Chapter-1: Introduction

1.1 Cancer:

Cancer in general is still considered as the death-nail for the affected unless it is detected early and treated with total compliance with the standard therapeutic regime. It is amongst the most challenging global issues of our times and can affect to any individual without any discrimination.

According to the definition given by the National Cancer Institute USA, cancer is a name given to a collection of diseases that are related to each other. It occurs when normal cells during the course of division stops responding to the regulatory signals and continue to divide indefinitely in the human body.

Cancer can start anywhere in the human body except the hair and nails. In most tissues it results in solid tumors. In blood, it results into haematological malignancy. The key feature of tumor being malignant unlike benign (non cancerous) is it can invade surrounding tissue and can travel to distant parts of the body through a process called metastasis (Sudhakar 2009). For example if the cancer cells from the gallbladder have spread to liver through circulatory networks the cancer is still known as gallbladder cancer with metastasis in liver not liver cancer.

The nomenclature of the cancer depends on the primary site of the human body where the cancer first started. The most common type in solid tumors are squamous cell carcinomas (SCCs), which are made up of highly heterogeneous groups of cells, which can occur in human anatomical sites like oral, esophageal, cervical etc. and are the leading cause of cancer mortality worldwide (Dotto and Rustgi 2016).

1.2 A brief history of cancer research:

In the last 200 years of cancer research, the field has evolved from the days when Physicians could observe malignant tumors, weigh them, and measure them without having any understanding of the molecular basis of cancer cells. Today developments in whole genome, exome and transciptome sequencing of the cancer cells is unravelling its genetic basis and revolutionising modalities used in cancer prevention, diagnosis and treatment (DeVita and Rosenberg 2012). Right from the very beginning the ultimate aim of the cancer research is to dissolve the suffering of cancer patients worldwide. Few images of cancer patients as depicted in figure 1.

Figure 1. Individuals suffering from cancer. (Informed consent taken)

Rudolf Virchow in 1863 with the help of a microscope elucidated the cellular origin of cancer (Virchow 1863). In 1889, Stephen Paget, proposed the seed-and-soil hypothesis as the reason behind the cancer metastases (Paget 1889). Subsequent findings have strengthened his hypothesis. It is been found that the occurrence of metastases as secondary growth to a different site is determined by several intricate interactions between malignant cells (considered as the seed) and their organ microenvironment (considered as the soil) (Langley and Fidler 2011). This has contributed immensely to our knowledge related to the cross talk between the tumor cells and their micro environment. Table 1 shows several such milestones.

Year	Discovery or Event	Relative Survival Rate
1863	Cellular origin of cancer (Virchow)	
1889	Seed-and-soil hypothesis (Paget)	
1914	Chromosomal mutations in cancer (Boveri)	
1937	Founding of NCI	
1944	Transmission of cellular information by DNA (Avery)	
1950	Availability of cancer drugs through Cancer Chemotherapy National Service Center	
1953	Report on structure of DNA	35%
1961	Breaking of the genetic code	
1970	Reverse transcriptase	
1971	Restriction enzymes Passage of National Cancer Act	
1975	Hybridomas and monoclonal antibodies Tracking of cancer statistics by SEER program	50%
1976	Cellular origin of retroviral oncogenes	
1979	Epidermal growth factor and receptor	
1981	Suppression of tumor growth by p53	
1984	G proteins and cell signaling	
1986	Retinoblastoma gene	
1990	First decrease in cancer incidence and mortality	
1991	Association between mutation in APC gene and colorectal cancer	
1994	Genetic cancer syndromes Association between *BRCA1* and breast cancer	
2000	Sequencing of the human genome	
2002	Epigenetics in cancer MicroRNAs in cancer	
2005	First decrease in total number of deaths from cancer	68%
2006	Tumor stromal interaction	

Table 1. Milestone discoveries and significant events in the cancer field and changing relative survival rates for cancer patients United States, 1863–2006.* Courtesy NEGM.
* Data are from the National Cancer Institute (NCI) program.

One landmark proposal came in 1914 from Theodor Boveri, said that cancer may be triggered by the chromosomal mutations (Boveri 1914). Through the last century of cancer research it has been proven that structural and chromosomal abnormalities are the hallmarks of malignant cells (Farkas, Jurányi et al. 2016). Table 1 lists out the most significant discoveries and events reported in the field of cancer in the last 2 centuries.

Cancer treatment has undergone a sea change in the last two centuries. Surgery was considered the first modality to treat cancer. The first evidence that surgery could cure cancer came in 1809 when Ephraim McDowell removed an ovarian mass without the use of anaesthesia.

Subsequently in 1846, anaesthesia (Warren 1846) and in 1867, antisepsis (Lister 1867) were introduced to surgical procedures that proved to be game changer (DeVita Jr and Rosenberg 2012). Figure 2 and 3 described in a precise way the timeline of the pivotal events in the field of cancer treatment and prevention respectively that changes the course of cancer management over a period of time. All these new discoveries have helped the cancer patients to live longer with dignity.

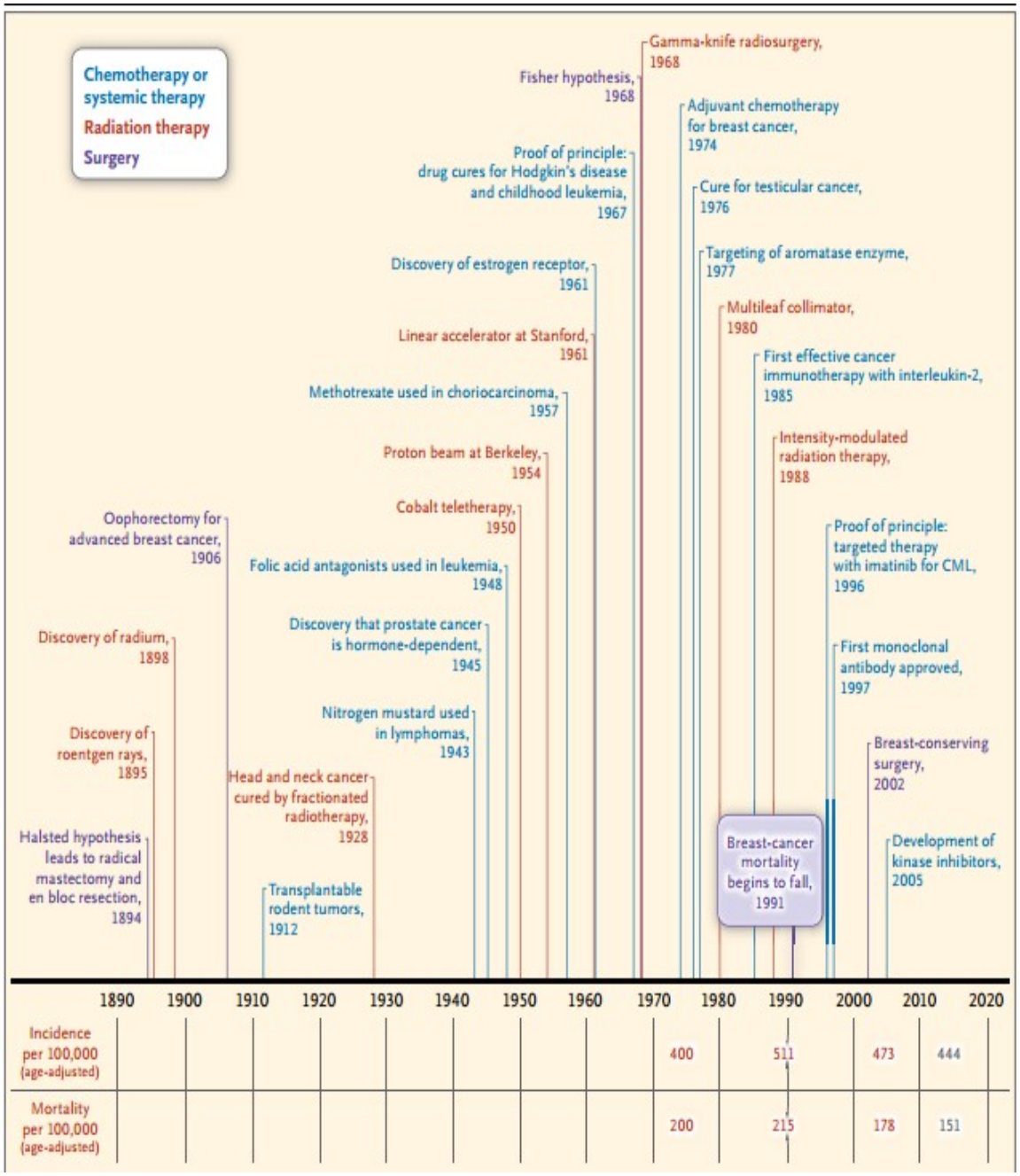

Figure 2. Timeline of pivotal events in cancer treatment. Courtesy: NEGM.; CML stands for chronic myeloid leukemia.

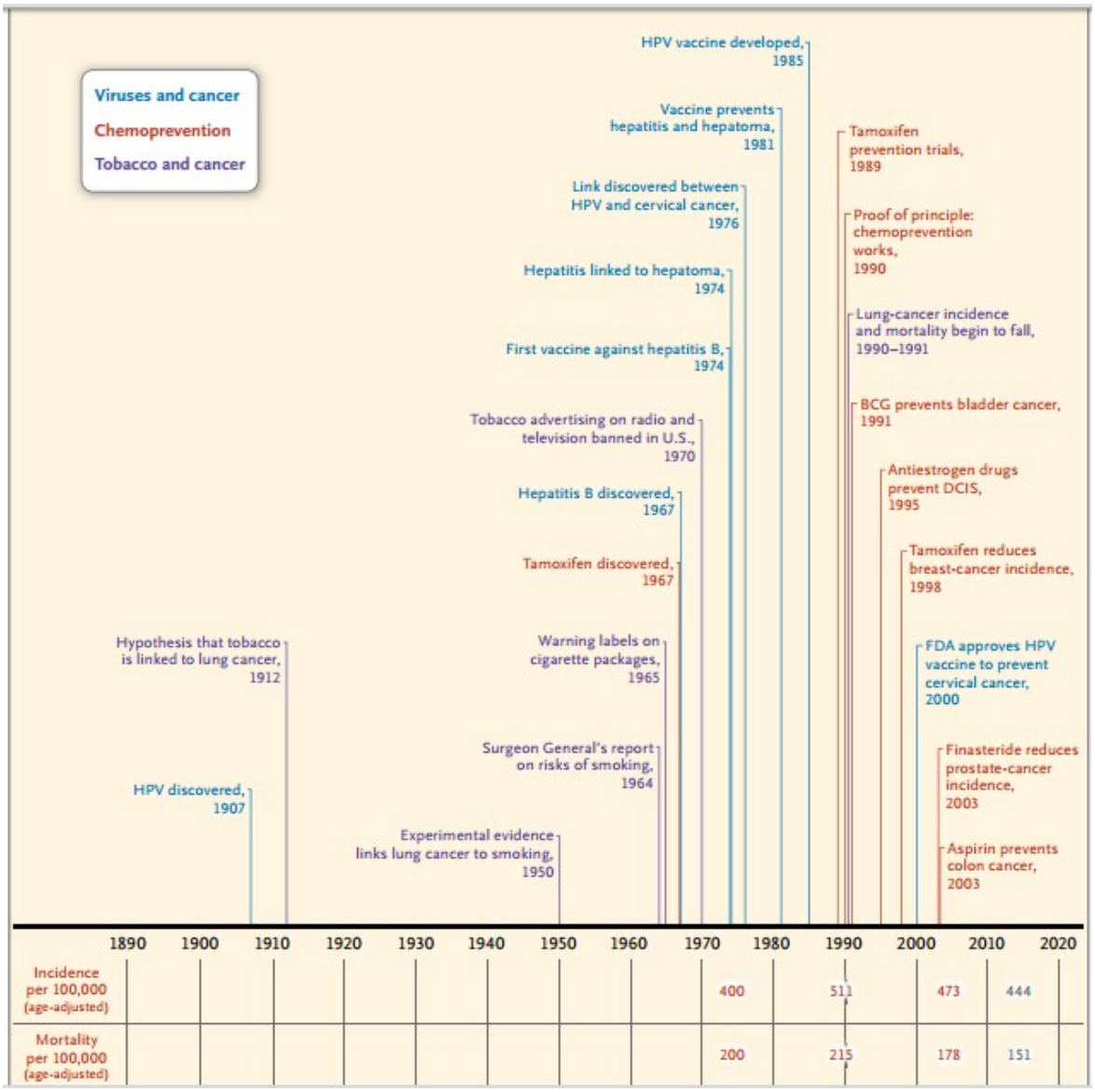

Figure 3. Timeline of Pivotal Events in Cancer Prevention. Courtesy: NEGM.; BCG: bacille Calmette–Guérin, DCIS ductal carcinoma in situ, HPV: human papillomavirus, and FDA: Food and Drug Administration.

1.3 Hallmarks of cancer:

Past and current research in cancer has established six perspectives, which are known as hallmarks that comprises of biological alterations in the human body due to the complex development of cancer. The six hallmarks of cancer according to Dr. Weinberg are sustaining proliferative signalling in the human tumor, escaping growth suppressors,

sustaining replicative immortality, preventing cell death, activating angiogenesis, and initiating local invasion and metastasis (Hanahan and Weinberg 2000, Hanahan and Weinberg 2011). There are two principal, underlying these hallmarks, one is genome instability that brings novel diversity in individual genome, and inflammation that can trigger several biological pathways that ultimately leads to activation of different hallmarks of cancer (Hanahan and Weinberg 2011).

1.4 Esophageal cancer:

Among the malignancies of gastrointestinal tract, esophageal cancer is known for its uniqueness as it comprises of two distinct histopathological types, adenocarcinoma and squamous cell carcinoma. Both types have their specific characteristics in terms of etiology, epidemiology, molecular, therapeutic and prognostic aspects. The life time risk of developing esophageal cancer 0.8 percent for men and 0.3 percent for women and it gradually increases with age (Enzinger and Mayer 2003). Esophageal adenocarcinoma is associated with gastro-oesophageal reflux and obesity, whereas esophageal squamous cell carcinoma (ESCC) is associated with use of tobacco and alcohol (Vaughan, Davis et al. 1995, Lagergren and Lagergren 2010).

Carcinoma started in the tissue that lines the inner or outer surface of the body like squamous cell lining of the esophagus. The principal precursor lesion of ESCC cancer is epithelial dysplasia (Kuwano 1998). Microscopically, this lesion represents an accumulation of atypical cells. Studies have shown that ESCC develops through a progressive sequence from mild to severe dysplasia, to carcinoma in situ, and finally to invasive carcinoma (Kuwano, Kato et al. 2005). ESCC is similar to head and neck cancer in their histology and association with tobacco and alcohol consumption.

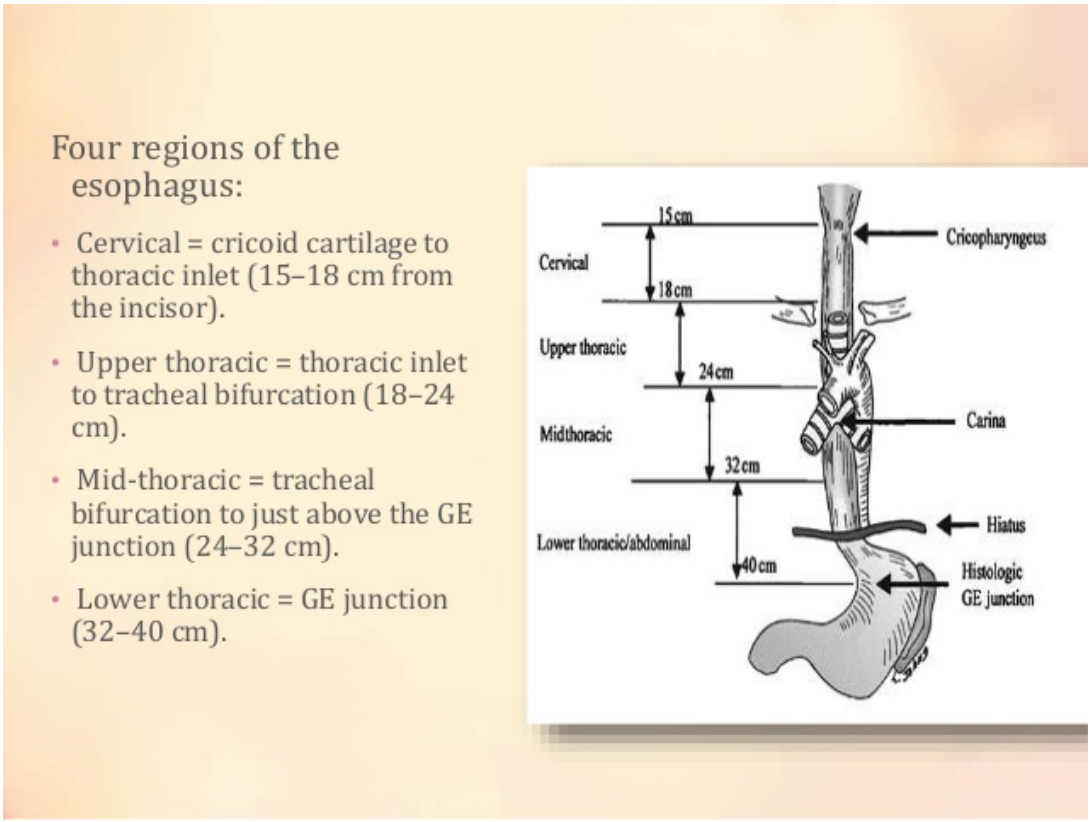

Figure 4. Four distinct region of esophagus in human body

(Source: http://www.slideshare.net/ruwidaalorfy/esopageal-cancer)

There are four regions of esophagus as described in Figure 4. Cervical esophagus starts from the cricoid cartilage to thoracic inlet, upper thoracic region starts from thoracic inlet to tracheal bifurcation. Mid thoracic esophagus starts from tracheal bifurcation to just above the gastro-esophageal (GE) junction and lower thoracic esophagus region is around the area of GE junction.

Dysphagia is the common symptom associated with esophageal cancer (Pennathur, Gibson et al. 2013). It warrants the need for endoscopy to further evaluate the symptom to either rule out esophageal cancer diagnosis or to establish it through histopathology reporting. Standard staging of esophageal cancer is required after the

confirmatory diagnosis that helps in selection of the best treatment modality. American Joint Commission on Cancer (AJCC) based tumor-node-metastasis (TNM) staging designed to be assessed to tailor made the treatment protocol in esophageal cancer as described in Figure 5 and Table 2.

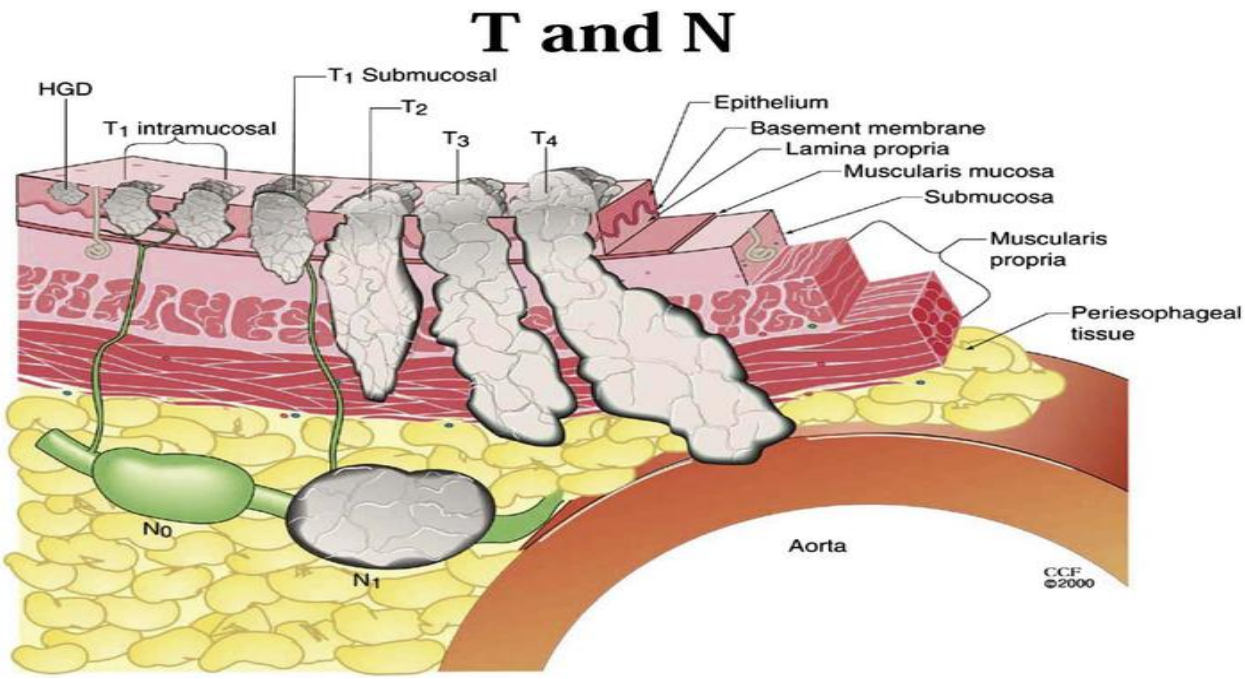

Figure 5. A pictorial representation of different stages in esophageal cancer (Source Absi A et al. 2013).

American Joint Commission on Cancer (AJCC) Staging for Esophageal Cancer
TNM Definitions
Primary Tumor (T)
TX: Primary tumor cannot be assessed
T0: No evidence of primary tumor
Tis: Carcinoma in situ
T1: Tumor invades lamina propria (T1a) or submucosa (T1b)
T2: Tumor invades muscularis propria
T3: Tumor invades adventitia
T4: Tumor invades adjacent structures
Regional Lymph Nodes (N)
NX: Regional lymph nodes cannot be assessed
N0: No regional lymph node metastasis
N1: Regional lymph node metastasis

N1a: One to three nodes involved
N1b: Four to seven nodes involved
N1c: More than seven nodes involved
Distant Metastasis (M)
MX: Distant metastasis cannot be assessed
M0: No distant metastasis
M1: Distant metastasis
• Tumors of the lower thoracic esophagus: M1a: Metastases in celiac lymph nodes M1b: Other distant metastases
• Tumors of the midthoracic esophagus: M1a: Not applicable M1b: Nonregional lymph nodes and/or other distant metastases
• Tumors of the upper thoracic esophagus: M1a: Metastases in cervical nodes M1b: Other distant metastases
AJCC Stage Groupings
Stage 0
Tis, N0, M0
Stage I
T1, N0, M0
Stage IIA
T2, N0, M0
T3, N0, M0
Stage IIB
T1, N1, M0
T2, N1, M0
Stage III
T3, N1, M0
T4, any N, M0
Stage IV
Any T, any N, M1
Stage IVA
Any T, any N, M1a
Stage IVB
Any T, any N, M1b

Table 2. American Joint Commission on Cancer (AJCC) Staging for Esophageal Cancer.

The principal precursor lesion of ESCC carcinoma is epithelial dysplasia that over the period of time advances from mild to severe. Under the microscope, dysplasia appears as an accumulation of atypical cells as described in figure 6. Studies have shown that ESCC develops through a progressive sequence from mild to severe dysplasia, to carcinoma in situ, and finally to invasive carcinoma along with the changes in molecular markers encompassing those alterations (Kuwano, Kato et al. 2005).

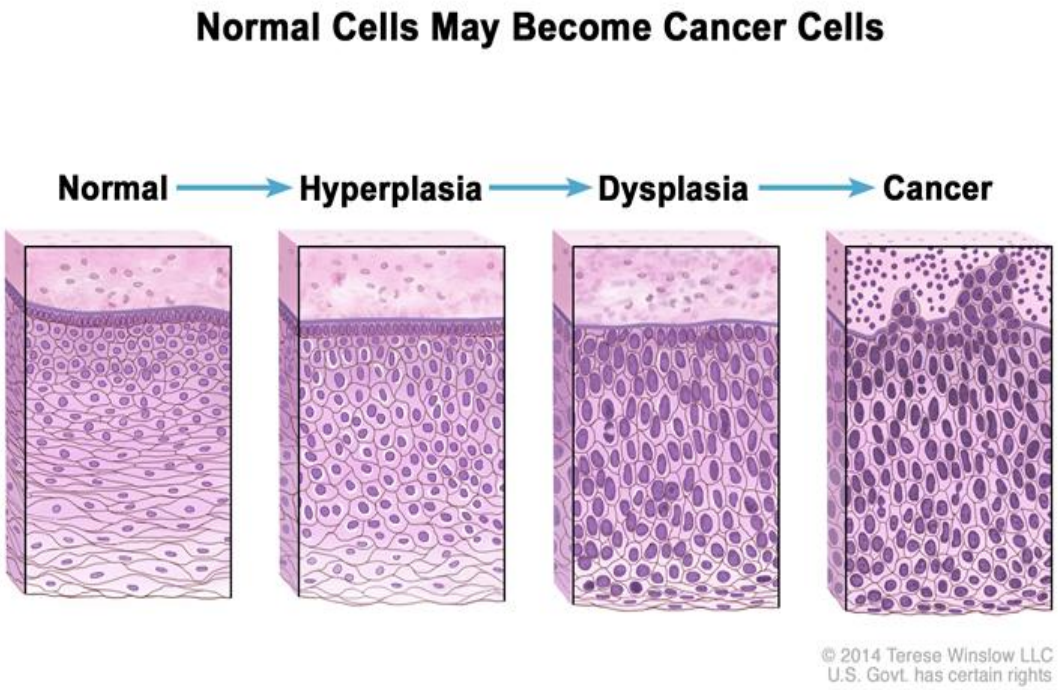

Figure 6. A representation of squamous cell carcinoma progression. Courtesy NCI USA.

1.5 Distribution of esophageal cancer:

With over 4,50,000 new cases annually, esophageal cancer is the 8th most common incident cancer in the world (Kamangar, Dores et al. 2006). In Western countries, the epidemiology of esophageal cancer has changed considerably over the past decades with a rise in the ratio of adenocarcinoma to squamous cell carcinoma. 16,640

new cases and 14,500 deaths from esophageal cancer was reported in 2010 in United States (NCI, 2011). Figure 7 depicts the age-standardized incidence and mortality (Both Sexes) GLOBOCAN data of esophageal cancer in major countries and regions in 2012.

Although the prevalence of gastroesophageal reflux is increasing in Asia, the prevalences of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) have remained low in most Asian countries (Chang, Cook et al. 2011).

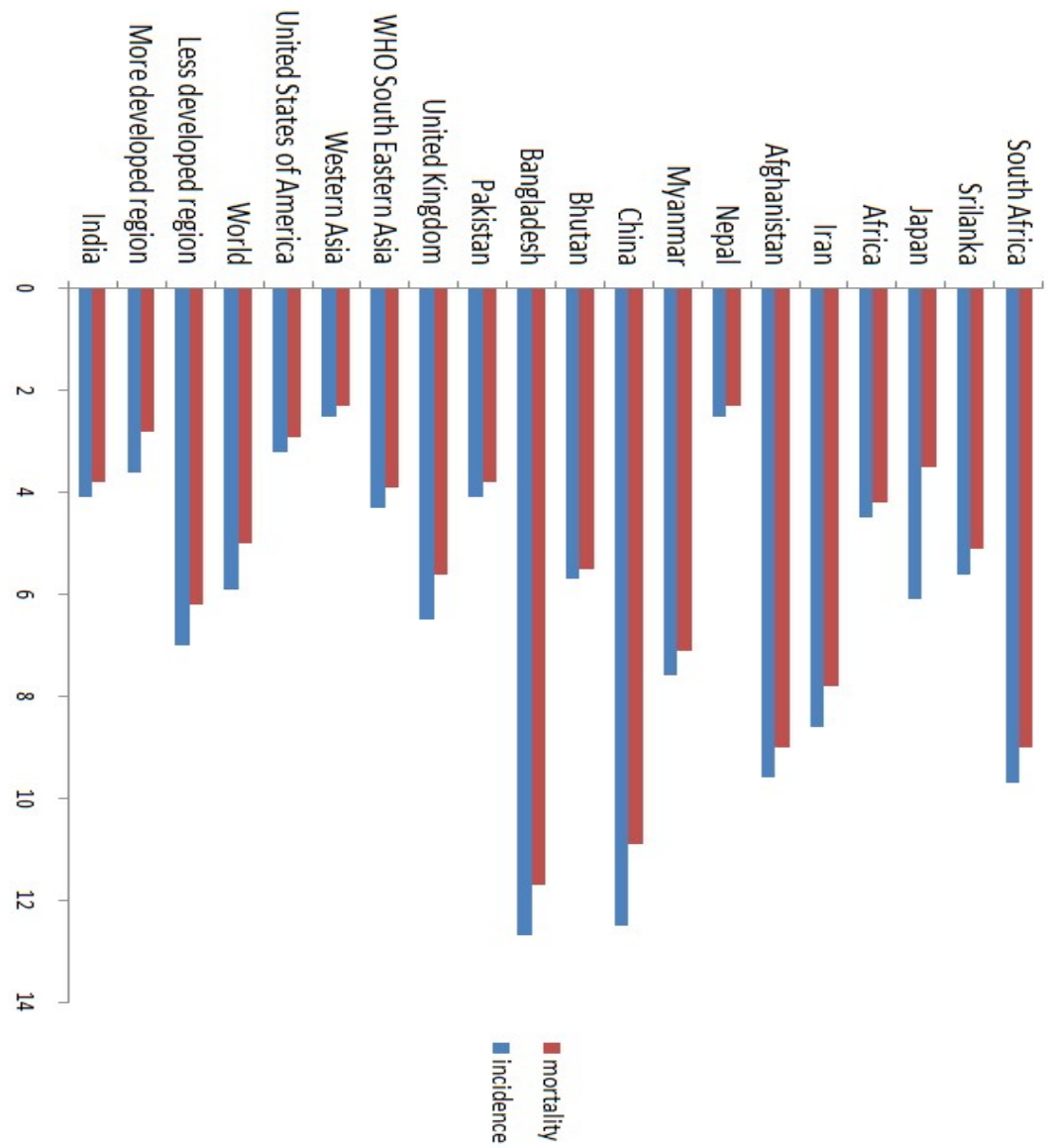

Figure 7. Comparison of estimated age-standardized incidence and mortality (Both Sexes) of esophageal cancer in major countries and regions in 2012. (GLOBOCAN 2012)

The age adjusted incidence rates (AAR) per 100,000 persons for males and females from Assam in Northeast India have been reported to be 32.6 and 21.1 in the district of Kamrup and 15.7 and 8.1 in the district of Dibrugarh as compared to AAR of 4.7 and 3.1 in Delhi (ICMR 2004).

1.6 Risk factors for esophageal cancer development:

Besides smoking and alcohol consumption that are the major risk factors for developing esophageal cancer (Vaughan, Davis et al. 1995). Additionally, poor nutritional status and dietary habits such as consumption of unconventional smokeless forms of tobacco, chewing betel quid and consuming fermented preserved food are possibly risk factors for people from this region and might contribute to higher mortality rates (Phukan, Chetia et al. 2001).

In a recent study reported from Iran where they collected lifestyle and dietary information through a structured questionnaire on 300 cases of esophageal squamous cell carcinoma patients and 571 controls matched individually for age, sex, and neighbourhood suggest roles for red meat intake, drinking water source, and food preparation methods in the development of ESCC (Golozar, Etemadi et al. 2016). Second hand smoking is also indicated as a risk factor for ESCC development in a study based on high incidence region of Kashmir (Rafiq, Shah et al. 2016).

Consumption of large quantities of salted tea and hot food and beverage for a longer duration of time was shown associated with the high risk of ESCC development (Islami, Boffetta et al. 2009, Dar, Bhat et al. 2015). It was also observed that low intake

of dietary selenium may be the putative risk factor for ESCC (Cai, You et al. 2006). Lack of selenium was found associated with the activation of genes involved in DNA damage, cell cycle regulation and oxidative stress and decrease in the expression level of detoxification related genes (Cai, You et al. 2006). Genetic polymorphism also plays a role in ESCC development where individuals with aldehyde dehydrogenase-2 (ALDH2) Lys/Lys and X-ray repair cross-complementing 1 (XRCC1) 399Gln allele carriers may be prevented from its development if they follow absenteeism from alcohol and tobacco (Cai, You et al. 2006).

1.7 Esophageal squamous cell carcinoma (ESCC):

With the exception of the USA, esophageal squamous cell carcinoma is histologically the most prevalent type of esophageal cancer worldwide and has a multifactorial origin. ESCC affects more than 4,50,000 people worldwide (Pennathur, Gibson et al. 2013). In addition to environmental components (Crawford 1994), several genetic factors are associated with esophageal carcinogenesis, such as chromosomal aneuploidy, allelic deletions, activation of oncogenes and inactivation of tumor suppressor genes (Kuwano, Kato et al. 2005). At the cellular level, all these factors contributes to disorders in cellular proliferation, differentiation and apoptosis (Daigo and Nakamura 2008, Khushalani 2008). Squamous cell carcinoma (SCC) of the human esophagus has a multifactorial etiology involving several environmental and/or genetic factors.

Besides smoking and alcohol intake, dietary and environmental factors that cause chronic irritation and inflammation of the esophageal mucosa can predispose underlying conditions, such as achalasia, tylosis, esophageal diverticula and webs, Plummer-Vinson syndrome, and human papillomavirus (HPV) infection (Absi et al. 2010).

1.8 Treatment Modality in ESCC:

The presentation of ESCC is insidious; at diagnosis, more than 50 percent of patients have either unresectable cancer or radiographically visible metastases, rendering management problematic (Enzinger and Mayer 2003). Surgical intervention is considered to be the most effective way of controlling the progression of esophageal cancer and ensuring long term survival. However, in most patients, surgery alone or other single interventions fail to achieve desired results. This led to combined therapeutic intervention in terms of chemoradiotherapy with or without radical surgery (Daly, Karnell et al. 1996).

The most commonly utilized chemotherapy agents belong to the class of fluoropyrimidines, taxanes, and platinum compounds. Unfortunately, even with multimodal approach, current treatments result in poor overall 5-year survival rate of 25% to 28% (Walsh, Noonan et al. 1996, Urba, Orringer et al. 2001, Burmeister, Smithers et al. 2005, Gebski, Burmeister et al. 2007).

Heterogeneity in response to chemoradiotherapy may be due to several factors, including age, sex, ethnicity, and drug-drug interactions. In addition, genetic variations in pharmacokinetic, pharmacodynamic, and drug action pathways have been shown to be important in determining sensitivity or resistance to treatment (Evans and Relling 1999).

1.9 Research question in relevance to the better understanding of treatment outcome and prognosis in ESCC

The northeast India has the highest incidence of ESCC in India despite that our understanding on the life style or molecular factors related to ESCC prevalent in this region which is socio-economically poor and backward is limited.

A regional comprehensive cancer hospital where cases for this study was recruited sees number of ESCC patients, most of them at advanced level during the time of clinical presentation. At Cachar Cancer Hospital and Research Centre, Silchar, most of the esophageal cancer patients who come for the treatment are from Assam and the adjoining states of Tripura, Manipur and Mizoram. On an average, the hospital registers around 170 ESCC every year. Majority of the ESCC patients presented with advance stage and hence their prognosis was poor. Therefore, there is a need for investigating the factors that may be influencing ESCC prevalence and treatment response for better clinical outcome. Table 3 listed the percentage wise figure of esophageal cancer in the local hospital where the patient was recruited for the current study

Year	Total reported cancer	Percentage of esophageal cancer
2015	1721	11.2%
2014	1736	9.8%
2013	1700	9%
2012	1611	10%
2011	1510	10%

Table 3. Percentage of reported esophageal cancer patients in Cachar Cancer Hospital and Research Centre (CCHRC)that has seen from the last 5 year.

The use of tobacco and its associated product is widespread in the north eastern region of India. Therefore, tobacco use like smoking, alcohol intake and other life style factors might have association with certain molecular markers like ALDH1, HER2, p16 in addition to the cell cycle parameter that may be impacting the clinical behaviour of ESCC. There might be an association between epidemiological, cell cycle, biochemical parameter, and molecular marker that could treatment response and prognosis in ESCC.

Therefore, this study aims to investigate the possible associations between life style factors, molecular markers, biochemical and cell cycle parameters and their impact on the treatment outcome and prognosis in ESCC.

1.10 Objectives:

1. **Study of life style, epidemiological and biochemical factors in ESCC.**
2. **Treatment response and prognostic factor investigation based on molecular markers in ESCC.**
3. **Cell cycle analysis to determine its impact on prognosis and treatment response in ESCC.**
4. **Inter-relationship among epidemiological, biochemical and molecular factors, and their impact on clinical outcome in ESCC.**