Chapter 1: Introduction

Cancer is the word with so many meaning. The secrecy associated with cancer is so great that in some societies and cultures the word is barely used and the disease rarely discussed. There is big tragedy for world. Recently Cancer is extensively found worldwide. It is the leading cause of death and disability in the world, behind only heart disease. Based on the statistical research report of different groups, cancer accounts for one out of every eight deaths annually. More and more people die from cancer every year around the world than other diseases like AIDS, tuberculosis and malaria combined. Even cancer deaths accounts for six times the frequency of traffic fatalities on an annual basis, and 42 times the frequency of deaths from injuries suffered in war. Before years ago people believe that disease was widely thought to afflict only the elderly in affluent countries, where it was seen as a death sentence, cancer has now moved beyond high income countries of the developed world. It is expected that cancer will create worst situation in the low and middle income countries of the developing world due to the consequences of the growing burden of new cases and deaths. In the United States one out of every two men and one out of every three women will suffers from some type of cancer in the course of their lives (National Cancer Institute, SEER Cancer Review). One recent study estimated the overall lifetime risk of developing cancer is expected to rise from more than one in three to one in two in both sexes by 2015. Cancer is a global challenge for ingenious man. Continuously more & more new cases of cancer happen and more deaths from the disease occur today in the lower-income and middle-income countries that make up the developing world, than in developed high income countries. Many places of the world where cancer is growing fastest, but silence still with the disease is often the result of a complete or partial lack of truthful information for those affected by cancer ultimately may be undetected and untreated until it leads to death. Cause of death may remain undiagnosed due to the lack of meaningful information about it. Scenario is that the lack of treatment extends and even lack of the treatment for pain management for those affected by cancer over the entire course of their illness. Few countries with restrictions on the availability of narcotics mean they cannot be dispensed by health professionals. The silence mainly in those parts of the world
where cancer goes undetected, undiagnosed and untreated adds another dimension to the threat—these are the reasons for hidden epidemic, even increase in the cancer victims. Indeed, even when cancer is discussed in these developing countries, misinformation and superstition often fill the air—while the stigma associated with being a cancer patient still remains in many countries and in all income groups. Even while the world is awakening slowly to the growing burden of cancer—which is like a wave that is still building—far too little is being spent globally to manage the growing crisis. In the developed world, much spending on cancer research and cancer control is fragmented and uncoordinated. The expenditures associated with cancer management and control may represent a share of total health spending that is below the proportion of the total health burden represented by cancer. In the developing world, the crisis is worsening. Aid donors and recipients have ramped up spending to address the immediate needs created by the most challenging infectious diseases, but non-communicable disease spending—including that for cancer control—has not kept pace\textsuperscript{4,5}. Cancer and other non-communicable diseases are often hidden by the diminutive “other” in tallies of healthcare expenditures. Classifying the disease this way keeps it out of sight—and out of the line of targeted action. As a result, the wave continues to grow.

1.1 What is cancer?

Cancer is a broad term used for a group of chronic diseases characterized by the uncontrolled growth of abnormal cells within the body. Normal Body cell divide and replicate in well organized manner to replace worn-out cells or to repair some form of injury to tissues of the body. Healthy body cells have predictable lifespan, in that normal cell wear out and die. Cancer cells grow, divide and die in the unpredictable fashion as compare to normal cells. Cancer cells develop and divide which create more abnormal cells, which outlive normal cells. Characteristic of cancer cell is to spread and invade in other body organ (for example, spreading from the blood to the lymph nodes or from the lungs to the liver). Spreading and invasive property is called metastasis. Cancer classified on the basis of source of infection or site where initially abnormal cell developed—for example, metastatic liver cancer that has spread to the kidneys is still called liver cancer, not kidney cancer. Not all, but majority of cancers spread and result in death. Some cancers progress very slowly and normally do not
spread during their lifetime. However, frequently cancer growth and development by metastasis leads to invasion of and damage to other tissues and the crowding out of normal bodily functions that leads to death. Recently more than 100 types of cancers are reported. Classified on the basis of types of cells in which they develop. Solid tissue and organ is major site of affection in most cancers. In these cases, abnormal cancer cells damage normal tissue, generally damage by clumping together to form tumors. Left behind Other class of cancers involves the presence of cancer cells throughout the circulatory or lymphatic system or in the bone marrow, such as leukemia, lymphomas and multiple myeloma, respectively. These cancers may not be tumor forming but it can be lethal as other cancer types. Tumor with metastasis property is responsible for life threatening condition. Another type of tumors, benign tumors do not metastasis, are not life threatening and not considered as cancer. Presence of malignant tumors only consider as a cancers. Biological reason for cancer disease still not fully understood because of the mechanism of disease for cancers is quite complex. Many factors are responsible for cancer disease. Mutation in functional genes and other damages either of which may be caused by internal or environmental factors disturb the key pathways operate within the cell ultimately leads to uncontrolled cell proliferation.

**Genes who play major roles in the genesis and spread of cancer**

- **Oncogenes** are mutant forms of the genes for proteins that regulate the cell cycle that cause normal cells to proliferate out of control and convert to cancer cells.

- **Tumor suppressor genes** encode proteins that normally restrain cell division. Mutation in one or more of these genes can lead to tumor formation.

The factors that affect carcinogenesis—the formation of cancer—include broad range chemicals, tobacco, dietary factors, infectious disease and radiation. International Agency for Research on Cancer (IARC, WHO) extensively worked on cancer prevention had identified more than 100 chemical, act as a carcinogens. Complexity of the cancer biochemistry, not every exposure leads to cancer. Sometimes disturbance in the homeostasis of internal factors of the body that may lead to cancer include hormonal imbalance, immune conditions, inherited genetic anomalies and metabolic pathway interruption. Again complex interaction of an individual’s behavior with the environment and genetic makeup is not fully understood. Also
exposure to internal and environmental risk factors and the relatively prolonged latency period between the onsets of the disease create difficulty in tracing causality. Perhaps, people mistakenly regarded exclusively cancer as a disease of old age because much of the damage to genetic material that leads to the disease occurs near old age which is the time that cells are programmed to die. Cancer disease primarily affecting older people but it can also strike at any age, depending on the type of cancer and exposure to different risk factors. It is soaking report that cancer is the second-leading cause of death for children up to the age of 14 in United States (CDC. Data & Statistics Feature: Cancer in Children.). Some cancers types only found in newborns or young adults. Other factors like lifestyle responsible for lifestyle cancers which caused by complex exposure to environmental risk factor or industrial carcinogens with obesity, tobacco and alcohol consumption. Region specific incidence for certain type of cancer. The global variation for various type of cancer due to exposure to risk factor related to environmental or lifestyle. The reality is that cancer can strike almost anyone at any time at any age.

Despite the Extensive steps towards cancer control, cancer burden continuously raising worldwide. Cancer is the leading cause of death, accounting for 8.2 million deaths in 2012(WHO GLOBOCON2012 fact sheet). Increasing life expectancy among much of the world’s population despite this increase in cancer cases due to the variety of reasons like adoption of western lifestyles in major population of the developing world, exposure with environmental risk factors leads to increasing burden of cancer, especially in the least and less developed countries. Fact is that more than half of the cases and 60% death from the cancer occur in developing country. Above 200 types of cancers are there but four cancers: breast cancer, lung cancer, prostate cancer and large bowel cancer accounts for half of the total victims. Based on previous data, it is estimated that 26.4 million cancer cases, 17 million cancer deaths and 75 million people living with the disease by the year 2030.

It is time to fight against cancer and need of work together globally for cancer control and prevention. Worldwide many organizations associated with mission of cancer control with different type of preeminent effort like investigate cancer etiology and other risk factors like identification of biological, nutritional and behavioral modifiers. Community based research to find way to cancer control and prevention. Development of new cancer surveillance and screening method that can be readily
translated into clinical practice is needed. Invention of new types of treatment is required to cure cancer.

1.2 Cancer treatment

Choices for the treatment of cancer depend on type of cancer and the stage of the tumor, e.g. where cancer occurs, tumor mass, spread speedily in to neighboring tissues and whether it shows metastasis or not. Based on different parameter different treatment options can be preferred. Some types of treatments are available for cancer. On the basis of use it can be classified into:

- Surgery
- Radiotherapy
- Hormonal therapy
- Biological therapy
- Chemotherapy

**Surgery**

Surgery is oldest form of treatment used for cancer in ancient time. Before invention of modern medicine like anesthesia and antisepsis, surgery was not fully developed and performed with great discomfort and risk to the patient. With the time variety of drugs and other health techniques invented due to that now a day’s surgery offers the great chance for treatment of many types of cancer. More number of people treated for cancer by surgery compare to other treatment. It is estimated that about 60% of people with cancer will have some type of surgery.

*Surgery can be recommended for following type of objective.*

**Prevention of tumor growth**

Removal of infected tissue that is not malignant but is known to be associated with the development of malignancy, e.g. removal of infected part of the organ or whole organ in breast cancer, removal of infected tissue in colon cancer.

**Diagnosis**

Examination of tissue samples for laboratory testing to confirm diagnosis and cancer identification.

**Staging**

Laparoscopy or laparotomy use to determine the extent of disease e.g. surgical incision into the abdominal cavity to find stages of colon cancer.
**Curative**
Primary cancer treatment starts by removal of the tumor as the hope for cure the cancer.

**Palliative**
Prolong life without any complications, to control pain and to improve quality of life by palliative surgery in cancer patient.

**Supportive**
To help with treatment e.g.- placement of a vascular line to assist chemotherapy treatment.

**Restorative**
It is immensely used for post cancer treatment to restore a person’s appearance or the function of an organ or body part, e.g. surgery for oral cancer, breast reconstruction and prosthetic implantation.

**Radiation therapy**
Radiation therapy uses high-energy radiation or particle to such as X-rays or gamma rays to shrink the tumors and kill the cancer cell. It is one of the oldest cancer treatments and most widely used because of inexpensive cancer therapies compare to other cancer therapy and it is estimated that more than 50% of all people with cancer will receive radiation at some stage in their treatment. Radiation is considered to be a local treatment because only cells in the area being treated are affected. It can be used in different phases of disease with modification in treatment parameter e.g. radiation can be used in an attempt to cure or control disease in early-phase of the cancer; it can be used prior to surgery to minimize the risk of cancer recurrence. In advanced disease, radiation therapy can be used to treat symptoms such as cancer pain. Radiation is most commonly used for external radiation therapy, i.e. radiation therapy administered using a machine that focuses radiation on the cancer site. It is also applied for internal radiation therapy e.g. brachy therapy by placing radioactive particles in tumors or radioisotope therapy by injection of radioactive liquids. In certain types of cancer, radiation therapy can be used in combination with other treatment like surgery or chemotherapy.

Radiation therapy show number of side effect because it can destroy cancer cells as well as affect the surrounding healthy cell. These non-specific side effects vary in
incidence depending on type of cancer and dose of radiation, and also differ from person to person.

**They include:**
- Fatigue
- Hematological toxicity
- Stomatitis
- Loss of appetite
- Skin burns
- Hoarseness
- Hair loss
- Difficulty in swallowing
- Nausea and vomiting
- Diarrhea

**Hormonal (endocrine) therapy**
Hormone therapy controls the growth of hormone-sensitive tumors by altering the body’s ability to produce hormones or by interfering with hormone action. Tumors that are hormone-insensitive do not respond to hormone therapy. Interfere with hormone production or hormone action by using some drugs or surgical removal of hormone producing glands to kill cancer cells or slow tumor growth, e.g. ovarian ablation in breast cancer. Drug can be used to alter the action or production of male or female hormones and is used to slow the growth of prostate, breast and endometrial cancers. It is selective for some type of cancer like breast cancer and prostate cancer. Examples of drugs which are commonly used for hormonal therapy e.g. tamoxifen, fadrozole, anastrozole, exemestane, fulvesterant etc...

Hormonal therapies are associated with fewer side effects but it can be used for some specific types of cancer.

**However, some side effects related to hormonal therapy are:**
- Flushes and sweats
- Nausea, diarrhea and indigestion
- Weight gain
- Changes in menstrual cycle
- Muscle cramps
- Mood changes
• Allergic reactions
• Headaches
• Thrombosis

Long term use of some drugs increased incidence of some cancer e.g. extensive use of tamoxifen increase risk for development of endometrial cancer.

**Biological therapy (immunotherapy)**

Biological therapy also known as immune therapy refers to any therapy that is related to components of the immune system, the body’s natural defense mechanism against disease. It is target specific cancer treatment compare to chemotherapeutic and other agents, which generally tend to affect both healthy and cancer tissues. Biological therapy is therefore the use of treatments that promote or support to generate powerful immune response or use components of the immune system as a basis in order to kill tumor cells or repress disease growth.

**Chemotherapy**

The word chemotherapy is broad term used for treatment of any disease by drugs, but mostly it is generally used for drug treatment for cancer disease. Chemotherapeutic drugs are often describing as anticancer and cell killing. Chemotherapeutic agent acts by killing rapidly reproducing cells. However, there is not an absolute difference in terms of reproduction between cancer cells and normal cells. Therefore, each time that chemotherapy is given, a balance between destroying the cancer cells to cure or control the cancer, and sparing the normal cells to minimize undesirable effects has to be found. It is important to understand that cytotoxic chemotherapy is not specific to cancer cells.

Wide range of chemotherapeutic agents is used for treatment and can be broadly classified. Classification based on specific mechanism of action and also use of chemotherapeutic agent at different stages of cancer disease. That’s why; wide range of combination can be made for treatment of wide range of cancer. This explains the complexity of many of the standard chemotherapeutic regimens used in clinical practice, which often employ three or four drugs sequentially or in combination.

**Different targets are set for use of chemotherapeutics:**

• Treatment for the cancer
• Reduce tumor size prior to surgery
• Improve quality of life instead of prolonging life
- Prevention of the disease

Application of chemotherapy for different situation:

Therapeutic agent used before surgery for primary disease this type of treatment termed as

**Adjuvant chemotherapy:**

Primary disease can be treated by various methods like surgery; radiation etc. to remove the cancer, there may still be some cancer cells left behind that can’t be seen. Post treatment to kill those unseen cells, it is known as adjuvant treatment e.g. use of hormone therapy after radiation for prostate cancer.

**Neo-adjuvant chemotherapy:**

Chemotherapy can be used before surgery or radiation termed as neo-adjuvant chemotherapy. It makes easier and more affective surgery or radiation therapy by means of decrease in cancer tumor size by shrinking. Neo-adjuvant chemotherapy also used to kill small deposits of cancer cells that cannot be detected by rational method like scans or x-rays.

**Metastatic:**

Where chemotherapy is used to cure disease, prevent disease spread or for symptom palliation depending on the type of cancer and extent of metastasis. Several courses of therapy may be administered to patients with metastatic disease, with first-line therapy being the initial choice and usually the therapy that is considered to be most active and second-line and further therapy being administered when first-line therapy fails or the disease recurs.

Chemotherapy given by vast number of routes, selection of the routes based on location of the tumor and types of the agent:

- Intravenously
  - Orally
  - Intra-arterially
  - Intraperitoneally
  - Intravesically
  - Topically
  - Intramuscularly
  - Subcutaneously
Intrathecally
Intralesionally.

Among these all, most common route is the intravenous route. Whole body gets affected by chemotherapeutic agents administered by this route, eradicate cancer cells but often killing actively dividing healthy cell. That is why, development of more suitable methods of administrate chemotherapeutic agent is challenging for researcher. In some extent, scientist got succeed by invention of suitable routes to achieve highest cure with lowest side effect e.g. treatment of liver cancer by intra-arterial administration and tumors of central nervous system by intrathecal. Extensive research is going on to develop best routes for chemotherapeutic treatment, in spite of this, chemotherapy generate some side effects:

- Most common and less chronic digestive system diseases like nausea, vomiting, taste change, constipation, diarrhea, stomatitis, oesophagitis, appetite and weight loss.
- Hair loss is found after chemotherapy and fatigue due to the muscle damage.
- Bone marrow suppression leads to hematological adverse effects and chance of infection.
- Chemotherapeutic agents have adverse effect on vital organs of the body like heart, liver, kidney and urinary damages.

Often, a complication created by combination of one or more of these side effects limits the individual dose or cumulative dose of chemotherapeutic agent that can be administered. Implementation of various strategies to reach the limits of cumulative dose administration of chemotherapeutic agents that can be increase the possibility of highest curative effect on the cancer. Combination of two or more drugs with chemotherapeutic agent e.g. drugs for antinausea and the use of cytokines to stop the bone marrow suppression. Toxicity related to the amount of drug that can be administered is main barrier to use chemotherapy and it is also apply to radiotherapy. Therefore, chemotherapy employs valuable nursing time and economic resources for intensive patient care to ensure that toxicity is within acceptable limits. Thus, need of development of specific anticancer therapy specifically to kill cancer cell while sparing normal body cell with lowest level of toxicity to the patient and will thus use fever resources (Adams, 2000; Berrino, Gatta, Chessa, Valente, & Capocaccia, 2014; Greenwald, 2007; Kramer, Gohagan, & Prorok, 1999).
1.3 Different types of chemotherapy drugs

Chemotherapy drugs can be classified based on factors such as mechanism of action, their chemical structure, and their relationship to another drug. Chemotherapy drugs with more than one mechanism of action, they may belong to more than one class. Prediction of side effects can be possible by knowing how the drug works. Based on these all information helps oncologists can decide which drugs are likely to work well together and to decide the use of more than one drug and to plan exactly when each of the drugs should be given.

**Alkylating agents**

Alkylating agents one of the biggest class of the drugs, directly damage DNA to prevent the cancer cell from reproducing. These agents act nonspecifically on all phases of the cell cycle.

Alkylating agents are broadly used to treat many types of cancers, including lymphoma, leukemia, Hodgkin disease, multiple myeloma, and sarcoma, as well as cancers of the lung, breast, and ovary. Alkylating agent can create serious side effects in bone marrow. In rare cases, this can eventually lead to acute leukemias.

**There are different subclasses of alkylating agents, including:**

- Nitrogen mustards: such as mechlorethamine (nitrogen mustard), chlorambucil, cyclophosphamide (Cytoxan®), ifosfamide, and melphalan
- Nitrosoureas: which include streptozocin, carmustine (BCNU), and lomustine
- Alkyl sulfonates: busulfan
- Triazines: dacarbazine (DTIC) and temozolomide (Temodar®)
- Ethylenimines: thiotepa and altretamine (hexamethylmelamine)

The platinum drugs (cisplatin, carboplatin, and oxalaplatin) are sometimes grouped with alkylating agents because they kill cells in a similar way.

**Anti-metabolites**

Anti-metabolites are a class of drugs that interfere with metabolic process for synthesis of DNA and RNA by substituting for the normal building blocks of RNA and DNA. These agents are specifically damage cells during the S phase of cell cycle. They are commonly used to treat leukemia, breast, ovary and the intestinal tract cancer, as well as other types of cancer.
Examples of anti-metabolites include:

- 5-fluorouracil (5-FU).
- 6-mercaptopurine (6-MP)
- Capecitabine (Xeloda®)
- Cladribine
- Clofarabine
- Cytarabine (Ara-C®)
- Floxuridine
- Fludarabine
- Gemcitabine (Gemzar®)
- Hydroxyurea
- Methotrexate
- Pemetrexed (Alimta®)
- Pentostatin
- Thioguanine

Anti-tumor antibiotics

Anti-tumor antibiotics are a class of drugs that interfere with enzymes involved in DNA replication. These drugs affect all phases of the cell cycle. Variety of cancers can be treated by this class of drugs. Lifetime small dose limits are more preferred instead of high doses because it can permanently damage the heart if given in high doses.

It could be divided into two sub classes include:

Anthracyclines include:

- Daunorubicin
- Doxorubicin (Adriamycin®)
- Epirubicin
- Idarubicin

Other anti-tumor antibiotics

Anti-tumor antibiotics that are not anthracyclines include:

- Actinomycin-D
- Bleomycin
- Mitomycin-C
Mitotic inhibitors
Mitotic inhibitors are known as plant derivatives, because compounds derived from natural products. They acting on mitosis or inhibit enzymes from making proteins needed for cell reproduction. These drugs work during the M phase of the cell cycle but can damage cells in all phases. These drugs are known for toxic side effect to damage peripheral nerve system, which can be a dose-limiting side effect.

Examples of mitotic inhibitors include:

- Taxanes: paclitaxel (Taxol®) and docetaxel (Taxotere®)
- Epothilones: ixabepilone (Ixempra®)
- Vinca alkaloids: vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®)
- Estramustine (Emcyt®)

Corticosteroids
Corticosteroids are class of natural hormone or hormone-like drugs that are use for treatment of some types of cancer e.g. leukemias, lymphoma and multiple myeloma, as well as other illnesses. When these drugs are used for cancer treatment to slow infected cell their growth, they are considered as chemotherapy drugs. Corticosteroids are also commonly used to prevent nausea and vomiting caused by chemotherapy.

Examples include:

- Prednisone, methylprednisolone (Solumedrol®), and dexamethasone (Decadron®).

Miscellaneous chemotherapy drugs
Some chemotherapy drugs act in slightly different ways and with completely different mechanism of action so, these do not fit well into any of the other categories.
Examples include:

- L-asparaginase, which is an enzyme, and the proteosome inhibitor bortezomib (Velcade®).

Alkylating agents were one of the earliest classes of among all other class of drugs used to treat cancer. Mechanism of action of alkylating agent makes it special for treatment purpose for vast number of cancer treatment. That’s why; it creates a center of interest for researcher for development of new potent anti cancer drugs. Among all class of alkylating agent, great importance to develop new nitrogen mustard drugs
with best curative capacity with lowest side effect for chemotherapy treatment in future.

1.4 DNA-Alkylating Agents
Compounds that alkylate DNA have long been of interest as anticancer drugs. Many different types of chemicals are able to alkylate DNA, and several are used as anticancer drugs, but the most important classes of such agents in clinical use are the nitrogen mustards. The first DNA alkylating agents used in the cancer therapy was mechlorethamine (1), and the related compounds chlorambucil (2), melphalan (3) and cyclophosphamide (4), temozolomide (5) and estramustine (6) remain in use today. Other DNA alkylating agents of importance are the bismethanesulfonates (7), the cisplatin (8) and the anthramycine (9) derivatives (Figure 1). The majority of the clinically employed alkylating agents behave as electrophilic traps for macromolecular nucleophiles. There are several nucleophilic groups in DNA such as N-1 and N-3 of adenine bases, N-3 of cystosine, and in particular N-7 of guanine. Drug with two alkylating groups can react with a guanine on each chain and cross-links the strand such that they disrupt the replication of transcription leading to the cell death. Thus binding of alkylating agents to the cellular DNA is considered to be the lethal event associated with anticancer activity.

Nitrogen Mustards
As noted above, the N-mustards were among the very earliest class of anticancer agents developed, and perhaps most extensively studied of the DNA alkylating agents\(^{26}\). The pronounced cytotoxicity of the N-mustard derivatives is attributed to their ability to induce inter strand cross-links between the two strands of DNA thereby inhibiting replication. The overall process of DNA alkylation by N-mustard is a two-step process (Figure 2). The nitrogen atom is able to displace a chloride ion intra-molecularly to form the highly electrophilic aziridinium ion. Alkylation of DNA can then take place via nucleophilic attack on that intermediate by DNA\(^{16}\). For N-mustards, the regiospecificity of alkylation of DNA is largely governed by electronic and stearic properties of DNA. Therefore, they target DNA at the most electronegative sites, with mono adducts occurring primarily at the N-7 of guanines\(^{17}\) and the inter strand cross-links between the N-7 positions of guanines in each strand at 5’-GNC sequences\(^{18}\).
Figure 1 Chemical Structure of some DNA-Alkylating agents

Despite their clinical importance, the usefulness of many DNA-alkylating drugs is often limited by a number of pharmacological deficiencies resulting from the intrinsic chemical reactivity of the agent. For example a drawback common to all DNA alkylating agents is their (necessarily) high chemical reactivity. This can result in loss of drug by reaction with other cellular nucleophiles, particularly proteins and low molecular weight thiols. This makes them vulnerable to cellular resistance mechanisms such as increased levels of glutathione (Millard et al., 1990; Wang AL, 1985). Other limitations, particularly for mustards, are a lack of intrinsic DNA binding affinity of the core $N$, $N$-bis(2-chloroethyl)amine pharmacophore, and a requirement for bi-functional cross-linking of DNA to be fully cytotoxic. These characteristics lower their potency, and produce carcinogenicity due to the formation of high ratio of genotoxic mono adducts to cross-links (up to 20:1)$^{19,20}$. There is also evidence that the major guanine N-7 adducts formed by mustards and other “simple” alkylators is readily repaired, which may also result in lower cytotoxicity. These drawbacks place severe restrictions on the potential utility of DNA-reactive agents$^{21,22}$. 

\[
\begin{align*}
1 & \quad \text{Me-}N\text{Cl} \\
2 & \quad \text{O-}\text{OH} \\
3 & \quad \text{O-}\text{NH}_2 \\
4 & \quad \text{O-}N\text{Cl} \\
5 & \quad \text{O-}\text{NH}_2 \\
6 & \quad \text{HO} \\
7 & \quad \text{Me-}O\text{SO}_3 \text{Cl} \\
8 & \quad \text{Cl-}\text{Pt-NH}_2 \\
9 & \quad \text{Me-}O\text{SO}_3 \text{NH}_2
\end{align*}
\]
DNA-directed Nitrogen Mustards

The therapeutic utility of the alkylating agents would be greatly enhanced if they could be more precisely targeted to defined geometric sites of DNA. One design for achieving this is to link “simple” mustards and other alkylators with DNA affine carrier molecules which possess an intrinsic reversible DNA-binding capability. The use of DNA-affinic carriers with their own defined binding geometry makes it possible to alter both the region and sequence specificity of alkylation compared with that of the “simple” mustards (or other alkylators). Moreover, the side reactions mentioned above would be reduced by specifically targeting the drug in the vicinity of the DNA. Attachment of N-mustards to the DNA-intercalating carriers goes back to the work of Creech et al\textsuperscript{23}, who originally suggested that the attachment to acridine carriers might serve to target the reactive center to DNA. Since then, many reports have been found in the literature based on the DNA-directed alkylating agents described as follows.

Creech et al\textsuperscript{23–26} have synthesized several mono- and bi-functional nitrogen mustards attached to the heterocyclic nuclei such as acridine, benz[c]acridine and phenanthridine nuclei through aminoalkyl side chain or amide linkage for antitumor studies (Figure 3).
It demonstrated that the presence of DNA-intercalating nuclei increased the antitumor effectiveness of the mustard moiety against Ehrlich ascites tumors in vivo and prolonged the survival time (relative to a control of 16 days) by a factor of at least 2.6 at the optimal dose. A striking observation was that, contrary to the common nitrogen mustards which required bi-functionality for good antitumor activity, some of the heterocyclic nitrogen mustards were almost equally effective as mono- or di-functional variants. In case of amide linkage, it was revealed that the entire molecule functioned as a unit in its action on the ascites tumors rather than through the formation of glycine mustard or glycine half-mustard by hydrolysis of the amide linkage.

Later work showed that N-mustards linked with DNA intercalators could also drastically modify the pattern of DNA alkylation by the N-mustard. The DNA-binding properties of the *para* substituted aniline mustards in which the mustard is covalently linked to 9-aminoacridines and 4-aminoquinazolines were studied (Figure 4)\(^{27,28,29}\). For N-mustards linked to the acridine by a short alkyl chain through a *para* O-or S-link group (17), 5′-GT sequences was the most preferred sites at which N-7-guanine alkylation occurred. For analogues with longer chain lengths, the preference of 5′-GT sequence diminishes in favor of N-7-adenine alkylation at the complementary 5′-AC sequence. The in vivo antitumor activities of these compounds have also been evaluated\(^{27,28}\). Compounds having a (CH\(_2\))\(_n\)O and (CH\(_2\))\(_n\) showed to
have higher activity (ILS values of 50-60%) and much greater potency (optimal doses of 20-30 mg/kg) than either chlorambucil (ILS 33% at an optimal dose of 225 mg/kg) or any of the untargeted mustards, using a single-dose protocol. In contrast, compounds in the \((\text{CH}_2)_n\text{S}\) and \((\text{CH}_2)_n\text{SO}_2\) series showed only minimal in vivo activity\[^{27}\]. In the series of compounds bearing CO-NH, CO or NH-CO linker, CONH-linked compound was the most potent (optimal doses of 20-30 mg/kg) having ILS values of 58% against P388 lymphocytic leukemia in vivo\[^{28}\]. The antitumor activity of N-mustards linked to amsacrine (clinical antileukemic drug and DNA-intercalating agent) is also reported (e.g., \(^{19, 20}\)\[^{30}\]). The aniline mustards of varying reactivity attached either off the 4-carboxamide (19) or at the 1’-position of the 9-anilino ring (20). Interactions of 19 and 20 with calf thymus DNA showed that 19 gave only one adduct, resulting from alkylation at guanine N-7 in the major groove. In contrast, the primary adduct (57%) of 20 resulted from alkylation at adenine N-3 in the minor groove\[^{31}\]. Although, the patterns of DNA alkylation were broadly similar, the comparison of their cytotoxicity in wild-type and DNA repair-deficient lines indicated that they were considerably more cytotoxic than analogous untargeted mustards. The 4-linked analogues (19) showed slightly higher in vivo antileukemic activity than the corresponding 1’-linked analogues (20)\[^{30}\].

However, most of the recent work has used DNA minor groove binders as carriers to construct DNA-directed mustards, because these offer much higher region-and sequence selectivity than intercalators. For examples, the prototype of distamycin A derivatives (tallimustine) bearing a benzoyl nitrogen mustards group (Figure 5) was synthesized for antitumor evaluation and found to have a broad spectrum of antitumor activity in experimental tumor models.\[^{32-35}\] Previous studies have shown that tallimustine (21) possess a high preference for alkylation of adenines located in the 5’-TTTTGA-3’ sequence\[^{36,37}\]. The tallimustine derivatives without a reactive halogen group or difluoro mustard or the diol derivatives showed a very low level of cytotoxic potency while the presence of a dibromo mustard significantly increased cytotoxicity (83-fold higher than tallimustine)\[^{37}\]. The isosteric tallimustine derivatives (e.g., 22)\[^{38}\] containing one or more pyrazolic ring showed on L1210 four-fold reduced cytotoxicity with respect to tallimustine but superior in vivo antileukemic activity.
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\[
\text{NH} \left( \text{CH}_2 \right)_n \text{X} \text{Cl} \quad \text{Cl}
\]

\( n = 2 \) to 5

\( \text{X} = \text{CH}_2, \text{O}, \text{S}, \text{CO}, \text{CO-NH}, \text{NH-CO}, \text{SO}_2 \)

18

19

20

Figure 4

21

22

23 a-e

\( a: R^1 = \text{H} \ \text{HCl}, \ R^2 = \text{H} \)

\( b: R^1 = \text{H} \ \text{HCl}, \ R^2 = \text{CH}_3 \)

\( c: R^1 = \text{CH}_3 \ \text{HCl}, \ R^2 = \text{CH}_3 \)

\( d: R^1 = \text{H}, \ R^2 = \text{CN} \)

\( e: R^1 = \text{H}, \ R^2 = \text{CH}_3 \)

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\( R^1 = \text{H} \ \text{HCl}, \ R^2 = \text{H} \)

Figure 5
The cinnamic N-mustard derivative of distamycin A (PNU-157911, 23a-e)\textsuperscript{39} a vinylogue of tallimustine, showed very good antileukemic activity, significantly superior to that of tallimustine. In the case of compound 23a, the replacement of the amidino group with basic or non-basic amidino moieties of different nature led to compounds 23b-e, in which the potent cytotoxicity of the parent amidino derivative was fully maintained and in some cases even increased\textsuperscript{38}. The cinnamic acid mustard derivative of 22 (compound 24)\textsuperscript{40} appeared to be 20-fold more cytotoxic than 22 (IC\textsubscript{50} = 14.2±0.52 vs 306±56 nM for 24 and 22, respectively) and maintained an in vitro potency equivalent to that of tallimustine’s vinylogue 21.

![Chemical structures 25, 26, 27](image)

Figure 6

Denny et al\textsuperscript{41} have reported a series of bis(benzimidazole) analogue of Hoechst 33258 (25) bearing nitrogen mustard moieties linked by a variable-length polymethylene chain (Figure 6). These agents efficiently cross-linked the cellular DNA and exhibited potent cytotoxicity (up to 85-fold more potent than chlorambucil), with IC\textsubscript{50} value of 10 nM against the P388 cell culture for the C3 analogue 26. Studies on bisbenzimidazoles where the mustard was directly attached to the phenyl ring, but the benzimidazole DNA binding chromophores were altered by changing the heteroatoms, showed that analogues (e.g., 27)\textsuperscript{42} retaining the cytotoxic effects with higher reversible DNA binding. The DNA minor groove binding ligands based on polybenzamide moiety bearing either one or two mono-functional mustards have been reported (Figure 7)\textsuperscript{43}. The antitumor evaluation and DNA interaction study showed that these agents possessed significant cytotoxicity against murine p388 leukemia cells in culture with high degree of DNA inter strand cross-linking ability.
Compounds with two alkylating functions were the most cytotoxic, with 28a being 1000-fold more potent ($IC_{50} = 0.007$ nM) than the chlorambucil in vitro.

In contrast, the other mono-functional compounds are more than 10-fold less cytotoxic except the compound 29c which showed the comparable cytotoxicity with $IC_{50}$ value of $0.027\mu$M. Despite the large variation in vitro cytotoxicity, all of these compounds showed broadly similar potency in vivo, with optimal doses in a single dose protocol of about 5-10 mg/kg.

Synthesis and antitumor studies of a number of amidine analogs of chlorambucil and melphalan have been reported (Figure 8)\textsuperscript{44-46}. In case of chlorambucil analogues, the 5-[4-(N-alkylamidino)phenyl]-2-furancarboxamide and the chlorambucil moiety were linked by a $\text{-NH}(\text{CH}_2)_2\text{NH-}$ chain (30a-e). Evaluation of the cytotoxicity of these compounds employing a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and inhibition of $[3\text{H}]$-thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that these compounds were more active than chlorambucil\textsuperscript{44,45}. The degree to which these compounds inhibited cell growth breast cancer cells was directly correlated to DNA-binding affinity. Moreover, these studies showed that cyclic amidine analogs of chlorambucil are potent catalytic inhibitors of topoisomerase II but not topoisomerase I. The highest degree of DNA binding and cytotoxicity in both MDA-MB-231 and MCF-7 breast cancer cells was observed for the compound, which possess a 4,5-dihydro-1$H$-imidazo moiety (30e). On the other hand, among the amidine analogues of melphalan
(31a-e)\textsuperscript{46}, compound 31b which possessed a N-cyclopropylamidine function was the most cytotoxic with IC\textsubscript{50} value of 10 µM and showed strong binding to the minor groove AT-rich sequences. From these results, it was suggested that the simultaneous DNA-binding and Inhibition of Topo-II activity may lead to increased anti-proliferative activity.

![Figure 8](image_url)

Recently, Su et al. reported alkyl N-mustard-9-anilinoacridine conjugates (Figure 9), in which the N-mustard pharmacophore was linked to the anilino ring of the 9-anilinoacridine via various length of alkyl chain (O-ethyl (O-C\textsubscript{2}) or O-butyl (O-C\textsubscript{4})) spacer at C3’ or C4’ position (e.g. 36 (BO-0742))\textsuperscript{47} or with acridine ring at 4-postion (e.g., 37, BO-0944)\textsuperscript{48}. The 9-anilinoacridines, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA, 32)\textsuperscript{49,50}, AHMA-alkylcarbamates (e.g., 33)\textsuperscript{51,52}, 5-(9-acridinylamino)toluidines (34)\textsuperscript{52} and 5-(9-acridinylamino) anisidines (35)\textsuperscript{53} were used as the carriers. The results showed that all compounds exhibited potent in vitro cytotoxicity against human lymphoblastic leukemia cells (CCRF-CEM) in culture. Studies on the structure-activity relationships of these N-mustards indicated that the antitumor activity was slightly affected by the length of the spacer and the location of the N-mustard pharmacophore. Among these agents, compound 36 (BO-0742) exhibited significant cytotoxicity against CCRF-CEM, with 107-fold higher potency than its parent analogue, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA, 32)\textsuperscript{49,50}. 
Additionally, it also exhibited a significant cytotoxic effect against drug-resistant sublines, such as those resistant to vinblastine and taxol, CCRF-CEM/VBL and CCRF-CEM/taxol, respectively. Remarkably, compound 36 at one-tenth of the taxol’s therapeutic dose resulted in complete tumor remission in nude mice bearing human breast carcinoma MX-1 xenografts. Furthermore, 36 yielded xenograft tumor suppression of 81-96% using human T-cell acute lymphoblastic leukemia CCRFCEM, colon carcinoma HCT-116, and ovarian adenocarcinoma SK-OV-3 tumor models. BO-0742 was about 10-fold less toxic to human normal hematopoietic stem cells (CFU-E, BFU-E and CFU-GM) than leukemic CCRF-CEM indicating that this agent has low toxicity to human bone marrow. Further studies suggested that the main mechanism of action of compound 36 is primarily through its DNA cross-linking activity rather than its inhibitory effect on DNA-topoisomerases. However, BO-0742 is chemically unstable and has a short half-life in rat (<25 min.) which put restriction on its utility as therapeutic agent.

All these studies clearly demonstrated that alkylating agents (N-mustard) ‘targeted’ to DNA by attachment to DNA-affinic carriers (either intercalators of minor groove binders) have generally shown altered sequence-selectivity of DNA alkylation, higher cytotoxicity and enhanced in vivo antitumor efficacy compared with the corresponding ‘untargeted’ mustards.
N-mustard prodrugs for ADEPT or MDEPT

One of the strategies to overcome the high reactivity and poor pharmacokinetic properties of N-mustards is to prepare N-mustard prodrug, which can be activated selectively at tumor site after enzymatic hydrolysis. Springer et al. have synthesized a series of N-mustard prodrug by attaching the aniline mustards to L-glutamic acid moiety through a urea, carbamate (38, Figure 10) or carboxamide (39, CMDA) linker for antibody-directed enzyme prodrug therapy (ADEPT). After enzymatic cleavage by bacterial enzyme carboxypeptidase G2 (CPG2), they can be transformed into their corresponding active metabolite phenol or aniline N-mustard drugs. It has been demonstrated that these prodrugs were effective substrates of the enzyme and showed to have improved therapeutic efficacy in CPG2-expressing xenografts. The prodrugs, 40 and 41 were also synthesized by linking the aniline N-mustard to the trigger unit tyramine or 3-hydroxytyramine via a urea or carbamate linker, respectively, for melanocyte-directed enzyme prodrug therapy (MDEPT). Upon exposure to tyrosinase, these conjugates can release the active aniline or phenol N-mustard. Since the trigger tyramines were found to be the substrates for tyrosinase by oximetry, the prodrug 42 was also prepared for MDEPT. Similarly, the 5-aziridinyl-2,4-dinitrobenzamides (i.e., 43, CB 1954), 2,4-dinitrobenzamide 5-N-mustards (44) and related derivatives can be used as prodrugs for gene-directed enzyme prodrug therapy (GDEPT) with the E. coli nfsB nitroreductase (NTR) as activator enzyme in hypoxia conditions. This suggested that the cytotoxic 2-hydroxyamine metabolite for forming DNA cross-linking may be generated in the presence of NTR.
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From these studies one can envisage that the urea, carbamate, or carboxamide linker is capable of lowering the reactivity of aniline or phenol N-mustard pharmacophore resulting in formation of rather stable N-mustard derivatives.

Hope of the better treatment of cancer by investigation of potent anticancer agent is on prime focus for all medicinal chemistry people. On the basis of previous work here our effort to construct biologically active molecules containing aniline mustard moiety as one of the active group integrated with biologically active scaffold like coumarin and pyrimidine.

Figure 10 Structure of some N-mustard prodrug

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