INTRODUCTION
1. CANCER

Cancer medically known as a malignant neoplasm is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental controls. The Greek physician, Hippocrates (460-370 BC) is considered the “Father of medicine” because he invented the word cancer. The terms carcinos and carcinoma were used by Hippocrates to describe non-ulcer forming and ulcer-forming tumors. This Greek term was later translated into the word cancer by the Roman physician Celsus (28-50 BC). Another Roman physician named Galen described tumors using the word oncos which is now commonly used as a part of the name for oncologists (The history of cancer, 2012). It is currently believed that cancer is a multi-gene, multi-step disease that originates from a single abnormal cell with a mutated DNA sequence. The slightly irregular stage is reached when the uncontrollable proliferation of these abnormal cells is followed by a second mutation. A tumor is created due to the consecutive rounds of mutation and the selective expansion of these cells (figure 1) (Momma H. 2010).

Fig 1: Mutations leads to Loss of normal growth control and develops cancer.

There are thirty trillion cells in a normal and healthy body. These normal cells only reproduce when instructed to do so by other cells in their surrounding area. The reason for this collaboration is so that each tissue maintains size and structure suitable to the body’s needs (Eva Bianconi et al., 2013). In contrast, cancer cells do not follow this collaboration. They follow their own internal instructions for reproduction and they have the ability to migrate from the site where they started. They invade tissues and forming tissues that are nearby. These malignant cells that make up tumors allow
them to become increasingly aggressive overtime. Furthermore, they disrupt the tissues and organs that are needed for the survival of the organism as a whole (D. A. Warrell et al., 2005).

1.1. EPIDEMIOLOGY OF CANCER

Global Scenario: Despite of prominent progress in therapeutics and diagnostics, cancer still remained as an unsolved problem and is the 2\textsuperscript{nd} most common disease subsequent to cardiovascular disorders causing maximum mortality worldwide, (Shalini et al.,2011) (Imran Ali et al.,2011). In 2012, an estimated 14.1 million new cases of cancer occurred worldwide with 7.4 million (53\%) in males and 6.7 million (47\%) in females, giving a male: female ratio of 10:9 (table 1). More than half of cancers occurring worldwide are in less developed regions.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
CANCER WORLDWIDE & Males & Females & Persons & Year\textsuperscript{2} \\
\hline
Number of new cases per year & 7,427,148 & 6,663,001 & 14,090,149 & 2012 \\
Incidence rate per 100,000 population\textsuperscript{2} & 205.4 & 165.3 & 182.3 & \\
Number of deaths per year & 4,653,132 & 3,547,998 & 8,201,030 & 2012 \\
Mortality rate per 100,000 population\textsuperscript{2} & 126.3 & 82.9 & 102.4 & \\
\hline
\end{tabular}
\caption{Table 1: Incidence of Cancer worldwide. (world cancer factsheet. 2012)}
\end{table}

The four most common cancers occurring worldwide are lung, female breast, bowel and prostate cancer (figure 2). In 2012, an estimated 8.2 million people died from cancer worldwide. More than 6 in ten cancer deaths worldwide occur in less developed regions of the world. Lung cancer is the most common cancer in men worldwide. More than 1 in 10 of all cancers diagnosed in men is lung cancer (Cancer Facts & Figures 2012)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Fig 2: Most Commonly Diagnosed Cancers, Worldwide, 2012 Estimates.}
\end{figure}
Comparison of Prevalence of Cancers in India with Global Scenario:
Cancer accounts for about 23% and 7% deaths in USA and India, respectively. By 2020 the world’s population is anticipated to be 7.5 billion and approximations foretell that about 15 million new cases will be registered with deaths of about 12 million cancer patients (IARC WHO, 2013). Thus, efforts have been made to study the status of cancers in India and comparison with global scenario for the year 2002 as shown in Figure 3.

**Fig 3: Comparison of cancer; Indian scenario with USA [Fenley et al, 2001]**

High incidences of oral and esophageal cancer in Indian populations were observed as compared to USA which can be attributed to the extensive use of tobacco by Indians. On contrary to this, high rate of lung, colon, stomach, liver, bladder, kidney cancers and melanoma of the skin cancers was reported in USA as compared to India (Imran Ali et al., 2011)

**Scenario of Cancer in India:** In India, cancer is the second most common cause of death, growing yearly with rate of 11%. There are about 2-2.5 million cancer cases reported in total with the yearly summation of 7-9 lacs new cases (Park et al., 2009). A data of cancer patients was compiled from 2004 to 2010 in India and shown in Figure 4. Above graph clearly portrays that the number cancer patients including both genders increased continuously up to year 2010. Furthermore, a prevision of cancer patients has also been made for year 2015 and 2020. One in five Indian men dies between age 30 and 69 due to tobacco-related cancers (Jha P. 2011; WHO, 2010). In India, even though the most prevalent cancers- breast, cervical and oral cancers are largely preventable, more than 70% of the cases are detected at later stages when it is too late for effective treatment (Smith, et al., 2009). As per WHO
studies, the total mortality (males and females) due to cancer in India is estimated to reach 6-7 lacs by 2015.

![Fig 4: Year wise total cancer prevalence in India [ICMR, 2006(14); ICMR, 2009(15)].](image)

**1.2 ETIOLOGY OF CANCER**

Cancer is caused by **internal factors** that accounts for about 5-10% and by **environmental/acquired factors** that accounts for about 90-95% (Figure 5) (Preetha A. et al, 2008).

![Fig 5: The role of genes and environment in the development of cancer.](image)
1. **Hereditary Factors:** Certain inherited mutations in the genes **BRCA1** and **BRCA2** cause more than 75% risk of breast cancer and ovarian cancer and hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) which is present in about 3% of people with colorectal cancer (Nancie P. et al., 2013)

2. **Environmental Factors:** External and lifestyle factors, such as smoking, alcohol consumption, dietary habits, infections, etc have a profound influence on the development of many types of cancers.

   A. **Tobacco:** Increased tobacco consumption leads to development of various cancers. It results in 25–30% of all deaths from cancer and 87% of deaths from lung cancer. Tobacco contains carcinogen like benzopyrene diol epoxide which has a straight etiological involvement with lung cancer (Knut-Olaf H. et al, 2010).

   B. **Alcohol:** Heavy alcohol consumption causes many cancers including that of mouth, liver, colorectal etc (Preetha A. ET. Al, 2008)( 20–60% of cancers of upper aero-digestive tract are attributable to alcohol, and up to 75% of these tumors can be prevented by avoiding use of alcohol (Eric S. et al.,1999; Preetha A. et. el, 2008). Globally, the attributable fraction of cancer deaths due to alcohol drinking is reported to be 3.5% (Boffetta P. et al., 2006).

   C. **Diet:** The extents to which diet conduce to cancer death varies significantly according to the type of cancer (Bryan et al., 2005) like high intake of meat is risk factor particularly for gastrointestinal tract cancer. Many carcinogens like pyrolysates, heterocyclic amines are formed during the charcoal cooking process of meat induces carcinogenesis. Food additives like nitrosamines, dioxins and nitrites are also found to be associated with cancer progression. High incidences of prostate and breast cancer are reported due to intake of bisphenol migrates into food which is kept in plastic containers. Refined flours and fatty acids found in many foods and beverages triggers inflammations that leads to cancer development (Preetha A. et. al, 2008).

   D. **Obesity:** Obesity has been linked with numerous cancers counting gastric, colon, prostate, breast, endometrium, gallbladder, renal cell, esophagus, liver and pancreas (Fatima et al., 2009; Ana I. et al., 2014). Findings from this study suggest that of all deaths from cancer in the United States, 14% in men and 20%
in women are attributable to excess weight or obesity. Obesity has direct association with many signaling and inflammatory cascades that contributes to cancer progression. For instance, hyperglycemia has been shown to activate NF-κB, which could link obesity with cancer (Eugenia et al., 2004).

E. Infectious Agents: Worldwide, an estimated 17.8% of neoplasms are associated with infections; this percentage ranges from less than 10% in high-income countries to 25% in African countries (WHO, 2010; Andrew Mathews, 2012). Viruses like Human papillomavirus, HIV, Epstein Barr virus, HCV, HBV, etc account for most infection-caused cancers. Almost all viruses related to cancer have been demonstrated to trigger the inflammatory marker, NF-κB (Bharat, 2006).

F. Environmental Pollution: Environmental pollution comprises of outside air pollution by carbon particles and inside air pollution by volatile substances like 1,3-butadiene and benzene, smoke of tobacco, cosmetics, pharmacy drugs, formaldehyde and other carcinogenic metals (GCO , 2013).

G. Radiation: Radiation accounts for up to 10% of total cancer that includes thyroid cancers, some leukemia & lymphomas, skin cancers, breast carcinomas etc. Ionizing and non ionizing radiation from radioactive substances, ultraviolet (UV), pulsed electromagnetic fields are carcinogenic that causes DNA damage and induces cancer in humans (Cosmin et al., 2014).

H. Asbestos: Asbestos is a known human carcinogen, when breathed in, asbestos fibers get trapped in the lungs where they accumulate and cause inflammation leading to elevated risk of lung cancer (Patrick, 2013) Mesothelioma is also found to be associated with asbestos exposure. Along with this some earlier reports suggests that other cancers including cancers of the kidney, throat, gallbladder, colorectal, esophagus are also linked with asbestos.

Hormones: Insulin-like growth factors and many others growth hormones are significant factors involved in proliferation, apoptosis and differentiation of cancerous cells ultimately leading to carcinogenesis. Sex hormones play crucial role in causing sex-related cancers such as breast, endometrium, testis, ovary, prostate, etc (Ramadevi et al., 2014).
1.3 GENETIC MUTATIONS LEADING TO CANCER: “Process of Carcinogenesis”

Cancer forms when genes within a normal cell are damaged and mutated. There are two basic types of genetic mutations: acquired and germline.

1. **Acquired mutations** are not inherited but are the damage acquired in genes during person’s life may be due to UV radiation, viruses, tobacco etc. These mutations are the most common cause of many cancers (Frederica, 1996).

2. **Germ-line mutations** are the inherited mutation that passes from generation to generations thus present in each cell of person’s body. These types of mutations accounts for only about 5% to 10% of all cancers.

In normal human cells there is a steady accumulation of mutations with time. These mutations arise spontaneously and are due to endogenous factors or processes that damage DNA (Deman et al., 2001). A small fraction of these mutations converts a normal cell into a cell that is initiated towards the development of cancer. These initiated cells are more susceptible to the mutagenic effects of exogenous carcinogenic agents than to the mutagenic effects of endogenous factors. A series of mutations must occur in the same cell in order for cell to become cancerous.

**Carcinogenesis** is a multistep process resulting from the accumulation of multiple genetic alterations that collectively give rise to the transformed phenotype (Rina et al., 2008). Present day oncology recognizes three main phases in cancer development: a) initiation, b) promotion and c) progression.

(a) **Initiation** involves one or more stable cellular changes arising spontaneously or induced by exposure to a carcinogen. This is considered to be the first step in carcinogenesis, where the cellular genome undergoes mutations, creating the potential for neoplastic development (UNSCEAR 1993(34), Cox 1994), which predisposes the affected cell and its progeny to subsequent neoplastic transformation. The transformed cell undergoes continuous division with fidelity to the transformed karyotype and, possibly, with further mutations, before a malignant lesion is manifested (P Uma Devi, 1982).

(b) **Promotion:** The transformed (initiated) cell can remain harmless, unless and until it is stimulated to undergo further proliferation, upsetting the cellular balance. The subsequent changes of an initiated cell leading to neoplastic transformation may involve more than one step and requires repeated and prolonged exposures to
promoting stimuli. This may result in an enhancement of cellular growth potential and/or an uncoupling of the intercellular communication processes that restrict cellular autonomy and thereby coordinate tissue maintenance and development (P Uma Devi, 1982).

(c) **Progression:** is the process through which successive changes in the neoplasm give rise to increasingly malignant sub-populations. Mutations and chromosomal aberrations are thought to be involved in tumour progression (Marileila, 2010). The process may be accelerated by repeated exposures to carcinogenic stimuli or by selection pressures favoring the autonomous clonal derivatives. The initiated cells proliferate causing a fast increase in the tumor size. As the tumor grows in size, the cells may undergo further mutations, leading to increasing heterogeneity of the cell population (P Uma Devi, 1982).

As the tumor progression advances, the cells lose their adherence property, detach from the tumor mass and invade the neighboring tissues (Tracey et al., 2013). The detached cells also enter the circulating blood and lymph and are transported to other organs/tissues away from the site of the primary growth and develop into secondary tumors at the new sites. These form the distant metastases, resulting in widely spread cancers.

There are about 25,000 genes in each cell but mutation within single gene will not result in cancer growth. Cancer occurs when there is damage in the particular genes involved in carcinogenesis which are normal constituents of the human genome and
their products play a central role in the physiological regulation of cell proliferation and differentiation (Susan, 2007). Tumour development is caused by regulation errors or structural changes in this network. All aetiological factors in carcinogenesis target regulatory genes of cell cycle, invasion and metastasis. The following genes are involved in carcinogenesis which can be grouped into two classes:

**Oncogenes:** Cells contain many normal genes that are involved in regulating cell proliferation which are known as proto-oncogenes and some of these genes can be mutated by different genetic mechanisms (point mutations, translocations, duplications, amplifications, deletions, etc) thus promoting uncontrolled cell proliferation are known as oncogenes. Such mutations are dominant or gain-of-function mutations. Therefore, only one copy of the gene needs to be mutated in order to promote cancer (B Sadikovic et al., 2008) Mutations in these genes are almost always acquired (not inherited) e.g. Her2-neu, Ras, Myc, Src, Htert.

**Tumor Suppressor Genes:** They can be defined as genes which encode proteins that normally inhibit the formation of tumours. Their normal function is to inhibit cell proliferation, or act as “brakes” for the cell cycle. Mutations in tumour suppressor gene contribute to the development of cancer by inactivating that inhibitory function Mutations of this type are termed loss-of-function mutations. But most tumor suppressor gene mutations are acquired, not inherited (Bert Vogelstein et al., 2013) e.g. p53, RB, APC etc.

### 1.4 TYPES OF TUMORS

As cancer cells divide and replicate themselves and they often form into a clump of cancer cells known as a tumour. Depending on whether or not they can spread by invasion and metastasis, tumors are mainly classified into two types as below:

1. **Benign:** The term "benign" implies a mild and non-progressive disease. Benign tumours do not spread to other parts of the body and the cells do not invade other tissues because they typically are surrounded by an outer surface that inhibits their ability to behave in such a manner. They can usually be removed and do not come back in most cases. Common examples of benign tumours include Adenomas, Fibroids, Papilloma, Osteopath moles and uterine fibroids (Joshi et al., 2012). However there are some neoplasms defined as
'benign tumours' include tumours which produce a "mass effect" or tumours of endocrine tissues, which may overproduce certain hormones. Such tumours inspite of lacking the invasive properties of a cancer, may still produce negative health effects.

2. **Malignant (Cancerous):** Malignant is a medical term used to describe cancer as a severe and progressively worsening disease. Malignant tumour is characterized as having limitless growth potentials which is capable of invading and spreading to distant tissues (*metastasis*). Illustration of malignant tumors includes hematological malignancies like leukemia & lymphomas and solid malignancies like Carcinomas & sarcomas (Salvador et al., 2012)

![Malignant versus Benign Tumors](image)

**Fig 7: Developmental Stages of Tumor and tumour types**

1.5 **HALLMARKS OF CANCER**

Cancer is a disease, with the accumulation of mutations that promote the immortalization of proliferating cells and disrupt the mechanisms which should stop this from occurring can cause cancer. Genetic instability itself is therefore a mode of (carcinogenic) action that has the potential to promote unscheduled genetic alterations and cause cancer. In view of the fact that, genome governs all cellular functions, any kind of genetic damage can potentially allows activation of other modes that leads to cancer progression (figure 8). Hanahan and Weinberg suggested ten hallmarks of cancer which endows with a firm basis for understanding the biology of cancer (Hanahan D and Weinberg R, 2011).
1. Sustaining Proliferative Signaling

Normal cells need external growth factors for growth and proliferation. These signals are transmitted through receptors that pass through the cell membrane and maintain the normal tissue architecture and function of the cells. Cancer cells lose the capacity to grow and divide normally and they do not require external growth factors for proliferation (Harvey et al., 2000).

For example, Glioblastomas can produce their own platelet-derived growth factor (PDGF). Alternatively, in many cancer cells receptors can be over expressed. For example, HER2/neu receptor is over expressed in stomach and breast cancer. Where as in other cases mutated receptors can send signals without the presence of growth factors at all.

2. Insensitivity to anti-growth signals

The growth of normal cells of the body is under the control of growth inhibitors found within extracellular matrix and on surface of cells present in neighboring environment. Cancer cells have the ability to disrupt the negative feedback mechanisms and proliferate uncontrollably (Zvia et al., 2010) For example, the growth inhibitor retinoblastoma protein (pRB), prevents the inappropriate transition from (G1) to S. If pRB is damaged through a mutation in its gene or by interference from HPV cervical cancer is caused.
3. **Evading Apoptosis**

The normal cells of the body undergo apoptosis (also known as programmed cell death) when the cells are damaged or mutated. But the cancer cells are characteristically able to bypass this mechanism of cell death. The p53 tumor suppressor protein elicits apoptosis in response to DNA damage, and is a major mechanism of cancer control. Mutation of p53 is found in >50% of cancers (Soussi T and Wiman KG, 2007).

4. **Limitless Reproductive Potential**

The non-cancerous cells of the body die after a limited number of cell divisions. These cells of the body have an intrinsic property called the Hayflick limit that limits their multiplication to about 65 doublings (Harry Rubin, 2002) at which point they achieve a phase of senescence. Cancer cells are capable of overcoming this limit and continue to grow and divide uncontrollably. This limit can be overcome by disabling their pRB and p53 tumor suppressor proteins, which allows them to continue doubling until they reach a stage called crisis.

5. **Inducing Angiogenesis**

Like normal tissues, tumors require sustenance in the form of nutrients and oxygen as well as an ability to evacuate metabolic wastes and carbon dioxide. The process of angiogenesis produces tumor-related neovasculature. Few angiogenesis governing factors like signaling proteins vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (TSP-1) regulates mechanism of new blood vessel formation (Areck et al., 2010).

6. **Activating Invasion and Metastasis**

Cells in general are attached to its surrounding by adhesion molecules such as CAM (cell adhesion molecules), integrins, proteins and enzymes. Alterations of the adhesion molecules, integrins, proteins or enzymes may lead to metastasis and can be defined as the ability of cancerous cells to break off from its primary site and form tumors at a different part of the body (Myrthala et al., 2010). For example, N-CAM becomes altered and allows metastases in Wilm's tumor, neuroblastoma, and small cell lung cancer.
7. Genome Instability and Mutation

In normal cells when there is alteration in cell division, particular genes are triggered that either represses the mutagenic molecules or activate the DNA repair mechanism where as in the cancer cells multiple alterations in the genome serve as the foundation of many oncogenic processes. Several mutations in the genome cancer cell arise due to increased sensitivity to mutagenic agents, breakdown of one or more of the cell’s DNA repair mechanism mediated by genes such as p53 or BRCA1, or due to a combination of these factors.

8. Characteristic Tumor- Promoting Inflammation.

The tumor microenvironment is often infiltrated by innate and adaptive immune system cells that enable tumors to mimic inflammatory conditions seen in normal tissues. The current molecular cancer research indicates that the tumor – associated inflammation might aid in the tumor growth. Early inflammation releases chemicals into the tumor microenvironment and leads to genetic mutations that enable and accelerate the formation of tumor (Sergei et al., 2010).

9. Reprogramming Energy Metabolism

In aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria whereas glycolysis is more preferential whereas comparatively slight amount of pyruvate is dispatched to the oxygen-consuming mitochondria during anaerobic circumstances. Metabolism of glucose can be enhanced in Cancerous cells by upregulating the glucose receptors followed by glycolysis for pyruvate and imports lactate as an energy source as a part of the citric acid cycle. For example, Isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are recurrently mutated in gliomas and leukemia’s (Rob A. and Tak W., 2013).

10. Evading Immune Destruction

Immune surveillance is an essential cellular process that proactively prevents tumor formation in the body. The interactions between the immune system and the malignant cells play an important role in tumorigenesis. The failure of the immune system detect and reject transformed cells may lead to the development of cancer. Tumors use multiple mechanisms to escape from immune-mediated rejection and many of these mechanisms are now known on a cellular and molecular level (Igney F and Krammer P, 2002).
1.6 CLASSIFICATION OF CANCER

Cancers may be classified by their primary site of origin or by their histological or tissue types. Cancers are also classified individually according to their grade and stage. Classification of cancer is as follows:

A. Classification by site of origin:

By primary site of origin, cancers may be of specific types. For example, lung cancer, liver cancer, oral cancer, breast cancer, brain cancer etc. The most common tumors in India are breast, prostrate, oral and lung. Each tumor site and type represents its own specific set of clinical manifestations (Nguyen DV and Rocke DM, 2002; Robert J. and I.e.-Ming, 2010).

B. Classification by tissue types:

Cancers can be categorized into six main types on the basis of tissue of their origin:

1. Carcinoma: This type of cancer originates from the epithelial layer of cells that form the lining of external parts of the body or the internal linings of organs in body. As, epithelial tissues are most abundant in the body found in the skin to the covering and lining of organs and inner passages like gastrointestinal tract cancers of epithelial tissue (carcinomas), reports approximately for about 90% of all tumours. Carcinomas generally influence organs like bladder, breast, prostate, lungs, etc. Carcinomas are of two types – adenocarcinoma and squamous cell carcinoma. Development of Adenocarcinoma takes place within an organ or gland and squamous cell carcinoma originates in squamous epithelium (Ana R. et al., 2006) Adenocarcinomas disseminate very fast and may affect mucus membranes and are first seen as a thickened plaque-like white/colorless mucosa.

2. Sarcoma: These malignancies initiates in connective and supportive tissues counting bones, fat, muscles, and cartilage. Osteosarcoma is bone cancer that usually affects the youths. Sarcomas seems resembling to the tissue in which they grow (Lane KL et al.,1997) Other examples include chondrosarcoma (of the cartilage), leiomyosarcoma (smooth muscles), rhabdomyosarcoma (skeletal muscles), Mesothelial sarcoma or mesothelioma (membranous lining of body cavities), Fibrosarcoma (fibrous tissue), Angiosarcoma or
hemangioendothelioma (blood vessels), Liposarcoma (adipose or fatty tissue), Glioma or astrocytoma (neurogenic connective tissue found in the brain), Myxosarcoma (primitive embryonic connective tissue) and Mesenchymous or mixed mesodermal tumor (mixed connective tissue types) (Isaac R et al., 2005).

3. Leukemia: This is a group of cancers that are grouped within blood cancers. Bone marrow where blood cells are formed is found to be affected by these types of cancers, resulting in production of excessive immature white blood cells that fail to perform their usual actions and the patient is often prone to infection (Harvey et al., 2000). Various forms of leukemias are as follows:

- Acute myeloid leukemia (AML) – these are malignancy of the myeloid and granulocytic white blood cell series seen in childhood.
- Chronic myeloid leukemia (CML) – this is seen in adulthood.
- Acute lymphoid leukemia (ALL) – these are malignancy of the lymphoid and lymphocytic blood cell series seen in childhood and young adults.
- Chronic lymphoid leukemia (CLL) – this is seen in the elderly.
- Polycytemia Vera or erythremia – this is cancer of various blood cell products with a predominance of red blood cells.

4. Lymphoma: Lymphomas are solid cancers that may precisely affect lymphatic sites like brain, abdomen etc (Muhammad Wasif Saif et al., 2010). Which are referred as extra-nodal lymphomas. Lymphomas may be of two types – Hodgkin’s lymphoma and Non-Hodgkin’s lymphomas. Reed-Sternberg cells present within sample tissue typically discriminates Hodgkin lymphoma from Non-Hodgkin lymphoma.

5. Myeloma: Myeloma originates in the plasma cells of bone marrow and is capable of producing various antibodies in response to infections (Kah-Whye P. et al., 2009). It is a type of blood cancer.

6. Mixed Types: The type components may be within one category or from different categories. Some examples are adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, etc (David J Grignon, 2004).
C. Classification by grade

Cancers can also be classified on the base of grade. The anomalousness of particular cells with respect to surrounding normal tissues determines the grade of the cancer (Michael M. Shen and Cory Abate-Shen, 2010). Cells differentiated appropriately strongly bear resemblance to typical specialized cells and fit in to low grade tumors while undifferentiated cells are extremely anomalous with respect to surrounding tissues thus belongs to high grade tumors. Higher anomalies are associated with higher grades ranging from grade 1 to grade 4 discussed as below:

- Cells differentiated properly having minor anomaly falls in category of grade one.
- Moderately differentiated cells with little more abnormality falls in grade two.
- A poorly differentiated and extremely anomalous cell comes under grade three.
- Completely undifferentiated, naïve and immature cells falls in grade four.

D. Classification by stage

Cancers are also classified individually according to their stage and universally accepted method of classification is defined as TNM staging where T is tumor size, N is the degree of regional spread or node involvement, and M is distant metastasis (Donald et al., 2003).
T0 means lack of tumor evidences whilst T 1 to 4 suggests growing tumor size and association.

N0 show complete absence nodal involvement, N 1 to 4 signifies higher level of involvement of lymphatic nodes and evaluation of nodal involvement cannot be done is indicated by Nx.

M0 denotes no reported distant spread while dissemination far-away is implied by M1.

On the basis of TNM classification, stages can be alienated as follows:
- Zero stage: Carcinoma confined to cellular surface.
- I stage: Tumour restricted to the tissue of origin.
- II stage: Cancer limited with local spread.
- III stage: Local and regional widespread.
- IV stage: Advancement of tumour showing invasion and metastasis.

1.7 DIAGNOSIS OF CANCER

Various tests are carried out to screen whether cancer prevails or not. Some of the tests that are carried out are as follows (Sharon E et al., 2008).

1. Laboratory Test:

- **Complete Blood Count (CBC):** The common blood test is carried out to determine if red blood cells (RBCs), white blood cells (WBCs), and platelets having usual counts and visual aspects to differentiate among the diverse types of white blood cells and determine their relative percentages in the blood. It assists in detection of variety disorders associated with production, functioning and destruction of blood cells. It may also be used to monitor cell production and cell maturity in diseases such as leukemia.

- **Metabolism related tests:** measures the blood levels of sodium, potassium, calcium, chlorine, carbon dioxide, blood urea, creatinine, and protein and liver enzymes.

- **Blood protein testing:** A test to examine various proteins in your blood (electrophoresis) can aid in detecting certain abnormal immune system proteins (immunoglobulin) that are sometimes elevated in people with multiple myeloma.
Table 2: Details of Bio-markers associated with various malignancies.

- **Tumor Marker**: A tumor marker is a substance found in the blood, urine, or body tissues that can be higher amount in cancer, among other tissue types. Raised levels of numerous tumour markers are found to be related with progression of particular disease and they are used to detect the presence of cancer. These biomarkers can be created by the malignant or by non-malignant cells in response of tumour occurrence (James D. Brooks, 2012)(table 2).

2. **Imaging**: Imaging tests are used to determine whether the cancer has spread to other areas in the body and to evaluate the size and location of the tumor. Imaging tests alone are usually not specific enough to diagnose cancer (Samir S Taneja, 2004). Imaging is done by mainly following three ways:

- **Transmission Imaging**:
  
  (a) **X-rays**: are diagnostic tests that use invisible electromagnetic energy beams to produce images of internal tissues, bones, and organs on film

  (b) **Computed tomography scan**: CT scan uses a mixture of X-rays and computer technology to create cross-sectional metaphors called slices. A CT is much more precise and provides thorough snapshots of the body, together with bones, organs etc. than routine X-rays procedures.

  (c) **Mammogram**: A mammogram is an X-ray examination of the breast that helps in diagnosis of disease in women with or without complaints of pain, nipple discharge, lump etc in breast.
• **Reflection Imaging:**
  Reflection imaging refers to the type of imaging where visual image is generated by computerized analytical procedure when high-frequency sounds waves "bounce" off the various types of body tissues and structures at different pace, depending on the mass of the tissues or organ being studied (Metin N et al., 2009).

  **(a) Ultrasound or sonography:** produces sonograms that aids in visualization and assessment of proper functioning of internal organs and blood flow through vessels is the most commonly used type of reflection imaging. This technique is widely accepted for detection of cancer including that of liver, bladder, kidney, etc.

• **Emission Imaging:**
  Emission imaging occurs when tiny nuclear particles or magnetic energy are detected by a scanner and analyzed by computer to produce an image of the body structure or organ being examined.

  **(a) Magnetic resonance imaging (MRI)** is a diagnostic procedure that uses radio waves, huge magnets, and a processor all together to construct in depth pictures of tissue structures within the body. An MRI checks normal functioning of our vital body organs, detects numerous malignancies and infections. It also measures extent of bone and joint injuries and blood flow.

  **(b) Positron emission tomography (PET)** is a specific nuclear medication method where very small quantity of radioactive matter is utilized to study metabolic pattern of different body tissues in order to get their structural and functional details and also evaluate advancement of treatment, to spot out certain conditions. Hence, PET may identify biochemical variations within tissue that may be due to initiation of particular disease progression at earlier than any other imaging protocols.

3. **Biopsy:** A biopsy is a test where a small sample of tissue or cells is taken from the suspected cancer for examination. There are different types of biopsies which include:

  **(a) Bone marrow biopsy** is a process where anesthesia is given and small piece of solid sample is collected from bone marrow located at back of hip bone with help of specialized needle, for diagnosis of blood cancers. A core biopsy of the bone may be undertaken simultaneously to check that whether cancer that originated somewhere else in body has spread to marrow.
(b) **Endoscopic biopsy** uses Endoscopes can be inserted through the mouth or through a tiny surgical incision. Endoscopes that are thin, lighted, flexible tubes with cameras in order to view any abnormal areas of the internal body organs and pinch off tiny samples of the tissue using forceps that are part of the endoscope (Philip Hunter, 2007). Examples of endoscopic biopsy procedures include cystoscopy, bronchoscopy and colonoscopy.

(c) **Needle biopsy:** is often used on tumors that your doctor can feel through your skin, such as suspicious breast lumps and enlarge lymphatic nodules. Needle biopsy along with other imaging protocols is utilized to collect tissue samples from a suspected body organ which can't be sensed via skin.

There are various types of Needle biopsy procedures as follows:

- **Fine-needle aspiration:** may be the first type of biopsy done on smaller tumors that the doctor can feel through the skin. The doctor uses extremely thin, hollow needle linked with syringe to collect a small amount of fluid and cells from the suspicious area for examination and further testing.

- **Core needle biopsy:** is very similar to a fine needle biopsy with the only difference in needle size. The bigger needle is utilized to remove more volume of tissue for the ease to study sample by diagnostician.

- **Vacuum-assisted biopsy:** uses vacuum suction to accumulate a tissue through a particular hollow needle thus providing doctors with multiple samples from same biopsy site without introducing needle another time.

- **Image-guided biopsy:** combines an imaging procedure — such as X-ray, computerized tomography (CT), magnetic resonance imaging (MRI) or ultrasound — with a needle biopsy, permitting physician to approach suspected areas which can't be sensed via skin. Using synchronized metaphors, doctors can be certain about the position of the needle (Kirsten et al., 2010)

(d) **Surgical biopsy** involves a surgeon who makes incision in skin and takes out suspected tissue from the body for diagnosis. There are two kinds of surgical biopsies (Vincent Ki and Coleman Rotstein, 2008):

- **An incisional biopsy** eliminates a piece of soft tumor tissue, may be from muscle or fat, to distinguish between benign lumps and cancerous tumors.
• **An excisional biopsy** takes away lumps seen in breast or small lump that are enough to be entirely and simply detached during single course of action.

4) **Molecular screening:** The diversity of genomic alterations involved in malignancy had led to the development of a variety of assays for complete tumor profiling (Salvador et al., 2012) The new molecular diagnostics when integrated into existing histo-morphological classifications in surgical pathology provides additional stratification for a more accurate cancer prognosis. Several Cytogenetic procedures that study the chromosomes in the tissue sample with the aim to identify any chromosomal changes which are peculiar to known cancer types are as follows:

(a) **FISH technique** is a molecular cytogenetic technique in which probes are used to confirm presence or absence of specific DNA sequences on chromosomes. It is used in diagnosis of blood disorders or cancer which is due to specific genetic alterations on the chromosomes.

(b) **DNA microarray analysis** is equipped to measure the expression levels of large number of genes concurrently.

(c) **Immunocytochemistry (IHC):** is used to detect antigens or protein expression on a fixed tissue section by means of an antibody that is specific for the antigen/protein. The antibody antigen reaction is visualized by linking the antibody to an enzyme that catalyzes a color producing reaction or to a substance that fluoresces. IHC serves as an adjunct to regular histological exam of a tissue sample and is being routinely used to detect the presence of antigens, proteins, and biomarkers in neoplastic tissue samples. It has been employed largely for the detection of estrogen and progesterone receptors on breast tissues, to detect oncogenes and tumor suppressor gene products on tumor samples as well as to characterize leukemia’s and lymphomas (Richard W. Burry, 2011)

(d) **PCR** is a quantitative technique that permits amplification and analysis of target DNA Regions in tumor samples (Gang Wang et al., 2004).

(e) **Flow cytometry** is a technique that is used to examine and differentiate cells based on certain physical and chemical properties. A sample of blood or tissue cells in suspension is passed through the flow cytometer and the scatter emitted by the cell where it meets the light is analyzed to better characterize
the cell (Byron F. and Eric A., 2004).

(f) Electron microscopy is used when specific cellular or intracellular structures need to be examined (Cynthia S. and Sara E., 2009).

1.8 TREATMENT OF CANCER

Surgery, radiation and chemotherapy are the oldest treatment modalities for malignancies. These therapies may be used either alone or in combination with other therapies. Since each patient and each cancer is different, treatment must be individualized. The exact treatment choice or combination of choices will depend on the patient, the disease and the stage of the disease as well as other considerations such as performance status, and comorbid conditions. Other cancer treatment options include targeted therapy, immunotherapy, hormone therapy and marrow transplantation (Leisha A. Emens, 2010).

1. Surgery:

Surgery is the primary treatment for all the solid cancers and may play a role in prolongation of survival. Surgery of localized cancer takes away the entire mass of the tumor and in some cases it is the only treatment needed to eliminate cancer (Harvey et al., 2000). On the whole benign tumors are removed by surgery, but not always possible. Surgery plays vital role in the prevention, diagnosis, staging, cure and palliation. Surgery forms the basis of therapy for early cancer in which case it is employed as local treatment for undersized tumour, to decrease the massiveness of the disease, and for exclusion of metastatic tumors. Even though late stage cancers are mainly treated by chemotherapy, surgery could offer palliation in advanced cancers. Cryosurgery has been also used to destroy abnormal exterior tumors tissue by producing extreme cold with help of liquid nitrogen.

2. Chemotherapy:

Cytotoxic agents still form the basis of course of therapy for many cancers. It is either given as a therapeutic purpose or it may also be given lifelong to the patient. Chemotherapy is often used in conjugation with other cancer treatments such as radiation therapy or surgery. Chemotherapy also known as chemo is a type of cancer treatment that uses drugs to destroy cancer cells. The growth pattern of individual neoplastic cells may greatly affect the overall behavior of tumors and their responses to specific types of cancer therapy (Leisha A. Emens, 2010). The cell cycle gives us an insight into the kinetic behavior of dividing cells. The four distinct phases of the
cell cycle are: G1, G2, S and M phases. It works by causing hindrance in cell division cycle of cancer cells. Treatment schedules for chemotherapy vary widely. Patients may receive chemo in cycles.

**Table 3: list of some chemotherapeutic agents commonly utilized.**

3. Radiation therapy:

Radiation therapy is the administration of high frequency radiation like X-rays, gamma rays and charged particles to kill cancer cells in cancer patient for the purpose of cure, palliation or as an adjunct to surgical treatment. Radiation therapy can either damage DNA directly or create charged particles (free radicals) within the cells that can in turn damage the DNA and thus cells stop dividing or die. Confirmation of malignancy by pathological assessment, auxiliary progress and staging is required to be done prior to radiation treatment (Leonard Fass, 2008). It is often used in combination with surgery for eradication of small, limited human cancers. Preoperatively, radiation therapy may be given to shrink inoperable tumors or to destroy unrecognized peripheral projections of the tumor. This method is applicable to advanced tumors of the head and neck, colorectum and bladder. On the other hand, radiation therapy can be given post operatively to eradicate residual disease or to control subclinical disease in the wound or in the lymph nodes. Radiation therapy is also used for palliation in instances like cancers of the central nervous system and pathological metastasis to the bones (Todd Pawlickia and Arno J. Mundt, 2007).

4. Stem cell transplantation:

Bone marrow transplantation and peripheral blood stem cell transplantation are procedures that restore stem cells that were destroyed by high doses of chemo
and/or radiation therapy (Jeevani T, 2011). After above mentioned treatment, the patients receive the gleaned stem cells, which locomote themselves to the bone marrow and commence to produce new blood cells.

5. Hormone therapy:

Certain hormones can inhibit the growth of some malignant cells. Hormonal treatment for malignancies is nothing but the utilization of medication to block the effects of hormones. Doctors use hormone therapy for people with cancers that are hormone sensitive or hormone dependent. Hormone therapy involves changing the levels or activity of certain hormones that can cause certain cancers to cease growing or even undergo cell death. Surgical removal of the endocrine organs, such as orchiectomy and oophorectomy is also considered as a form of hormonal therapy. Examples of hormonal therapy for cancers include use of antiestrogens and LHRH analogues for breast cancer as well as use of antiandrogens and LHRH analogues for prostate cancer. Steroids (prednisone, dexamethasone) are used either alone or in combination with other cytotoxic agents for the treatment of leukemias, lymphomas and multiple myelomas. Steroids are also used in the management of hypercalcemia as well as for the tissue swelling that accompanied tumors of the lungs and the airway obstruction (Mark A. et al., 2011).

7. Gene therapy:

Cancer gene therapy is anchored on the premise that many cancers are due to genetic alterations that eventually lead to malignant changes in tissues. Gene therapy involves the transfer of genetic material into a cell to alter the cellular phenotype transiently or permanently. Gene transfer can be performed in-vitro or in-vivo. Different vectors exist for gene delivery into cancerous cells. Viruses (such as retroviruses) serve as a perfect tool for transmission of genetic material. Gene therapy for treatment of malignant neoplastic diseases is evolving and is largely still undergoing studies (Anirban M. and Ralph H., 2008).

8. Immunotherapy (biologic therapy or biotherapy):

Immunotherapy is a type of cancer treatment designed to boost the body's natural defenses to combat against cancer. It employs materials either prepared by the body or by research lab to reinstate immunity. Even though it is not well defined that how immunotherapy cures malignancy, but it possibly functions either by discontinuing or retarding tumour growth, preventing cancer from diffusing to other
body part, or increasing the immune functioning for abolishing tumour cells. Different kinds of immunotherapies are discussed as below (Narendra Kumar et al., 2012)

1) **Non-specific immunotherapies**: helps the immune system to destroy cancer cells. Non-specific immunotherapies are generally given later on or simultaneously with chemotherapeutic or radiation treatments with few exceptions. Two widespread non-specific immunotherapies are:
   - **Interferon** help the immune system fight cancer and may slow the growth of cancer cells. Eg. Interferon alpha (Roferon-A [2a], Intron A [2b], Alferon [2a]), is the most common type of interferon used in cancer treatment.
   - **Interleukins** help the immune system produce cells that destroy cancer. Eg. Interleukin-2, IL-2, or aldesleukin (Proleukin), is used to treat kidney cancer and skin cancer, including melanoma.

2) **Specific immunotherapy**: Cancer vaccines are another method used to help the body fight against disease. A vaccine divulges the immune structure to a protein (antigen) that triggers the immune system to recognize and destroy that protein or related materials. Prevention vaccines and treatment vaccines are two major kinds of tumour associated vaccines. For example, Gardasil is a vaccine that prevents a person from being infected with the human papillomavirus (HPV), a virus known to cause cervical cancer and some other types of cancer. At this time, sipuleucel-T (Provenge) is the only treatment vaccine approved in the United States.

6. **Targeted therapy:**
Recent advances in genetics and molecular cellular biology has led to exponential increase in our understanding of the molecular events that either initiate or sustain cancer growth. Whereas traditional chemotherapeutic agents may not differentiate between normal and cancer cells, the newer biological agents target specific molecular pathology (pathways and aberrant genes) in tumour cells. Monoclonal antibodies or small molecules inhibitors can be considered as Targeted curatives which can be utilized solely or by merging with other chemotherapeutics, surgery or radiation therapy (Michael M. Shen and Cory Abate-Shen, 2010).
   - **Antiangiogenesis drugs** are designed to prevent the formation of new blood vessels, thereby stopping or slowing the growth or spread of tumors.
   - **Monoclonal antibody** drugs are relatively new innovation in tumour inter-
vention. This Works in different manner like when a monoclonal antibody attaches to cancer cell, it can make the cancer cell more detectable to the immune system, obstructs proliferation signaling, and prevents angiogenesis, deliver radiation to cancer cells (Ralf A. and Bernd C., 2008).

<table>
<thead>
<tr>
<th>Agent</th>
<th>Molecular target</th>
<th>Disease indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab (Herceptin)</td>
<td>ERBB2</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>VEGFR</td>
<td>Metastatic colorectal cancer</td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>EGFR</td>
<td>Metastatic colorectal cancer</td>
</tr>
<tr>
<td>Alemtuzumab (Campath)</td>
<td>CD52</td>
<td>B-cell Chronic lymphocytic leukemia</td>
</tr>
</tbody>
</table>

Table 4: list of some common monoclonal antibodies used as anti-cancerous drugs

c. Tyrosine kinase inhibitors  A tyrosine-kinase inhibitors (TKI) are most common anti-cancerous drugs that inhibit enzymatic class of protein tyrosine kinases which are responsible for the activation of many other downstream proteins by various signaling pathways (Reason Wilken et al., 2012). The proteins are activated by phosphorylation mechanism.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular target</th>
<th>Uses/Disease indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>26S proteosome</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>brc-abl,PDGFR</td>
<td>CML, Philadelphia positive ALL</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>HER1/EGFR</td>
<td>NSCLC, pancreas</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>EGFR</td>
<td>NSCLC</td>
</tr>
<tr>
<td>Imatinib</td>
<td>brc-abl,PDGFR</td>
<td>CML, GIST</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>EGFR, HER2</td>
<td>HER-2 positive metastatic breast cancer</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>brc-abl,PDGFR</td>
<td>CML</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR, PDGFR, RAF-1</td>
<td>renal cell cancer</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR, PDGFR, RET, c-kit</td>
<td>renal cell cancer, GIST</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>mTOR</td>
<td>renal cell cancer</td>
</tr>
</tbody>
</table>

Table 5: list of most common TKIs used as anti-cancerous drugs.