CHAPTER 1

GENERAL INTRODUCTION
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Cancer can be defined as a multifactorial disease in which a group of abnormal cells grow uncontrollably defying all the normal rules and regulatory mechanisms of cell growth and division (Hejmadi 2010). The word cancer is derived from Latin, which literally means crab and the Greek word is *karkinos* which also means crab. The term originated from the observation of Hippocrates (the father of modern medicine) that the blood vessels around malignant tumors looked like the claws of a crab, and hence named it cancer. The advancement in cancer research provided an insight into cancer cell’s autonomy, and now the cancer has been defined as a disease which involved changes or mutation into the cell genome. The mutated cell genome produced proteins which disrupt the cellular balance leading to their uncontrolled division and subsequently form cancer.

**HISTORY**

Paleopathologic findings have indicated that tumors already existed in animals in prehistoric times, long before men appeared on the Earth. The earliest written description of cancer, a breast cancer, was found in the Edwin Smith Papyrus that was written approximately 3000 BC. The Ebers Papyrus, dated circa 1500 BC, contains the first reference to a soft-tissue tumor, a fatty tumor, and includes reference to possible cancers of the skin, uterus, stomach, and rectum (Ebbell 1937). The Sumerians, Chinese, Indians, Persians, and Hebrews of the same epoch were used herbal remedies such as tea, fruit juices, figs, and boiled cabbage to treat cancer, but in advanced cases, they did not hesitate to resort to solutions and pastes of iron, copper, sulfur, and mercury to control the disease. Many of these concoctions remained in external and internal use, in various concentrations, for more than 3000 years (Wolff 1928; Castiglioni 1931). Although sporadic application of chemical agents was introduced by the Egyptians and Greeks before the Common Era (BCE), the earliest systematic therapeutic use of
chemicals was initiated in the 16th century by Paracelsus (1493-1541) who introduced mercury, lead, sulfur, iron, zinc, copper, arsenic, iodine, and potassium as internal remedies (Paracelsus 1562). The word metastasis was introduced by Joseph Recamier (1774-1852), a French gynecologist, in 1829. By watching the growth and spread of cancers, he was able to identify blood vessel invasion by cancer with the naked eye. In 1838 Johannes Muller described cancers as special groupings of abnormal cells and stroma. He attributed cancer to the formation of new cells in diseased organs with potential to be destructive and to spread to other parts of the body by vascular invasion. He associated cancer with aging and identified tumor necrosis (apoptosis) as a sign of regression. He microscopically distinguished epithelial and connective tissue tumors. Muller divided malignant epithelial tumors for carcinoma simplex (squamous carcinoma), carcinoma alveolare (adenocarcinoma), carcinoma fasciculatum (spindle cell carcinoma), carcinoma medullare (medullary carcinoma), and carcinoma melanodes (malignant melanoma).

With regards to malignant connective tissue tumors, Muller described infiltrating fibrous tumors (desmoid tumor and fibrosarcoma), cystosarcoma of the breast, chondrosarcoma, and osteosarcoma of bones. In 1846, Virchow coined the terms “hyperplasia” and “metaplasia” and recognized that both conditions are potential precursors of cancer, and that cancer cells have marked difference both in size and shape as compared to benign cells. The embryonal characteristics were proposed by Julius Cohnheim in 1877. In addition twenty years later, Moritz Wilhelm Hugo Ribbert, a Zurich pathologist, proposed that mechanical irritation like chronic inflammation and trauma can also lead to the development of cancer especially in the epithelial and connective tissue cells (Cohnheim 1877; Ribbert 1904). Schistosoma haematobium was accused to be the only microorganism which caused cancer (bladder) for decades until the discovery of Clonorchis sinensis, a causative factor for bile cancer (Harrison 1889). Despite the
fact that the field of medicine had made great strides in some ancient civilizations, the progress in cancer treatment has been meager, which is even dreaded today due to the persistent view that cancer is an incurable disease (Diamandopoulus 1996; Kardinal 1979).

**CHARACTERISTICS OF CANCER AND CANCER CELLS**

In normal condition, adult human body constitutes approximately $10^{15}$ cells, of which approximately $10^{12}$ are formed, divide and differentiate each day to replace the dead and worn out cells, these cells will enter the active proliferative phase only after receiving the mitogenic growth signals, and cannot multiply in the absence of these signals. However, cancer cells have lost the need of these stimulatory signals and therefore can proliferate whether these signals are present or not and produce their own growth factors mimicking the normal growth factors which make them independent of the normal growth factors (Fedi *et al.* 1997; Hanahan and Weinberg 2000).

Till date there are more than 100 distinct types of cancers and tumors and each specific organ has subtypes, in spite of this diversity, human cancers share several fundamental properties. A set of six characteristic properties of cancers has been proposed by Hanahan and Weinberg and called it the ‘hallmarks of cancer’ (Hanahan and Weinberg, 2000). These capabilities comprised of

i. **Self-sufficiency in growth signals**: Tumor cells show a greatly reduced dependency on exogenous growth stimulation as they generate their own growth signals by altering the extracellular growth signals, transducers of those signals and intracellular circuits that translate those signals into action thereby disrupting the normal homeostatic mechanism within a tissue. Examples include the production of PDGF (platelet-derived growth factor) and TGFα (tumor growth factor α) by glioblastomas and sarcomas, respectively.
ii. **Insensitivity to anti-growth signals:** Tumor cells are able to evade the antiproliferative signals by disrupting the retinoblastoma protein (pRb) pathway which blocks the cells from advancing through G1 phase of the cell cycle, thereby allowing the cells to proliferate, rendering cells insensitive to antigrowth factors (Weinberg 1995).

iii. **Evasion of apoptosis:** Cancer cells can acquire resistance to apoptosis through mutation of the p53 tumor suppressor gene which is evident in more than 50% of human cancers (Harris 1996).

iv. **Limitless replicative potential:** The three common acquired capabilities of cancer cells such as the growth signal autonomy, insensitivity to antigrowth signals and resistance to apoptosis can lead to an uncoupling of a cell’s growth program from signals in its environment. This can be achieved by upregulating the expression of telomerase enzyme.

v. **Sustained angiogenesis:** The formation of new blood vessel is a prerequisite for the rapid clonal expansion of tumor cells, so the tumor cells appear to activate the angiogenic switch by changing the balance of angiogenesis inducers such as VEGF and countervailing inhibitors such as thrombospondin-1 (Hanahan and Folkman 1996).

vi. **Tissue invasion and metastasis:** At the time of development of most of human cancers the tumor cells move out and invade adjacent tissues where they may succeed in finding new colonies by a process known as metastasis. About 90% of human cancer deaths are caused by metastases (Sporn 1996). One of the reasons for this capability is the loss of function of E-cadherin due to mutational inactivation, transcriptional repression or proteolysis of the extracellular cadherin domain in majority of epithelial cancers (Christofori and Semb 1999).
CAUSES AND MECHANISM OF CANCER

In humans, carcinogenesis is a multifactorial and complex mechanism. It can be divided into four stages: tumor initiation, tumor promotion, malignant conversion, and tumor progression. Tumor initiation is an irreversible change caused by chemical carcinogens by forming DNA-adducts resulting in the activation of proto-oncogenes, inactivation of tumor suppressor genes and genomic instability. Tumor promotion occurs when the initiated cells undergo clonal expansion which will undergo further genetic changes and increased malignancy. Tumor promoters are not carcinogenic alone but they can act as mediators for tumor initiator and they can increase the latency period of tumor formation as well as increase the number of tumors in tissues. Examples of tumor promoters include dioxin, benzoyl peroxide, dichlorodiphenyltrichloroethane (DDT), phenobarbital, cigarette-smoke condensate, Ultraviolet light, etc. Malignant conversion occurs with further genetic changes which can be due to infidelity during DNA synthesis leading to the transformation of a preneoplastic cell into the malignant phenotype. Tumor progression is the transformation of a malignant tumour with the accumulation of further mutations and selection of mutated cells and subclones. The mutations affect additional oncogenes and tumour suppressor genes. Metastasis may also occur where tumor cells are able to secrete proteases that allow invasion beyond the immediate primary tumor location (Schulz 2005).

The initiation and progression of cancer depends on exogenous factors (chemical, physical, or biological carcinogens) as well as endogenous factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These factors can act together or in a sequence, by different mechanisms at different stages of tumor development resulting in abnormal cell behavior and excessive proliferation resulting in metastasis. The
lifestyle, environment and age have a profound effect in the development of cancer. In 1950, the attendees of an international symposium sponsored by the World Health Organization learned that people who migrated to other countries, developed types of cancer common to their adopted countries, rather than their homelands. This implied that most cancers were caused by exposures in the environment, such as smoking, alcohol consumption and exposure to various carcinogens rather than inherited genetic factors. Genetic predisposition increases the chances of earlier development of cancer in less than 10% of cases such as childhood leukemias, retinal cancers etc. (Higginson 1992; Hejmadi 2010).

**Chemical carcinogens**

Chemical carcinogens can be grouped into organic and inorganic compounds. Inorganic compounds comprised of nickel, cadmium, or arsenic which could be encountered in the workplace or present as contaminants in water whereas organic compounds comprised of aliphatic compounds, like nitrosamines, which occur in smoked and pickled foods, or trichloroethylene, which is used for cleaning. Aromatic compounds like benzopyrenes and arylamines are generated from natural sources by burning of coal and fuels, and are among the many carcinogens in tobacco smoke. Nitrosamines are thought to contribute to stomach cancer whereas arylamines cause bladder cancer, in particular. Natural compounds produced by plants and mold such as Aflatoxin B1 is implicated as a carcinogen in liver cancer. Medical drugs used in cytostatic tumor therapy like cyclophosphamide, nitrogen mustards, and platinum compounds are also carcinogenic. Various hormones and hormone-like compounds from natural and pharmaceutical sources also influence the development of cancers in specific tissues e.g. in the breast and prostate. Reactive oxygen species produced at increased rates during certain
physiological processes such as immune defense and inflammation can also be mutagenic (Schulz 2005).

**Physical carcinogens**

Depending on its’ dose and absorption any electromagnetic radiation can act as a carcinogen. Ultraviolet radiation (UVR) can be divided into three wavelength ranges based on their differences in photochemistry and biological importance such as UVA (320 to 400 nm), UVB (290 to 320 nm) and UVC (240 to 290 nm). Among them, UVB is mainly responsible for skin cancer through direct photochemical damage to DNA as it overlaps the upper end of the DNA and proteins’ absorption spectra whereas UVA is photocarcinogenic and involved in photoaging but is weakly absorbed by the DNA and proteins. The relevant chromophores may therefore involve reactive oxygen species (ROS), which secondarily cause damage to DNA. Hence, although UVA and UVB light constitute a minute portion of the emitted solar wavelengths, they are primarily responsible for the Sun’s pathologic effects. γ-radiation from natural, industrial, and iatrogenic sources (e.g., used in X-ray diagnostics) can penetrate the body and become carcinogenic to the extent to which it is absorbed, damaging DNA and cells by direct absorption but also indirectly by generating reactive oxygen species. Radioactive β-radiation and specifically α-radiation is most dangerous when nuclides are ingested or incorporated, eg. cesium, uranium, and plutonium. The effect of radioactive isotopes depends also on their distribution in the body. For instance, radioactive iodine is accumulated in the thyroid gland and therefore causes specifically thyroid cancers, whereas radioactive cesium isotopes tend to become enriched in the urinary bladder (Devita 2001; Schulz 2005).
Biological carcinogens

Biological carcinogens include certain bacteria and viruses like human papilloma virus HPV16 and HPV18 that cause cervical and other genital cancers which can also influence the development of cancers of the skin and of the head and neck. Herpes simplex virus (HHV8) is involved in Kaposi sarcoma, hepatitis B virus in liver cancers, and Epstein Barr virus in lymphomas. HIV facilitate the development of cancers mostly by interfering with the immune system, but HTLV1 (human T-cell leukemia virus) causes a rare leukemia by direct growth stimulation of T-cells. Bacteria such as Helicobacter pylori causes stomach cancer through infection. Parasites like Schistosoma trematodes are also known to cause urinary bladder cancer (Schulz 2005).

Endogenous carcinogens

Endogenous factors alone may result in development of cancer or they can act along with exogenous agents as cancer modulators. The detoxification mechanism might not be able to remove the carcinogenic compounds such as nitrosamines, aromatic amines, quinones, reactive aldehydes and ROS generated during normal metabolism leading to the formation of cancer. However, inefficient DNA repair mechanism, genetic and even epigenetic errors during fetal development, ageing and chronic inflammation could all lead to the development of cancer at some point of life. Prolonged hormonal stimulation of a particular target organ can also lead to cancer such as breast and other reproductive organs which is mainly controlled by mutation of genetic sequence that encoded protein. (Kufe et al. 2003; Schulz 2005)
CLASSIFICATION OF CANCER

Cancers are classified based on their degree of malignancy and histological subtype which is a prerequisite for better prognosis and appropriate treatment. The various classifications or characterizations include:-

**Staging:**

Cancer can be classified depending on the extension of tumor. Investigation by visual inspection, palpation and various imaging techniques (ultrasound, X-rays, scintigraphy, computer tomography, magnetic resonance, and positron emission tomography) prior to surgery to detect changes in tissue shape density, changes in metabolism and blood flow is called clinical stage and denoted by ‘c’ whereas inspection of the tumor site and histopathological investigation of the specimen post surgery is called pathological stage and denoted by ‘p’.

The most widely used systematic staging system is the TNM classification where the extent of the primary tumor is normally described by T1-T4, where increasing numbers describe larger and/or more invasive tumors. The system varies for different tumor sites. The presence of cancer cells in lymph nodes is denoted by N0, N1, and in some cancers also N2, with N0 meaning none detected. The presence of metastases is indicated by M0 meaning none detected, M1, or in some cancers also M2. After surgery, to know whether all of the local tumor growth has been removed R value is used. R stands for resection margin, so R0 means that the tumor seems to be wholly contained within the removed specimen. In all categories, the affix ‘x’ is used for ‘not determined/unknown’.

**Grading:** To further estimate the degree of malignancy grading system is being used. The most prevalent system is G grading, which usually ranks from G0 to G4. The designation G0 typically denotes normal differentiation and no cellular atypia, as would be found in a benign
tumor. At the other end, G4 would be assigned to cancers with a cellular morphology completely different from the normal tissue and pronounced atypia of the cells and nuclei. The grades G1-G3 are called well-differentiated, moderately and poorly differentiated tumors.

**Histological classification**: Histological typing of tumors is performed by evaluating their morphology. Routine procedures use a variety of specific stains developed over centuries in anatomy and pathology to highlight particular cell types as well as extracellular structures like basement membranes, fibers or mucous. Increasingly, tumor classification by histopathological investigation is being improved by specific molecular markers. Immunohistochemical staining with antibodies directed against specific antigens of the presumed tissue of origin, e.g. cytokeratins, or tumor-specific antigens, eg. carcinoembryonic antigen, is often performed. For leukemias, analysis of subtypes can be determined by antibody staining followed by flow cytometry (Schulz 2005; Webber et al. 2014; American Cancer Society, 2015).

**CANCER EPIDEMIOLOGY**

Cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases in 2012 (Farley et al. 2012) and it is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. In 2017, 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the United States (Siegel et al. 2017). Globally, nearly 1 in 6 deaths is due to cancer and the number of new cases is expected to rise by about 70% over the next 2 decades. Approximately 70% of deaths from cancer occur in low- and middle-income countries. Around one third of deaths from cancer are due to the 5 leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco and alcohol use. Tobacco use is the most important risk factor for cancer and is responsible for approximately 22% of cancer deaths (GBD 2015). Cancer causing
infections, such as hepatitis and human papilloma virus (HPV), are responsible for up to 25% of cancer cases in low- and middle-income countries (Plummer et al. 2012). Late-stage presentation and inaccessible diagnosis and treatment are common. In 2015, only 35% of low-income countries reported having pathology services generally available in the public sector whereas more than 90% of high-income countries reported treatment available services. The economic impact of cancer is significant and is increasing; in 2010 the total annual economic cost of cancer was estimated at approximately US$ 1.16 trillion (Stewart 2014).

TREATMENT

For the treatment of cancer a different range of therapies are available. Surgery, irradiation or drugs can be employed, or a combination of these. The choice of therapy depends on the type of cancer; surgery or radiation treatment is chosen for localized cancers. In contrast, leukemias, lymphomas, and metastatic or locally advanced carcinomas and soft tissue cancers require drug chemotherapy, which is in some cases supplemented by radiotherapy or surgery of primary cancers or metastases. Conversely, adjuvant treatment can be given where surgery is followed by chemotherapy or irradiation to attack residual local tumor or metastases or neoadjuvant treatment can be given where chemotherapy can be applied before surgery to shrink the tumor mass and facilitate its complete resection. The standard chemotherapy regimen for a cancer is usually designated as ‘first-line’, if it fails, ‘second-line’ therapy can be attempted (Schulz 2005).

Surgery

Surgery is the oldest treatment for cancer and forms the mainstay of treatment of solid tumors till today. It is most effective in the treatment of localized primary tumor and associated regional lymphatic. This is accomplished by en bloc surgical procedures that attempt to
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encompass gross and microscopic tumor in all contiguous and adjacent anatomic locations. Intuitively, it appears logical that surgery should have little role in disease management once a neoplasm has spread from the primary location to a distant site. However, advances in surgical techniques and a better understanding of the patterns of spread of individual cancers have allowed successful resections for an increased number of patients (Kufe et al. 2003).

Radiotherapy

Radiotherapy refers to the treatment of benign and malignant tumor with ionizing radiation (IR). With the discovery of X-rays by Wilhelm Conrad Röntgen from Germany in 1895 and radium by Marie Curie, the clinical usefulness of radiation as a cancer therapy has been established (Baskar 2012). Along with surgery and chemotherapy, radiation therapy or radiotherapy gains an important modality for the treatment of cancer because of its highly cost effective single modality treatment and accounts only about 5% of the total cost of cancer care (Ringborg 2003). With the advancement in imaging techniques, computerized treatment planning systems, radiation treatment machines (with improved X-ray production and treatment delivery) as well as improved understanding of the radiobiology of radiation therapy, there has been a great progress in this field (Bernier 2004). Radiotherapy can be delivered primarily with high-energy photons (γ-rays and X rays) and charged particles (electrons) and other therapeutic modalities include neutrons (Vynckier 1998) and protons (Miller 1995). The radiation randomly affects the molecules of the cell where the main target is the deoxyribonucleic acid (DNA) which can result in single- and double-strand breaks (DSBs) in the sugar-phosphate backbone of the DNA molecule (Dizdaroglu, 1992; Lomax et al., 2013), damage caused to the cellular and nuclear membranes and other organelles may also play an important role. Cross-links between DNA strands and chromosomal proteins also occur. Depending on the type of radiation the
mechanism of DNA damage also differs. For example, X and γ radiation are indirectly ionizing where DNA damage is caused by the short-lived, hydroxyl free radicals produced primarily by the ionization of water components of the cell (Ward 1988; Desouky et al., 2015), however protons and other heavy particles are directly ionizing and damage DNA directly (Phillips 1997). Radiation damages both normal cells as well as cancer cells, so the main goal of radiation therapy is to maximize the radiation dose to abnormal cancer cells while minimizing exposure to normal cells. Since, normal cells usually repair themselves at a faster rate and retain its normal function status than the cancer cells which are generally not as efficient as normal cells in repairing the radiation damage resulting in the differential cancer cell killing (Begg 2011).

Chemotherapy

Chemotherapy is any drug which is used to treat any disease. But the word is commonly used in cancer therapy. The term ‘chemotherapy’ was coined by Paul Ehrlich while he was working on the treatment of infectious diseases by using antibiotics. In the early 1900s, George Clowes at Rosewell Park Memorial Institute used this idea to induce tumor model in rodents to screen the potential anticancer drugs. Alkylating agents, the first class of chemotherapeutic drugs to be used in the clinical setting were a product of the secret gas program of the United States in both world wars where the military seamen were exposed to mustard gas in World War II which led to the observation that alkylating agents caused marrow and lymphoid hypoplasia (Alexander 1944; Hersh 1968). This observation led to the direct application of such agents in humans with hematologic neoplasms, including Hodgkin's disease and lymphocytic lymphomas, at the Yale Cancer Center in 1943 (Marchall 1964; DeVita and Chu, 2008). During the same year, the significant proliferative effect of folic acid against leukemic cell growth in children with lymphoblastic leukemia had also been reported by Sidney Farber and the antifolates like
aminopterin (the predecessor of methotrexate) was found to kill tumor cells by blocking DNA replication (Farber, 1948). This led to the discovery of many drugs capable of blocking different functions of cell growth and replication. This was the beginning of the chemotherapy research. The use of methotrexate in 1956 treated metastatic cancer for the first time after the discovery of DNA. There are more than 100 different types of chemotherapeutic drugs for the treatment of different types of cancers, which are used either alone or in combination (Colvin 2003).

The chemotherapeutic drugs can be classified according to their mechanism of action such as the alkylating agents, antimetabolites, plant alkaloids, antitumor antibiotics, hormonal agents and targeted cancer therapies. The alkylating agents form covalent bonds with amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules such as DNA, RNA or proteins which impaired their function. In DNA the electron-rich nitrogen at the 7 position of guanine is particularly susceptible to alkylation. Alkylating agents depend on cell proliferation for activity by killing a fixed percentage of cells in a given dose, but are not cell-cycle phase–specific. The alkylating agents are classified according to their chemical structures and mechanisms of covalent bonding; this drug class includes the nitrogen mustards used against cancer of the hematopoietic system, nitrosoureas used against a variety of brain tumors and platinum complexes like cisplatin is used against testicular, ovarian, bladder cancers etc. (Colvin, 1990; Pazdur et al. 2007).

Antimetabolites are structural analogs of the naturally occurring metabolites involved in DNA and RNA syntheses. Antimetabolites exert their cytotoxic activity either by competing with normal metabolites for the catalytic or regulatory site of a key enzyme or by substituting for a metabolite that is normally incorporated into DNA and RNA. Because of this mechanism of action, antimetabolites are most active when cells are in the S phase and have little effect on cells
in the G\textsubscript{0} phase. Consequently, these drugs are most effective against tumors that have a high growth fraction. The antimetabolites can be divided into folate analogs, purine analogs, adenosine analogs, pyrimidine analogs, and substituted ureas. These drugs are most effective against tumors that have a high growth fraction (Devita et al. 2001; Pazdur et al. 2007).

Plant alkaloids inhibit tubulin polymerization thereby disrupt the assembly of microtubules which result in mitotic arrest at the metaphase stage and eventually leading to the death of cells. They include vinca alkaloids (vincristine, vinblastin) derived from the periwinkle plant \textit{Vinca rosea}, taxanes including paclitaxel and docetaxel (Taxotere) which are semisynthetic derivatives of extracted precursors from the needles of yew plants. The other class of plant based drugs that inhibit topoisomerases include epipodophyllotoxins such as etoposide, a semisynthetic epipodophyllotoxin extracted from the root of \textit{Podophyllum peltatum} (mandrake), and camptothecin derived from the Chinese ornamental tree \textit{Camptotheca acuminata} and its semisynthetic analogs including irinotecan (CPT-11 [Camptosar]) and topotecan (Hycamint). Plant alkaloids are effective against different types of cancers (Pazdur et al. 2007).

Antitumor antibiotics intercalate DNA and inhibit topoisomerases I and II resulting in spontaneous oxidation and formation of free oxygen radicals that cause strand breakage and finally cell death. They include bleomycin and anthracycline which is produced by the \textit{Streptomyces verticillus} and \textit{Streptomyces percetus} var \textit{caesius} (Umezawa et al., 1966; Arcamone et al., 1969).

Hormonal agents such as estrogen inhibitors, androgen inhibitors, gonadotropin-releasing hormone agonists, aromatase inhibitors and glucocorticoids bind to their respective hormone receptors thereby suppressing their action. Estrogen inhibitor such as tamoxifen could bind to the estrogen receptors of breast tumor inducing competitive inhibition and suppresses the
production of insulin-like growth factor 1 (IGF 1) and transforming growth factor alpha (TGF-α) (Kufe et al. 2003).

Targeted cancer therapies interfere with specific proteins involved in tumorigenesis. There are three main types of targeted cancer therapies; 1) monoclonal antibodies, 2) small molecule inhibitors and 3) immunotoxins (Baudino 2015). Monoclonal antibodies deregulate the functions of the cancer cell by the disruption of protein function and possible downstream signaling, antibody-dependent cytotoxicity and complement dependent cytotoxicity. Avastin (bevacizumab, Genentech) is a monoclonal antibody that targets VEGF by inhibiting VEGF signaling. Small molecule inhibitors competitively bind to the active or inactive ATP binding site of a tyrosine kinase. They are used to target proteins that have become either unregulated or upregulated during cancer progression, such as BCR-ABL. Gleevec (imatinib mesylate, Novartis) is a small molecule inhibitor targeted against the BCR-ABL tyrosine kinase domain, which is used in the treatment of several different cancers, including Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML), and c-Kit positive gastrointestinal stromal tumors (de Jong, 1997; Deininger, 2003). Immunotoxins are a new class of drugs that involves modifying monoclonal antibodies or growth factors which binds to a cell surface protein and becomes internalized via clathrin-coated pits and elicit apoptosis of the targeted cell (Alewine, 2015). Ontak (denileukin diftitox, Ligand Pharmaceuticals) is an interleukin-2 (IL-2)/DT fusion protein produced by creating recombinant DNA (rDNA) that fuses together a DT gene that has a truncated binding region and an IL-2 gene is used to treat patients with cutaneous T-cell lymphomas (CTCLs) (Foss, 2005). Activated T-cells highly express high affinity IL-2R on their cell membrane and thus, IL-2R becomes a potential target in T-cell lymphomas. The bacterial toxin, DT, results in ADP-ribosylation of elongation factor-2 (EF-2), which causes EF-2 to
become inactive, preventing protein synthesis (Carroll, 1987). DT is extremely potent and only one molecule needs to enter the cytosol of a cell to cause an apoptotic response (Kreitman, 2006).

However, cancer therapy have their own limitations as they also damage the normal dividing cells especially the rapidly regenerating tissues, such as hair follicles and can also lead to drug resistance as well induction second malignancies, (Wu et al. 2008; Morton et al., 2014) necessitate the need to utilize alternative concepts or approaches to treat the cancer.

**AIM OF THE STUDY**

*Helicia nilagirica* Bedd. (Family: Proteaceae) locally known as Pasaltakaza is a medium-sized tree, which grows up to 12 meters high. It is widely distributed in Sri Lanka, southern India, Burma (Myanmar), Indochina, Japan, Taiwan, and Thailand. It is also found scattered in lowland to montane rain forests, up to 2,000-3,350 m altitude. Some species prefer habitats along streams but other species are found on hilltops or ridges (Khamyong et al., 2004). This tree has been used as a folk medicine since time immemorial in Mizoram, India by the Mizo tribe. Its decoction prepared by boiling the leaves or bark is used to treat various stomach ailments including peptic ulcers, indigestion, mouth ulcer, urinary tract infection and gynaecological disorders. It is also used in scabies and other skin diseases (Sawmliana, 2003). The fruits of *H. nilagirica* have been used as a medicine to cure cough and cold in Sikkim (Chauhan, 2001). The scientific evaluation regarding its medicinal or other properties is scarce Except that, it has been shown to possess anti-inflammatory activity in rat cotton pellet granuloma model (Lalawmpuii et al., 2014). This indicates the need to systematically evaluate its anticancer properties. The main objective of present investigation is to evaluate the anticancer activity of *Helicia nilagirica* extracts *in vivo* and *in vitro* by carrying out the following investigations:
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1. Activity guided fractionation of different extracts of *Helicia nilagirica*.

2. Evaluation of anticancer activity of the various extracts in different cultured neoplastic cell lines and in tumor bearing mice.

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