# Chapter 1

# INTRODUCTION

## 1.1 Introduction

Cancer refers to a group of diseases which may affect any one or more number of organs in the body. Malignant tumours and neoplasm are the other medical terms used for cancer. Cancerous cells has the tendency to multiply rapidly which can grow beyond their usual boundaries, moreover, it can invade the surrounding tissue or organ and can spread throughout the body. This process is called as Metastasis [1].

Cancer is the principle reason of mortality in economically developed countries and the second leading cause of death in developing countries. The burden of cancer is increasing in economically developing countries as a result of population ageing and growth as well as, numerous factors enlisted below [2].

Key Facts of Cancer

- As per the statistics of 2015, 14 million new cases have been diagnosed with cancer and 8.8 million deaths worldwide occurred due to cancer.
- Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year.
- The most frequent types of cancer differ between men and women.
- About 30% of cancer deaths are due to the some behavioural and dietary risks.
- Consumption of tobacco leads to increased risk factor for developing cancer causing 22% of global cancer deaths and 71% of global lung cancer deaths [1].

Causes of Cancer

- Smoking
- Alcohol consumption
- Overweight
- Lack of Physical Activity
- Infection
- Sunlight (increased UV radiation)
- Occupational Exposure to cancerous agents
- Environmental Exposure [3].

**Pathophysiology of Lung Cancer:**

Lung cancer is a disease characterized by uncontrolled cell growth in tissues of the lung. If left untreated, this growth can spread beyond the lung in a process called metastasis into nearby tissue and, eventually, into other parts of the body. Most cancers that start in lung, known as primary lung cancers, are carcinomas that derive from epithelial cells. The main types of lung

cancer are *small cell lung carcinoma* (SCLC), also called oat cell cancer, and *non-small cell lung carcinoma* (NSCLC) [4].

**Symptoms of Lung Cancer**

The symptoms of lung cancer are persistent cough, coughing up blood, breathlessness, wheezing, hoarseness, chest or shoulder pain, tiredness and weight loss [5].

**Currently available treatment options of Lung Cancer:**

The class of drugs used for treatment of lung cancer are alkylating agents eg. Ifosfamide, antimetsbolites eg. Gemcitabine, antibiotic eg. Mitomycin, plant derived products eg. Vinblastine, miscellaneous agents eg. Cisplatin [6]

Gemcitabine and Vinorelbine are first line agents used in the treatment of lung cancer and both show good cytotoxicity against lung tumour.

Gemcitabine, a nucleoside analog related to cytarabine, is a novel pyrimidine antimetabolite that shows significant cytotoxicity against lung tumours [7].

As with fluorouracil and other analogues of pyrimidines, the triphosphate analogue of Gemcitabine replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process arrests tumour growth, as only one additional nucleoside can be attached to the "faulty" nucleoside, resulting in apoptosis.

Another target of Gemcitabine is the enzyme ribonucleotide reductase (RNR). The diphosphate analogue binds to RNR active site and inactivates the enzyme irreversibly. Once RNR is inhibited, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair, and cell apoptosis is induced [8].

Gemcitabine is marketed as “Gemzar” by Eli Lilly and Company. “Gemzar” is a white to off white solid lyophilized powder and it is supplied in vials of either 200 mg or 1 gm. The recommended dose of Gemzar is 1000 mg/m$^2$ intravenously over 30 minutes on Days 1, 8, and 15. It is formulated with mannitol and sodium acetate. Hydrochloric acid or sodium hydroxide may have been added for pH adjustment [9].

Our another drug of interest is Vinorelbine. Vinorelbine (5'nor-anhydro-vinblastin) is a semisynthetic vinca alkaloid that is manufactured from alkaloids extracted from the rosy periwinkle, Catharanthus roseus. Vinorelbine induces cytotoxicity by inhibiting the polymerisation of tubulin dimers into microtubules, which in turn disrupts mitotic spindle formation and prevent cell division. This promotes apoptosis in cancer cells [10].

Vinorelbine is marketed by Abbott Healthcare under the name Navelbine. Navelbine is available in vial containing vinorelbine tartarate equivalent to 10 mg (1 ml vial) or 50 mg (5 ml vial) and formulated in water for injection. Navelbine is administered intravenously at a

dose of 30 mg/m², once a week. It is given by intravenous infusion over a period of 6 to 10 minutes [11].

Both Gemcitabine hydrochloride (Vd: i.v. 50 L/m2) and Vinorelbine tartarate (Vd: i.v. 25.4-40.1 L/kg) due to their non-specific distribution throughout the body, are associated with serious side effects like anaemia, thrombocytopenia, cardiac arrhythmia, alopecia etc. [12, 13]. Development of a targeted drug delivery systems containing these drugs reduces its distribution to the other organs and tissues resulting into decrease in the side effects. Various nanocarrier based delivery systems for targeting these drugs to the lung tumor have been reported. However, Gemcitabine loaded nanocarriers are found to have limitations like failure to achieve desired vesicle size and also low drug entrapment efficiency in case of liposomes [14], low drug loading capacity and low stability of the formulation in aqueous media in case of polymeric micelles [15], in the dendrimers based formulation the selection of suitable vector is tedious process [16] and for carbon nanotubes it may show toxicity by bioaccumulation [17]. Vinorelbine has also been entrapped in nanocarriers but they are having some of the disadvantages such as from the liposomes the drug gets rapidly released thus it eliminates the benefits of liposomal encapsulation [18], the polymeric nanoparticles show slight decline in drug entrapment efficiency [19], dendrimers are having the risk of bioaccumulation [20]. Thus a tumor specific targeted drug carrier is required which eliminates these limitations of the presently available systems.

**Types of Targeted Drug Delivery Systems to Lung Tumour**

- Passive Tumour Targeting: Passive targeting is achieved via generalised Enhanced Permeation and Retention (EPR) effect. EPR effect is achieved by virtue of leaky vasculature of tumour. Novel carrier systems bearing anticancer drugs have no tumour selectivity in passive targeting. In passive targeting, drug delivery to the tumour occurs via EPR effect during blood circulation. Polymer carriers bearing physically entrapped or chemically conjugated drugs are strategy for passive tumour targeting.
- Active Tumour Targeting: Cancer cells often display increased cell surface expression of proteins that may be found at low levels on normal cells (tumour-associated antigens), as well as proteins that are found exclusively on cancer cell surfaces (tumour-specific antigens). Active drug targeting is usually achieved by chemical attachment to a targeting component that strongly interacts with antigens (or receptors) displayed on the target tissue, leading to preferential accumulation of the drug in the targeted organ, tissue, or cells [21].

**Types of Nanocarriers for drug delivery**

- Polymeric Nanoparticles: Drugs are conjugated to side chain of a linear polymer with a linker.
- Polymeric micelles: Amphiphillic block copolymers assemble and form a micelle with hydrophobic core and hydrophilic shell.
- Dendrimers: Radially emerging hyperbranched synthetic polymer with regular pattern and repeated units.
- Liposomes: Self assembling close colloidal structures composed of lipid bilayers.
- Viral Nanoparticles: molecular cages derived from the assembly of viral structural proteins.
- Carbon Nanotubes: Carbon cylinders composed of benzene ring [22].

**Advantages of Nanocarriers**

- Increased drug localization at tumour site.
- Protect drug from degradation and from premature clearance.
- Retain drug at target site for desired period of time.
- Facilitate cellular uptake and intracellular trafficking.
- Biocompatible and biodegradable [23].

**Disadvantages of Nanocarriers**

- Nanocarriers taken up by reticuloendothelial system (RES).
- Short circulation time.
- Failure to achieve desired particle size.
- Drug leakage before reaching targeted site [24].

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

Spherulites are obtained by shearing or grinding of lipidic lamellar phase and surfactants and are characterized by their concentric multilamellar internal structure. Multilamellar arrangement of spherulites is because of use of amphiphillic lipids. These lipids arrange themselves in alternating concentric bilayers in aqueous medium providing high yield in encapsulating the hydrophilic drugs. For drug targeting purpose spherulites must have the desired size range and long circulation characteristics [25].

Unlike liposomes, spherulites have the potential to encapsulate various types of lipophilic or hydrophilic agents. Moreover, their lamellar structure confer them a higher stability. Furthermore, spherulites have the ability to maintain the internal multilamellar structure while having an ideal size range which is required for efficient targeting to desired site or organ.

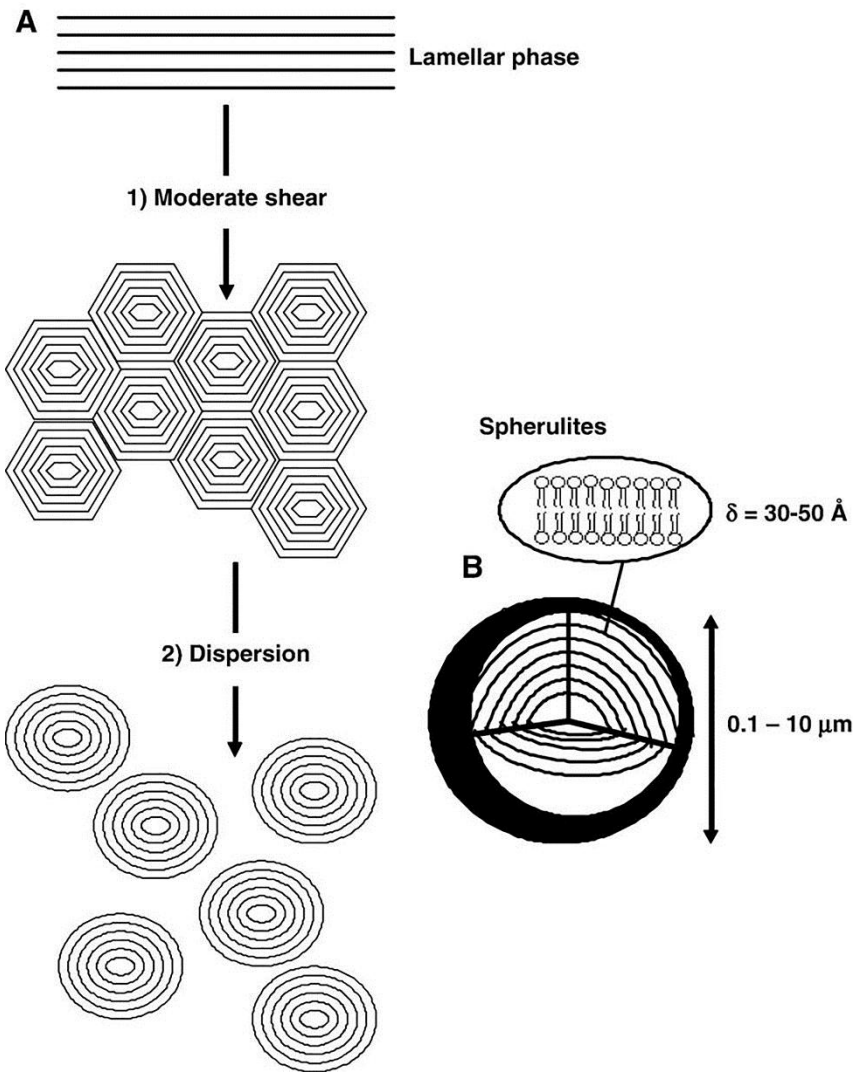


**Figure 1.1:** Graphical representation of Spherulites assembly [25].

Spherulites are also called as Lyotropic Lamellar Phases or Onion phases. Spherulites are made of surfactants, water and sometime an additional hydrophobic component (cosurfactant) [26].

**The important properties of Spherulites**

- High stability and protection of the incorporated molecule against enzymatic degradation.
- Ability to incorporate both hydrophilic and lipophillic 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.
- Have desired size in nanometric range which is required for the Enhanced Permeation and retention effect for drug targeting to the tumour [27].

### 1.2 Hypothesis

The drug loaded Spherulites are expected to overcome the limitations of existing nanocarrier formulations like instability, drug leakage during circulation and low entrapment efficiency. The drug loaded spherulites, by virtue of their nano size will be targeted to the tumor due to the Enhanced Permeation and retention (EPR) effect. PEGylation will increase the circulation half-life of spherulites.

### 1.3 Aim and Objectives

Our aim is to develop a spherulites formulation loaded with an anticancer drug Gemcitabine hydrochloride and Vinorelbine tartrate separately in order to target it to the lung tumour and decrease its distribution to other tissues and organs resulting into decreased side effects.

### 1.4 Plan of Work

i) Literature review, obtaining excipients and drugs.
ii) Preformulation studies to confirm the purity of drugs and ensure of drug-excipient compatibility.
iii) Analytical Methods development for estimation of Gemcitabine Hydrochloride and Vinorelbine Tartrate.
iv) Primary optimization of parameters.
v) Preparation and optimization of Gemcitabine Hydrochloride loaded spherulites.
vi) Preparation and optimization of Vinorelbine Tartrate loaded spherulites.
vii) To characterize the prepared formulations for Vesicle size, zeta potential, % entrapment efficiency, % Loading (w/w) and Morphology by TEM and SEM
viii) To determine the *in vitro* release profile of drugs from developed formulations.
ix) To perform the stability studies of prepared formulations as per ICH guidelines.
x) To perform the cytotoxicity studies and apoptosis study.
xi) To perform *in vivo* studies- acute toxicity study, pharmacokinetic study, biodistribution study by organ isolation and biodistribution study by gamma scintigraphy.

### 1.5 References

1. Cancer. Available online: http://www.who.int/mediacentre/factsheets/fs297/en/. (Accessed on April 30, 2018).
2. Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. CA: a cancer journal for clinicians, 68(1), 7-30.

3. Martin-Moreno, J. M., Soerjomataram, I., & Magnusson, G. (2008). Cancer causes and prevention: a condensed appraisal in Europe in 2008. European Journal of Cancer, 44(10), 1390-1403.
4. Lung Carcinoma, Merk professional edition, online edition, 15-08-2007. (Accessed on April 30, 2018).
5. Lung Cancer, Scottish Intercollegiate Guidelines Network, ISBN 9781905813100 (2007).
6. B.I. Sikic, Antineoplastic agents, VI Chemotherapy, 638-656.
7. Shirai, T., Hirose, T., Noda, M., Ando, K., Ishida, H., Hosaka, T., Ozawa, T., Okuda, K., Ohnishi, T., Ohmari, T., Horichi N., Adachi M. (2006), Phase II study of the combination of gemcitabine and nadaptalin for advanced non-small-cell lung cancer. Lung Cance, 52, 181-187.
8. Cerqueira, N. M., Fernandes, P. A., & Ramos, M. J. (2007). Understanding ribonucleotide reductase inactivation by gemcitabine. Chemistry-a European Journal, 13(30), 8507-8515.
9. Available online: www.rxlist.com/gemzar-drug.htm; (Accessed on April 30, 2018).
10. Faller, B. A., & Pandit, T. N. (2011). Safety and efficacy of vinorelbine in the treatment of non-small cell lung cancer. Clinical Medicine Insights: Oncology, 5, CMO-S5074.
11. Available online: www.rxlist.com/navelbine-drug.htm; (Accessed on April 30, 2018).
12. Gemcitabine, BC Cancer Agency Cancer Drug Manual, Revised on 1 June 2011.
13. Vinorelbine, BC Cancer Agency Cancer Drug Manual, Revised on 1 March 2008.
14. Pitrubhakta, A. B., Shinde, A. J., & Jadhav, N. R. (2012). Design, development and characterization of PEGylated liposomes of gemcitabine hydrochloride. Der. Pharm. Lett, 4, 314-329.
15. Kim, S., & Park, K. (2010). Targeted delivery of small and macromolecular drugs.
16. Chen, Y., Wang, G., Kong, D., Zhang, Z., Yang, K., Liu, R., ... & Xu, Y. (2012). In vitro and in vivo double-enhanced suicide gene therapy mediated by generation 5 polyamidoamine dendrimers for PC-3 cell line. World journal of surgical oncology, 10(1), 3.
17. Yang, F., Jin, C., Yang, D., Jiang, Y., Li, J., Di, Y., ... & Fu, D. (2011). Magnetic functionalised carbon nanotubes as drug vehicles for cancer lymph node metastasis treatment. European Journal of Cancer, 47(12), 1873-1882.
18. Zhigaltsev, I. V., Maurer, N., Akhong, Q. F., Leone, R., Leng, E., Wang, J., ... & Cullis, P. R. (2005). Liposome-encapsulated vincristine, vinblastine and vinorelbine: a comparative study of drug loading and retention. Journal of controlled release, 104(1), 103-111.

19. Wan, F., You, J., Sun, Y., Zhang, X. G., Cui, F. D., Du, Y. Z., ... & Hu, F. Q. (2008). Studies on PEG-modified SLNs loading vinorelbine bitartrate (I): preparation and evaluation in vitro. International journal of pharmaceutics, 359(1-2), 104-110.
20. Oliveira, J. M., Salgado, A. J., Sousa, N., Mano, J. F., & Reis, R. L. (2010). Dendrimers and derivatives as a potential therapeutic tool in regenerative medicine strategies—a review. Progress in Polymer Science, 35(9), 1163-1194.
21. Park, J. H., Lee, S., Kim, J. H., Park, K., Kim, K., & Kwon, I. C. (2008). Polymeric nanomedicine for cancer therapy. Progress in Polymer Science, 33(1), 113-137.
22. K. Cho, X. Wang, S. Nie, Z. Chen, D.M. Shin, Therapeutic nanoparticles for drug delivery in cancer, Clinical Cancer Research 14 (2008) 1310-1316.
23. Lammers, T. G. G. M., Hennink, W. E., & Storm, G. (2008). Tumour-targeted nanomedicines: principles and practice. British journal of cancer, 99(3), 392.
24. Brannon-Peppas, L., & Blanchette, J. O. (2012). Nanoparticle and targeted systems for cancer therapy. Advanced drug delivery reviews, 64, 206-212.
25. Simard, P., Hoarau, D., Khalid, M. N., Roux, E., & Leroux, J. C. (2005). Preparation and in vivo evaluation of PEGylated spherulite formulations. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1715(1), 37-48.
26. Redkar, M., Hassan, P. A., Aswal, V., & Devarajan, P. (2007). Onion phases of PEG-8 distearate. Journal of pharmaceutical sciences, 96(9), 2436-2445.
27. Didier, R. O. U. X., & CSO, C. P. The Spherulites®: an innovative encapsulation system for active ingredients.