Virus Nanoparticles & Different Nanoparticles Affect Lung Cancer- A New Approach

Ranajit Nath[1], Ratna Roy[2], Soubhik bhattacharyya[3], Sourav Datta[4]

[1,3,4]Department of Pharmaceutics, NSHM Knowledge Campus, Kolkata- Group of Institutions, Kolkata, West Bengal, India

[2]Department of Pharmacology, NSHM Knowledge Campus, Kolkata- Group of Institutions, Kolkata, West Bengal, India

ABSTRACT

In the past, few decades cancer has become a worldwide problem to mankind. Lung cancer is the most life-threatening among all cancer types. Non-small cell lung cancer (NSCLC) is the main reason for approximately 80% to 90% of deaths. Lack of early detection and incompetent conventional therapies is the leading cause for poor prognosis and overall survival rate of lung cancer patients. Immense progress in the field of nanotechnology and nanomedicine has given inspiration to the development of an alternative strategy in the treatment of lung cancer. The unique physicochemical properties of the nanoparticles likeability to cross the different biological barriers, effectiveness in delivering hydrophobic drugs which are difficult to incorporate in the body, and targeting in the particular disease sites have given rise to enormous advantages for nanoparticulate systems for the early diagnosis and active delivery of drugs for a better treatment for lung cancer. Recently, many formulations of nanocarriers like lipid-based, polymeric and branched polymeric, metal-based, magnetic, and mesoporous silica are being used in this treatment. Innovative strategies have been employed to utilize the multicomponent, three-dimensional structure of nanoparticles and modify it and construct a new structure moiety that has multifunctional capabilities. Developing such designs permits simultaneous drug delivery of chemotherapeutics as well as anticancer gene therapies to site-specific targets. In lung cancer, nanoparticle-based therapeutics is now breaking the ground in the diagnosis, imaging, screening, and treatment of primary and metastatic tumors. This review emphasizes the pathogenesis of lung cancer and its treatment by nanotechnology.

Keywords : Lung Cancer, Pathogenesis, Virus Nanoparticles, Nanoparticles, Treatment

I. INTRODUCTION

Day by day cancer is taking devastating forms in the whole world. Lung cancer is one type of carcinoma where the uncontrolled and abrupt growth of lung cells begins by forming malignant tumours. In 2018, there are 18.1 million cases were reported as per International Agency for Research on Cancer (IARC)[1]. As per 2012 global lung cancer statistics, 1.8 million new cases and 1.6 million deaths were

recorded[1]. According to Malaysian National Cancer Registry (2007), estimated lung cancer cases with female 29.2% whereas with males it was 70.85%[1]. The infection of lung cancer is instigated by the activation of oncogenes or by the inactivation of tumor suppressor genes that leads to abnormal multiplication and growth of the cells in the lungs[2]. There are several factors that may lead to alteration of genes that may be inherited from parents or acquired by prolonged exposure to carcinogens.

A. TYPES OF LUNG CANCER:

According to World Health Organization, lung cancer is classified into two categories: they are small cell lung cancer (SCLC) & non-small cell lung cancer (NSCLC). SCLC is less common and more life-threatening; the mean survival is of 4 months if left untreated[3]. It has extreme deathly roots which lead to rapid growth rate, early metastasis, and fast metabolism. SCLC derives from neuroendocrine tumors and is studded with neurosecretory vesicles, neurofilaments. It accounts for almost 10% to 15% of lung cancers and is not susceptible to conventional chemotherapy and radiation therapy.

I. Non-small cell lung cancer:

NSCLC can be further subclassified into the epidermoid, large cell, Broncho-alveolar, adenocarcinoma, and squamous cell carcinoma[4].

i. Adenocarcinoma:

Back in the year 2015 before the WHO classification, adenocarcinoma was explained as carcinoma containing an acinar/tubular structure formed in mucus-secreting glands[5]. From the year 2015 onwards after the WHO classification adenocarcinomas are categorized into three types they are: adenocarcinoma in situ (AIS, preinvasive lesion), minimally invasive adenocarcinoma (MIA) & (overt) invasive adenocarcinoma based on the extent of invasiveness. The disease-free survival rate of AIS and MIA when completely eradicated is 100%[6].

a) Adenocarcinoma in situ:

According to the new IASLC/ATS/ERS adenocarcinoma classification Adenocarcinoma in situ is described as carcinoma in glandular proliferation[7] of the diameter of ≤3 cm formed from lepidic growth, not from lymphatic, vascular or pleural invasion[5]. If the tumor size is greater than 3 cm, then it is called as "lepidic predominant adenocarcinoma, suspect AIS" because these tumors are uncommon and lacking appropriate characterization[5]. In most cases the tumor cells are mainly the non-mucinous type with a proliferation of type II pneumocytes or Clara cells, but sometimes the tumor cells are unique the mucinous consists of tall columnar goblet cells having abundant apical mucin. If these lesions are completely eradicated from the body, then there is a chance of the patients having 100% 5- year disease-free survival (DFS) which is reported[8]. From the CT it is observed that these lesions generally consist of a ground-glass nodule if non-mucinous and a solid nodule if mucinous AIS[7].

b) Minimally invasive adenocarcinoma:

Minimally invasive adenocarcinoma is defined as a lepidic predominant tumor with a diameter of ≤3 cm and an invasion size of≤5 mm[7]. Even though the tumor size and invasion size are observed in the definition of MIA, the existence of lymphovascular invasion, pleural invasion, or tumor necrosis can be an elimination factor for an MIA diagnosis. If the tumor size is much greater than 3 cm with an invasion size of ≤5 mm, it is called as "lepidic predominant adenocarcinoma, suspect MIA" because these tumors are abnormal and lack adequate characterization[5]. There are very little data that says patients with MIA have a near 100% 5-year disease-

free survival[7,8]. In Most of these cases the tumors are non-mucinous, but very rare mucinous cases may happen[7]. From the CT it is seen that MIA with non-mucinous type typically shows a ground-glass nodule with a solid component measuring 5 mm or less. But mucinous MIA shows a solid nodule on CT[7].

c) Invasive Adenocarcinoma:

From the year 2015 in WHO classification, Invasive adenocarcinomas is now included as the predominant subtype. This classification is decided best after studying thorough histologic subtyping to calculate the percentages of the various histologic subtypes within a tumor in a semiquantitative method[9]. LPA composed of tumors mainly classified as mixed subtype tumors containing a predominant lepidic growth pattern of type II pneumocytes or Clara cells (formerly known as non-mucinous BAC) that have an invasive component greater than a diameter of 5 mm. The other major subtypes are acinar, papillary, micropapillary, and solid with mucin-predominant adenocarcinomas. The micropapillary predominant subtype is now added into this class because of the result observed in multiple studies that it is related to poor diagnosis in early-stage adenocarcinomas[7,10,11]. Signet ring and clear cell carcinoma subtypes are no longer considered as histologic subtypes, but they are now registered as cytologic features where the percentage are identified[7]. In spite of the fact that clear and signet ring cell cytologic alterations are mainly found in the solid subtype, they can also show acinar or papillary patterns as well[7].

d) Variants of Invasive Adenocarcinoma:

The variants of lung adenocarcinoma are divided into invasive mucinous adenocarcinoma (formerly mucinous BAC), colloid adenocarcinoma, fatal adenocarcinoma, and enteric adenocarcinoma[7]. The term "mucinous bronchioloalveolar carcinoma (BAC)" is no longer used due to the reason that most mucinous BACs are excluded from the variants and incorporated into invasive components. Therefore, the term "invasive mucinous adenocarcinoma (IMA)" renamed for mucinous BAC. IMA and mucinous AIS are accurately classified on the basis of invasiveness[5]. Invasive mucinous adenocarcinomas (formerly mucinous BAC) are now separated from the non-mucinous invasive adenocarcinomas due to repeated correlation with KRAS mutation, deficiency of thyroid transcription factor 1 (TTF-1), and persistent multicentric lung lesions. From the analysis, it is seen that these tumors have different amounts of lepidic, acinar, papillary, or micropapillary growth containing columnar cells with abundant apical mucin and small basally oriented nuclei[7]. Enteric adenocarcinoma is expressed as adenocarcinoma with a principal component that resembles adenocarcinoma emerging in the colorectum and frequently shows CDX2immunoreactivity[5].

ii. Squamous Cell Carcinoma:

In the year 2015 according to WHO's classification, Squamous Cell Carcinoma is divided into keratinizing SqCC, non-keratinizing SqCC, and basaloid SqCC. Before this classification, basaloid SqCC was grouped under the variant of large cell carcinoma. However, basaloid SqCC immunohistochemically exhibits "SqCC markers" (e.g., p40, CK5/6, and p63) and for that reason categorized as SqCC.

In the United States among all the lung cancers diagnosed, Squamous cell carcinoma accounts for approximately 20% of it[7]. After the diagnosis is seen that two-thirds of squamous cell carcinomas are central lung tumors, whereas the remaining one-third are peripheral[12,13]. However, from the late reports document it is found out that an increase in the rate of squamous cell carcinomas is found in the periphery, exceeding 50%[14]. The morphologic characterizes that recommend squamous differentiation which includes intercellular bridging, squamous pearl formation, and

individual cell keratinization. In well-mutated tumors, these features are readily evident; whereas, in poorly mutated tumors they are difficult to detect[15]. Squamous cell carcinoma emerges most frequently in segmental bronchi and in addition to that lobar and mainstem bronchus are also involved[16]. Squamous cell carcinoma can be divided into the papillary, clear cell, small cell[17] and basaloid subtypes[13]. However, this subtyping is out of date requires proper updating because it does not cover up the morphologic spectrum of appearances of lung squamous cell carcinoma and it does not authorize meaningful harmonization between clinical, prognostic, or molecular features. For example, the small cell variant apparently should be eliminated, because most of these cases would better be classified as basaloid variants and the term small cell generate confusion between the true small cell carcinoma. The exophytic endobronchial growth pattern is repeatedly examined in papillary squamous cell carcinomas[18,19].

Several articles have suggested alternative approaches to sub-classifying pulmonary squamous cell carcinoma[20–22]. These approaches include the identification of an alveolar space-filling variant, which correlates to favourable diagnosis. Funai and colleagues studied and concludes that among the SqCC cases there are 5 cases with 100% disease-free survival[20], and Watanabe and colleagues researched and found that in the patient the alveolar space-filling ratio of 70% or more also had a 100% disease-free survival[22]. However, this pattern very rarely occurs in only a few cases and is more often seen only focally; in a study from North America, the prognostic significance could not be shown. Maeshima and colleagues explained minimal tumor cell nests as large (>6 tumor cells), small (2–5 cells), and single cell. From this study, it is seen that the single-cell infiltrating tumors had the worst prognosis. The tumors related to a background of usual interstitial pneumonia and lymph node metastases also had a poor prognosis[21]. Furthermore, work is needed to develop a strong practical approach to subclassification of squamous cell carcinoma and to associate better histologic predictors of prognosis[9].

II. Small cell lung cancer:

In the United States cancer infection is increasing day by day, about 30,000 new cases are detected every year. Among all the cancer types SCLC consists 14% of them[23]. Among all the SCLC types roughly two-thirds of SCLC present as a perihilar mass. SCLC is usually located in a parabronchial location with infiltration of the bronchial submucosa and parabronchial tissue. Bronchial obstruction is generally occurred due to circumferential compression, but very occasionally endobronchial lesions may occur. Because the diagnosis is usually established on transbronchial biopsy or cytology, is it unusual to encounter SCLC as a surgical specimen. Substantial lymph node metastases are very frequent[24]. The characteristic feature of this tumor is white-tan, soft, and friable and often shows extensive necrosis. In advanced cases, the bronchial lumen may be blocked by extrinsic compression. In 5% of cases, SCLC appears as a solitary coin lesion[25,26].

In the year 1981 WHO classification are SCLC are classified into three subtypes: (1) oat cell carcinoma, (2) intermediate cell type, and (3) combined oat cell carcinoma. However, in the year 1988, the IASLC suggested to eliminate the category of intermediate cell type because expert lung cancer pathologists could not expand this subclassification and crucial differences in survival could not be found out during the study. They even suggested including the category of mixed small cell/large cell carcinoma because these patients seemed to have a poor prognosis than other patients suffering from SCLC[27]. The category of combined SCLC was reserved for SCLC with a combination of adenocarcinoma or squamous cell carcinoma[27]. In 1999 WHO classification rejected the class of mixed small cell/large cell carcinoma because

there was inadequate data information in reproducibility for this subtype and lack of confirmation that these patients had a worse prognosis[13,28]. Therefore in the year 2004 according to WHO classifications there are only 2 types of SCLC: SCLC (with pure SCLC histology) and combined SCLC (with a mixture of any non–small cell type)[29].

SCLC has a characteristic morphological appearance. The tumor cells are small in size and have a round to fusiform shape and scant cytoplasm and finely granular nuclear chromatin, nucleoli may be absent or unobtrusive[29]. Nuclear moulding and smearing of nuclear chromatin as a result of crush artefact may be noticeable. There is huge necrosis in the area of infection with high in mitotic rates approximately 80 mitoses per 2 mm2 areas[23,24,29]. The growth pattern generally consists of diffuse sheets, but rosettes, peripheral palisading, organoid nesting, streams, ribbons, and rarely, tubules may be present[30]. Basophilic encrustation of vessel walls, also known as the Azzopardi effect, is often seen in necrotic areas[30]. SCLC is reliably detected in small biopsies and cytology specimens. After chemotherapy, mixtures of the large cell, squamous, giant cell, or adenocarcinoma may be seen in 15% to 45% of SCLC[31,32].

B. CAUSES OF LUNG CANCER:

➤ Tobacco Smoke and Lung Cancer:

Tobacco smoking is one of the predominant causes of lung cancer. The smoke of tobacco consists of more than 60 carcinogens, out of these more than 20 carcinogens are directly correlated in lung cancer development[33]. Among these carcinogens the most infectious compounds are the polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrsosamino)- 1-(3-pyridyl)-1-butanone, both of them can lead to gene alterations through DNA adduct formation[34]. There are two groups of enzymes that are involved in DNA adduct formation:

P450 enzymes which are encrypted by CYP family genes; and glutathione S-transferases (GSTs). The CYP450 enzymes help in the activation of the carcinogens. The metabolically activated intermediates which are formed are secreted or can bind to DNA which leads to DNA adduct formation. Whereas, GSTs helps in the detoxification of the intermediates of carcinogens, thus, acts as an inhibitor against adduct formation. In the majority of cases, these adducts which are formed from the CYP450 enzyme are repairable, but sometimes the damage is acute enough to cause apoptosis. Chronic exposure to these compounds often leads to alterations in critical genes such as p53 or RAS, which lead to the initiation and development of the disease. Tobacco smoke is another cause for the induction of oxidative DNA lesions[35]. 8-oxoguanine is a major oxidative lesion that is responsible for G-to-T transversion, leading to alteration of critical genes involved in lung cancer pathogenesis. 8-oxoguanine is restored by 8-oxoguanine DNA N-glycosylase 1 (OGG1) and, thus, the different forms in OGG1, with the decrease in enzymatic activity of OGG1 are maybe correlated with increased risk for lung cancer. Although it is generally accepted that tobacco smoke causes lung cancer, but not everyone who smokes are susceptible to lung cancer. Epidemiologic studies have shown that smokers are 14 times more susceptible to develop lung cancer than non-smokers, but only about 11% of heavy smokers may be infected with lung cancer in their lifetime[36]. As a result, from the research studies, it is proposed that genetic factors may make prone people to lung cancer development. These are studies have surveyed to find the relationship between polymorphic variants of the genes involved in tobacco smoke metabolism and DNA repair pathways, including P450 and GST family genes and OGG1, and the risk for lung cancer, but the results of these studies are undecided[37]. However, from a research study it is seen that low activity of OGG1 is linked with an increase in the threat of lung cancer, recommending that people with low OGG1 activity

are good volunteers for smoking termination programs[38].

> ### Inherited Susceptibility to Lung Cancer:

From the research studies and survey, scientists came to a conclusion that 2.5-fold increased risk identified in a family history of lung cancer after ceasing the tobacco smoke, proposed that genetic factors other than those have a direct link with metabolizing carcinogens from tobacco smoke are also responsible for a person's susceptibility to lung cancer[36]. From a current large-scale linkage analysis (52 pedigrees) by the Genetic Epidemiology of Lung Cancer Consortium recommended that a major autosomal susceptibility locus for inherited lung cancer is found in 6q23-25[39]. This area contains many potential genes of interest, including SASH1, LATS1, IGF2R, PARK2, and TCF21[39,40]. If any mutations occurred in one of the genes in this region that makes these families more susceptible to lung cancer, it could be used to screen the wide range of the population to recognize people with the susceptible allele. The peoples with such a genetic combination could be chosen for the programs conducted for early detection and prevention[35].

> ### Genomic instability in lung cancer:

Almost all the solid tumours show genetic instability at two definite levels: large-scale chromosomal instability (CIN) and microsatellite instability (MSI)[41]. CIN is defined as losing or gaining of whole chromosomes or large portions of it. The basic mechanisms of CIN have not been fully explained; but it is found out that mutations in mitotic checkpoint gene, such as BUB1, are clearly linked with the CIN phenotype in colon cancer, but they uncommon in lung cancer[35,42].
MSI is explained as a modification in the DNA sequence of any length attributable to addition or removal of the microsatellite one- to four-base DNA

repeating units within a tumour[43]. The most common and frequent used method for MSI study is a polymerase chain reaction-based method, where DNA sequence alterations are identified by comparing the electrophoresis patterns of polymerase chain reaction products targeting microsatellite loci between tumour and normal samples from the same candidate. From the studies, it is concluded that frequencies of MSI ranging from approximately 2% to 70% in both small cell lung cancers (SCLCs) and NSCLCs. The wide difference in the studies is attributable to different microsatellites and different methods of analysis. Sozzi et al. analysed the usefulness of detecting microsatellite mutations in plasma or serum DNA from patients with NSCLC as a non-invasive strategy for early detection[44]. From the analysis they found that altered DNA sequences (either MSI or loss of heterozygosity) could be found in 43% of blood samples from patients with stage I disease, with no alterations found in control cases, recommending that identifying these mutated DNA sequences may be useful as a tool for diagnosis and early detection screening[35].

> ### Abnormalities in growth-stimulatory signalling pathways: Proto-oncogenes

There are several elements to each of the growth signalling pathways involved in lung cancer, we will give emphasis on those proteins that are frequently affected by genetic mutations in cancer. It is now understood that these mutated proteins steer the affected cells toward transformation, also "addict" the cells to their unusual function. This concept is called as "oncogene addiction" and represents a cellular physiologic state where, there is continued presence of the uncommon function, although oncogenic, also becomes required for the tumor to survive[45]. This means that if the function is eliminated or inhibited (e.g., by a targeted drug or other therapies), the tumor cells will die. By contrast, normal cells, which are not addicted to the variant protein, are less sensitive to

the drug; thus, the targeted drugs have great tumor cell specificity. The most important example of this concept is EGFR TK mutation. Tumors with mutations in EGFR are dependent on survival signals transduced by mutant EGFR and, thus, are particularly sensitive to this[46]. These findings have led to massive genome wide sequencing efforts targeting thousands of genes to find additional mutated oncogene targets for rational therapeutics design. Whether this approach will be greatly useful for targeting genes frequently overexpressed but not mutated in lung cancers, such as MYC, remains to be determined.

C. PATHOGENESIS OF LUNG CANCER:

Pathogenesis in lung cancer is more alike to other cancers, lung cancer is started by activation of oncogene or inactivation of tumor suppressor genes[2]. One of the reasons are carcinogens which cause alteration in these genes which persuade the development of cancer[47]. Modification in the K-ras proto-oncogene is the main cause for 10-30% of adenocarcinomas [11]. The involvement of the EML4-ALK tyrosine kinase fusion gene is responsible for 4% of non-small cell lung carcinomas[48]. Genetical changes-such as mutations in DNA methylation, histone tail modification, or mRNA regulation may lead to the inactivation of tumor suppressor genes[49]. Epidermal growth factor receptor (EGFR) controls cell proliferation, apoptosis, angiogenesis, tumor invasion in the body [50]. Alterations and amplification of EGFR gene are common in non-small cell lung carcinoma which layout the base of treatment with EGFR-inhibitors[48]. Other genes which are frequently variated or amplified are c-MET, NKX2-1, LKBI, PIK3CA, and BRAF[50]. The mechanism of action involves abnormally activation of stem cells. In proximal airways, stem cells that exhibit keratin[51] which are more prone to be affected, typical to squamous-cell lung carcinoma. In middle airways, implicated stem cells include club cells neuroepithelial cells that indicate club cell secretory

protein[52]. Small–cell lung carcinoma may be originated either from these cell lines[53] or neuroendocrine cell[54] and may express CD44[55]. The evolution of lung cancer required alteration from epithelial to mesenchymal cell type. This may happen through the activation of signalling pathways such as Akt/GSK3Beta, MEK-ERK, Fas and Par6[55].

D. DESCRIPTION OF NANOTECHNOLOGY:

In recent trends, nanotechnology and nanomedicine have become an important sector flourishing in the area of biomedical science that has been used to find a solution for different biological problems including therapeutics and diagnostics. Lately, the use of nanotechnology has been very common for the treatment of various diseases including cancer, diabetes, bacterial infections, cardiovascular diseases, etc[56]. Due to multiple disadvantages in the conventional therapeutic strategies for lung cancers, scientists and researchers are now focusing on the development of the nanoscale therapeutic agents, whereas the delivery system which includes liposomal nanoparticles, polymeric nanoparticles, metal nanoparticles, and bio-nano particles for the treatment of cancer. In lung cancer, these theranostic applications of nanoparticles have been huge success rate due to their small size, which enables them to target are tumor cells and specifically accumulate into it, due to enhanced permeability and retention (EPR) phenomenon[57]. Besides, nanoparticles are easy to handle and administer and it is revealed that nanoparticles have high drug loading due to large surface area and volume. In addition to that, nanoparticles have good biocompatibility and overcoming clearance by the kidney relieving long circulation holds an edge over conventional therapeutic treatments. Further, several nanoparticles exhibit multifunctional abilities like imaging, diagnostics, therapeutics, sensing that helps researchers to show interest in these nanomaterials

and use them for multifunctional biomedical applications in lung cancer theranostic[58].

This review focused on the pathogenesis of lung cancer and its treatment by nanotechnology.

ROLE OF NANOTECHNOLOGY IN THE TREATMENT OF LUNG CANCER

Particles which have at least one dimension in the nanometre scale or in other words dimension less than 100 nm are termed as nanoparticles[59]. Nanoparticles have been a major attraction in nano biomedical technology due to its unique property of being present in smaller size which contains a huge amount of energy and because of this, the particle is able to adsorb and transport hydrophobic and hydrophobic macromolecules to the target site[3]. Nanoparticles can positively alter the biodistribution increasing therapeutic efficiency and a reduction in the non-specific toxicity of potent anticancer drugs. They possess a superior biocompatibility, the ability to protect nucleic acids from degradation, and the ability to deliver therapeutic genes to cancer cells in-vivo which makes nanoparticles the ideal vehicle for delivery of such ingredients[60]. In addition to this, nanoparticles offer several advantages such as specific targeted drug delivery, improved stability and eradicates toxicity, active and passive drug targeting due to reduction of size, controlled release, requirement of lesser amount of drugs and enhanced rapid onset of therapeutic action[3]. Over the past few years, there has been an explosive development of nanoparticle-based medicines like liposomes, micelles and simple nanoparticles to name a few. The ability to design nanoparticles as personalized medicine is quite attractive and found to be ideal for the lung cancer treatment regimen. Combinational approaches are documented extensively in recent years with a complex balance between targeting moieties and anti-cancer agents. However, even with all the advantages provided by the nanoparticles, the journey of nanoparticle based drug delivery formulations for lung cancer therapy to the clinic is extremely challenging. This is because firstly synthesis of nanoparticles having a specific size, with the ability to avoid unnecessary metabolism and residence at the target site for sufficient time is a tricky and complicated process. Besides, a lack of established rules and regulations for the evaluation of nanoparticulate systems such as manufacturing, functional and safety evaluation exists currently. It should be kept in mind that nanoparticles are complex and contain multi-component compared to the conventional anti-cancer drugs which exist as a single component. Hence, modifications of standard testing of nanoparticles should be done keeping the above point in mind[61].

Currently, there are three types of nanoparticle systems that have been used in the treatment of Lung Cancer. These are (I) Natural and semi-synthetic nanoparticles, (II) Synthetic (organic) Nanoparticles, (III) Synthetic (In-Organic) Nanoparticles[3].

1.1 Natural and Semi- synthetic nanoparticles:

Natural and semi-synthetic nanoparticles owing to their high biocompatibility and biodegradability have found significant application in lung cancer therapy.

a. Virus Nanoparticles:

A virus is an infectious agent with a size range of about 20 to 400 nm that consists of an encapsulated genome or in simpler terms a nucleic acid and a protein coat which can multiply in living cells of animals, plants, or bacteria. The protein coat that protects the genetic material of a virus is known as a capsid. The basic difference between a virus and virus nanoparticles is that virus nanoparticles consist of virus protein coat or capsid or the shells but the genomic material is absent. Since the genomic material of a virus is the root cause of its infectivity a virus nanoparticle that is devoid of any genomic

material is incapable of replicating and is non-infectious. The protein coat or capsid of the viral nanoparticles functions as a protective sheath which protects the viral nanoparticles from various extreme environmental factors such as extreme temperatures, a range of pH and from various harsh and corrosive chemicals. These viral nanoparticles are asymmetrical in structure, are polyvalent and monodispersed and have the combined advantages of being biodegradable and non-immunogenic[62]. An interesting fact about these virus nanoparticles is that they have been found to conjugate covalently to a diversity of moieties ranging from drugs, reagents used for targeting, probes for imaging and numerous inorganic nanoparticles. This shows that these have the potential to effectively deliver therapeutic proteins and other necessary ingredients into target locations such as the cancer cells, in their non-infectious form. Since the viruses can self- assemble themselves naturally from various monomers of coat proteins and is capable of encapsulating nucleic acids that are negatively charged, this property of the virus can be taken advantage of to trap artificial therapeutic nucleic acids and other polyanions[63]. There are generally two types of viruses: Mammalian Virus and Plant Virus. The mammalian virus in general comes with a significantly higher risk of infection and therefore is purposefully not used. On the other hand, plant viruses do not show infectivity against mammalian cells and therefore are considered to be an alternative in biomedical applications of virus nanoparticles. In addition to this plant virus have been known to have an awfully small probability for genetic recombination with mammalian viruses and is not known to be inherently capable of targeting any biological unit until or unless they are modified chemically. The plant virus capsid vectors that are commonly employed are mainly of five types: (i) Cowpea mosaic virus (CPMV), (ii) Tobacco mosaic virus (TMV), (iii) Cowpea chlorotic mottle virus, (iv) Canine parvovirus, (v) Bacteriophages Qβ and MS2. Virus Nanoparticles provide numerous advantages against synthetically designed nanoparticles such as they have well-defined dimensions, they can possibly evade the immune system, and they are biocompatible and biodegradable[64]. The most widely studied viral vectors are the CPMVs capsid proteins. Their structure consists of icosahedrons which have a spherical average size of 28.4 nm, and are made up of 60 identical subunits. They remain stable in temperatures up to 60 °C and in a pH range of 3.5–9 [65]. The exterior surface of the capsid of CPMVs does not show the presence of a cysteine group, therefore it can be concluded that any naturally occurring thiol groups are absent in them. Although, due to the presence of 60 identical subunits in the CPMVs capsid amino acid modifications can be established into 60 positions on the surface of the virus. Therefore, while a single cysteine (HO_2 $CCH(NH_2)$ CH_2SH) can produce 60 reactive thiols on the capsid, adding two cysteines can produce 120 reactive thiols on the viral capsid surface[65]. The production of these thiol' groups on the exterior surface of the capsid of CPMVs can be used to promote the attachment of thiols-reactive gold nanoparticles through strong covalent bonds between sulphur and gold. This theory can be used to deliver high-contrast agents to the effected zones. CPMVs are believed to have the potential for in situ vaccine delivery in lung cancer therapy.

b. Liposomes and Solid Lipid nanoparticles

• Liposomes:

Liposomes comprise of an aqueous core which is surrounded by the bilayer lipid structure which separates the inner aqueous core from the bulk outside. Liposomes are made up of phospholipids, phosphatidylcholine, and cholesterol, and their characteristics are much dependent on the nature of the materials they are composed of[3]. Among the non-viral carriers that are used for drug and additional macromolecules delivery into various targeted cells, the liposomes are the carriers that most

widely studied. Due to the unique property of having high hydrophobicity in the lipid bilayer, a range of molecules such as drug molecules, proteins, nucleic acids and plasmids and loaded into the liposomal moiety. Liposome can enhance the solubility of hydrophobic drugs in the bloodstream by incorporation of such drug tightly into its phospholipid bilayer. Targeting of antibodies and proteins, which are specific to a certain receptor or cancerous antigen, to specific cells can be improved by their possible conjugation to the outer surface of the liposomes. Due to their powerful biocompatibility properties, the popularity of liposomal formulations as delivery vehicles for anticancer medication is increasing day by day. However, presently only two such liposomal formulations are available that has approved by the FDA, and they are (i) DOXIL and (ii) Marqibo. DOXIL is a liposomal formulation of doxorubicin used for its administration in the ovarian cancer while Marqibio is a liposomal formulation containing vincristine sulphate mainly used for its administration in cases of lymphoblastic leukaemia. Lipusu is another liposomal formulation which contains PTX and is available in the market for clinical trials[60]. Liposomes may have the potential for drug and genes delivery in the lung cancer treatment, however there are only a limited number of examples for liposomal formulations being used as potential drug delivery systems in the treatment of NSCLCs. Cisplatin, a promising drug in the treatment against NSCLCs is often reported as nephrotoxic in higher doses. Boulikas with his group developed a formulation in which cisplatin was conjugated to the liposomal surface which they name lipoplatin. Lipoplatin was found to reduce the nephrotoxicity from cisplatin significantly in the rat tumor models[66]. This shows that lipoplatin can be used for the treatment of lung cancer using cisplatin to effectively bypass the nephrotoxicity reported to be caused by the drug. Recently a report already claims for lipoplatin to have successfully completed its phase III clinical trial testing[67]. Besides this, in phase I clinical trials PTX delivery to NSCLC cells has also shown a remarkable improvement to the therapy. During the preclinical trials PTX liposomal formulation has also been able to successfully target the lung cancer cells and drug resistance was also significantly reduced. The current objective of the researchers worldwide is to develop an increased number of liposomal formulations for the treatment of stage IV cancer. Liposomes have also found utility in the field of immunotherapy for the delivery of vaccines for cancer. Liposomes are able to encapsulate tumor-associated antigenic (TAAs) stimuli in their aqueous core or can embed them into the bilayer or just attach them to the outer surface[68]. A study done by Sangha and North revealed BLP25 or Biomera Liposomal Protein 25 offered very sufficiently good results in the treatment of advanced stage NSCLCs. There are a number of variations of liposomes such as (i) cationic liposomes which consist of charged heads and hydrophobic skeletons made up of carbon, and (ii) archaeosomes which are made up of glycerolipids. But these variations are yet to show any successful results in the clinical trials of lung cancer.

- **Solid Lipid Nano-Particles (SLN):**

Solid lipid nanoparticles or SLN are spherical shaped lipid-based drug delivery systems with a diameter range from 50-1000 nm which consists of the solid lipid core made up of physiological lipid which is dispersed in water or in aqueous surfactant solution which forms a mono-layered phospholipid coating around the solid lipid core. This Solid lipid core of the SLNs, which is hydrophobic in nature, is usually made of triglycerides, beeswax, carnauba wax, cetyl alcohol, and cholesterol[69]. In fact, solid lipid nanoparticles are just structural variations of liposomes and nanoemulsions but have added stability within the biological systems because of the presence of a solid lipophilic core. SLNPs are frequently used as colloidal carrier systems for prolonged circulation of chemotherapeutic drug and genes that are

hydrophobic in nature in the bloodstream. This is because of the hydrophobic environment that is present inside the core of the solid lipid nanoparticles. In the treatment of endobronchial cancer, cationic SLNPs complexes of p53 have been administered to the target sites via intra-tracheal instillation or aerosol inhalation. The principal reason behind the occurrence of endobronchial cancer is the mutation of p53 tumor suppressor gene which causes a loss in the ability of the gene to induce growth inhibition resulting in programmed cell death or apoptosis. Hence, the disease condition can be treated if a wild type p53 gene could be transferred to the tumor region that is usually found to be present in the lower respiratory airways. Cationic SLNPs contain one or two hydrophobic fatty acid side chains and a linker amino group which is hydrophilic, these results into cationic SLNPs having amphiphilic nature. A stable complex is formed due to the interaction of this hydrophobic amino group and the negatively charged DNA plasmids which make it possible for the transfer of p53 gene to the cells effectively[70]. Repeated administration of the formulation can ensure direct contact of the complexes to deeper layers of epithelial tissues that are lining the endotracheal tube through the removal of superficial apoptotic cells. Choi et al. had a different approach to the p53 gene delivery through SLNPs. Using SLNPs delivery shuttles they were able to deliver p53 tumor suppression genes effectively to null H1299 lung cancer cell lines[71]. SLNPs can successfully unload therapeutic macromolecules in to the specific target sites by exploiting the hyperaemia effects present in the tumor micro environment as the drug or genes are loaded into the SLNPs while at low temperatures whereas they are unloaded at higher temperatures. When compared to liposomes, Solid Lipid nanoparticles can provide a number of advantages like reduced leakage of the drug into the blood circulation, creating hindrance to drug degradation by hydrolysis, increased amount of drug circulation and controlled delivery of drug which is because of the drug

molecules being immobilized by the solid lipid matrix. Therefore, SLNPs have the potential to become a promising anticancer drug delivery system specifically for the eradication of impenetrable lung cancer cells, in the future.

c. Chitosan nanoparticles:

Chitosan is a biodegradable polysaccharide which is extracted from marine natural sources such as crustacean shells. It is nitrogenous and is obtained through deacetylation of chitin and contains glucosamine and N-acetyl-glucosamine monomers which are linked through β-, 4 glycosidic bonds. It is biocompatible and shows mucoadhesive nature which is because of it being cationic and due to which it can interact with the negatively charged sites present on the cell lines through electrostatic bonds. In addition to this, it can enhance the permeation of the macromolecules which it encapsulates through tightly bound epithelial cells which makes chitosan an attractive element in the field of therapeutic drug delivery. Since the nucleic acids are anionic in nature they are usually transported by chitosan nanoparticles to the lung cancer cells. Lysozyme which is the most abundantly present enzyme in the lung cells can readily degrade chitosan due to which chitosan can be easily excreted out of the system after the delivery of drug and genes to the cells. Boca et al. developed chitosan–coated triangular Ag nanoparticles which due to the strong resonance of Ag nanoparticles in the near infrared region, acted as photothermal agents against NSCLC human cell lines. The cancer cells were shown to efficiently take in the chitosan-coated triangular AgNPs and these AgNPs were also observed to have low cytotoxicity against the normal embryonic cells[72]. Galbeiti and his group formulated folic acid bound chitosan conjugated on the surface of microcapsules made of polyvinyl alcohol, named as (MC-Chi-FA). They encapsulated camptothecin into this conjugate of (MC-Chi-FA) and successfully targeted the tumor cells of epithelial origin[73].

Ventura and his co-workers prepared 2, 2'-difluorodeoxycytidine loaded chitosan microspheres which showed remarkable in vitro antitumoral activity against A54, human lung cancer cells[74]. Lv et al. synthesized N-((2-hydroxy- 3-trimethylammonium) propyl chitosan chloride (HTCC) nanoparticles into which they loaded PTX which is a water soluble mitotic cell cycle arresting drug. They were able to successfully treat the Lewis lung cancer cells in xenografted mouse models and the study also revealed that there was a reduction in the tumor volume with respect to time after the administration due to the successful PTX loaded HTCC nanoparticle accumulation in the site of the tumor[75]. Okamoto et al. used chitosan nanoparticle vectors of low molecular weight to prepare gene powders containing pCMV-Luc, which is a luciferase expression plasmid driven by the cytomegalovirus promoter. Successful transfection of pCMV- Luc genes to the lung cancer cells was achieved and in addition, the chitosan gene powder demonstrated an elevation in the activity of pulmonary luciferase[76]. From all the above research it can be said that the chitosan nanoparticles or better be called as chitosan-modified nanoparticles are promising drug delivery systems for drugs and genes etc and along with this chitosan-modified nanoparticle can also boost the sustained delivery of medicinal entities.

1.2 Synthetic (Organic) nanoparticles:

A major disadvantage of natural or semi synthetic nanoparticles is that they frequently get degraded too fast and fall prey to hepatic first-pass metabolism before they can even achieve the delivery of macromolecules mainly to lung cancer cells. To rectify these limitations nanoparticles are attempted to be prepared synthetically.

- **Polymer Nanoparticles:**

Synthetic polymer nanoparticles are synthesized from synthetic biodegradable and biocompatible polymers which are listed as follows:

i. poly(lactic acid) (PLA),
ii. poly(glycolic acid) (PGA),
iii. poly(lactic-co-glycolic) acid (PLGA),
iv. poly(L-glutamic acid),
v. poly(ε-caprolactone) (PCL),
vi. poly(amino acids),
vii. poly(ethylene glycol) (PEG),
viii. poly(alkyl cyanoacrylates),
ix. N-(2-hydroxypropyl) methacrylamide copolymer,
x. Poly (styrene maleic anhydride) copolymer.

These are colloidal particles in micellar form with a size range of 50-300 nm. The Polymer nanoparticles can be further sub-classified into spherical polymer micelles and worm-like polymer micelles. PNPs are shown to have significantly enhanced the chemotherapeutic and radiotherapeutic efficacies of a number of anticancer medications. Jung et al. demonstrated that the therapeutic index of chemotherapy in vivo and in vitro A549 cells in xenografted models was improved with taxanes loaded PEG-modified PLA nanoparticles[77]. In vivo studies have revealed that PLGANPs are prone to rapid excretion from the body despite them being excellent drug or gene carriers having a high target specific efficiency toward cancer cells. To solve this problem involving the PLGA nanoparticles, they are coated with PEG to increase their circulation time into the bloodstream. This was done by Sengupta et al. to successfully treat NSCLC-type bronchogenic cancer in mice where they loaded combretastatin-A4 within the outer lipid envelope and entrapped DOX to the PLGA NPs core. When the delivery system reached the tumor site, the antiangiogenesis agent combretastatin-A4 caused the cytoskeletal structure

to crumple resulting in a rapid vascular shutdown. Side by side the PLGA nanoparticles were entrapped into the tumors and gradually released the cytotoxic drug DOX which killed the neoplastic cells by the initiation of apoptosis[78]. Zhou et al. prepared a heparin conjugated PEI system and demonstrated the possible delivery of pIL gene for having inhibitory effects against lung metastasis of B16- F10 malignant melanoma in a murine model[39]. Jere et al. aiming to achieve efficient delivery of Akt1 siRNA to A549 lung cell line by performing chitosan grafting on poly(ethyleneimine). They were able to successfully inhibit the proliferation of tumor cells and tumorigenesis[79]. Benfer and Kissel applied cationic PLGA for the delivery of negatively charged siRNA which functions as a gene-silencing agent for inhibition of protein synthesis which is crucial for tumor growth in H1299–EGFP cells[80].

Synthetic polymer nanoparticles provide many advantages including ease of synthesis, lower viscosity, small aggregate size, extended shelf life with increased blood circulation. They also enable permeation through pores of small sizes, reduction in toxic side effects and targeting.

1.3 Synthetic (Inorganic) nanoparticles:

Despite natural and organic nanoparticles being highly biocompatible, they fail to improve the increasing mortality rate due to the lung cancer. Synthetic inorganic nanoparticles display a few attractive intrinsic properties which noticeably boost their therapeutic value compared to the natural and synthetic organic nanoparticles. Inorganic nanoparticles is believed to improve the lung cancer treatment regimen in various ways, if are developed in a proper mechanism[81]. Several synthetic inorganic nanoparticles have found their applications in the field of lung cancer therapy, some of which are:

- Gold Nanoparticles
- Magnetic Nanoparticles
- Quantum Dots
- Silica Nanoparticles
- Lanthanide Nanoparticles

II. CONCONCLUSION & FUTURE PERSPECTIVES

Nanoparticle-based medicine has unlimited prospective along with novel applications continuously evolving for use in cancer diagnosis, detection, imaging, and treatment. These new approaches are already helping to identify the main disadvantages in the traditional anticancer agents such as nonspecific targeting, low therapeutic efficiencies, unwanted side effects, and drug resistance as well as exceeding their precursors with the potential to detect early metastasis. The ability of nanoparticles is that they can be modified for a personalized medicine strategy makes them ideal for the treatment of lung cancer. Enormous nanoparticle-based experimental therapeutics for lung cancer used as a combinational approach in balancing the design with targeting and identifying moieties and anticancer agents. In general, nanoparticles with multicomponent structures allow design flexibility in drug delivery of poorly water-soluble molecules and imparting the ability to overcome biological barriers and selectively target desired sites within the body. Despite the enormous amount of research and a mammoth quantity of studies, nanoparticles have yet to realise their full potential. This is evidenced by the scarcity of clinically approved therapies regardless of the activeness of the research activities being done in this field. Nanoparticles right now are facing a major revolution as an increasing amount of researchers are abandoning old ideas and accepting the new emerging concepts. This shift of concepts works in favour of improvising the research field of nanotechnology more and more as an increasing amount of knowledge is being continuously gained from the neighbouring fields like immunology and molecular biology. We

believe that the potential of the nanoparticles in cancer therapy will increase with a deeper understanding of the heterogeneity of tumours, the utility of the enhanced permeability and retention (EPR) effect, transport of Nanoparticles to tumours, nano-bio interactions, the tumour microenvironment and prevention of metastasis. The introduction of more sophisticated drug delivery systems in the near future will allow the superior release of therapy and more skilful targeting to the disease site. Machine learning assisted predictions of effective nanomedicine is believed to play a sizable role in the future of nanoparticle-assisted drug delivery. There remain a huge number of challenges regarding the nanotechnology usage in the diagnosis and treatment of lung cancer but the widespread view has concluded that nanotechnology has a huge potential in revolutionising not only the field of cancer therapy but the medicinal field as a whole. As it stands, Nanotechnology in the field of cancer treatment is prepared to conquer the present challenges and rise up as a greater influence on the cancer diagnosis and treatment therapies that are currently available in the clinics. In the upcoming decades, the nanotechnology especially the nanoparticles-based drug delivery system will be promoted from a promising potential to a major treatment choice against lung cancer and various other diseases.

III. REFERENCES

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