug delivery systems-based formulation approaches.

Conventional treatment approaches of lung cancer offer not so satisfactory results, and a compromised life associated with potential side effects of chemotherapy, radiation therapy, and surgical resection. In the absence of targeted therapies, systemic administration of chemotherapy exposes normal cells to these potent chemotherapeutic drugs resulting in the inhibition of their growth making patient weak and could eventually leads to death.

Hypothesis and rationale of the study

The intranasal delivery of Gemcitabine and Epigallocatechin-3-gallate (GEM-EGCG) co-loaded solid lipid nanoparticles (SLNs), developed and optimized using a Quality by Design (QbD) approach, will facilitate efficient pulmonary deposition, enhance the localized and sustained release of both therapeutic agents at the primary site of lung tumors, improve the

pharmacokinetic limitations of EGCG, and induce synergistic anticancer activity through complementary mechanisms of action. This targeted delivery approach is expected to minimize systemic drug distribution, thereby reducing off-target toxicity commonly associated with conventional chemotherapy, while simultaneously improving therapeutic efficacy against primary and micrometastatic lung cancer lesions[50], [52]. The thin wall lining of the alveoli is surrounded by small vessels (which are basically divisions from the pulmonary arteries) and allow easy permeation. The large surface of the alveoli permit accumulation of significant amount of drug in short span of time with further distribution to the rest of the body resulting in a systemic effect of the drug which is beneficial in case of micrometastasis. Since lung tissues are the first point of contact following intranasal administration, this route enables direct targeting of the tumor site, enhancing local drug concentration and therapeutic efficacy. A sustained release of drug further controls systemic distribution, thereby minimizing off-target effects and associated side effects. Additionally, inhalation therapy holds promise in addressing micrometastasis or distant metastasis due to the drug's simultaneous interaction with the lymphatic system, as the alveoli are closely associated with extensive lymphatic drainage.The alveoli, where gas exchange occurs, are surrounded by a dense network of lymphatic capillaries. This anatomical proximity allows inhaled drugs to diffuse into interstitial fluid, which drains into lymphatic vessels; reach regional lymph nodes, where micrometastases often reside; and interact with immune cells, potentially enhancing immunotherapy effects. This dual accessto both lung tissue and lymphatic pathways makes inhalation therapy uniquely suited for targeting early metastatic spread[36].

Key Components in the Hypothesis:

- Intranasal delivery – utilizes the lung’s large surface area and rich vasculature for rapid and localized drug absorption, bypassing hepatic first-pass metabolism. Drugs

administered intranasally can travel down the nasopharynx and trachea, reaching the lungs directly. This is especially useful for targeting lung tumors or premalignant lesions with localized therapy. No needles, no infusions are required, just a spray or drop.This makes it ideal for long-term chemoprevention, especially in high-risk individuals like former smokers or patients with early-stage NSCLC.

- GEM-EGCG co-loaded SLNs – combines a potent chemotherapeutic (GEM) with a natural compound (EGCG) known for its multi-targeted anticancer activity and protective effects on healthy tissue.
- Optimization via QbD – ensures systematic, reproducible, and scalable formulation with controlled critical quality attributes (e.g., particle size, zeta potential, entrapment efficiency).
- Efficient pulmonary deposition and sustained release – targets drug accumulation at the disease site, ensures prolonged retention, and reduces dosing frequency.
- Improved EGCG pharmacokinetics – overcomes EGCG's poor bioavailability and instability by encapsulating it in lipid nanoparticles.
- Synergistic anticancer activity – hypothesizes a combined therapeutic effect from GEM and EGCG via inhibition of cell proliferation, induction of apoptosis, anti-angiogenic effects, and modulation of multiple oncogenic pathways.
- Reduced systemic toxicity – focuses on minimizing the adverse effects of chemotherapy by limiting systemic exposure and enhancing lung-specific delivery.

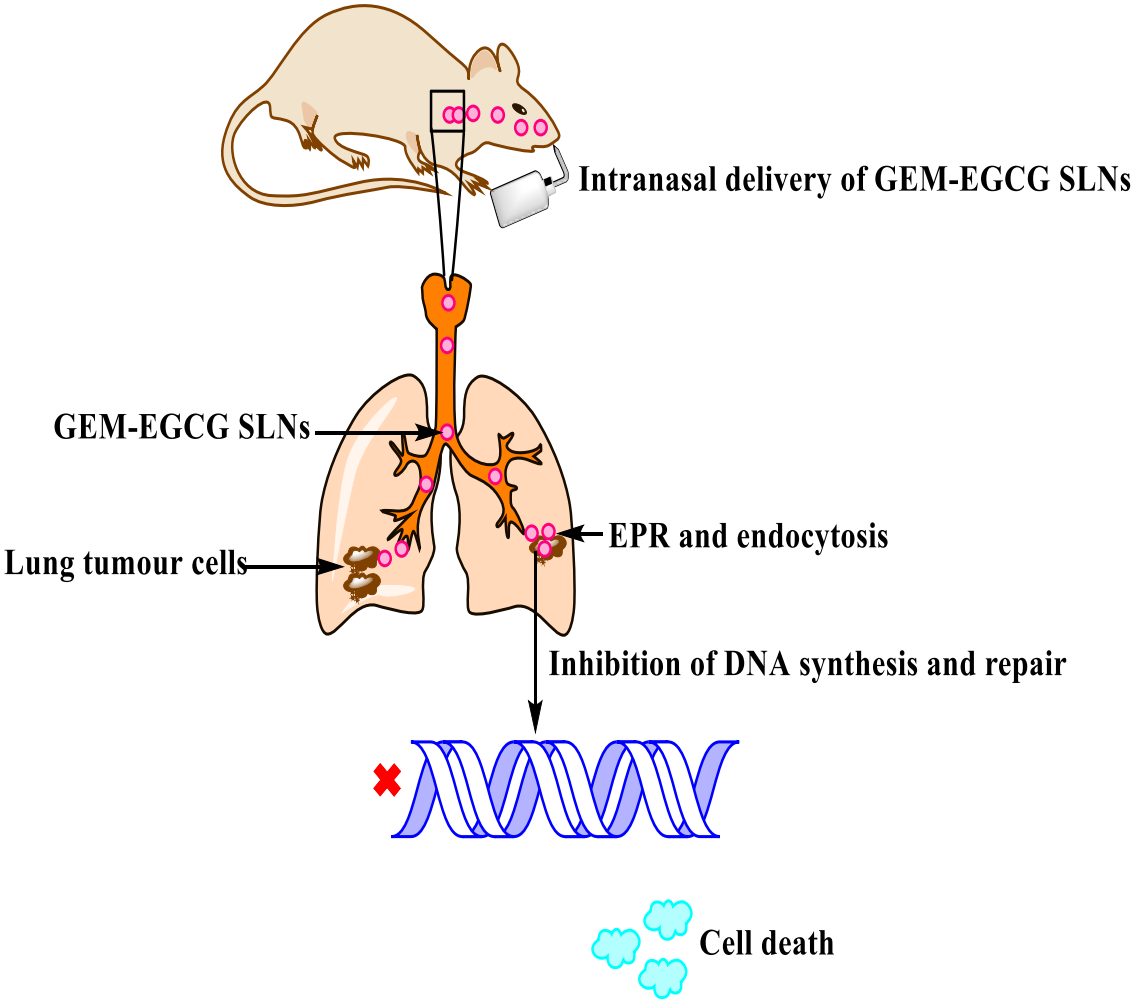

Figure 4: Diagrammatic representation of delivery of gemcitabine and epigallo-catechin-3-gallate loaded solid lipid nanoparticles (GEM-EGCG SLNs) in the mice model of lung cancer and its mechanism of action.

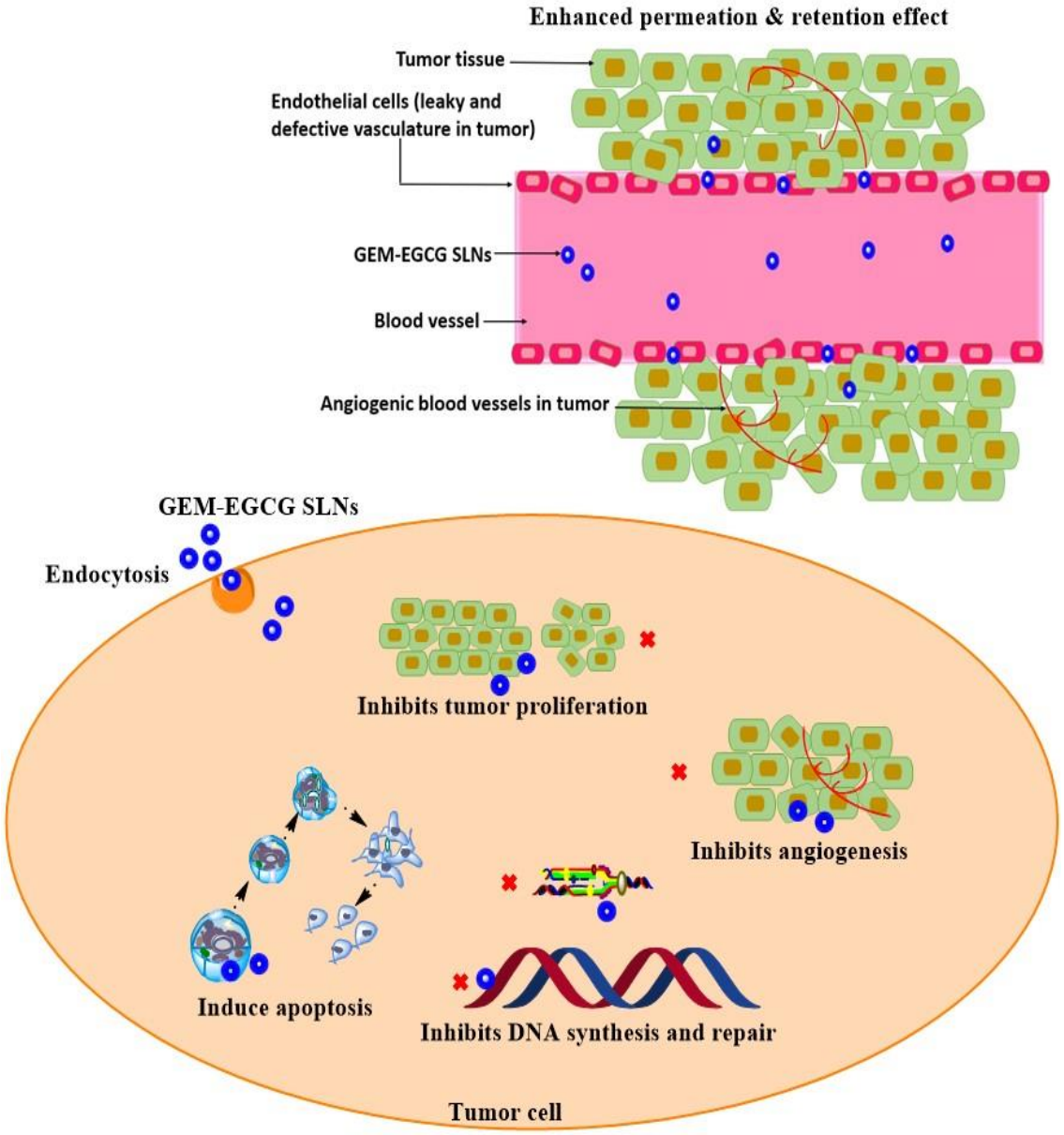

Figure 5: Putative mechanism of GEM-EGCG SLNs as anticancer agent: upon reaching the tumor site GEM-EGCG SLNs are selectively internalized by the endocytosis [27] and enhanced permeability & retention effect (EPR) [28] into the tumor cells. GEM-EGCG SLNs exhibit their anti-cancer effect by induction of apoptosis [29], [30], [31], inhibition of tumorigenesis and proliferation along with inhibition of angiogenesis in tumor cells [32], [33], [34]. GEM-EGCG SLNs also inhibit DNA synthesis and its repair [35] (abbreviations: GEM-EGCG SLNs- gemcitabine and epigallocatechin-3-gallate loaded solid lipid nanoparticles).