CHAPTER -2
CURRENT PROBLEM
Cancer Treatment:

Treatment facilities are also mostly limited to urban areas of the country. There are no uniform protocols for management, and the availability and affordability of cancer treatment shows wide disparities. The majority of patients with cancer present to a cancer treatment center in late stages of the disease (80% are advanced) and this adds to the already high morbidity, mortality and expenditure. Treatment results are about 20% less than what is observed for similar conditions in more developed countries, mostly due to late diagnosis and inappropriate treatment. Pediatric cancers are highly curable but this has not been achieved in India due to lack of access to quality care and lack of support systems.

- **Pain relief and palliative care** - Oral morphine is the mainstay for cancer pain relief and is still not widely available in the country. There is a serious limitation of manpower for providing palliative care.

- **Finances** - The funds for the cancer programme are mainly from the Government and need to be augmented. Private initiatives are few and are unlikely to cater to a large population across different socioeconomic strata, as it is often not a financially viable venture.

Existing cancer treatment methods may be divided into four main categories:

- Surgery
- Radiation therapy (including photodynamic therapy)
- Chemotherapy (including hormonal therapy and molecular targeted therapy)
- Biological therapy (including immune therapy and Gene therapy) (Sausville and Longo 2005)

We may use these treatment methods singly or in combinations based on the assessment of the drug toxicity and anti-tumour efficacy (Humes 2001).

Nowadays, India is growing with a good progress rate and probably will become a developed country within a few decades resulting into its participation in the world development. Therefore, it is important to study the status of cancers in India so that advance measures may be taken to control this havoc in near future. In view of these facts, attempts have been made to study the status of cancers in India including its
causes, preventive measures, effect on Indian economy and comparison with global scenario. (Imran Ali, Wasim A et al., 2011).

**Anti-Cancer Peptides from Bacteria**:

Cancer is a leading cause of death in the world. The rapid development of medicine and pharmacology allows to create new and effective anticancer drugs. Among modern anticancer drugs are bacterial proteins. Until now has been shown anticancer activity among others azurin and exotoxin A from *Pseudomonas aeruginosa*, Pep27anal2 from *Streptococcus pneumoniae*, diphtheria toxin from *Corynebacterium diphtheriae*, and recently discovered Entap from *Enterococcus* sp. A lot of antitumour peptides, including some of bacterial peptides are characterized by low molecular weight and hydrophobicity. These features appear to be important for the penetration stage of these peptides into tumour cells, the surface of which differs in some of the characteristics from normal cells. Bacterial peptides are a specific group of anticancer drugs, now widely studied. Some of these are in clinical development what gives us hope for their pharmacological use in cancers treatment. (Tomasz M.Karpinski and Anna K.Szaradkiewicz, 2013).

Attempts to use bacteria or their products for the treatment of cancer dates back to turn of the XIX and XX century. William Coley (Coley, 1909) in the treatment of patients with unresectable tumours applied the treatment with bacterial culture supernatants of *Streptococcus pyogenes* and *Serratia marcescens*. This preparation called Coley’s toxins was used in approximately 1200 patients with malignancy, often yielding regression of the tumour and in 30 patients, supposedly a complete cure. Currently, it is assumed that the main factor responsible for therapeutic effect of Coley’s toxins was induction of enhanced tumour necrosis factor-α (TNF-α) secretion in the body of the patient. The antitumour efficacy of TNF-α was confirmed in an animal model, observing the inhibition of growth and even complete regression of the tumour (Gratia and Linz, 1931; Carswell et al., 1975).

Therapeutic peptides are a promising class of therapeutics that present many advantages over classical drugs like proteins, antibodies and small organic molecules. Therapeutic peptides have a higher affinity to the target and lower toxicity profile than
small size, peptides can be modified in order to increase its stability and potency. (Lu et al., 2006; Vileghe et al., 2010).

Peptides have already shown to be useful in many pathologies like diabetes, infective diseases (bacterial, fungal and viral) oncology and osteoporosis (Vlieghe et al., 2010). The majority of these peptides are derived from protein active sites although some are designed based on genetic, recombinant and chemical libraries (Duncan Patrick, 2008).

**Azurin**: Azurin, a secondary metabolite derived from bacterial species especially from *P. aeruginosa* function as a donor in terminal electron transfer process. (Pozdnyakova I, et al., 2001). Azurin, a redox protein recently fascinated biomedical researcher’s immense interest as an anticancer therapeutic agent which enters human breast cancer cells and induces apoptosis without any adverse effects in cancer patients. (Yamada T, et al., 2002). Azurin is also produced by other pathogenic bacteria besides *P. aeruginosa*. The azurin-like protein produced by meningitis-causing bacteria, *Neisseria meningitides* is termed Laz. Unlike most azurin produced by other bacteria, laz is not periplasmic but surface expressed. It has an additional 39 amino acid lapidated tail in its N-terminal called H.8 epitope. Comparing to azurin, Laz is much more efficient entering glioblastoma cells and has higher cytotoxicity level against these cells. This suggests that H.8 epitope is important in disrupting entry barriers to glioblastoma cells. The cloning of the H.8 epitope in other toxic drug candidates might be very promising in the treatment of brain tumours (Fialho et al., 2008).

It is involved in the denitrification process (Edward et al., 1992); Webb and Loppnow, 1999). It is acting as an electron transfer shuttle in *Pseudomonas aeruginosa* and other bacteria. In fact, considering its anticancer activity, studies revealed that azurin preferentially enter breast cancer cells and induce apoptosis (Yamada et al., 2002b) cell cycle arrest (Chaudhari et al., 2007) and inhibits angiogenesis (Mehta et al., 2011).

The presence of copper ion gives this protein a number of features, including an intense blue color, a high reduction potential and a small parallel hyper fine coupling in the electron spin resonance spectrum (Adman, 1991). In particular, azurin’s p28 peptide, corresponding to aminoacids 50 to 77 which include the PTD (Protein Transduction
Domain) responsible for cell entry is able to induce apoptosis and inhibit angiogenesis and recently ended Phase I clinical trials (CDG, 2011; Mehta et al., 2011; Yamada et al., 2009).

Another azurin derived peptide, p26, corresponding to aminoacid 88 to 113, was identified as being structurally similar to ephrin B2, the Eph B2 ligand. P26 is able to competitively bind to Eph B2 inhibiting cellular signaling pathways that ultimately contribute for cancer growth (Chaudhari et al., 2007).

Recently, P-cadherin, which is associated with breast cancer patients with poor prognosis (Paredes et al., 2007; Ribeiro et al., 2010), was identified as a new target molecule for azurin (Fialho, 2009).

**Nanotechnology:**

One of the ongoing and important objectives of biomedical sciences is to find an effective strategy for cancer treatment. For this to become a reality, laboratories worldwide are performing research to find effective methods to eradicate tumour cells. These activities have resulted in a number of drugs, some of which are effective if properly targeted. We are now facing these severe challenges of drug delivery and targeting. Nanotechnology can greatly help us to meet these challenges.

Az protein was recently utilized in nanoelectronics. Az is able to enter malignant cells more readily than healthy cells and ultimately destroys the cells. Az may be considered as an effective anti-cancer agent when targeted appropriately. (Kianoosh Keyhanian et al., 2010).

A study by Kuipers et al., 2009 showed that Az as an anticancer candidate with the special features presented in this report, and with the assistance of nanotechnology methods potentially may help us to unravel some problems of cancer treatment and may overcome some of the therapeutic challenges. Some limitations of the peptides instability in vivo have been recently addressed as the researchers reported the synthesis of “thioester-bridged Az peptide fragment” which is aimed to resist the proteolytic degradation.
If Az is used with appropriate nanoparticles, we can handle not only the tumour cells drug resistance but also other dilemmas like drug toxicity and problems in drug targeting, drug release and drug dosage adjustment could be eliminated. Therefore, by applying nanotechnology for the delivery of Az joined with other nanoparticles, more effective elimination of cancer cells may be achieved.

These are number of outstanding challenges in using nanotechnology for cancer treatment by Az. There exist several nanoengineering and biophysiochemical problems with materials at such a small scale to be solved. Materials could have different and sometimes peculiar behaviour and interactions with Az in nanoscale. Also, because of large nanomaterials surface relative to their volume, surface effects are much more serious in nano systems than they are in large systems. Therefore further studies seem essential to determine the interaction between various nanotechnology platforms and Az and their combines effect on healthy cells and tissues before their use. Nevertheless, the fact that we are potentially able to overcome chemotherapeutic limitations of Az through nanotechnology is undeniable.

Even the source of Az in most the studies was from either soil isolates or particular ATCC strains. Clinical isolates will have more compatibility with human cells / tissues when compared to soil isolates in invading the targeted cells. Not much light is thrown in this area in spite of so many successful studies to treat cancer using Az. Our study is based on the isolation of \textit{P. aeruginosa} clinical isolates from Immunocompromised patients and extraction of azurin from those strains and to check how efficient the Az is on selected cancer cell lines.