Chapter-I

Introduction
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INTRODUCTION

Cancer is one of the most frightening diseases and even 4 decades after President Nixon’s declaration of the “war on cancer” in 1971; it still is challenging the entire scientific community.

1.1 Environmental carcinogenesis

Cancer incidence rates are escalating at an alarming pace. By 2020, the world population is anticipated to increase up to 7.5 billion; of this number, about 15 million new cancer cases will be diagnosed, and around 12 million cancer patients will be lost to the clutches of death (Bray and Moller, 2006). This increase can be largely attributed to environmental contaminants as it is estimated that as many as two-thirds of all cancer cases are linked to environmental causes (NCI, 2004). The association between the environment and the occurrence of particular kinds of cancer were first recognised when Dr John Hill, a London physician, published his conclusion on the relationship between nasal cancer and the use of snuff in 1761 (Hajdu, 2011). Since then hundreds of confirmed and suspected environmental carcinogens have been identified. For instance, asbestos exposure is linked mainly to lung cancer, whereas exposure to benzidine, a chemical found in many dyes, is associated with bladder cancer. In contrast, smoking is associated with cancers of the lung, bladder, mouth, colon, kidney, throat, voice box, esophagus, lip, stomach, cervix, liver, and pancreas (NCI, 2004). Every year, at least 200,000 people die worldwide from cancers related to their workplace (WHO, 2007). Figure 1.1. shows various cancers that have been linked to environmental carcinogens. While data and studies abound, our current understanding of the relation between cancer and the environment is still very limited. Understanding environmental carcinogenesis is critical to its effective management.

One of the environmental hazards are pesticides whose exposure has been associated with brain/central nervous system (CNS), breast, lung, ovarian (female spouses), pancreatic, kidney, testicular, colon, and stomach cancers, as well as Hodgkin and non-Hodgkin lymphoma, multiple myeloma, and soft tissue sarcoma (Reuben, 2010).

1.2 Pesticides - As environmental mutagen and carcinogen

Pesticides are used to eliminate or control unwanted or harmful insects, plants, fungi, animals, or microorganisms in order to protect food crops and other plants, are environmental mutagens that are in use since 2000 BC (Fishel, 2009). The first known pesticide was elemental sulfur dusting used in ancient Sumer about 4,500 years ago in...
ancient Mesopotamia (Sharma and Anmol, 2010). Use of synthetic pesticides became widespread in 1940s (Daly et al., 1998). 1940s and 1950s have been considered as the start of the "pesticide era" (Murphy, 2005) and since 1950 their use has increased 50-fold with an annual consumption of industrial pesticides being approximately 2.3 million tonnes these days (Miller, 2002).

Figure 1.1. Various cancers that have been linked to environmental carcinogens. The carcinogen linked to each cancer is shown inside bracket.

(Anand et al., 2008)

Although about 75% of the total pesticide consumption of the world is in developed countries, but use in developing countries is also increasing rapidly and over the last few decades pesticides have become a common household item in rural areas of the developing world (Miller, 2004).
Undoubtedly, chemical pesticides have contributed appreciably to the human health sector by increasing agricultural yield by controlling pests and also by protecting from insect-borne diseases (malaria, dengue, encephalitis, filariasis, etc.) (Abhilash and Singh, 2009). But, besides these beneficial effects there are certain deleterious consequences of pesticide exposure on non-target species including human beings. Pesticide-induced environment spoiling effects first came into light in 1960s when it was discovered that dichlorodiphenyltrichloroethane (DDT), first synthetic pesticide of modern age, was preventing many fish-eating birds from reproducing thereby posing a serious threat to biodiversity. This menace was well expounded on by Rachel Carson in the book called ‘Silent Spring’. Although the agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants, but it is still being used in many developing countries including India to prevent malaria and other tropical diseases (Lobe, 2006).

1.2.1 Indian scenario: Use of pesticides

India is primarily an agrarian society with agriculture accounting for 14.6 percent of the country's gross domestic product (GDP) in 2009-10, and 10.23 percent (provisional) of the total exports. Furthermore, agriculture sector provides employment to 55 percent of the total work force in India (Emerlson Mose, 2012). A large portion of Indian population is engaged in agriculture and is consequently exposed to the pesticides used in agriculture. At present, the Indian pesticide industry encompasses more than 125 basic producers of large and medium scale and more than 500 pesticide formulations. According to WHO, India has now become the second largest industry in the whole of Asia and twelfth largest in the entire world with a production of 90,000 metric tons of pesticides annually (WHO, 2009; Abhilash and Singh, 2009).

Though only 20% of the world's agrochemicals are used by developing countries, they endure 99% of deaths due to pesticide poisoning (Kesavachandran et al., 2009). Main reasons for this scenario are unsafe methods of pesticide application and handling practices. These safety issues are further worsened by the illiteracy and poverty which prevail in most farming communities of developing countries like India. Furthermore, most class-I technical grade pesticides which are either banned or strictly regulated in the developed world, are easily available in developing countries at places that even lack the resources for their safe application (Eddleston et al., 2002). Table 1.1. shows some of the pesticides banned/severely restricted in some countries of world but are still being used in India. Even in the 1990s more than 70% of the total pesticides used in
agricultural applications in India comprised of formulations that are either banned or severely restricted in the other countries (Abhilash and Singh, 2009).

Table 1.1. Banned/ restricted pesticides in other countries, still widely used in India.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Pesticides</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Benzene Hexachloride (BHC)</td>
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<tr>
<td>2.</td>
<td>DDT</td>
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<tr>
<td>3.</td>
<td>2,4-Dichlorophenoxyacetic acid (2,4-D)</td>
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<tr>
<td>4.</td>
<td>Dichlorovos</td>
</tr>
<tr>
<td>5.</td>
<td>Dimethoate</td>
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<td>6.</td>
<td>Endosulfan</td>
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<td>7.</td>
<td>Methyl Parathion</td>
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<tr>
<td>8.</td>
<td>Lindane</td>
</tr>
<tr>
<td>9.</td>
<td>Monocrotophos</td>
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<tr>
<td>10.</td>
<td>Mancozeb</td>
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<tr>
<td>11.</td>
<td>Paraquat</td>
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</table>

(Kuruganti and Sudhakar, 2005)

1.2.2 Adverse effects of pesticides

Exposure to pesticides both occupationally and environmentally causes a range of human health problems. It has been observed that the pesticides exposures are increasingly linked to rising incidence of cancer, chronic kidney diseases, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and behavioral disorders (Abhilash and Singh, 2009) and especially among people like farmers, pesticide applicators, crop duster pilots, and manufacturers who have high exposures to pesticides (Gutman, 2009).

Pesticides have been categorized into various classes by WHO (WHO, 2000) on the basis of their toxic effects:

- **Class Ia** - extremely hazardous
- **Class Ib** - highly hazardous
- **Class II** - moderately hazardous
- **Class III** - slightly hazardous
Class U - unlikely to present acute hazard

1.2.3 Pesticides and cancer

It is very imperative that every pesticide new or in use undergoes complete assessment for potential toxic and carcinogenic risks even before getting introduced into the market. In India all pesticides have to essentially go through the registration process with the Central Insecticides Board & Registration Committee (CIB & RC) before they can be made accessible for use or sale. While in US Environmental Protection Agency (EPA) is the agency that registers and ranks pesticides on the basis of possible carcinogenic risks posed by them. Around 1,400 pesticides have got registered by EPA. Though all have not got tested yet, exposure to these chemicals has been associated with brain/central nervous system (CNS), breast, lung, ovarian (female spouses), pancreatic, kidney, testicular, colon, and stomach cancers, as well as Hodgkin and non-Hodgkin lymphoma, multiple myeloma, and soft tissue sarcoma (Reuben, 2010). Due to these carcinogenic risks a number of pesticides have been banned or their use has been restricted. These comprise ethylene oxide, amitrole, some chlorophenoxy herbicides, DDT, dimethylhydrazine, hexachlorobenzene, hexamethylphosphoramidate, chloredene, lead acetate, lindane, mirex, nitrofen, and toxaphene (NCI, 2004). Some of the pesticides banned in India due to their toxic effects, are mentioned in Table 1.2.

Table 1.2. List of Pesticides banned in India.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Pesticides</th>
<th>Sl. No.</th>
<th>Name of Pesticides</th>
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<tbody>
<tr>
<td>1.</td>
<td>Aldrin</td>
<td>16.</td>
<td>Pentachloro nitrobenzene</td>
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<tr>
<td>2.</td>
<td>BHC</td>
<td>17.</td>
<td>Pentachlorphenol</td>
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<td>3.</td>
<td>Calcium Cyanide</td>
<td>18.</td>
<td>Phenyl Mercury Acetate</td>
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<td>5.</td>
<td>Copper Acetoarsenite</td>
<td>20.</td>
<td>Tetradifon</td>
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<td>6.</td>
<td>Dibromochloropropane</td>
<td>21.</td>
<td>Toxaphene</td>
</tr>
<tr>
<td>7.</td>
<td>Endrin</td>
<td>22.</td>
<td>Phosphamidon 85% SL</td>
</tr>
<tr>
<td>8.</td>
<td>Ethyl Mercury Chloride</td>
<td>23.</td>
<td>Methomyl 12.5 % L</td>
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<tr>
<td>12.</td>
<td>Methomyl 24% Formulation</td>
<td>27.</td>
<td>Ethylene Dibromide</td>
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</tr>
<tr>
<td>14.</td>
<td>Nitrofen</td>
<td>29.</td>
<td>Trichloro Acetic Acid</td>
</tr>
<tr>
<td>15.</td>
<td>Paraquat Dimethyl Sulphate</td>
<td>30.</td>
<td>Endosulfan</td>
</tr>
</tbody>
</table>

(Adapted from CIBRC, 2011)

But still there are many pesticides present in the market that have not yet been tested for their potential risks. Because of inadequate evidences most of the harmful pesticides are still listed in the *Report on Carcinogens* as likely to be cancer-causing, rather than as known carcinogens and are being used unrestrictedly. ‘Mancozeb’, the pesticide selected for this research work, is also one among such pesticides that have many animal studies accrediting them as carcinogen but lack of adequate information about carcinogenic potential in humans. Inspite of being listed on the highly hazardous pesticide (HHP) list by pesticide action network (PAN) (PAN HHP, 2009) and being referred to as class B2 probable human carcinogen by USEPA (2005a), mancozeb is very much in use with an annual consumption of ~5.6 million pounds (USEPA, 2005b). Thus, there is an urgent need for studies evaluating the mancozeb’s neoplastic potential in human system.

### 1.3 Skin - major site of pesticide induced toxicity and cancer

Humans are exposed to pesticides (found in environmental media such as soil, water, air and food) by diverse routes such as inhalation, ingestion and dermal contact (Abhilash and Singh, 2009). In classic work environments, dermal absorption is the most common route of pesticide poisoning (Wolfe et al., 1966; Spiewak, 2001). Farmers get dermally exposed to pesticides while spraying, mixing, loading the pesticide, sowing pesticide preserved seeds, weeding and harvesting previously sprayed crops as well as while cleaning the equipment, disposing of empty containers (Yoshida, 1989; Spiewak, 2001).

The most frequent clinical form of pesticide-related skin maladies is contact dermatitis, both allergic and irritant, although pesticide exposure sometimes might lead to urticaria, erythema multiforme, ashy dermatosis, occupational acne, porphyria cutanea tarda, hair and nail disorders, and skin cancer (Spiewak, 2001).

### 1.4 Role of RNA interference (RNAi) in determining the underlying mechanisms of pesticide induced toxicity

Uncovering pathways underlying pesticide-induced toxicity is very crucial as this mechanistic information can be combined with other data such as exposure levels to inform a risk assessment for the pesticide. Such mechanistic studies usually include the
exaggerated action or inhibitions of cellular pathways via the overexpression or inhibition of cellular proteins. RNAi-based gene silencing technique for specific inhibition of protein expression may be a valuable tool for elucidating the relationships between phenotypic changes and target gene functions in response to xenobiotic-induced cytotoxicity. Tumor necrosis factor receptor I (TNFRI) is a gene whose signaling has been implicated in xenobiotic-induced toxicity. RNAi inducible TNFRI knockdown cells were employed by Saito et al. (2006) for the analysis of Tumor necrosis factor (TNF)α-induced cytotoxicity.

1.4.1 RNAi

RNAi is an endogenous, ubiquitous, and evolutionarily conserved mechanism for regulating the gene expression, observed in all eukaryotes, from yeast to mammals. Human cells have to manufacture the correct proteins at the right time and in the appropriate amounts to remain healthy. This requires stringent regulation and one of the important ways by which cells accomplish this control is by the mechanism "RNA interference," a form of gene silencing through which double-stranded RNAs silence cognate genes, thus impeding the production of specific proteins by disrupting the flow of genetic information from the cell's nucleus to the protein-building machinery in cytoplasm.

The RNAi pathway is thought to be an ancient mechanism of cellular RNA-based immune defense for protecting the host and its genome against viruses and rogue genetic elements that use double-stranded RNA (dsRNA) in their life cycles. Like our natural adaptive immune response, the RNAi mechanism is extremely specific in targeting these RNA molecules. In fact, they are identified for degradation by well-defined complementarity rules between the targeting small RNA and target messenger RNA (mRNA).

1.4.2 Mechanism of RNAi

Two major types of small RNAs that provide target specificity to the silencing machinery are, short interfering RNAs (siRNAs) and microRNAs (miRNAs). Classical RNAi mechanism has been depicted in Figure 1.2. Upon introduction, the long dsRNAs enter a cellular pathway that is commonly referred to as the RNAi pathway. First, the dsRNAs get processed into 20-25 nucleotide (nt) small interfering RNAs (siRNAs) by a ribonuclease (RNase) III-like enzyme called Dicer (initiation step). Then, siRNAs assemble into endoribonuclease-containing complexes known as RNA-induced silencing complexes (RISCs), unwinding in the process. The siRNA strands subsequently guide
the RISCs to complementary RNA molecules, where they cleave and destroy the cognate RNA (effector step). Cleavage of cognate RNA takes place near the middle of the region bound by the siRNA strand. This cleavage is a catalytic event, involving repeated cleavage of multiple copies of the target mRNA, leading to reduced copies of target mRNA and ultimately to inhibition of the target protein. Therefore, RNAi therapeutics developed to harness the siRNA pathway typically involves delivery of sufficient synthetic siRNA into the cell cytoplasm to trigger this catalytic process.

**Figure1.2.** RNAi mechanism
(http://flyembryo.nhlbi.nih.gov/images/mechanismRNAi.png)

### 1.4.3 Major applications for RNAi in mammalian systems

- Validating hypotheses of gene function

Prediction about mammalian gene functions are usually made by first identifying differentially expressed genes and then searching the databases for the known functions of homologous genes in model organisms like Drosophila, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*. RNAi allows researchers to knock down expression of a targeted gene and observe the phenotypic consequences of partial to full loss of function.
• Functional screening and target detection

Cellular pathways that have been discerned biochemically or genetically can be further scrutinized employing RNAi. siRNAs libraries targeting large collections of genes facilitate screening experiments that tie genes to cellular function.

• Target validation

Once a potential therapeutic target has been identified through a variety of biochemical or genetic methods, including screening using RNAi, its validation as a therapeutic target is a must. For this validation siRNAs can be used to reduce its expression and if the desired phenotype results, it provides confidence that an inhibitor against that target should have therapeutic value.

• siRNAs as therapeutics

siRNAs have the potential to act as therapeutic products. RNAi-based drugs currently in pre-clinical development include those targeting respiratory syncytial virus, hepatitis C, HIV, Huntington’s disease and several other neurodegenerative disorders (Check, 2005). Phase I clinical trials are underway for several siRNA-based drugs targeting age-related macular degeneration (AMD), and Phase II trials have recently started for one siRNA candidate for AMD (www.sirna.com/sirnaproduct/sirna-027.html;www.acuitypharma.com/press.asp).

1.5 Need for preventive agents against pesticide-induced toxicity

It is estimated that as many as two-thirds of all cancer cases are linked to environmental causes (NCI, 2004) but the good news is that a large number of cancers can be prevented. To achieve this, identification of hazardous carcinogenic pesticides is required. Future agricultural practice should aim to diminish pesticide use to a minimum so that these cancers can be evaded. Since such action may take some years. In the meantime, exploring natural plant products present in food consumed by human population, which have lately been proved to have cancer chemopreventive and anti-mutagenic properties, for attenuation of pesticide-induced damage seems to be a very promising approach.

1.5.1 Chemoprevention

As long ago as 480 BC, Hippocrates recognized that several aspects of what we now call “lifestyle” must come together to produce a healthy body. He said, “Positive health requires a knowledge of man’s primary constitution and the powers of various foods, both those natural to them and those resulting from human skill.” What
Hippocrates called “man’s primary constitution,” we today call “genetics,” and we can infer that foods “resulting from human skills” can be equated with today’s diet.

By 2020, the world population is anticipated to increase up to 7.5 billion; of this number, about 15 million new cancer cases will be diagnosed, and around 12 million cancer patients will be lost to clutches of death (Bray and Moller, 2006). For maximum impact on the cancer problem, societies must change their priority from detection and treatment to prevention. Dr. Peter Boyle, from IARC said, “The rapid increase in the global cancer burden represents a real challenge for health systems worldwide. However, there is a clear message of hope: although cancer is a devastating disease, it is largely preventable” (ACS, 2008). Although there is no ‘magic bullet’ that can absolutely surmount cancer, many diseases might be avoidable. The time worn maxim, “an ounce of prevention is worth a pound of cure,” still holds true, as validated by the significant decline in mortality caused by coronary heart disease since 1973, which resulted chiefly from recognizing the precursors to coronary heart disease and executing preventive measures to diminish risk (Sporn, 1996). The fact that only 5–10% of all cancer cases are due to genetic defects and that the rest 90–95% are due to lifestyle and environment offers great opportunities for averting cancer (Anand et al., 2008).

1.5.2 Currently available chemopreventive drugs and their limitations

The first Food and Drug Administration (FDA) approved chemopreventive agent was tamoxifen, for reducing the risk of breast cancer. With this agent although incidence of breast cancer gets reduced by 50% in women at high risk, but there is an increased risk of serious side effects such as uterine cancer, blood clots, ocular disturbances, hypercalcemia, and stroke (http://www.fda.gov/cder/foi/appletter/1998/17970s40.pdf). The second chemopreventive agent to reach clinical level was finasteride, for prostate cancer, which could decrease the incidence by 25% in men at high risk. Side effects of this agent include erectile dysfunction, lowered sexual desire, impotence and gynecomastia. Celecoxib, a cycloxygenase (COX)-2 inhibitor is another approved agent for the prevention of familial adenomatous polyposis (FAP). But, the chemopreventive benefit of celecoxib is at the cost of its severe cardiovascular harm. (http://www.fda.gov/cder/drug/infopage/cox2/NSAIDdecisionMemo.pdf).

These serious side effects of such FDA approved chemopreventive drugs is a matter of great concern when considering long-term administration of them to healthy people who may or may not develop cancer (Anand et al., 2008). Hence, there is an imperative need for agents which are safer and effective in preventing cancer. Diet derived natural products show tremendous potential for this purpose.
1.5.3 Usage of dietary phytochemicals: as safe chemopreventive agents

In 1976, when, Dr. Michael Sporn coined the term “chemoprevention”, referring to the activity of natural forms of vitamin A in preventing the development and progression of epithelial cancer (Sporn, 1976), he instigated a novel field in cancer research. According to him, chemoprevention is the use of pharmacologic or natural agents to inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells in which such damage has already taken place.

Phytochemicals are non-nutritive components in the plant-based diet that possess substantial health promoting properties. Vegetables, fruits, and whole grains contain a wide variety of phytochemicals (e.g. terpenes, organosulfides, isothiocyanates, indoles, dithiolthiones, polyphenols, flavones, tannins, protease inhibitors, and non [vitamin A]-active carotenoids) (Figure 1.3.) that have the potential to modulate cancer development (Fresco et al., 2006; Seifried et al., 2007). The NCI has identified about 35 plant-based foods that possess cancer-preventive properties. These include garlic, soybeans, ginger, onion, turmeric, tomatoes and cruciferous vegetables (for example eg. broccoli, cabbage, cauliflower and Brussels sprouts). Over 4000 different flavonoids have been tested for their medicinal properties. These include polyphenolic compounds which occur naturally in fruits, vegetables and other plant derived products. The polyphenols are consumed in considerable amounts by human beings, at least 1 g/person on a daily basis (Hertog et al., 1993). However, they are generally non-toxic and show a wide spectrum of biological activities, such as anti-allergic, anti-inflammatory, anti-oxidative, free radical scavenging, anti-mutagenic, enzyme modulating activities etc. (Marzouk et al., 2007). They may, therefore, have beneficial health effects and can be considered as possible chemopreventive agents against various diseases including cancer.

[6]-Gingerol, one such dietary compound with well reported chemopreventive properties, has been selected in this research work, for exploration of its protective potency against mancozeb-induced damages. [6]-Gingerol is a pharmacologically active component of ginger (Zingiber officinale) that has commendable anti-oxidant, anti-inflammatory and cancer chemopreventive properties (Shukla and Singh, 2007).

1.6 Current cancer treatment modalities and their limitations

While prevention and timely diagnosis can help in reducing the global cancer burden, there is also an urgent need to find better and effective therapeutic approaches for 28 million people around the world living with cancer (IARC, 2008) so that we can help them win their battle against this dreadful disease. Four primary modalities employed to treat cancer are surgery, radiation, chemotherapy, and biologic therapy.
Figure 1.3: Different types of phytochemicals, found in fruits and vegetables, and their structures
Surgery which is the oldest of these treatment modalities plays a key role in the diagnosis and treatment of cancer and remains the treatment preference for majority of solid tumors diagnosed in the early stages. Cancer patients often have poor nutrition due to anorexia and the catabolic influences of tumour growth, and these factors may inhibit or slow recovery from surgery, patients may be neutropenic or thrombocytopenic or may have clotting disorders; thus increased risk of sepsis and hemorrhage. Importantly, probability of relapse of the disease is very high, if the disease is malignant at the time of surgery, practically making eradication of cancer impossible.

Radiation therapy was first used for cancer treatment in the late 1800s and continues to be a mainstay in the management of cancer. One limitation of this therapy is that besides exterminating the tumor cells, radiation therapy injures normal noncancer cells also. Another major limitation of radiotherapy is that the cells of solid tumors can outgrow their blood supply, causing a hypoxia state. As oxygen is a potent radiosensitizer, tumor cells in a hypoxic milieu may become up to 2 to 3 folds more resistant to radiation damage than those in normal oxygen conditions (Harrison et al., 2002).

Although very efficacious in treating many cancers, surgery and radiation are local modes of treatment and thus can treat only a specific localized region of the body. But since most cancer patients have metastatic disease at diagnosis, localized therapies generally fail to wholly exterminate the cancer. Moreover, systemic diseases such as leukemia cannot be treated with a localized modality.

Chemotherapy, the third type of available treatment modalities, can be the solution in such type of cases. It accesses the systemic circulation and thus can treat the primary tumour as well as any metastatic disease. Chemotherapeutic drugs can be majorly categorized into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents (Takimoto and Calvo, 2008).

Biological therapy is a relatively new addition to the aforementioned family of cancer treatments. The objective of biologic therapy is to boost up body's natural immune defense and its capability to fight cancer by utilizing substances that occur naturally inside the body. Biological response modifiers used for this therapy include interferons, interleukins, colony-stimulating factors, monoclonal antibodies, vaccines, gene therapy, and nonspecific immunomodulating agents. Like other forms of cancer treatment, biological therapies can cause a number of side effects like fever, chills, nausea, vomiting, appetite loss, fatigue, bone pain and blood pressure alterations while monoclonal antibodies usage may result in allergic reactions and cancer vaccines can cause muscle aches and fever. This aspect of cancer therapy is relatively new and will require explicit research to minimize side-effects.
However, as practice is, most commonly used therapy for cancer is chemotherapy. But, inspite of a number of chemotherapeutic treatments shown to be effective at inhibiting or eliminating cancer in preclinical studies, clinical applications are frequently limited due to the severe adverse side effects inflicted by anticancer drugs, inability of drugs to selectively target diseased cells and development of drug resistance. Most chemotherapy agents kill cancer cells by disrupting the cell division or metabolic process and cells are destroyed once they embark on division and replication. Thus, these agents are effective on cancer cells, which usually undergo rapid growth through cell division, but the drugs also destroy healthy, non-cancerous cells as they undertake ordinary division. This damage is mainly apparent in fast-growing normal cells, such as blood cells of bone marrow, digestive tract tissue, hair follicles, and reproductive organ cells. Damage to specific organs cardiotoxicity (heart damage), Hepatotoxicity (liver damage), nephrotoxicity (kidney damage) and ototoxicity (damage to the inner ear), producing vertigo may also occur.

Due to these toxicities the level of a drug needed to successfully eliminate all malignant cells is often intolerable by the patients while the levels that can be tolerated are not sufficient therapeutically. Consequently, chemoresistance and ensuing tumor recurrence are often the result of such therapies. That’s the reason novel targeted therapies that can interfere with specific molecular signaling pathways affecting cancer cell survival are being discovered as prospective treatment modes to make cancer cells more sensitive to chemotherapy. Such targeted therapies that chemosensitize the cancer cells towards chemotherapy proffer the benefits of lowering the amounts of required drug doses thereby reducing the toxic side effects and enhancing the destruction of resistant cells while avoiding healthy cells where there is little or no expression of the targeted entity.

“New drugs will not necessarily eradicate tumors, but when used in combination with other agents, may turn many cases of rapidly fatal cancer into ‘manageable’ chronic illness,” says Dr. Bernard W. Stewart (WHO, 2003).

1.6.1 How can targeted therapies supercharge cytotoxic agents?

Lately, oncologists have begun to surmise that chemotherapy has attained a plateau of effectiveness as a primary treatment modality, even if the dose-limiting toxicity and side effects can be efficiently restrained. In an effort to enhance the efficacy, combination of targeted therapy with conventional chemotherapeutics has become a routine way of testing multiple new agents in early phase clinical trials.

While only 0.6% (3 of 497) of phase I/II trials during the period of 1998–2001 involved combination treatments of targeted and chemotherapeutic agents, by 2005–2009
percentage of such trials using this approach reached 8.6% (99 of 1,145) and the trend is continuously rising (Bagnyukova et al., 2010). Actually, cancer cells can stimulate multiple pro-survival signaling cascades to maintain their viability in the situations of the DNA damage and oxidative damage inflicted by chemotherapy and radiation; use of targeted agents that disrupt these pro-survival signaling cascades can potentiate tumor cell killing (Bagnyukova et al., 2010).

Among such novel therapies the concurrent use of cancer drugs and RNAi-mediated gene silencing is a paradigm providing strategies to inactivate essential genes promoting neoplastic growth.

1.6.2 RNA interference: An emerging cancer treatment strategy

Tremendous potential of RNAi in human therapeutics has been recognized with the 2006 Nobel Prize for Medicine awarded to Craig Mello and Andrew Fire for their discovery of RNAi, and has resulted in major investments in RNAi-based drug development by large pharmaceutical and biotech companies.

Cancer cells generally possess two important anomalies—cell cycle dysregulation ensuing in unrestrained growth and resistance to death as an outcome of abnormalities in one or more apoptosis related proteins (Nam and Parang, 2003). Thus, RNAi approach can be instrumental in cancer therapy by knocking down the expression of a cell cycle gene and/or an anti-apoptotic gene in the cancer cells thereby suppressing tumor growth and exterminating the cancer cells.

1.6.3 Advantages of RNAi therapeutics platform

- RNAi therapeutics operate upstream of protein production by eliminating its encoding mRNAs, thus it is gaining tremendous interest in therapeutics. Instead of trying to prevent a flood by swabbing the floor, with RNAi we can check the flood by turning off the tap. Furthermore, since each mRNA is the template for the translation of many proteins, relatively lesser amounts of an RNAi therapeutic should be enough for abating protein function.
- RNAi being an endogenous biological pathway allows for the development of safe and efficacious drugs thus minimizing the risk of adverse side effects often associated with the use of large amounts of drugs.
- RNAi opens up many novel targets for drug development as through RNAi mechanism, dsRNAs can be used to target any protein-coding mRNA irrespective of the protein structure and localization, many genetically verified, hitherto reckoned “undruggable targets” for RNAi-based drug development.