Chapter 2

LITERATURE REVIEW

2.1 Anatomy and Physiology of Human Lungs

Lungs are vital organ of body which has balloon like structure and its main purpose is exchange of gases. Inhalation of air, called inspiration, breath enters the airway and travels down to alveoli, which are the smallest functional unit of lungs for exchange of gases. Oxygen enters the blood through diffusion process and carbon dioxide diffuses out from alveolar-capillary membrane. This is the process of respiration. During breathing out i.e. exhalation, gas travels from the alveoli towards upper airway and is exhaled out through the nose and mouth. The exchange of gas between the atmosphere and lungs is called as ventilation. The continuous process of respiration i.e. inhalation and ventilation i.e. exhalation of breath depends on airway system of an individual [1].

2.1.1 Anatomy of Lungs

2.1.1.1Airways

The upper part of respiratory tract comprises of the nasal and oral cavities. Pharynx and larynx are located in the throat. The main purpose of the upper respiratory tract is to moisten and warm the inspired air prior entering to the lungs.

The inspired air is conditioned in nasal passages. The nasal cavity turbinates the air and mucous membrane filter and humidify the inhaled air.

Inhaled air is filtered by the strong hairs present in nostrils. The inhaled air gets warmed and humidified due to presence of network of blood capillaries in the mucous membrane.

Below the larynx, the airways are subdivided into three parts:

- Trachea, bronchi and terminal bronchioles. They are known as conduction airways and helps in air passages.
- Respiratory bronchioles where conduction and exchange of gas takes place.
- Alveolar ducts, sacs, and alveoli, where exchange of gas with pulmonary capillary blood takes place [2].

2.1.1.2 Tracheobronchial Tree

In adults, the diameter and length of Trachea are about 2.0-2.5 cm and 11 cm, respectively. Its extension is from 6^{th} cervical vertebra to the 5^{th} thoracic vertebra. At the level of the carina the division of trachea into left and right main stem bronchi takes place. This further subdivides into lobar bronchi followed by sub segmental bronchi.

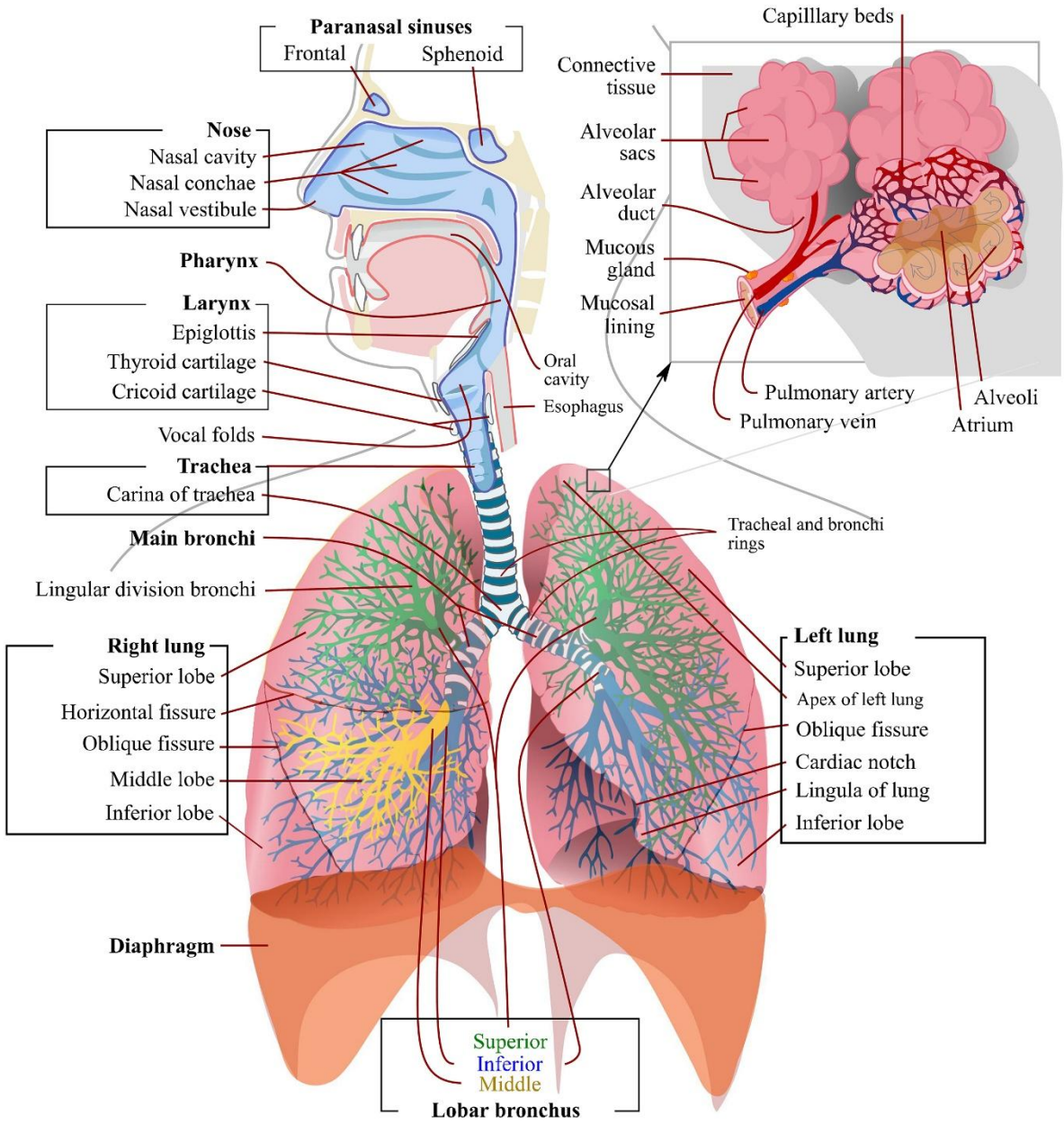


Figure 2.1: A complete, schematic view of the human respiratory system with their parts and functions. (Image retrieved from: http://bcrt.ca/interesting-facts-about-the-respiratory-system-kids-to-parents-information/ (Accessed on May 11, 2018))

The airway system is similar to the roots of a tree which are branched from larger to smaller. Similarly, trachea consists of branched structure form larger airways to smaller and is known as tracheobronchial tree. The airways further in the lungs i.e. from trachea to the alveoli, resulting in about 23 generations of branching airways in an adult.

Airways walls are composed of smooth muscles throughout the tracheobronchial tree, however, it varies with the size of airways. In the trachea and large bronchi, a band of muscle connects the opening of U-shaped cartilages that support the airways. As the airways divide further the smooth muscles becomes more prominent with muscle fibers surrounding in both directions of the walls. The contraction of smooth muscle results in decrease in the diameter of diameter and length of the peripheral airways. Smooth muscle are present to the level of the respiratory bronchioles.

Respiratory tract from below the larynx till the bronchioles is lined by ciliated columnar epithelial cells. The cilia lining the columnar epithelium follow a rhythmic pattern of movement by which most of the debris and particles that enter the airways are removed [3].

2.1.1.3 Bronchial and Pulmonary Circulation

Blood supply to the lungs can be divided into two components

- Bronchial circulation
- Pulmonary circulation

Metabolic demands of lung tissue is fulfilled by bronchial circulation and is connected to the aorta. Entire tracheobronchial tree to the level of bronchioles is perfused by bronchial circulation.

Blood supply to respiratory bronchioles i.e. pulmonary circulation commences from right ventricle and oxygenated blood comes into left atrium, completing the pulmonary circulation loop. Pulmonary circulation supplies blood to bronchioles and alveoli, where gas exchange occurs. Some of the blood from bronchial and pulmonary circulation mix together in the airways near terminal bronchioles [4].

2.1.1.4 Mechanics of Respiration

Thorax protects the vital organs by virtue of its rigid structure enough flexible to permit chest expansion while breathing. Bones, cartilage and supportive tissues provide it the rigidity. Thorax is composed of various joints. The 12 pairs of ribs acts as the structural foundation for the chest. During inspiration, the chest and lungs expand in all three planes: front to back, horizontal, and longitudinal. On inhalation, the ribs move anteriorly, vertically, and expand as a result of the shrinkage of the diaphragm and accessory muscles of inspiration [5].

2.1.1.5 Muscles of Respiration

2.1.1.5.1 Primary

Diaphragm is the key muscle in the process of respiration. It have two dome-shaped hemidiaphragms. Thorax and abdomen are separated by diaphragm. The diaphragm shrinks and compresses and inclines toward the abdomen in inspiration, causing increase in lung size

longitudinally. During exhalation, the diaphragm relaxes and retains its dome-shaped configuration [6].

2.1.1.5.2 Accessory

These muscles assists the diaphragm to increase the thoracic volume. These muscles are

- Sternocleidomastoid
- Trapezius
- Intercostal
- Rhomboid muscles.

Accessory muscles are dormant while relaxed breathing. However, they start participating while performing intense physical activity. The abdominal muscles too remain dormant in relaxed breathing; however, they play important part in forced exhalation, coughing, performing physical activity or sneezing [7].

2.1.1.6 Mechanics of Lung Function

Lungs and surrounding chest wall function similar to a pump. The lungs and chest wall are separated by parietal and visceral pleura. The pleural space has a characteristic pressure which is called as pleural pressure and it varies onto the intensity of breathing.

At resting state the pressure in entire airway nearly equal to atmospheric pressure.

At the end of relaxed expiration, the lungs contract interiorly whereas the chest wall expand outwardly. This generate an opposing force and creates a subatmosperic pressure of approximately 5 cm of water. The magnitude of pressure varies with each breathing cycle.

During inhalation, contraction of diaphragm takes place and at the same time lungs expand, which creates a negative pressure. This difference in pressure gradient causes inhaled air to fill the lungs.

During the end of relaxed inspiration, the air volume inside lungs increased causing augmentation in pressure in pleural cavity and alveoli, which stops the air flow into lungs. During exhalation, the respiratory muscles relax and the lungs contract, causing elevation in alveolar pressure than at the airway opening. This difference in pressure gradient is responsible for outward flow of air from lungs [8].

2.1.2 Physiology of Respiration

2.1.2.1 Lung Volumes and Capacities

Tidal Volume [TV]

Tidal volume is defined as the sum of volume of inhaled air and exhaled air.

Inspiratory Minute Volume or V_I

Total volume of air inhaled over a minute.

Similarly, the expiratory minute volume is total volume of air exhaled over a minute.

Inspiratory Reserve Volume [IRV]

The maximum volume of air that can be inhaled after normal inhalation.

Inspiratory Capacity [IC]

The sum of the tidal volume and inspiratory reserve volume compartments comprise the inspiratory capacity. This is represents maximal inhaled volume originating from the end of the resting exhaled tidal volume.

Expiratory Reserve Volume [ERV]

The maximum volume of air exhaled further after the end of normal tidal volume is the volume of air remaining in the lungs after maximal exhalation is the residual volume [RV].

Functional Residual Capacity [FRC]

The sum of the expiratory reserve volume as the residual volume compartments.

Total Lung Capacity

The volume of air in the lungs following maximal inspiration is total lung capacity.

Vital Capacity

The maximal volume of air exhaled from the lungs after a maximal inspiration or the maximum volume of air inhaled following maximal exhalation is the vital capacity.

$$Vital\ Capacity = Inspiratory\ reserve\ volume + Tidal\ Volume + Expiratory\ reserve\ volume$$

The resting volume in the lungs can be altered many factors. The factors can be less elasticity of lungs, increase in the functional residual capacity (FRC).

The FRC also decreases when the posture of patient changes (caused by impingement of the abdomen on the diaphragm) [9].

2.1.2.2 Pulmonary Gas Exchange

The key function of the lungs is to provide oxygen to, and remove carbon dioxide from, the blood circulation in the pulmonary capillaries. In process of breathing, alveolar air and blood flowing through pulmonary capillaries come into close contact separated only by a very thin membrane which is referred as alveolar-capillary membrane. Alveoli are richly perfused, they receive blood through pulmonary capillaries which comes from right ventricle. Oxygen from alveoli diffuses into blood while carbon dioxide diffuses out. The carbon dioxide accumulated in alveoli is exhaled. The oxygen combined with hemoglobin is circulated in the body via the systemic circulation. During circulation oxygen diffuses out from systemic capillaries to the

various tissues of the body. Whereas, carbon dioxide which is a metabolic byproduct diffuses into the systemic capillaries. Capillaries transport the blood back to the lungs through venous blood vessels, right atrium and right ventricle. Circulatory loop completes when gas exchange takes place in lungs [10, 11].

2.2 Cancer

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. These abnormal cells are termed cancer cells, malignant cells, or tumor cells. These cells can infiltrate normal body tissues. Many cancers and the abnormal cells that compose the cancer tissue are further identified by the name of the tissue that the abnormal cells originated from (for example, breast cancer, lung cancer, colon cancer). Cancer is not confined to humans; animals and other living organisms can get cancer. Figure 2.2 is a schematic that shows normal cell division and Figure 2.3 shows, how when a cell is damaged or altered without repair to its system, the cell usually dies.

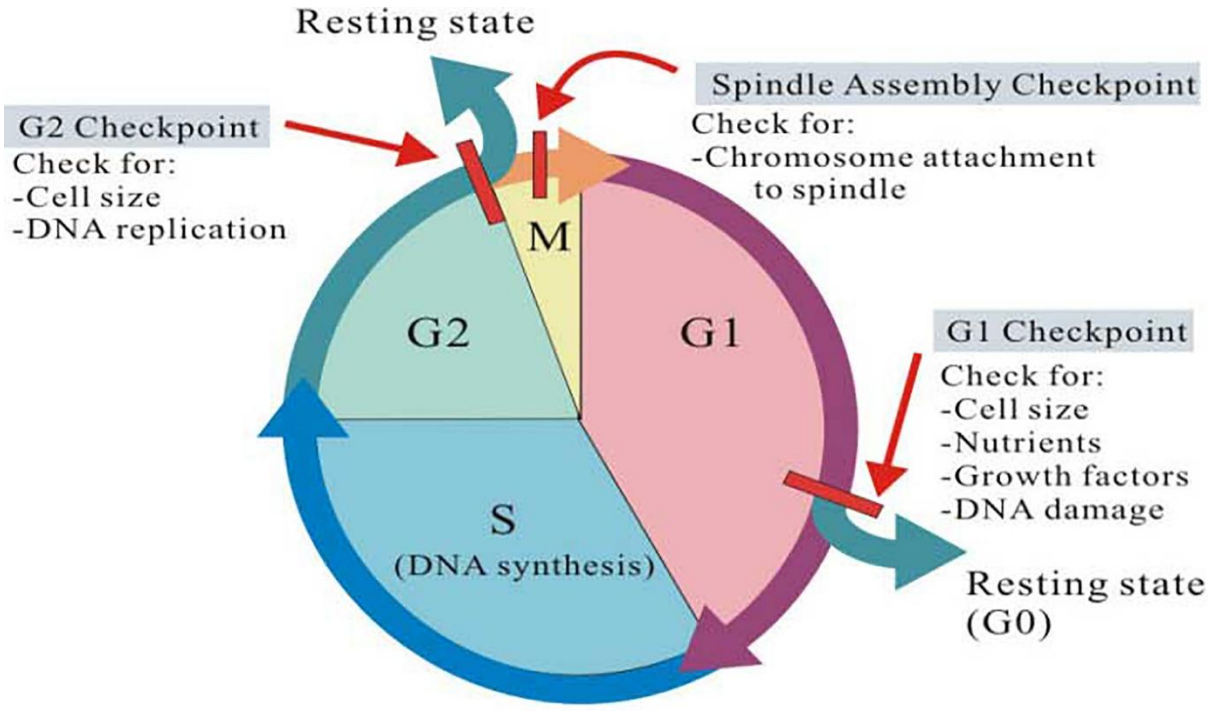


Figure 2.2: Schematic representation of normal cell cycle.

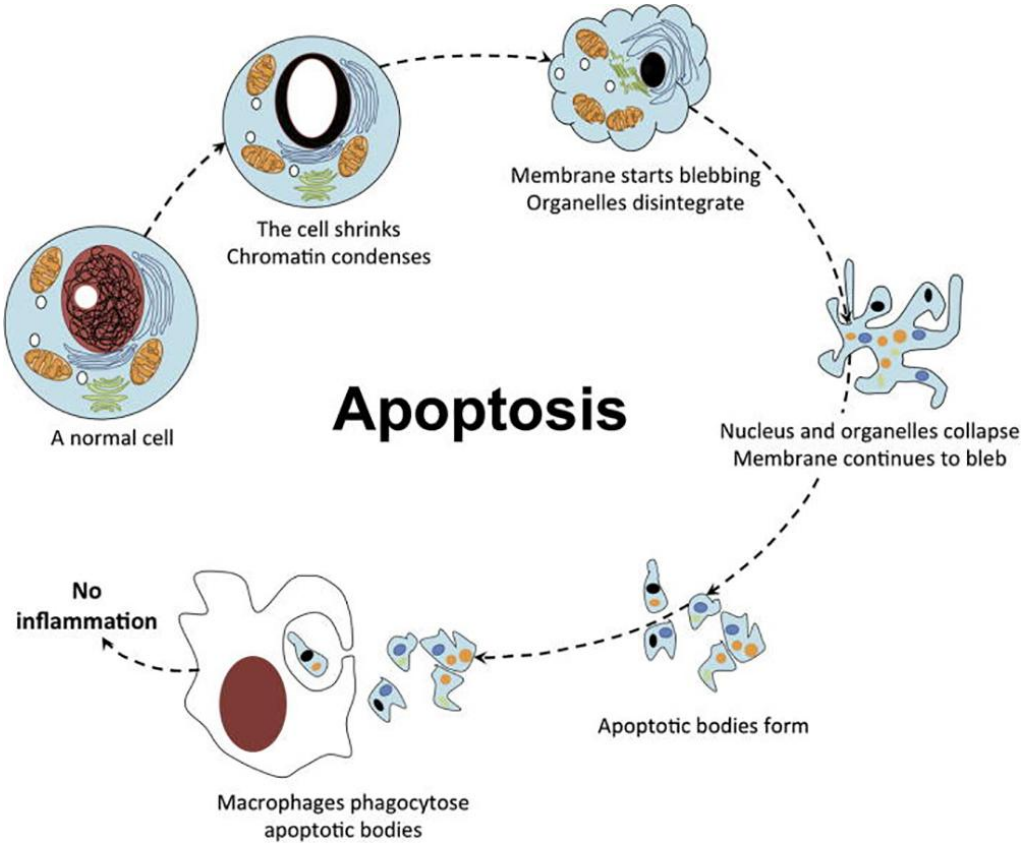


Figure 2.3: Cellular death due to damage or alteration in normal cell cycle [12].

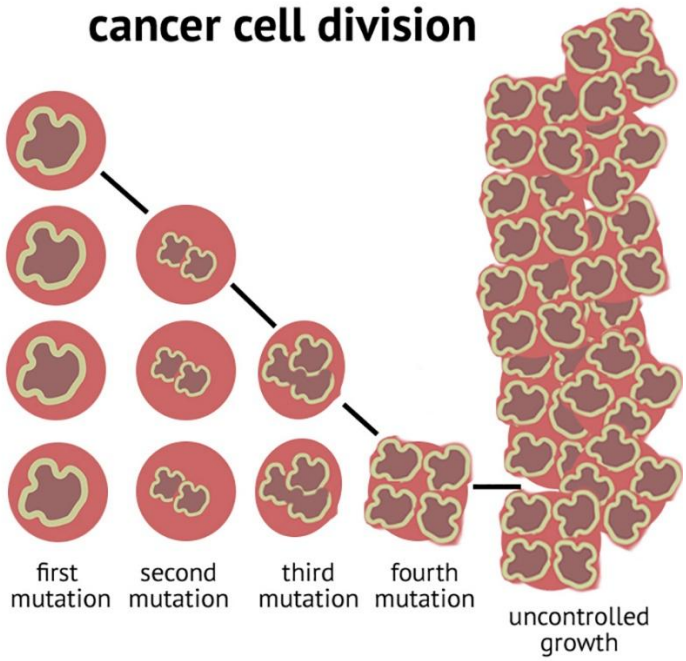


Figure 2.4: Cancer cell division. (Image retrieved from: https://www.philpoteducation.com/mod/book/view.php?id=779&chapterid=1123# (Accessed on May 11, 2018)).

Figure 2.4 shows, what occurs when such damaged or unrepaired cells do not die and become cancer cells and show uncontrolled division and growth -- a mass of cancer cells develop. Frequently, cancer cells can break away from this original mass of cells, travel through the blood and lymph systems, and lodge in other organs where they can again repeat the uncontrolled growth cycle. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastasis [13].

2.2.1 Types of Cancer

There are over 200 types of cancer. The list of more specific types of cancers found in each general category is given below; it is not all inclusive and the cancers listed in quotes are the general names of some cancers. Figure 2.5 shows the cancer of specific organs.

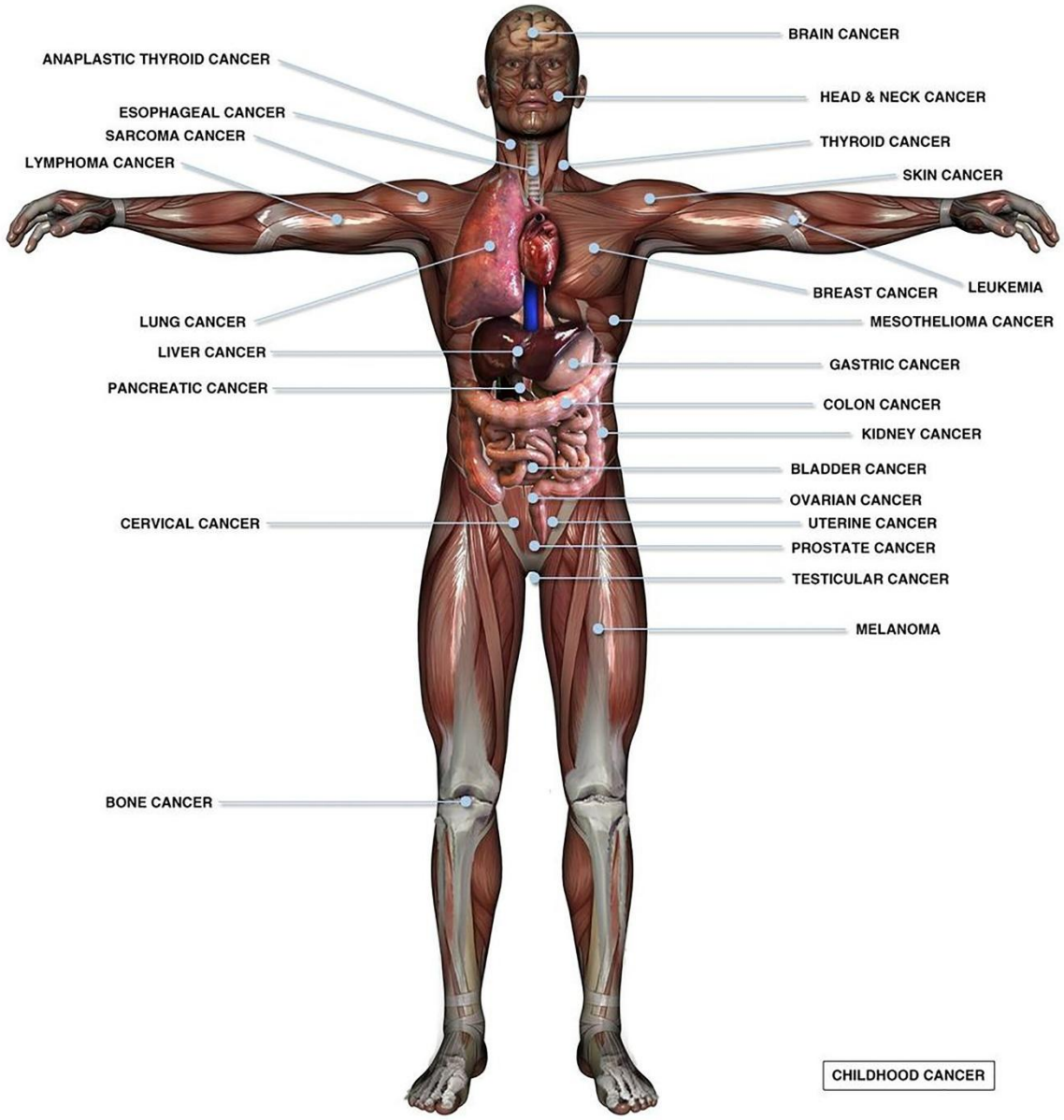


Figure 2.5: Types of cancer. (Image retrieved from: http:// jeux-fille. biz/ 9663_inner_parts_of_body_cancer_and_the_human_body_an_inside_look / (Accessed on 11th May 2018))

Carcinoma: Cancer that begins in the skin or in tissues that line or cover internal organs: "skin, lung, colon, pancreatic, ovarian cancers," epithelial, squamous and basal cell carcinomas, melanomas, papillomas, and adenomas.

- Sarcoma: Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue: "bone, soft tissue cancers," osteosarcoma, synovial sarcoma, liposarcoma, angiosarcoma, rhabdosarcoma, and fibrosarcoma.

- Leukemia: Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood: "leukemia,"lymphoblastic leukemias, myelogenous leukemias, T-cell leukemia, and hairy-cell leukemia.
- Lymphoma and myeloma: Cancers that begin in the cells of the immune system: "lymphoma," T-cell lymphomas, B-cell lymphomas, Hodgkin lymphomas, non-Hodgkin lymphoma, and lymphoproliferative lymphomas.
- Central nervous system (CNS) cancers: Cancers that begin in the tissues of the brain and spinal cord: "brain and spinal cord tumors," gliomas, meningiomas, pituitary adenomas, vestibularschwannomas, primary CNS lymphomas, and primitive neuroectodermal tumors.

Not included in the above types listed are metastatic cancers; this is because metastatic cancer cells usually arise from a cell type listed above and the major difference from the above types is that these cells are now present in a tissue from which the cancer cells did not originally develop. Consequently, if the terms "metastatic cancer" is used, for accuracy, the tissue from which the cancer cells arose should be included [14].

2.2.2 Cancer Statistics

Every sixth death in the world is due to cancer, making it the second leading cause of death (second only to cardiovascular diseases) [15]. In 2016, 8.9 million people are estimated to have died from the various forms of cancer. The Institute for Health Metrics and Evaluation (IHME) put relatively small error margins around this global figure: the lower and upper estimates extend from 8.75 to 9.1 million [16]. Progress against many other causes of deaths and demographic drivers of increasing population size, life expectancy and particularly in higher-income countries aging populations mean that the total number of cancer deaths continues to increase. This is a very personal topic to many: nearly everyone knows or has lost someone dear to them from this collection of diseases. Figure 2.6 shows the world map indicating the share of population with cancer. Figure 2.7 shows share of total population with different forms of cancer, measured as the age-standardized percentage. This share has been age-standardized assuming a constant age structure to compare prevalence between countries and through time.

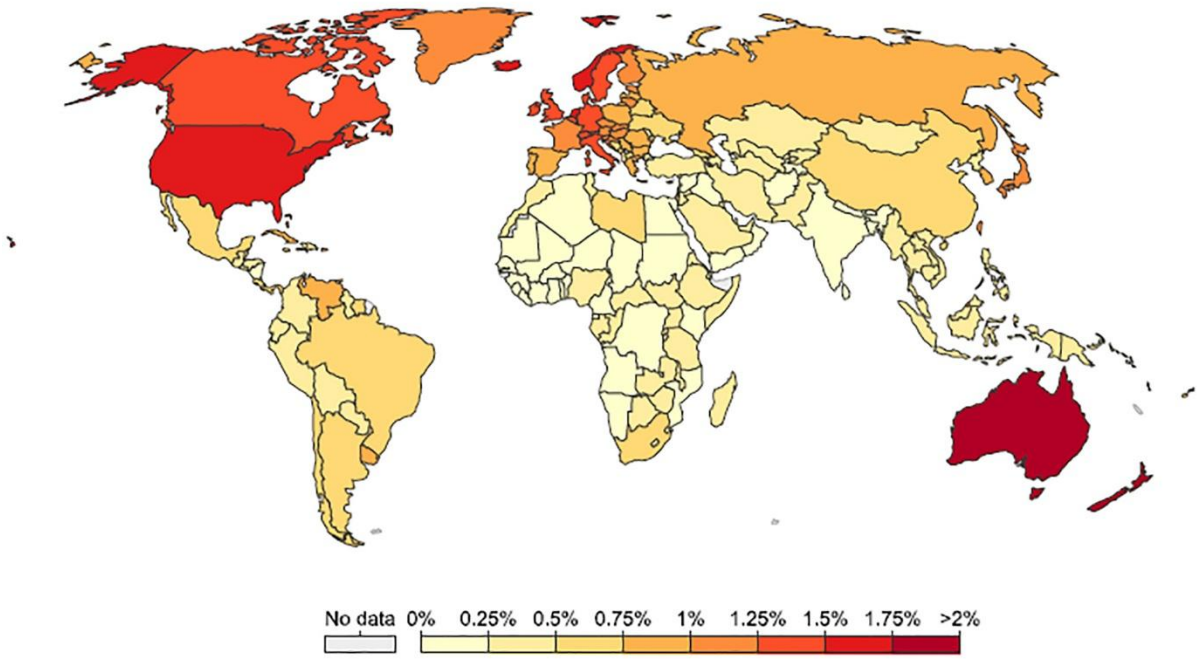


Figure 2.6: Share of population with cancer in 2016. (Image Retrieved from Max Roser and Hannah Ritchie (2018) - "Cancer". Published online at OurWorldInData.org. Available online: 'https://ourworldindata.org/cancer'. (Accessed on 11th May 2018).

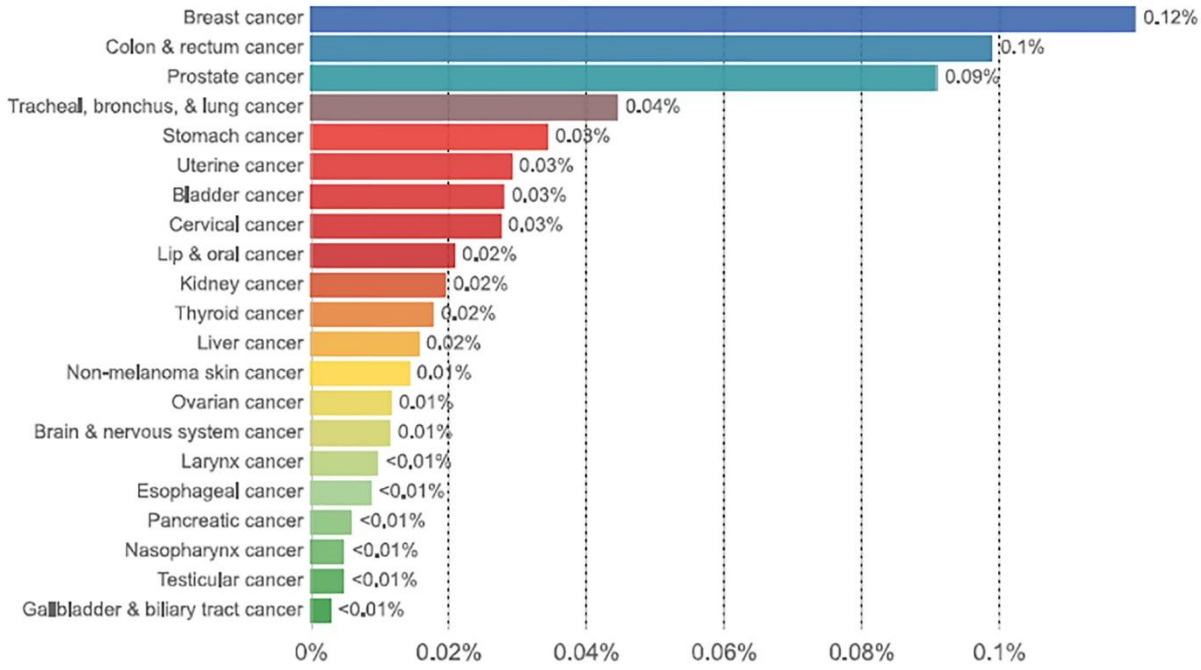


Figure 2.7: Share of population suffering from cancer types, World, 2016. (Image Retrieved from Max Roser and Hannah Ritchie (2018) - "Cancer". Published online at OurWorldInData.org. Available online: 'https://ourworldindata.org/cancer'. (Accessed on 11th May 2018).

Globally, it's estimated that 42 million people across the world suffered from any of the forms of cancer. This number has more than doubled since 1990 when an estimated 19 million had cancer. Total number of deaths in 2016 attributed to the range of cancer types. With more than double the number of attributed deaths of the next leading form, 'tracheal, bronchus, and lung cancer' claimed the largest number of lives at more than 1.7 million in one year. Next follow 'stomach cancer', 'colon and rectum cancer', and 'liver cancer', all with a similar number of deaths, at around 830,000 globally in 2016. In exploring patterns across various countries, we see that 'tracheal, bronchus, and lung cancer' is the leading form of cancer deaths across most high and middle-income countries. However, the leading form in lower income countries varies: colon and rectum; liver; cervical; stomach; breast and prostrate all top the list in several countries [17].

2.2.3 Risk factors and Causes of Cancer

Anything that may cause a normal body cell to develop abnormally potentially can cause cancer. Many things can cause cell abnormalities and have been linked to cancer development. Some cancer causes remain unknown while other cancers have environmental or lifestyle triggers or may develop from more than one known cause. Some may be developmentally influenced by a person's genetic makeup. Many patients develop cancer due to a combination of these factors. Although it is often difficult or impossible to determine the initiating event(s) that cause a cancer to develop in a specific person, research has provided clinicians with a number of likely causes that alone or in concert with other causes, are the likely candidates for initiating cancer. The following is a listing of major causes and is not all-inclusive as specific causes are routinely added as research advances:

Chemical or toxic compound exposures: Benzene, asbestos, nickel, cadmium, vinyl chloride, benzidine, N-nitrosamines, tobacco or cigarette smoke (contains at least 66 known potential carcinogenic chemicals and toxins), asbestos, and aflatoxin

Ionizing radiation: Uranium, radon, ultraviolet rays from sunlight, radiation from alpha, beta, gamma, and X-ray-emitting sources

Pathogens: Human papillomavirus (HPV), EBV or Epstein-Barr virus, hepatitis viruses B and C, Kaposi's sarcoma-associated herpes virus (KSHV), Merkel cell polyomavirus, Schistosoma spp., and Helicobacter pylori; other bacteria are being researched as possible agents.

Genetics: A number of specific cancers have been linked to human genes and are as follows: breast, ovarian, colorectal, prostate, skin and melanoma.

It is important to point out that most everyone has risk factors for cancer and is exposed to cancer-causing substances (for example, sunlight, secondary cigarette smoke, and X-rays) during their lifetime, but many individuals do not develop cancer. In addition, many people have the genes that are linked to cancer but do not develop it. Why? Although researchers may not be able give a satisfactory answer for every individual, it is clear that the higher the amount or level of cancercausing materials a person is exposed to, the higher the chance the person will develop cancer. In addition, the people with genetic links to cancer may not develop it for similar reasons (lack of enough stimulus to make the genes function). In addition, some people may have a heightened immune response that controls or eliminates cells that are or potentially may become cancer cells. There is evidence that even certain dietary lifestyles may play a significant role in conjunction with the immune system to allow or prevent cancer cell survival. For these reasons, it is difficult to assign a specific cause of cancer to many individuals. Recently, other risk factors have been added to the list of items that may increase cancer risk. Specifically, red meat (such as beef, lamb, and pork) was classified by the International Agency for Research on Cancer as a high-risk agent for potentially causing cancers; in addition processed meats (salted, smoked, preserved, and/or cured meats) were placed on the carcinogenic list. Individuals that eat a lot of barbecued meat may also increase risk due to compounds formed at high temperatures. Other less defined situations that may increase the risk of certain cancers include obesity, lack of exercise, chronic inflammation, and hormones, especially those hormones used for replacement therapy. Other items such as cell phones have been heavily studied. In 2011, the World Health Organization classified cell phone low energy radiation as "possibly carcinogenic," but this is a very low risk level that puts cell phones at the same risk as caffeine and pickled vegetables. Proving that a substance does not cause or is not related to increased cancer risk is difficult. For example, antiperspirants are considered to possibly be related to breast cancer by some investigators and not by others. The official stance by the National Cancer Institute is "additional research is needed to investigate this relationship and other factors that may be involved." This unsatisfying conclusion is presented because the data collected so far is contradictory. Other claims that are similar require intense and expensive research that may never be done. Reasonable advice might be to avoid large amounts of any compounds even remotely linked to cancer, although it may be difficult to do in complex, technologically advanced modern societies **[**14].

2.3 Lung Cancer

Cancer of the lung, like all cancers, results from an abnormality in the body's basic unit of life, the cell. Normally, the body maintains a system of checks and balances on cell growth so that cells divide to produce new cells only when new cells are needed. Disruption of this system of checks and balances on cell growth results in an uncontrolled division and proliferation of cells that eventually forms a mass known as a tumor. Tumors can be benign or malignant; when we speak of "cancer," we are referring to those tumors that are malignant. Benign tumors usually can be removed and do not spread to other parts of the body. Malignant tumors, on the other hand, often grow aggressively locally where they start, but tumor cells also can enter into the bloodstream or lymphatic system and then spread to other sites in the body. This process of spread is termed metastasis; the areas of tumor growth at these distant sites are called metastases. Since lung cancer tends to spread or metastasize very early after it forms, it is a very life-threatening cancer and one of the most difficult cancers to treat. While lung cancer can spread to any organ in the body, certain locations -- particularly the adrenal glands, liver, brain, and bones -- are the most common sites for lung cancer metastasis. The lung also is a very common site for metastasis from malignant tumors in other parts of the body. Tumor metastases are made up of the same types of cells as the original (primary) tumor. For example, if prostate cancer spreads via the bloodstream to the lungs, it is metastatic prostate cancer in the lung and is not lung cancer.

The principal function of the lungs is to exchange gases between the air we breathe and the blood. Through the lung, carbon dioxide is removed from the bloodstream and oxygen enters the bloodstream. The right lung has three lobes, while the left lung is divided into two lobes and a small structure called the lingula that is the equivalent of the middle lobe on the right. The major airways entering the lungs are the bronchi, which arise from the trachea, which is outside the lungs. The bronchi branch into progressively smaller airways called bronchioles that end in tiny sacs known as alveoli where gas exchange occurs. The lungs and chest wall are covered with a thin layer of tissue called the pleura.

Lung cancers can arise in any part of the lung, but 90%-95% of cancers of the lung are thought to arise from the epithelial cells, the cells lining the larger and smaller airways (bronchi and bronchioles); for this reason, lung cancers are sometimes called bronchogenic cancers or bronchogenic carcinomas. (Carcinoma is another term for cancer.) Cancers also can arise from

the pleura (called mesotheliomas) or rarely from supporting tissues within the lungs, for example, the blood vessels.

2.3.1 Causes and Risk factors of lung cancer

2.3.1.1 Smoking

The incidence of lung cancer is strongly correlated with cigarette smoking, with about 90% of lung cancers arising as a result of tobacco use. The risk of lung cancer increases with the number of cigarettes smoked and the time over which smoking has occurred; doctors refer to this risk in terms of pack-years of smoking history (the number of packs of cigarettes smoked per day multiplied by the number of years smoked). For example, a person who has smoked two packs of cigarettes per day for 10 years has a 20 pack-year smoking history. While the risk of lung cancer is increased with even a 10-pack-year smoking history, those with 30-pack-year histories or more are considered to have the greatest risk for the development of lung cancer. Among those who smoke two or more packs of cigarettes per day, one in seven will die of lung cancer. Pipe and cigar smoking also can cause lung cancer, although the risk is not as high as with cigarette smoking. Thus, while someone who smokes one pack of cigarettes per day has a risk for the development of lung cancer that is 25 times higher than a nonsmoker, pipe and cigar smokers have a risk of lung cancer that is about five times that of a nonsmoker.

Tobacco smoke contains over 4,000 chemical compounds, many of which have been shown to be cancer-causing or carcinogenic. The two primary carcinogens in tobacco smoke are chemicals known as nitrosamines and polycyclic aromatic hydrocarbons. The risk of developing lung cancer decreases each year following smoking cessation as normal cells grow and replace damaged cells in the lung. In former smokers, the risk of developing lung cancer begins to approach that of a nonsmoker about 15 years after cessation of smoking.

2.3.1.2 Passive smoking

Passive smoking or the inhalation of tobacco smoke by nonsmokers who share living or working quarters with smokers, also is an established risk factor for the development of lung cancer. Research has shown that nonsmokers who reside with a smoker have a 24% increase in risk for developing lung cancer when compared with nonsmokers who do not reside with a smoker. The risk appears to increase with the degree of exposure (number of years exposed and number of cigarettes smoked by the household partner) to secondhand smoke. It is

estimated that over 7,000 lung cancer deaths occur each year in the U.S. that are attributable to passive smoking.

2.3.1.3 Exposure to asbestos fibers

Asbestos fibers are silicate fibers that can persist for a lifetime in lung tissue following exposure to asbestos. The workplace was a common source of exposure to asbestos fibers, as asbestos was widely used in the past as both thermal and acoustic insulation. Today, asbestos use is limited or banned in many countries, including the U.S. Both lung cancer and mesothelioma (cancer of the pleura of the lung as well as of the lining of the abdominal cavity called the peritoneum) are associated with exposure to asbestos. Cigarette smoking drastically increases the chance of developing an asbestos-related lung cancer in workers exposed to asbestos; asbestos workers who do not smoke have a fivefold greater risk of developing lung cancer than nonsmokers, but asbestos workers who smoke have a risk that is fifty- to ninety-fold greater than nonsmokers.

2.3.1.4 Exposure to radon gas

Radon gas is a natural radioactive gas that is a natural decay product of uranium that emits a type of ionizing radiation. Radon gas is a known cause of lung cancer, with an estimated 12% of lung cancer deaths attributable to radon gas, or about 21,000 lung-cancer-related deaths annually in the U.S., making radon the second leading cause of lung cancer in the U.S. after smoking. As with asbestos exposure, concomitant smoking greatly increases the risk of lung cancer with radon exposure. Radon gas can travel up through soil and enter homes through gaps in the foundation, pipes, drains, or other openings.

2.3.1.5 Familial predisposition

While the majority of lung cancers are associated with tobacco smoking, the fact that not all smokers eventually develop lung cancer suggests that other factors, such as individual genetic susceptibility, may play a role in the causation of lung cancer. Numerous studies have shown that lung cancer is more likely to occur in both smoking and nonsmoking relatives of those who have had lung cancer than in the general population. It is unclear how much of this risk is due to shared environmental factors (like a smoking household) and how much is related to

genetic risk. People who inherit certain genes, like genes that interfere with DNA repair, may be at greater risk for several types of cancer.

2.3.1.6 Lung diseases

The presence of certain diseases of the lung, notably chronic obstructive pulmonary disease (COPD), is associated with an increased risk (four- to six-fold the risk of a nonsmoker) for the development of lung cancer even after the effects of concomitant cigarette smoking are excluded. Pulmonary fibrosis (scarring of the lung) appears to increase the risk about seven-fold, and this risk does not appear to be related to smoking.

2.3.1.7 Prior history of lung cancer

Survivors of lung cancer have a greater risk of developing a second lung cancer than the general population has of developing a first lung cancer. Survivors of non-small cell lung cancers (NSCLCs, see below) have an additive risk of 1%-2% per year for developing a second lung cancer. In survivors of small cell lung cancers (SCLCs, see below), the risk for development of second lung cancers approaches 6% per year.

2.3.1.8 Air pollution

Air pollution from vehicles, industry, and power plants can raise the likelihood of developing lung cancer in exposed individuals. Up to 1%-2% of lung cancer deaths are attributable to breathing polluted air, and experts believe that prolonged exposure to highly polluted air can carry a risk for the development of lung cancer similar to that of passive smoking.

2.3.1.9 Exposure to diesel exhaust

Exhaust from diesel engines is made up of gases and soot (particulate matter). Many occupations, such as truck drivers, toll booth workers, forklift and other heavy machinery operators, railroad and dock workers, miners, garage workers and mechanics, and some farm workers are frequently exposed to diesel exhaust [18].

2.3.2 Types of Lung Cancer

Lung cancers, also known as bronchogenic carcinomas because they arise from the bronchi within the lungs, are broadly classified into two types: small cell lung cancers (SCLC) and non-

small cell lung cancers (NSCLC). This classification is based upon the microscopic appearance of the tumor cells themselves, specifically the size of the cells. These two types of cancers grow and spread in different ways and may have different treatment options, so a distinction between these two types is important.

SCLC comprise about 20% of lung cancers and are the most aggressive and rapidly growing of all lung cancers. SCLC are strongly related to cigarette smoking, with only 1% of these tumors occurring in nonsmokers. SCLC metastasize rapidly to many sites within the body and are most often discovered after they have spread extensively. Referring to a specific cell appearance often seen when examining samples of SCLC under the microscope, these cancers are sometimes called oat cell carcinomas.

NSCLC are the most common lung cancers, accounting for about 80% of all lung cancers. NSCLC can be divided into several main types that are named based upon the type of cells found in the tumor:

- Adenocarcinomas are the most commonly seen type of NSCLC in the U.S. and comprise up to 50% of NSCLC. While adenocarcinomas are associated with smoking like other lung cancers, this type is observed as well in nonsmokers who develop lung cancer. Most adenocarcinomas arise in the outer, or peripheral, areas of the lungs.
- Bronchioloalveolar carcinoma is a subtype of adenocarcinoma that frequently develops at multiple sites in the lungs and spreads along the preexisting alveolar walls.

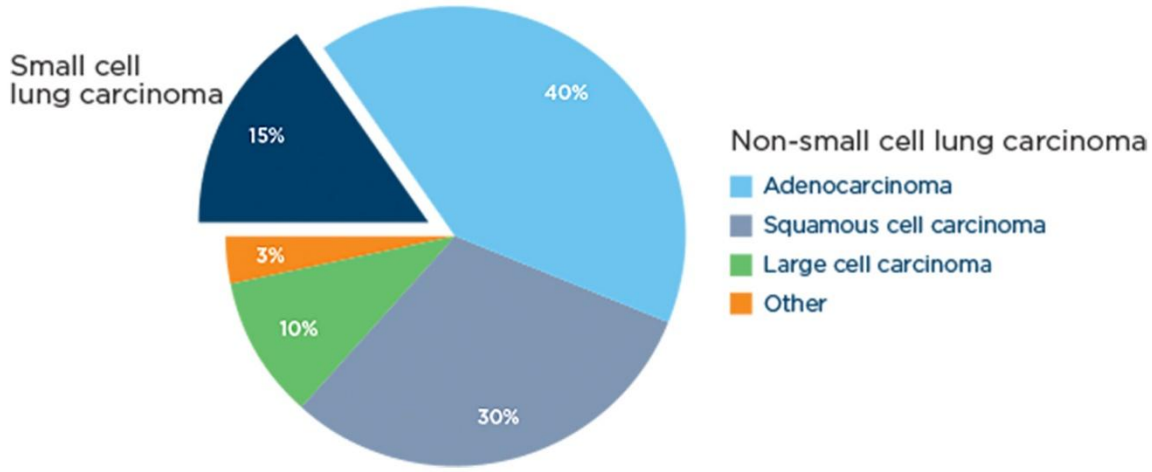


Figure 2.8: Types of Lung cancer (Image retrieved from: https://lungevity.org/for-patients-caregivers/lung-cancer-101/types-of-lung-cancer (Accessed on May 11, 2018)).

- Squamous cell carcinomas were formerly more common than adenocarcinomas; at present, they account for about 30% of NSCLC. Also known as epidermoid carcinomas, squamous cell cancers arise most frequently in the central chest area in the bronchi.
- Large cell carcinomas, sometimes referred to as undifferentiated carcinomas, are the least common type of NSCLC.
- Mixtures of different types of NSCLC also are seen.

Other types of cancers can arise in the lung; these types are much less common than NSCLC and SCLC and together comprise only 5%-10% of lung cancers:

- Bronchial carcinoids account for up to 5% of lung cancers. These tumors are sometimes referred to as lung neuroendocrine tumors. They are generally small (3 cm-4 cm or less) when diagnosed and occur most commonly in people under 40 years of age. Unrelated to cigarette smoking, carcinoid tumors can metastasize, and a small proportion of these tumors secrete hormone-like substances that may cause specific symptoms related to the hormone being produced. Carcinoids generally grow and spread more slowly than bronchogenic cancers, and many are detected early enough to be amenable to surgical resection.
- Cancers of supporting lung tissue such as smooth muscle, blood vessels, or cells involved in the immune response can rarely occur in the lung [19].

2.3.3 Treatment options for NSCLC

2.3.3.1 Surgery

Most stage I and stage II non-small cell lung cancers are treated with surgery to remove the tumor. For this procedure, a surgeon removes the lobe, or section, of the lung containing the tumor. Some surgeons use video-assisted thoracoscopic surgery (VATS). For this procedure, the surgeon makes a small incision, or cut, in the chest and inserts a tube called a thoracoscope. The thoracoscope has a light and a tiny camera connected to a video monitor so that the surgeon can see inside the chest. A lung lobe can then be removed through the scope, without making a large incision in the chest.

Advantages of Surgery

- Removal of a large volume of tumor can relieve mass effect, which may reduce symptoms instantly
- The removal of cancer cells that are producing blood-borne factors that stimulate the growth of cancer cells in other parts of the body
- Potential ability to remove all cancer cells in a small area (the patient may be cured with surgery alone)
- Ability to look at the cancerous tissue (pathology).
    - Tissue samples can be examined to decide the best treatment options for that particular patient.
    - If the patient has already been treated, the samples can be used to see how the cancer responded to previous treatment to see if more of that treatment should be given or if the treatment needs to be changed.
- Convenience for the patient (since the surgery is performed once over the course of a day while the patient is asleep)

Disadvantages of Surgery

- Inability to kill microscopic disease around the edges of the tumor may leave tumor cells in the patient after surgery.
- The patient must be able to tolerate the surgery and anesthesia (i.e. have minimal medical problems, have good lung function, not be on certain medications)

- Some damage to nearby normal tissues (e.g. removing ribs or normal lung tissue to reach a lung tumor)
- Complications from surgery (e.g. infection, and others that are site-specific)
- Inability to remove cancer in other parts of the body (i.e. metastatic disease)
- Removal of an organ which may affect the patients quality of life (e.g. breast, larynx, bowel)
- Inability of a surgeon to discern cancer cells from normal cells with the naked eye (particularly after chemotherapy or radiation have been delivered to the site) [20].

2.3.3.2 Chemotherapy and Radiation

For people with non-small cell lung tumors that can be surgically removed, evidence suggests that chemotherapy after surgery, known as "adjuvant chemotherapy," may help prevent the cancer from returning. This is particularly true for patients with stage II and IIIA disease. Questions remain about whether adjuvant chemotherapy applies to other patients and how much they benefit. For people with stage III lung cancer that cannot be removed surgically, doctors typically recommend chemotherapy in combination with definitive (high-dose) radiation treatments. In stage IV lung cancer, chemotherapy is typically the main treatment. In stage IV patients, radiation is used only for palliation of symptoms. The chemotherapy treatment plan for lung cancer often consists of a combination of drugs. Among the drugs most commonly used are cisplatin (Platinol) or carboplatin (Paraplatin) plus docetaxel (Taxotere), gemcitabine (Gemzar), paclitaxel (Taxol and others), vinorelbine (Navelbine and others), or pemetrexed (Alimta). There are times when these treatments may not work. Or, after these drugs work for a while, the lung cancer may come back. In such cases, doctors often prescribe a second course of drug treatment referred to as second-line chemotherapy. Recently, the concept of maintenance chemotherapy has been tested in clinical trials, either as a switch to another drug before the cancer progresses; or to continue one of the drugs used initially for a longer period of time. Both of these strategies have shown advantages in selected patients.

2.3.3.3 Chemotherapy before Other Treatments (Neoadjuvant Treatment)

Receiving chemotherapy before radiation or surgery may help people with lung cancer by shrinking the tumor enough to make it easier to remove with surgery, increasing the effectiveness of radiation and destroying hidden cancer cells at the earliest possible time. If a tumor doesn't shrink with chemotherapy, the medication can be stopped right away, allowing the doctor to try a different treatment. In addition, research shows that people with lung cancer are much more able to cope with the side effects of chemotherapy when it is given before

surgery. Sometimes, a short trial period of treatment with the drug shrinks the tumor before surgery. If that is the case, then continued treatment with the same drug after surgery is more likely to benefit the patient. Because many lung cancer specialists around the world are giving chemotherapy to their patients before surgery, patients should discuss it with their doctor.

2.3.3.4 Targeted Treatments

One of the most exciting developments in lung cancer medicine is the introduction of targeted treatments. Unlike chemotherapy drugs, which cannot tell the difference between normal cells and cancer cells, targeted therapies are designed specifically to attack cancer cells by attaching to or blocking targets that appear on the surfaces of those cells. People who have advanced lung cancer with certain molecular biomarkers may receive treatment with a targeted drug alone or in combination with chemotherapy. These treatments for lung cancer include:

Erlotinib (Tarceva and others). A targeted treatment called erlotinib has been shown to benefit some people with non-small cell lung cancer. This drug blocks a specific kind of receptor on the cell surface—the epidermal growth factor receptor (EGFR). Receptors such as EGFR act as doorways by allowing substances in that they can encourage a cancer cell to grow and spread. Lung cancer cells that have a mutation on the EGFR are likely to respond to treatment with erlotinib instead of chemotherapy. For patients who have received chemotherapy, and are in need of additional treatment, erlotinib can be used even without the presence of the mutation [21].

Afatinib (Gilotrif). In 2013, the FDA approved afatinib for the initial treatment of metastatic NSCLC in patients with the same EGRF gene mutations or deletions as those who can be treated successfully with erlotinib [22].

Gefitinib (Iressa). In 2015, the FDA approved gefitinib for the first-line treatment of patients with NSCLC whose tumors harbor specific types of EGFR gene mutations, as detected by an FDA-approved test [23].

Bevacizumab (Avastin). Just like normal tissues, tumors need a blood supply to survive. Blood vessels grow in several ways. One way is through the presence of a substance called vascular endothelial growth factor (VEGF). This substance stimulates blood vessels to penetrate tumors and supply oxygen, minerals, and other nutrients to feed the tumor. When tumors spread throughout the body, they release VEGF to create new blood vessels.

Bevacizumab works by stopping VEGF from stimulating the growth of new blood vessels. (Because normal tissues have an established blood supply, they are not affected by the drug.) When combined with chemotherapy, bevacizumab has been shown to improve survival in people with certain types of non-small lung cancer, such as adenocarcinoma and large cell carcinoma [24].

Crizotinib (Xalkori). A treatment that has shown benefits for people with advanced non–small cell lung cancer who have the ALK gene mutation. Crizotinib works by blocking ALK and stopping the growth of the tumor [25].

Ceritinib (Zykadia). This was approved in 2014 for people with metastatic ALK-positive lung cancer who cannot tolerate crizotinib or whose cancer continued to grow while being treated with crizotinib [26].

Because the genes of cancer cells can evolve, some tumors may become resistant to a targeted treatment. Medications to meet those challenges are being studied now in clinical trials, which often offer important treatment options for people with lung cancer.

2.3.3.5 Immunotherapy

Immunotherapy has recently emerged as a new treatment option for certain lung cancers. While any cancer treatment can cause side effects, immunotherapy is generally well-tolerated; this is in part due to its mechanism of action. Our immune system is constantly working to keep us healthy. It recognizes and fights against danger, such as infections, viruses, and growing cancer cells. In general terms, immunotherapy uses our own immune system as a treatment against cancer. In March 2015, the FDA approved the immunotherapy nivolumab (Opdivo) for the treatment of metastatic squamous NSCLC which was unsuccessfully treated with chemotherapy. Nivolumab works by interfering with a molecular “brake” known as PD-1 that prevents the body’s immune system from attacking tumors. In 2016, the FDA approved a new immunotherapy called pembrolizumab (Keytruda) for the treatment of advanced NSCLC as an initial therapy. Its therapeutic activity is similar to that of nivolumab. Patients are tested for a protein known as PDL-1 and if a sufficient quantity is identified they may qualify for this treatment. Additional approaches to immunotherapy for lung cancer have shown promise in early clinical trials and are now in late-phase development. Treatments for NSCLC have advanced the furthest; however, a number of new immune-based treatments for SCLC are also in clinical development. These treatments fall into four main categories:

- **Monoclonal antibodies** are lab-generated molecules that target specific tumor antigens (a substance that the immune system sees as being foreign or dangerous).
- **Checkpoint inhibitors** target molecules that serve as checks and balances in the regulation of immune responses.
- **Therapeutic vaccines** target shared or tumor-specific antigens.
- **Adoptive T-cell transfer** is an approach in which T-cells (a type of white blood cell) are removed from the patient, genetically modified or treated with chemicals to enhance their activity, and re-introduced into the patient with the goal of improving the immune system's anticancer response [27, 28].

Advantages of Radiation, Chemotherapy, Targeted therapy and Immunotherapy

- Ability to kill many cancer cells throughout the entire body (including cancer cells in the main tumor, and other tumors in the body)
- Work together with radiation therapy (i.e. can kill more cells together than either therapy could do alone
- Ability to kill microscopic disease at the edge of the main tumor that may not be seen by the naked eye of a surgeon (thereby decreasing the chance that therecancer cells will be left behind at the time of surgery)
- Tailoring of the systemic treatment for each patient (e.g. specific hormonal therapies for breast cancers; targeted therapies for lung cancers), a backbone of personalized medicine
- Preservation of an organ (e.g. not removing a breast, larynx, or part of the gastrointestinal tract, which would have significant negative impact on a patients quality of life)

Disadvantages of Radiation, Chemotherapy, Targeted therapy and Immunotherapy

- Inability to kill a tumor alone (in most cases, systemic therapy must be used with either surgery or radiation therapy)
- Inability to deliver systemic therapy if the patient is on certain medications (e.g. blood thinners), or around the time of surgery, or has certain medical conditions (e.g. kidney failure, liver failure, heart disease)
- Systemic toxicities (since the therapies go through the entire body and may affect all normal tissues) The side-effects are therapy-dependent and can be different for different drugs
- Inability of the systemic therapy to get to the tumor (e.g. crossing the blood-brain barrier; going to a limb where there is poor circulation)

- Relatively uneven killing of cancer cells in tumors (akin to having hundreds of beach balls and randomly popping half of them — one does not know where the remaining beach balls [the living cancer cells] are still located;
- Relative inconvenience of systemic therapy (e.g. some forms of chemotherapy must be delivered daily, 5 days per week, for weeks; or they must be taken orally for years) [29, 30].

2.3.4 Novel drug delivery systems for the treatment of NSCLC

Several novel approaches are being developed or in developing stage to counter the side effects of current available treatment options. Novel drug delivery systems have emerged which are enlisted below. Figure 2.9 shows the assembly of novel drug delivery systems.

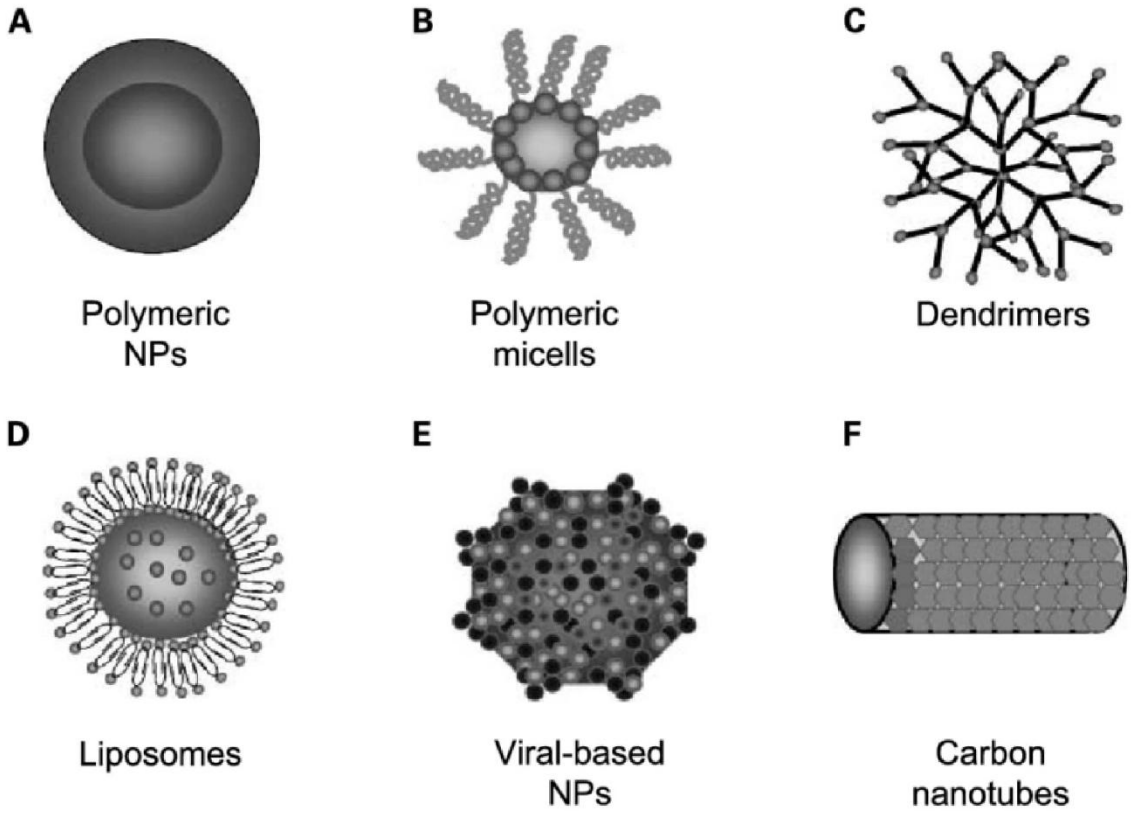


Figure 2.9: Graphical representation of novel drug delivery systems; A) Polymeric nanoparticles, B) Polymeric micelles, C) Dendrimers, D) Liposomes, E) Viral-based nanoparticles, F) Carbon nanotubes [31].

2.3.4.1 Polymeric Nanoparticles

Polymeric nanoparticles can be more bounded for two major types; nanocapsules and nanospheres. Nanocapsules are acting as a drug reservoirs, due to their vesicular structure, in which the retained active pharmaceutical ingredients are reserved in an aqueous or nonaqueous liquid core placed in the vesicle cavity and enclosed by the solidified polymeric shell. On the

other hand, nanospheres can be described as a solid/mass of matrix polymers. In other words, any nanosphere may portrayed as an entire polymeric spherical mass in which, as result, drug molecules may be trapped within the sphere centre or adsorbed at the mass surface [32].

Advantages

- Controlled and sustained release of the drug during transportation and at the site of localisation, altering the organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- Decreased toxicity and occurrence of adverse drug reactions.
- Better drug utilisation.
- Site-specific targeting can be achieved by attaching targeting ligands to the surface of the particles or through use of magnetic guidance.
- The system can be used for various routes of administration, including oral, nasal, parenteral, intra-ocular, etc.

Disadvantages

- Particle-particle aggregation makes physical handling of nanoparticles difficult in liquid and dry form [33].

2.3.4.2 Polymeric micelles

Polymeric micelles are core-shell structures synthesized form amphiphilic block copolymers. Various conventional characteristics of these polymeric micelles such as increasing the solubilization of poorly hydrophobic drugs, exhibiting sustained release profile, more importantly, protecting the encapsulated cargo from degradation by various enzymes and even metabolism make them favorable candidates for drug delivery purposes. These micelles are formed when the concentration of the polymer in solution exceeds a certain threshold concentration known as the Critical Micellar Concentration (CMC) and above a certain threshold temperature known as the Critical Micellar temperature (CMT) [34, 35].

Advantages

- High entrapment of Lipophilic drug molecules.
- Easy and precise control of micelle size.
- Sustained drug release.
- Bypass reticuloendothelial system.

Disadvantages

- Premature drug release and drug leakage.
- Failure in releasing drug at desired site.
- Failure to cross biological membranes.
- Micellar toxicities [36].

2.3.4.3 Dendrimers

Dendrimers are globular, nano-sized (1-100 nm) macromolecules with a particular architecture constituted of three distinct domains: i) a core at the center of dendrimer consisting of an atom or a molecule having at least two identical chemical functions; ii) branches, emanating from the core, constituted of repeat units having at least one branch junction, whose repetition is organized in a geometrical progression that results in a series of radially concentric layers called generations; and iii) many terminal functional groups, generally located at the surface of dendritic architecture. These surface groups are vital in determining the properties of dendritic macromolecules.

Advantages

- Monodispersity i.e. Uniform size distribution.
- Electrostatic properties of dendrimers are beneficial for crossing biological membranes.

Disadvantages

- Lengthy process of synthesis.
- Haematological toxicities.
- Polyvalency of dendrimers leads to high degree binding with plasma proteins.
- Non-specific interaction with cells and cellular components [37].

2.3.4.4 Liposomes

Confronted with excess water, phospholipids and other polar amphiphiles form closed concentric bilayer membranes, entrapping water and dissolved solutes (e.g. drugs) in the process. Lipid-soluble or lipid-bound drugs can also be accommodated into liposomal membranes by a variety of techniques, for instance by mixing such drugs with the lipids prior to forming liposomes. A wide array of phospholipids and other lipids (e.g. non-ionic surfactants), as well as lipids extracted from biological membranes, can be used to prepare liposomes or other lipid-based vesicles. Liposomal membranes can attain various degrees of fluidity depending upon phospholipids gel-liquid crystalline transition temperature (Tc).

Advantages

- Improvement and control over pharmacokinetics and pharmacodynamics.
- Decreased toxicity.
- Enhanced activity of drugs against intracellular pathogens.
- Liposomes can be made to be target selective.
- Enhanced activity against extracellular pathogens.

Disadvantages

- High cost of raw material.
- Short shelf life and stability.
- Low encapsulation of hydrophilic drug molecules.
- Premature clearance from systemic circulation [38].

2.3.4.5 Viral Nanoparticles

Viral nanoparticles are genetically encoded and self-assemble into discrete and monodisperse structures of precise shape and size. For many virus-based systems, the structures are known to atomic resolution and can be tailored at the atomic level. This level of quality control and structural engineering cannot yet be achieved with synthetic nanoparticles. Viruses have naturally evolved to deliver cargoes to specific cells and tissues a property that we as biomedical engineers, materials scientists, and chemists seek to mimic. Viruses can be tailored for desired applications using at least three approaches: (i) bioconjugate chemistries can be applied to link contrast agents, drugs, or targeting ligands to the exterior or interior capsid shell; (ii) disand reassembly protocols facilitate the encapsulation of artificial cargoes, i.e., drugs or contrast agents; (iii) genetic engineering allows the introduction of precise and reproducible modifications so that large quantities of identical particles can be manufactured, displaying targeting ligands or unique ligation handles for further modification through bioconjugation [39].

Advantages

- Enters cells efficiently.
- Viral genes absent

Disadvantages

- Hard to produce
- Induces immune response [40].

2.3.4.6 Carbon Nanotubes

Carbon nanotubes (CNTs) are essentially cylindrical molecules made of carbon atoms. CNTs are graphene sheets rolled into a seamless cylinder that can be open ended or capped, having a high aspect ratio with diameters as small as 1 nm and a length of several micrometers. CNTs made from a single graphene sheet results in a single-walled nanotubes (SWNT) while several graphene sheets make up multiwalled carbon nanotubes (MWNTs).

Advantages

- High stability in vivo because of their mechanical properties.
- Large surface area available for multiple functionalization.
- Capacity to easily pass biological barriers leading to novel biocompatible delivery systems.
- Empty internal space for encapsulation and transport of therapeutic and imaging molecules.
- Bulk production associated to low costs.

Disadvantages

- Non-biodegradable.
- Large surface area for protein opsonisation.
- Insolubility of as-produced materials – functionalisation is required for rendering the material compatible in physiological conditions.
- Strong tendency to aggregate.
- Toxicity and bioaccumulation.
- Extremely high variety of carbon nanotube types – standardization difficult [41].

The above mentioned novel formulation can be targeted specifically to desired site or organ. The targeting can be done by two ways; i) Passive Targeting, ii) Active targeting.

2.3.5 Passive Targeting

The Enhanced Permeability and Retention Effect (EPR), in murine solid tumors discovered that when polymer-drug conjugates were administered intravenously, 10-100 fold higher concentrations could be achieved in the tumor due to the well-noted EPR effect as compared to free drug administration. The permeability of the compromised vasculature and retention can lead to the accumulation of even macromolecules thus increasing their tumor concentration by 70-fold. The foremost advantage in treating cancer with advanced, non-solution based therapies is this very inherent leaky vasculature present in the pathologically compromised cancerous tissues. This leaky and defective vascular architecture created due to the rapid vascularization which is a vital cog to enrich the ever-growing malignant tumors, coupled with

poor lymphatic drainage allows the famous EPR effect. Various important factors such as circulation time, targeting and the capability to overcome barriers are heavily reliant on the shape, size and the surface area of these particles. Conventionally, a particle must be at least 10 nm in diameter to avoid clearance by first pass renal filtration. Passive targeting is largely possible through diffusion-mediated transport, which makes size a critically important factor. The optimal size range of 40-200 nm will ensure longer circulation time, increased accumulation within the tumor mass and lower renal clearance. Like particle size, particle shape also governs largely the route through which nanoparticles can be taken up within the tumor.

Surface characteristics also play a very important role in determining the extent of internalization of these nanoparticles into cells. Relatively, the surface can be modified by the polymer composition, thus governing an extra amount of hydrophobicity or hydrophilicity to these particles. Surface modification of these polymers by addition of Polyethylene Glycol (PEG) has been known to protect the nano-systems from opsonization and subsequent clearance by the Reticulo-endothelial (RES) system. Furthermore, increasing the molecular weight of PEG chains will also increase the circulation time of these nanoparticles. Particularly for negatively charged nanoparticles, this PEG shield will confer more protection and thus prevent immediate clearance of these particles. Passive targeting, thus can be regulated by modifying the size, shape or in some cases, the surface dimensions of these nanoparticles [42, 43].

2.3.6 Active Targeting

Active targeting employs some kind of strong interaction such as ligand-receptor or other molecular recognition to confer more specificity to the delivery system. Eventually, it reduces the unwanted non-specific interactions and localization of the drug in peripheral tissues. Active targeting takes advantage of over-expression of certain receptors such as folate on the tumor cell surface. Novel drug delivery systems can be tweaked with their surface chemistries to confer more specificity. Conventionally, targeted nanocarriers have an edge over their non-targeted counterparts by being more efficacious at the site of delivery and also reducing any potential undesirable toxicities [42, 43].

2.3.7 Long circulating of Novel carriers

PEGylation enhances the therapeutic efficacy of the drugs by bringing in several advantageous modifications over the non-PEGylated products. The systematic classification is illustrated in Figure 2.10. Increase in the serum half-life of the conjugate is the major way of enhancing

therapeutic potential of the PEGylated conjugate. PEGylation prolongs the circulation time of conjugated therapeutics by increasing its hydrophilicity and reducing the rate of glomerular filtration [44, 45].

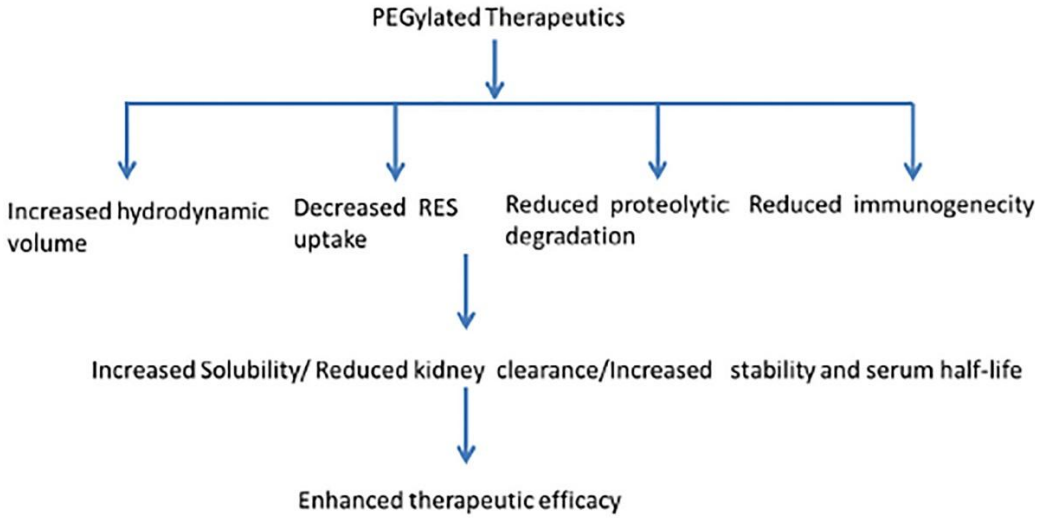


Figure 2.10: Advantages of PEGylation of a novel drug delivery system [46].

Few factors such as protection from reticuloendothelial cells, proteolytic enzymes and decreased formation of neutralizing antibodies against the protein by masking antigenic sites by formation of a protective hydrophilic shield are the key components of PEG molecule that attributes to the improved pharmacokinetic profile (PK) of the conjugates [47-50]. It has also been reported that PEGylation increases the absorption half-life of subcutaneously administered agents and is associated with a decreased volume of distribution [44]. PEG is a non-biodegradable polymer that puts limits on its use. It has been shown that PEGs (up to molecular weight 20 kDa) is primarily excreted through the renal system, whereas higher molecular weight PEG chains get eliminated by fecal excretion [51]. PEGylation proved to be the most promising approach for increasing the serum half-life of the conjugated therapeutics, which is related to enhancement of efficacy of the conjugate [52].

2.3.8 Previous work done on NSCLC drugs

2.3.8.1 Docetaxel

Bowerman et al. reported fabrication of Docetaxel loaded PLGA nanoparticles. Pharmacokinetic study revealed that nanoparticles showed increased area under the concentration (AUC) curve and circulation half-life ($T_{1/2}$). However, dose was needed to be doubled for nanoparticle formulation when compared with standard drug solution [53].

Zhang et al. reported preparation of Docetaxel loaded liposomes. Entrapment efficiency achieved was 36.4% and particle size was 277 nm [54].

Raza et al. reported preparation of docetaxel conjugation with multiwalled carbon nanotubes. Conjugation process comprised of using concentrated sulphuric acid and nitric acid. Drug loading was 62.8%. However, particle size achieved was in the range of 461.5 nm to 282.4 nm [55].

2.3.8.2 Gemcitabine

Joshi et al. reported preparation of Gemcitabine loaded PLGA nanoparticles. Entrapment efficiency was 56.48%. Preparation of nanoparticles was carried out in acidic environment at pH 3.5 [56].

Bornmann et al. reported preparation of Gemcitabine loaded liposomes. Mean particle size obtained was 36 nm with 47% entrapment of drug [57].

Das et al. reported preparation of carbon nanotubes loaded with Gemcitabine. Average particle size obtained was 188.7 nm with 41.7% entrapment efficiency [58].

Soni et al. reported preparation of 4.0G poly(propyleneimine) (PPI) dendrimers loaded with Gemcitabine. Entrapment efficiency was found to be 37.2%. Formulation exhibited rapid release of drug [59].

2.3.8.3 Paclitaxel

Hu et al. prepared Paclitaxel loaded polymeric nanoparticles. Particle size obtained was 168 nm and 96.83% entrapment efficiency [60].

Yang et al. reported preparation of Liposomes loaded with Paclitaxel. Liposomes were prepared with thin film hydration method. Hydration media used was comprised of Tween 80 surfactant [61].

Zhang et al. prepared paclitaxel loaded polymeric micelles from poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCL-PEG-PCL, PCEC) copolymers. Formulation exhibited particle size of 93 nm and 94.36% of entrapment efficiency [62].

Liu et al. reported the preparation of Paclitaxel loaded single-walled carbon nanotube. Size of carbon nanotube was found to be 132.2 nm [63].

2.3.8.4 Vinorelbine

Cryer et al. reported the preparation of polymeric nanoparticles encapsulating Vinorelbine. Particle size attained was 215 nm with 89.76% of encapsulation efficiency [64].

Zhigaltsev et al. reported preparation of Vinorelbine loaded liposomes. The drug loading process in liposomes was lengthy. Also, high lipid-drug ratio was used [65].

Lu et al. reported Vinorelbine loaded polymeric micelles. Particle size obtained was 17.2 nm with 99.8% encapsulation efficiency [66].

2.3.8.5 Pemetrexed

Eldin et al. reported preparation of Pemetrexed loaded liposomes. Size of liposomes obtained was in the range of 119-152 nm with encapsulation efficiency of 9.9-14.8% [67].

2.4 Spherulites: A Novel vesicular drug Delivery System for Tumour Targeting

Spherulites have been discovered during academic research at a CNRS (Centre National de la Recherche Scientifique) laboratory working on physics of liquid-crystal. Spherulites are multilamellar microvesicles (from 0.1 to 10 μm), with an internal structure of concentric spherical bilayers made of water and amphiphile, created by the controlled shearing of liquid-crystalline phases. Because the initial phase is a lamellar liquid-crystalline equilibrium, the structure resulting is uniform throughout the sample. This results in a high stability of the vesicles and acceptable reproducibility of process, at industrial scale.

2.4.1 Characteristics of Spherulites

Due to their unique structural properties and mode of manufacture, Spherulites are exceptionally suited to numerous applications: in protection, prolongation, enhancement of bioavailability, administration through alternate route, or vectorization of active substances.

The main asset of the Spherulites are:

- High stability, and protection of the incorporated molecule against enzymatic degradation.
- Ability to incorporate both hydrophilic and lipophilic active molecules with high encapsulation yield.
- Manufacture without use of organic solvents and with little stress (pressure, shear, temperature) allowing the encapsulation of fragile molecules like proteins.

For pharmaceutical applications Spherulites® are manufactured starting from already approved components, therefore limiting the toxicological issues. The final dosage can be either liquid (a dispersion of Spherulites in aqueous medium) or solid, after freeze-drying, spray-drying and other solid dosage manufacturing process [68].

2.4.2 Preparation of Spherulites

A lamellar phase can be defined as a structure comprising of alternating layers of surfactant and water [69]. This structure is highly advantageous because of its partial organization as a crystal while maintaining some degree of fluidity like a liquid. Lamellar phase is sensitive to mechanical inputs, such as shearing, mixing or stirring. Application of shearing stress is highly efficient in modifying the structure of lipidic lamellar phase. Thus, the onion-like structure results by controlled shearing of lyotropic lamellar phase. The three consecutive steps to prepare spherulites are as follows: i). Forming a lamellar phase, ii). Application of shearing stress to the lamellar phase and iii). Dispersion of sheared lamellar phase [70, 71].

2.4.3 Previous work done on Spherulites

Freund reported work performed on Spherulites. Spherulites were entrapped with radio coupled protein to assess the potential of formulation for its gastrointestinal delivery and biodistribution. Formulation was administered orally and intravenously. Spherulites were composed of amphiphillic lipid Soyabean Phosphatidylcholine, cholesterol and a non-ionic surfactant Polyoxyethylene alcohol. Formulation was prepared by controlled shearing of mixture of surfactant along with the radio coupled protein. Spherulites exhibited mean hydrodynamic diameter of 300 nm. The purpose of the study was to explore the biodistribution of spherulites along with their potential to protect the encapsulated active in *in vivo* [72].

Zhang et al. reported formulation of spherulites. The purpose of the work was to explore the potential of spherulites to encapsulate both low and high molecular weight actives. Model drugs Calcein as low molecular weight agent and FITC-labeled albumin as high molecular weight agent were used. Formulation was composed of Soyabean Phosphatidylcholine, cholesterol, Tween 80 or Brij S10. Spherulites were prepared by dissolving the lipid phase in mixture of ethanol and chloroform, followed by solvent evaporation. Dried lipid phase was then hydrated using buffer, subsequently, moderate shear was applied. Obtained spherulites exhibited size of 170 ~ 290 nm and encapsulation efficiency of 55 ~ 60% [73].

Dhanikula et al. reported spherulites prepared for the purpose of drug detoxification. Spherulites were composed of Soyabean Phosphatidylcholine, cholesterol and glycerol. Spherulites were prepared by dissolving lipid phase in ethanol followed by controlled shear [74].

Freund et al. reported preparation spherulites for exploring their stability profile *in vitro* and *in vivo*. Spherulites were composed of amphiphillic lipid Soyabean Phosphatidylcholine,

cholesterol and a non-ionic surfactant Polyoxyethylene alcohol. Lipid phase in presence of aqueous media was subjected to controlled shear. Spherulites exhibited size of 300±20 nm [75].

Crauste-Manciet et al. reported preparation of spherulites. Spherulites were composed in Egg lecithin and Tween 80. Formulation was prepared by manual hand shearing of lipid phase. Spherulites obtained exhibited size of 0.2-10 µm [76].

Redkar et al. reported preparation of spherulites. Spherulites were composed of PEG-8 Distearate. Lipid phase in presence of aqueous media was subjected to controlled shearing. Sumatriptan succinate was encapsulated in spherulites. Spherulites exhibited size of 10 µm [77].

As literature suggest that Spherulites are remained to explore for their drug delivery potential. In the present work we studied Spherulites by encapsulating anticancer actives and characterized them *in vitro* and *in vivo*.

2.5 Drug Profile

2.5.1 Gemcitabine Hydrochloride

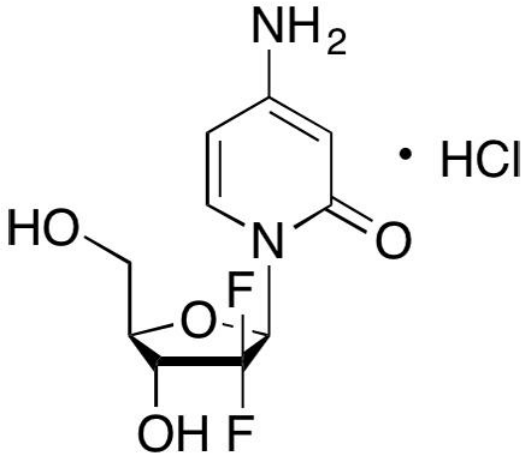
Category: Antineoplastic agent

CAS Number: 95058-81-4

Molecular formula: $C_9H_{12}ClF_2N_3O_4$

Molecular Weight: 299.659 g/mol

Structural Formula:



Chemcial name: 4-amino-1-((2R,4R,5R)-3,3–Difluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)pyrimidin-2(1H)-one.

Physicochemical Properties: Gemcitabine hydrochloride is a white to off-white crystalline powder. It is soluble in DMSO at 12.5 mg/mL with slight warming; very poorly soluble in ethanol; soluble in water at 25 mg/mL with slight warming; buffers, serum, or other additives may increase or decrease the aqueous solubility. The melting point of Gemcitabine hydrochloride is 258-278°C.

2.5.1.1 Mechanism of Action

Gemcitabine Hydrochloride is the hydrochloride salt of an analogue of the antimetabolite nucleoside deoxycytidine with antineoplastic activity. Gemcitabine is converted intracellularly to the active metabolites difluorodeoxycytidine di- and triphosphate (dFdCDP, dFdCTP). dFdCDP inhibits ribonucleotide reductase, thereby decreasing the deoxynucleotide pool available for DNA synthesis; dFdCTP is incorporated into DNA, resulting in DNA strand termination and apoptosis [78, 79].

2.5.1.2 Pharmacokinetics

Distribution

Gemcitabine hydrochloride gets widely distributed into tissues, also present in ascetic fluid. Volume of distribution for IV infusion < 70 min: 50 L/m^2 and for IV infusion 70-285 min: 370 L/m^2. About 10% drug gets plasma protein bound.

Metabolism

Gemcitabine hydrochloride gets metabolized intracellularly by nucleoside kinases to active metabolites gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP); also rapidly deaminated in the blood, liver, kidneys and other tissues. It undergoes deamination via cytidine deaminase to an inactive uracil metabolite (dFdU).

Excretion

Gemcitabine hydrochloride is mainly excreted through urine. 92-98% over one week (89% as dFdU, < 10% as gemcitabine) after a single dose of 1000 mg/m^2 given over 30 minutes. Terminal half life is IV infusion <70 min: 0.7-1.6 h; IV infusion 70-285 min: 4.1-10.6 h. Clearance is gender specific IV infusion < 70 min: 41-92 $L/h/m^2$ (male) and 31-69 $L/h/m^2$ (female).

2.5.1.3 Indications and Usage

Gemcitabine hydrochloride is used to treat certain types of cancer (including breast, lung, ovarian, pancreatic). It is a chemotherapy drug that works by slowing or stopping the growth of cancer cells. It is administered as intravenous infusion for 30 minutes. The dosage is based

on patient's medical condition, body size, and response to treatment. Gemcitabine hydrochloride is available in 200 mg, 1000 mg, and 2000 mg vials manufactured by Hospira Healthcare Corporation, Accord Healthcare Inc., Eli Lilly, Novopharm Limited, and Sandoz. [80]

2.5.1.4 Adverse reactions

Pale skin, easy bruising or bleeding, unusual weakness;

- Urinating less than usual or not at all;
- Nausea, upper stomach pain, itching, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes);
- Chest pain or heavy feeling, pain spreading to the arm or shoulder, nausea, sweating, general ill feeling;
- Sudden numbness or weakness, especially on one side of the body;
- Sudden severe headache, confusion, problems with vision, speech, or balance;
- Fever, chills, body aches, flu symptoms;
- White patches or sores inside your mouth or on your lips;
- Pain, swelling, or skin changes where the needle was placed;
- Hearing problems;
- Blood in urine; or
- Breathing problems.

Less serious side effects may include:

- Mild nausea, vomiting, upset stomach;
- Diarrhea or constipation;
- Swelling in your hands, ankles, or feet;
- Skin rash;
- Numbness or tingly feeling;
- Drowsiness; or
- Hair loss [81].

2.5.1.5 Problems associated with Gemcitabine hydrochloride:

- Major limit for the use of gemcitabine is represented by its rapid metabolic inactivation (deamination operated by deoxicytidine deaminase) responsible for its short half-life together with its low but still important systemic toxicity.
- The half-life and volume of distribution depends on age, gender and duration for infusion.

- The development of multidrug resistance in cells exposed to gemcitabine can limit its effectiveness. Gemcitabine HCl is efflux by the Pgp (P glycol protein) and resistance is observed by MDR gene (Multi drug resistance gene).
- High Dose of Gemcitabine Hydrochloride 1000 to 1200 mg/m^2 [82].

2.5.2 Vinorelbine Tartrate

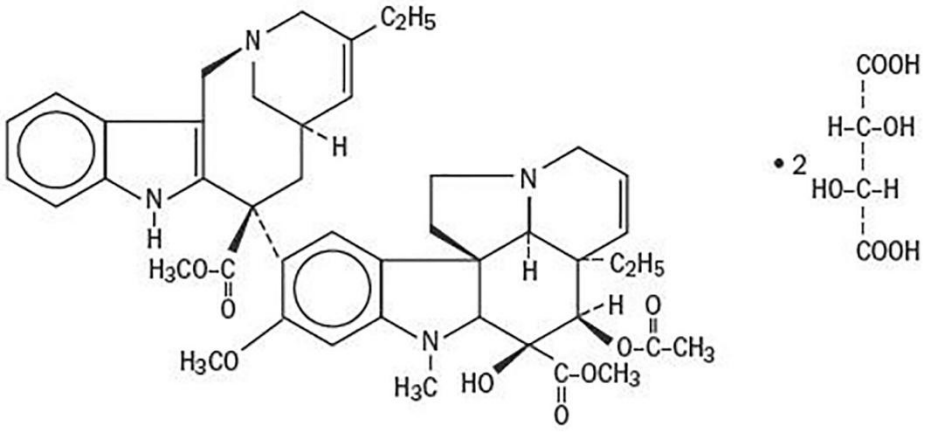
Category: Antineoplastic agent

CAS Number: 125317-39-7

Molecular formula: $C_{53}H_{66}N_4O_{20}$

Molecular Weight: 1079.119 g/mol

Structural Formula:



Chemcial name: Methyl(3aR,4R,5S,5aR,10bR,13aR)-4-(acetyloxy)-3a-ethyl-9 [(6R, 8S)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2H azacyclo decino[4,3-b] indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a octa hydro-1H indolizino [8,1-cd]carbazole-5-carboxylate dihydrogen bis [(2R,3R)-2,3- dihydroxy butanedioate].

Physicochemical Properties: Vinorelbine tartrate is a white to off white amorphous powder which is very hygroscopic. Vinorelbine tartrate is freely soluble in water and partially soluble in Methanol. It is insoluble in aprotic solvents. The melting point of Vinorelbine Tartrate is 208-222°C.

2.5.2.1 Mechanism of Action:

Vinorelbine Tartrate is the ditartrate salt of a semisynthetic vinca alkaloid derived from the leaves of the periwinkle plant (Vinca rosea) with antineoplastic properties. Vinorelbine binds to tubulin, thereby inhibiting tubulin polymerization into microtubules and spindle formation and resulting in apoptosis of susceptible cancer cells. Inhibition of mitotic microtubules

correlates with antitumor activity, whereas inhibition of axonal microtubules seems to correlate with vinorelbine's neurotoxicity. Compared to related vinca alkaloids, vinorelbine is more selective against mitotic than axonal microtubules in vitro, which may account for its decreased neurotoxicity. This agent is also a radiation-sensitizing agent [83].

2.5.2.2 Pharmacokinetics

Distribution

Vinorelbine exhibits high steady-state volume of distribution (VSS) ranging from 25.4 to 40.1 L/kg. It has high binding affinity towards human platelets and lymphocytes, which in cancer patients ranges from79.6% to 91.2%.

Metabolism

Vinorelbine undergoes extensive hepatic elimination, with large amounts excreted in feces. The metabolism results in formation of two metabolites vinorelbine N-oxide and deacetylvinorelbine, which have been identified in human blood, plasma, and urine. Deacetylvinorelbine is the primary metabolite of vinorelbine, and possesses similar antitumor activity to that of vinorelbine. The metabolism of vinorelbine is mediated by hepatic cytochrome P450 isoenzymes in the CYP3A subfamily.

Excretion

After intravenous administration of radioactive vinorelbine, approximately 18% and 46% of administered radioactivity was recovered in urine and feces, respectively. In a different study, 10.9% ± 0.7% of a 30-mg/m² intravenous dose was excreted as parent drug in urine.

2.5.2.3 Indications and Usage

Vinorelbine tartrate is indicated as a single agent, for the treatment of patients with metastatic NSCLC. It is also administered along with cisplatin for first-line treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). Vinorelbine tartrate is supplied in 1 mL (10 mg/1 mL) and 5 mL (50 mg/5 mL) by Pierre Fabre Pharmaceuticals, Inc. [84].

2.5.2.4 Adverse reactions

- Myelosuppression
- Pulmonary Toxicity and Respiratory Failure
- Constipation and Bowel Obstruction
- Extravasation Tissue Injury

- Neurologic Toxicity
- Hepatic Toxicity

2.5.2.5 Problems associated with Vinorelbine Tartrate

Long half-life of Vinorelbine tartrate enables it to remain in systemic circulation for extended period of time. It is responsible for increased side effects where healthy tissues get affected due to its non-specific biodistribution. Vinorelbine tartrate has high degree of plasma protein binding of 80-90% [85].

2.6 Excipient Profile

2.6.1 Soyabean Phosphatidylcholine

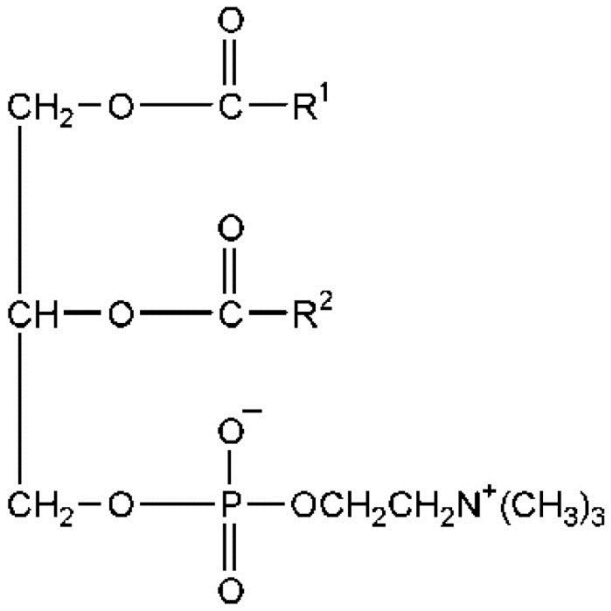
Category: Emollient; emulsifying agent; solubilizing agent.

CAS Number: 8002-43-5

Molecular formula: $C_{35}H_{66}NO_7P$

Molecular Weight: 643.887 g/mol

Structural Formula:



Storage instructions:

Soyabean Phosphatidylcholine decompose at extreme pH. They are also hygroscopic and subject to microbial degradation. Store in a cool, dry, well-ventilated area. Keep container tightly closed. Store at - 20 °C.

Handling:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Soyabean Phosphatidylcholine may be irritant to the eyes; eye protection and gloves are recommended.

Soyabean Phosphatidylcholine is Faint Yellow to Yellow to Brown powder. It comes under Generally Regarded As Safe excipient approved by USFDA.

2.6.2 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-2000] (DSPE PEG 2000)

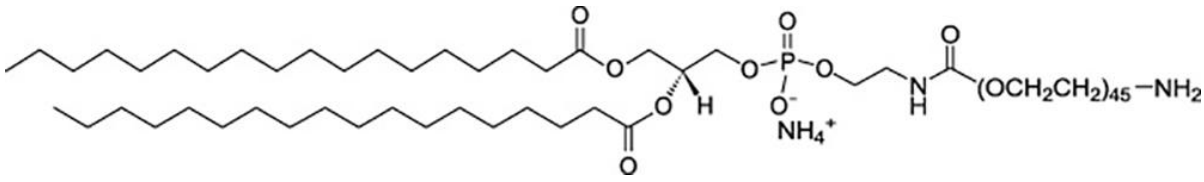
Category: PEGylating agent

CAS Number: 474922-26-4, 159156-98-6

Molecular formula: $C_{132}H_{266}N_3O_{54}P$

Molecular Weight: 2790.48 g/mol

Structural Formula:



Storage instructions:

Store in a cool, dry, well-ventilated area. Keep container tightly closed. Freeze. Keep cold. Store at - 20 °C.

Handling:

Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use with adequate ventilation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation.

DSPE PEG 2000 is White to off white powder which may contain lumps.

2.6.3 Cholesterol

Category: Organic alcohol present in body

CAS Number: 57-88-5

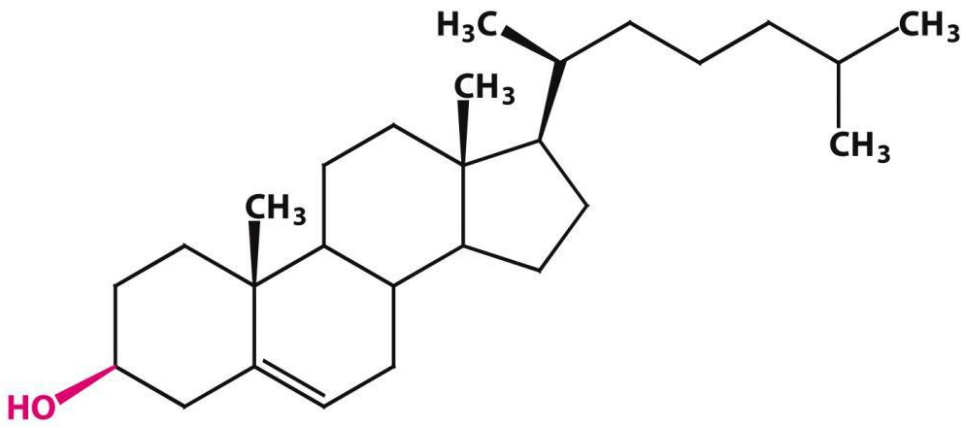
Empirical Formula: C27H46O

Molecular Weight: 386.65 g/mol

Chemical Name: (3β)-cholest-5-en-3-ol

Melting point: 148 °C

Structure:



It is a major component of all biological membranes; ~25% of total brain lipid is cholesterol. Cholesterol is a lipid that makes up about 20-25% of the structural components of the cell membranes. It determines the fluidity and permeability of the membrane, making it permeable to water but not to ions and protons. Cholesterol also regulates the functions of the transporters and signaling proteins present on the plasma membrane. The major sites of cholesterol synthesis are small intestine and liver.

Storage instructions: Store at -20°C. Store under Desiccating conditions. The product can be stored for up to 12 months. It should be stored in a well-closed container, protected from light.

Handling: Observe normal precautions appropriate to the circumstances and quantity of material handled. Rubber or plastic gloves, eye protection, and a respirator are recommended. May be harmful following inhalation or ingestion of large quantities, or over prolonged periods of time, owing to the possible involvement of cholesterol in atherosclerosis and gallstones. May be irritant to the eyes. When heated to decomposition, cholesterol emits acrid smoke and irritating fumes.

It comes under Generally Regarded As Safe excipient. It approved by USFDA for in vivo use. Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan color. It is freely soluble in Isopropyl myristate, Ether, Methanol, Benzene, Acetone, Ethanol, Chloroform and Hexane.

2.6.4 Potassium oleate

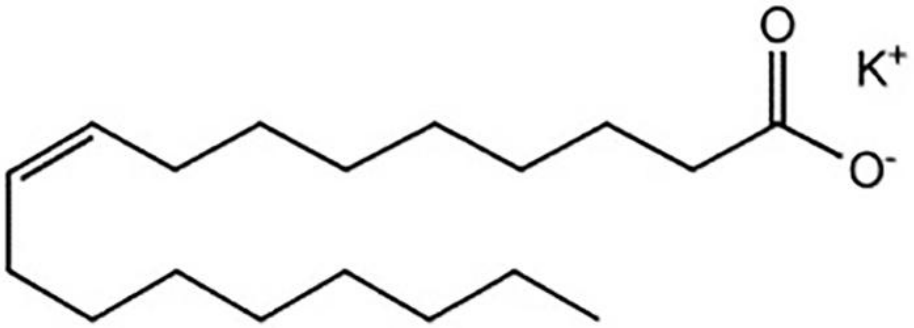
Category: Emulsifying agent, emollient

CAS Number: 143-18-0

Molecular formula: $C_{18}H_{33}KO_2$

Molecular Weight: 320.55 g/mol

Structural Formula:



Storage instructions:

Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Handling:

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.

Potassium oleate is Beige powder in appearance. It comes under Generally Regarded As Safe excipient approved by USFDA.

2.6.5 Mannitol

Category: Diluent; diluent for lyphilized preparations; sweetening agent;

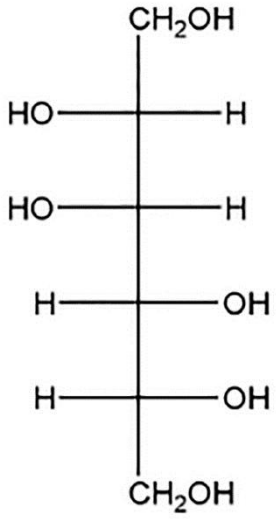
tablet and capsule diluent; tonicity agent.

CAS Number: 69-65-8

Molecular formula: $C_6H_{14}O_6$

Molecular Weight: 182.17 g/mol

Structural Formula:



Storage instructions:

Mannitol is stable in the dry state and in aqueous solutions. Solutions may be sterilized by filtration or by autoclaving and if necessary may be autoclaved repeatedly with no adverse physical or chemical effects.(28) In solution, mannitol is not attacked by cold, dilute acids or alkalis, nor by atmospheric oxygen in the absence of catalysts. Mannitol does not undergo Maillard reactions. The bulk material should be stored in a well-closed container in a cool, dry place.

Handling:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Mannitol may be irritant to the eyes; eye protection is recommended.

Mannitol occurs as a white, odorless, crystalline powder, or free-flowing granules. It has a sweet taste, approximately as sweet as glucose and half as sweet as sucrose, and imparts a cooling sensation in the mouth. It comes under Generally Regarded As Safe excipient approved by USFDA.

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