CHAPTER -1

INTRODUCTION
The term cancer refers to a group of diseases which share similar characteristics. Cancer can affect all living cells in the body, at all ages and in both genders. The causation is multifactorial and the disease process differs at different sites. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries (Ahmedin Jamal, DVM, Freddie Bray et al., 2011).

**Cancer In India:**

Among various diseases, cancer has become a big threat to human beings globally. As per Indian population census data, the rate of mortality due to cancer in India was high and alarming with about 8,06,000 existing cases by the end of the last century. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year. This is owing to the poor availability of prevention, diagnosis and treatment of the disease. All types of cancers have been reported in Indian population including the cancers of skin, lungs, breast, rectum, stomach, prostate, liver, cervix, esophagus, bladder, blood, mouth etc. The causes of such high incidence rates of these cancers may be both internal (genetic, mutations, hormonal, poor immune conditions) and external or environmental factors (food habits, industrialization, over growth of population, social etc.). In view of these facts, the present article describes the status of various types of cancers in India and its comparison at global level. Besides, attempts have been made to describe the main causes of cancer along with their preventive measures.

In addition to this, efforts have also been made to predict the effect of increasing number of cancer patients on the Indian economy (Imran Ali, Wasim.A et al., 2011).

A data of cancer patients was compiled from 2004 to 2010 in India and shown in **Figure 1**.
India has a National Cancer Control Programme which was established in 1975–76. This has contributed to the development of Regional Cancer Centres (RCCs), oncology wings in medical colleges and support for purchase of teletherapy machines. The District Cancer Control Programme was initiated but did not result in sustainable and productive activity.

Figure 1: Year wise total cancer prevalence in India [ICMR, 2006; ICMR, 2009].

Figure 2: Cancer prevalence in five metropolitan cities of India [Marimuthu, 2008].
The carcinogenic agents that people breathe, eat, drink and are otherwise exposed to, largely determine the occurrence of the disease. Personal habits such as the use of tobacco play a key role; people develop such habits in response to the social circumstances of life. Thus, the social origin of lifestyle must be considered in cancer prevention. The control of cancer requires the effective implementation of knowledge derived from more than two decades of successful research. It is now known that over one-third of cancers are preventable, and one-third potentially curable provided they are diagnosed early in their course. The quality of life of patients with incurable disease can be improved with palliative care.

Advanced technology is required in many situations and ongoing research initiatives might lead to better understanding of the disease and its control. (M.Krishna Nair, Cherian Varghese, R. Swaminathan : 2015).
*Pseudomonas aeruginosa* is an opportunistic pathogen capable of infecting virtually all tissues. It has been demonstrated that *Pseudomonas aeruginosa* produces at least two cytotoxic proteins against cancer cells. One protein is not known as a virulence factor or as an enzyme but another water soluble low molecular weight copper-containing redox protein named azurin (128 aa; 14 kDa) is involved in the electron transport chain. Besides its well documented function as a redox partner in electron transfer reactions, azurin has been found to have cytotoxic activity towards human cancer lines *in vitro* and *in vivo*. Azurin has cytotoxic activity against murine macrophage cell line J774 when its release was observed in the presence of eukaryotic proteins in the growth media [Yamada T, Hiraoka Y, Ikehata M, et al., 2004]. Besides azurin, cytochrome c551, believed to be a partner of azurin in electron transfer, also demonstrated somewhat reduced cytotoxic activity towards the macrophage cells. Since J774 is a tumour cell line, it was of interest to see if azurin would demonstrate similar cytotoxicity towards human cancer cells. Melanoma (UISO-Mel-2) Yamada T, Goto M, Punj V, Zaborina O et al., 2010] and breast cancer (MCF-7) were two models tested, where the efficacy of azurin to induce apoptosis and allow significant regression of these two cancers was noticed. In addition, intraperitoneal administration of azurin in nude mice xenografted with the two human cancer cells led to statistically significant tumour regression *in vivo*, with no apparent toxic effects to the animals. It has been demonstrated that azurin can enter preferentially into cancer cells and form a complex with the tumour suppressor p53, stabilizing it and inducing apoptosis. A chemically-synthesized 28-amino acid peptide derived from azurin (p28) has been approved by the FDA and entered Phase I clinical trials as an anticancer agent Fialho, A.M., das Gupta, T.K., Chakrabarty, A.M., et al., 2007].

Azurin has structural similarity with variable domains of immunoglobulins, thereby clearly demonstrating its single antibody-like structure. Interestingly, azurin has an ability to mediate high-affinity interactions with unrelated proteins relevant in cancer, conferring on it the property of a natural scaffold for therapeutic purposes. This effect has been already proven for the receptor tyrosine kinase EphB2-mediated cell signaling, since azurin, due to its interaction with this receptor, prevents its binding to the ligand.
ephrinB2 [Fialho, A.M., Stevens, F.J et al., 2007]. Azurin is a low molecular weight (14k Da), water-soluble blue copper protein produced by the bacterium Pseudomonas aeruginosa. It undergoes oxidation-reduction between Cu (I) and Cu (II), and transfers single electrons between enzymes associated with the cytochrome chain. Azurins and pseudoazurins participate in denitrification processes in bacteria [De Rienzo, F, et al., 2000].

Microbial based therapy of cancer is one of the emerging cancer treatment modalities. Over the past few years, important advances have been made to study and develop live bacteria, with or without additional cloned genes that encode toxins targeted specifically to cancer cells, or bacterial products with cancer killing ability. Various types of obligate anaerobic and facultative anaerobic bacteria can preferentially target cancer cells for growth in the hypoxic regions of the solid tumour. Attention has also recently been directed not only to the use of live bacteria, but bacterial proteins that can enter preferentially into cancer cells and disrupt their growth or kill them by multiple mechanisms [Bernardes, N et al., 2010].

Thus, the work is designed to identify the organism, Pseudomonas aeruginosa from immuno-compromised cancerous patients and identify the species using microbial staining, biochemical analysis, and DGGE as well as DNA sequence analysis which will enable to carry future research.

**Therapeutic Proteins / Peptides:**

Various peptides are nowadays used as therapeutic drugs (Lien and Lowman, 2003). The first commercial therapeutic peptide made available was Lypressin, a vasopressin analogue, developed by Novartis in 1970’s (Pichereau and Alllary, 2005). The so called therapeutic peptides have three main origins, they are isolated form nature (naturally occurring peptides or fragments or larger proteins) or developed based on chemical or genetic / recombinant libraries (Duncan Patrick, 2008; Sato et al., 2006)

Traditionally, therapeutic peptides are obtained from natural sources and clearly show some advantages over protein drugs such as antibodies (better tissue penetration
due to their smaller size) and small molecules (higher affinity/specificity with the target and lower toxicity profile). However, some disadvantages such as low in vivo stability, short half-life, lower potency comparing to antibodies and possible occurrence of immunogenic sequences have drawn back peptide industry (Sato et al., 2006).

Because of their length, peptides can be produced through chemical synthesis, recombinant DNA technology, cell-free expression systems, transgenic animals and plants or enzymatic synthesis. With chemical synthesis it is possible to use unnatural amino acids and conjugation of small molecules, which diversifies the produced peptides and increases their stability. (Vlieghe et al., 2010)

Advantages over other drugs:

As referred before, therapeutic peptides have several advantages over protein drugs, antibodies and small molecules. Comparing proteins and antibodies to peptide drugs, peptide drugs are more advantageous for several reasons: the complex conformation of proteins and antibodies made difficult its production and storage, whereas peptides don’t prompt conformational issues, are more stable and have lower manufacturing costs (synthetic versus recombinant production); the risk of immunogenicity posed by whole proteins and antibodies is very reduced in peptides sequences obtained from these proteins; because of their bigger size, proteins and antibodies have more restraints moving to its target; because of their smaller size, peptides have higher activity per mass than proteins and antibodies; because of peptide’s simpler intellectual property landscape, they have lower royalty costs (Lu et al., 2006; Vlieghe et al., 2010). Therefore, using short peptides derived from the interacting regions of proteins and antibodies and antibodies poses a great advantage over using the whole protein (Lu et al., 2006).

Compared to small organic molecules, therapeutic peptides also show several advantages. Because most therapeutic peptides represent small portions of functional regions of a protein, they are more efficient, selective and specific than organic
molecules. The shorter half-life of peptides reduces the risk of accumulation in tissues. Besides, degradation of peptides generates amino acids that do not pose a risk of systemic toxicity, whereas degradation of small organic compounds may lead to the formation of toxic substances (Vlieghe et al., 2010).

Nowadays most therapeutic peptides are chemically synthesized using the solid-phase peptide synthesis (SPPS) technique. Peptides produced through this technique often show higher purity comparing to recombinant techniques. SPPS also enables the use of unnatural amino acids and C-terminal amidation that confer higher stability to the peptide (Vlieghe et al., 2010).

Several strategies can be used in order to increase peptide stability. The most used modifications are the addition of polyethylene glycol (PEG) to the N or C-terminal, peptide cyclization, use of D-amino acids and unnatural amino acids. N-terminal modifications (acetylation and glycosylation), C-terminal modifications (amidation) and fusion of Fc domain of human gamma immunoglobulin (IgG) (Sato et al., 2006).