CHAPTER 1

INTRODUCTION

AND

REVIEW OF LITERATURE
INTRODUCTION

The term immunity means protection from disease and more specifically infectious disease. The cells and molecules responsible for immunity constitute the immune system and their collective and coordinated response to the introduction of foreign substance is the immune response. Immunology is the branch of science which deals with the understanding of the immune system and the process through which it works. Immunity present at birth is termed innate and it is the main first line defence against invading organisms. Its characteristics are that, it is present for life, has no specificity and no memory. In contrast, some types of immune responses are not present at birth but are gained as a part of our development. It is called acquired immuinity. It is absent at birth and hence specificity and memory. It is also known as adaptive immunity. Based on the component of the immune system specific immune responses are classified into humoral and cellular immune responses. Humoral immunity is mediated by molecules in the blood that are responsible for specific recognition and elimination of antigens, these are called antibodies. It can be transferred to unimmunized individuals by cell free portions of the blood. Cell mediated immunity is mediated by macrophages, NK cells and T lymphocytes. Cell mediated immunity can be transferred to naïve individuals with T lymphocytes from an immunized individuals but not with serum (15).
Immune defects in Cancer patients

Immunosuppression is the major drawback in cancer (1). It includes suppression of both T and B lymphocytes in spleen, bone marrow and thymus. In leukemic patients there is an abnormal rise in total WBC count and granulocyte count. The cytokines such as TNF-α; IL-1 and GM-CSF level were found to be elevated in patients with acute myeloid leukemia (17). Thrombocytosis has been seen in association with carcinomas, leukemia and Hodgkins disease. Anemia occurs very frequently in cancer patients. Patients with Bence Jones myeloma are characterised by an abnormal rise in IgM level in serum. It is a monoclonal tumour composed of identical cells, which produce homogenous myeloma protein; often in large quantities. The Iggs may be intact or a part of either light or heavy polypeptide but will not have immune activity. Eosinophilia is associated with non-leukemic neoplasms; particularly Hodgkins diseases, melanomas and other cancers (14). Gastric, lung; pancreas and brain tumours are associated with granulocytosis. The mechanism behind tumour-associated granulocytosis is most often due to the production of CSF by tumour cells. The colony stimulating factors in granulocytosis include G-CSF; GM-CSF; M-CSF; IL-3 and IL-1.

Immune defects in Carcinogenesis

Carcinogenesis is a multistage process derived by genetic damages and epigenetic changes. It includes activation of protooncogenes, inactivation of tumour suppressor genes and inactivation of antimetastatic genes. Oxygen free radicals are formed in association with the promotion of cancer. They have the potential to damage any nucleotide sequences in DNA and...
the mutations that result from this damage could occur at divergent loci of genome. Carcinogenic chemotherapeutic agents generally exhibit target organ specificity. The bonemarrow, lymphatic system and urinary bladder are most commonly affected (2). Carcinogenesis is mainly associated with severe immunosuppression. NK cell activity was found to be suppressed during carcinogenesis. There was a drastic decrease in total WBC count and bonemarrow cellularity in carcinogenesis (18).

Immune defects in chemo and radiotherapy

Most of the commonly used antineoplastic agents are capable of suppressing both cellular and humoral immunity (2). However, immunosuppression varies tremendously; depending upon the precise dose and shedule of drug administration and whether the drug is used alone or as a part of multidrug combination. However, the impact of immunosuppression on the natural history of cancer is unpredictable; it may in fact be a necessary part of the antineoplastic efficacy of some drugs (19). There is a marked decrease in all the host defense during treatment; however within 2-3 days there is a complete or nearly complete recovery of immune functions (14). Bonemarrow toxicity is the most dangerous form of toxicity for many of the antineoplastic drugs in clinical practise. It is accompanied by leukopenia, with an attendent high rate of infection; thrombocytopenia and granulocytopenia. It is also associated with bleeding; defects in polymorphonuclear leukocytes; platelets and further aggravate the bonemarrow suppression. Some drugs cause cummulative myelosuppression which may rarely lead to prolonged and severe pancytopenia. This is particularly seen in alkylating agents such as nitrosoureas and Mitomycin -C (18).
Tumour Immunology

A major focus of Immunology and Oncology research is the development of ways to augment host immune responses to tumours. If malignantly transformed cells express molecules that act as foreign antigens in the host, it is possible that a physiologic function of the immune system is to recognize and destroy these abnormal cells before they grow into tumours; or to kill tumours after they are formed. This theoretical role for the immune system is called immunosurveillance. If the concept of immunosurveillance is valid then the immune effector cells such as B cells, helper T cells, CTLs and NK cells must be able to recognize tumour antigens and mediate the killing of tumour cells. Immunosurveillance for tumour is often ineffective as indicated by the fact that lethal cancers arise in immunocompetent individuals. It is therefore likely that immune responses to tumour are often week (15).

A variety of clinical and pathologic evidence indicate that tumours can stimulate immune responses. A common histologic observation suggests that tumours may be immunogenic in the presence of mononuclear infiltrates, composed of T cells, NK cells; and macrophages surrounding many tumours. Another histopathologic indication that tumours stimulate immune responses is the frequent finding of lymphocytic proliferation in lymphnodes draining sites of tumour growth. Furthermore, there is often evidence of cytokine effects in tumours, such as increased expression of class II MHC and intracellular adhesion molecule - (1CAM-1) suggesting an active immune response at the site of the tumour. The spontaneous regression of some tumours also suggests that host immune responses to tumour cells can occur although there are many explanation for this phenomenon.
Immunotherapy in Cancer

The term immunotherapy describes a variety of agents and therapeutic approaches that continue to evolve from increased understanding of biology of tumour cells and their relationship to the environment. Strategies for the immunotherapy of cancer can be divided into active and passive approaches. Active immunotherapy refers to the immunization of the tumour-bearing host with materials designed to elicit an immune reaction capable of eliminating or retarding tumour growth. Active immunotherapy can be subdivided into nonspecific or specific immunization. Most early attempts at the immunotherapy of cancer used nonspecific active approaches to immune stimulation with adjuvants such as Bacillus Calmette-Guerin (BCG), Corynebacterium parvum and levamisole (18). Attempts at immunotherapy used for immunization include tumour cells or tumour-cell extracts alone or in vaccines, often in conjugation with immune stimulators such as BCG. The advent of recombinant cytokines provided a more selective means for stimulating the immune system. Treatment with the interferons or with IL-2 is a form of nonspecific active immunotherapy (2). Many studies have demonstrated that the tumour-bearing host is immunosuppressed by growing tumour; and attempts at active immunotherapy may therefore have intrinsic disadvantages. Recent efforts have concentrated on passive approaches to immunotherapy, which involve the transfer to the tumour-bearing host of previously sensitized immunologic reagents (e.g. cells or antibody) that have the ability to mediate antitumour responses directly or indirectly. The term adoptive immunotherapy usually denotes passive immunotherapy with cells (e.g. lymphocytes; macrophages). Recent efforts have been devoted to developing adoptive immunotherapies using lymphokine activated killer (LAK) cells; tumour infiltrating lymphocytes (TIL) or other means for in vitro stimulation of cells with antitumour
reactivity. The development of techniques for generating monoclonal antibodies has greatly improved the ability to obtain preparations with specific reactivity to human-tumour-associated antigens (18). These antibodies are being employed alone or conjugated with toxins or radiolabels in cancer treatment. In addition to active and passive approaches, the immune system can be used in a variety of indirect ways to mediate antitumour responses, including removal of blocking factors from serum or inhibition of essential tumour growth factors.

Immunomodulators of plant origin

The use of plants as a source of immunomodulatory material is still in its infancy. In fact a variety of materials from the plant source has been known to stimulate the haemopoietic system and act by the maturation of the immune cells. Some of the plants with known immunomodulatory activities are *Viscum album* which is being used in cancer therapy. The active component which stimulated the immune system was found to be a small peptide (5). It is reported that *Tinospora cordifolia* was found to possess immunomodulatory activity (6). Administration of Tinospora extract was found to enhance total WBC count, bone marrow cellularity and α-esterase positive cells in mice. *Asparagus racemosus* was also reported to possess immunomodulatory activity. The roots are used in dysentery, tumours, inflammations and diseases of eye (34). Ginseng plant is one of the important plants with immunomodulatory effect and it increases the nonspecific resistance of organism and modulates specific immune responses (8). Administration of curcumin (*Curcuma longa*) was found to enhance total WBC count; antibody titre; antibody forming cells; bone marrow cellularity and α-esterase positive
cells in mice (9). Rasayanas are nontoxic; preparations made from plants with known immunomodulatory activity is used in several Ayurvedic formulations (11). In general, medicinal herbs are known to promote immunity in several ways. They may boost NK activity; increase the phagocytic function of macrophages; induce interferon production or augment specific cellular and humoral immune response (30).

**Withania somnifera**

*Withania somnifera* is a plant which has drawn the interest of many researchers in several countries, either for its active principle or for the extremely important pharmacodynamic or pharmacotherapeutic properties. The fine powder from the dried roots of *Withania somnifera* called ‘Ashwagandha choorna’ is used in the Ayurvedic system of treatment. Besides these the powder of Ashwagandha root is widely incorporated in several Ayurvedic formulations. It increases fresh energy and vigor in a system worn out owing to any constitutional disease like syphilis, rheumatic fever etc or from over work and thus prevent premature decay. As nutrient and health restorative to the pregnant and old people a decoction of root or its powder with milk is recommended. The dried powder of the roots contain a bitter alkaloid somniferin. Withaferin A, is the most important alkaloid isolated so far from the leaves of *Withania somnifera* (12). Withanolide D, another steroidal lactone occuring in the leaves of *Withania somnifera* was also reported to possess antitumour properties (13).
Objectives of the study in general

Present study was mainly aimed to analyse in detail the immunomodulatory activity of *Withania somnifera* in mice. One of the important aims of this study was to detect the effect of Withania on Cytotoxic T Lymphocyte (CTL) production and its effects on various cytokines such as IFN-γ, IL-2; GM-CSF and TNF-α. Another important aim of this study was to assess the effect of Withania on the reversal of radiation induced toxicities. This study was also aimed to analyse the effect of Withania on the reversal of immunosuppression and severe urotoxicity induced by Cyclophosphamide. Another aim of the study was to reverse the chemically induced skin carcinogenesis in mice. The most important aim of this study was to analyse the adjuvant activity of Withania along with chemotherapy; radiotherapy; thermotherapy on solid tumour reduction induced by DLA cells. This study was also aimed at the reversal of ascites tumour induced by EAC cells using *Withania somnifera*. In brief the; present study was mainly aimed to analyse the immunomodulatory activity of *Withania somnifera* and its implication in the treatment of cancer.

In chapter I introduction & review of literature is discussed. Chapter II describes elaborately the materials and methods used in experiments. In chapter III, Immunomodulatory activity of *Withania somnifera*; especially humoral immune response is discussed in detail. Chapter IV mainly analyses the effect of *Withania somnifera* on the cell mediated immune response in mice. In chapter V the effect of *Withania somnifera* on cytotoxic T lymphocyte production and cytokines such as IFN-γ, IL-2, GM-CSF and TNF-α is discussed. Chapter VI
describes the effect of *Withania somnifera* on the reversal of radiation induced damages. Chapter VII illustrates the effect of *Withania* on the reversal of severe toxicity induced by chemotherapy. In chapter VIII the effect of *Withania somnifera* on the chemically induced skin carcinogenesis in mice model is discussed. Chapter IX deals with the effect of *Withania somnifera* along with chemo, radio and thermotherapy on solid tumour reduction is discussed. In Chapter X summary and conclusion of the thesis is described in detail.
Cancer can be described as an abnormal mass of tissues and it is the growth of cells in a disorganized fashion. Cancer is one of the major leading causes of death in the world. Several factors are involved in the causation of cancer. The multistage nature of the process of cancer development is a cyclical process of DNA damage; proliferation; clonal selection and progression. This process could potentially be modulated by chemicals that effects cellular enzyme systems, gene expression, signal transduction pathways, differentiation or interaction with surroundings and extracellular matrices. Physical factors such as ionizing radiations, x-rays, gamma rays and ultra violet rays are also involved in the causation of cancer. Chemical carcinogens such as dimethyl benzanthracene, methyl cholanthrene etc may cause cancer. Similarly psychological factors such as frustrations, mental tensions, worries may transform normal cells into cancerous cells. Immunological status of cancer patients are reduced by the disease itself.

The genes implicated in tumour initiation and progression are called protooncogenes. They are normal cellular genes with potential to contribute to the induction or progression of malignant tumours when their structure is altered. Tumour suppressor genes are capable of arresting the growth of tumour cells. Oncogenes are the genes which are capable of inducing and maintaining cell transformation. They encode proteins called oncoprotein which are very similar to normal production of protooncogenes except that they have lost important regulators constraints on their activity and they do not need any external activation signal (113). Mutation that after
the level of gene expression or the form of gene products in the signal transduction pathway have been shown to activate the oncogenic potential. Many of the proteins encoded by cellular oncogenes constitutively activate intracellular signalling pathways; used by the normal cell to process exogenous mitogenic stimuli (114). In this pathway, oncoproteins induce a cellular state similar to that experienced when a normal cell is exposed to mitogens. As a consequence, the oncogene bearing cell acquires growth autonomy, as it is no longer dependent on mitogenic stimuli from its surroundings. This state of cell proliferation autonomy may lead to malignancy (115).

The oncogenesis is a multistep process with sequential genetic events (117). The specific sets of genetic events that are pre-requisite for neoplastic change are generated by the accumulation of mutations following the action of carcinogens (116). Oncogenes and tumour suppressor genes have been proposed as targets for carcinogen. Some of the examples of oncogenes are Src, Myc, Fos, Ras etc and tumour suppressor genes are P53 and nm23. Carcinogenesis is a term implying all the process which leads to the development of cancer. The majority of human cancers are now believed to be caused or promoted by life style factors that are controllable at the individual or society level or both. The ideal way to tackle the cancer disease is to find measures to reduce the relative risk of carcinogenic exposure. An understanding of the ability of these agents to bring about undesirable changes such as DNA damages and mutation will decrease the cancer risk (117).
Different treatment modalities of cancer

Chemotherapy, radiotherapy, surgery, photodynamic therapy, endocrine therapy, thermotherapy and immunotherapy are the major treatment modalities of cancer. Chemotherapy and radiotherapy have severe toxic side effects out of which immunosupression is the major drawback which is highly relevant in the treatment of cancer.

Surgery

Surgery is the oldest treatment for cancer and until recently, was the only treatment that could cure patients with cancer. The surgical treatment of cancer has changed dramatically over the last several decades. Advances in surgical techniques and a better understanding of the patterns of spread of individual cancers have allowed surgeons to perform successful resections for an increased number of patients. The treatment of most tumours depends on two subsequent development in surgery, the first of these was the introduction of general anesthesia. The second major development is stimulating the widespread application of surgery resulted from the introduction of the principles of antisepsis. These developments in surgery free from pain and sepsis and have greatly increased its use for the treatment of tumours. Surgery is the main treatment modality for patient with metastatic disease. Many patients with few metastases to lung or liver or brain can be cured by surgical resection. Surgery is used to obtain histologic conformation of diagnosis, but primary local therapy is achieved through the use of a nonsurgical modalities such as radiation therapy. Cytoreductive surgery has led to the inappropriate use of surgery for reducing the tumour in some cases (127).
Chemotherapy

Cancer chemotherapy had its roots in the work of Paul Ehrlich, who coined the word chemotherapy. Alkylating agents, the first modern chemotherapeutic agents were a product of the secret war gas programme in both worldwars. There are four ways by which chemotherapy is generally used, as an induction treatment for advanced disease, as an adjunct to the local methods of treatment, as the primary treatment for patients who present with localized cancer, and by direct installation into sanctuaries. The term induction chemotherapy has been used to describe the drug therapy given as the primary treatment for patients who present with advanced cancer for which no alternative treatment exists. Adjuvant chemotherapy denotes the use of systemic treatment after the primary tumour has been controlled by an alternative method, such as surgery and radiotherapy. Primary chemotherapy denotes the use of chemotherapy as the initial treatment for patients who present with localized cancer for which there is an alternative, but less than completely effective treatment. This approach also has been called neoadjuvant chemotherapy, but the term primary chemotherapy is more accurate(15).

Chemotherapeutic agents are customarily divided into several classes. For two of the classes, the alkylating agents and the antimetabolites, as the names indicate the mechanism of cytotoxic action of the drugs in their classes. For the hormonal agents, the name designates the physiologic behavior of the drug and for the natural products, the name reflect the source of the agents. The drugs that do not fit easily into other categories are grouped together as miscellaneous agents. Some of the commonly used chemotherapeutic agents are shown in Table - 1 (125).
<table>
<thead>
<tr>
<th>Class &amp; type</th>
<th>Agents</th>
</tr>
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<tbody>
<tr>
<td><strong>A. Alkylating agents</strong></td>
<td></td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Chlorambucil,</td>
</tr>
<tr>
<td></td>
<td>cyclophosphamide,</td>
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<tr>
<td></td>
<td>ifosfamide, melphalan</td>
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<tr>
<td>Ethylenimine derivative</td>
<td>Thiotepa</td>
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<tr>
<td>Alkyl sulfonate</td>
<td>Busulfan</td>
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<tr>
<td>Nitrosurea</td>
<td>Carmustine, lomustine,</td>
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<tr>
<td></td>
<td>Semustine, streptozocin</td>
</tr>
<tr>
<td>Triazene</td>
<td>Decarbazine</td>
</tr>
<tr>
<td>Metal salt</td>
<td>Carboplatin, Cisplatin</td>
</tr>
<tr>
<td><strong>B. Antimetabolites</strong></td>
<td></td>
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<tr>
<td>Antifolates</td>
<td>Methotrexate, raltitrexed;</td>
</tr>
<tr>
<td></td>
<td>trimetrexate</td>
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<tr>
<td>Pyrimidine analogs</td>
<td>Azacitidine, cytarabine,</td>
</tr>
<tr>
<td></td>
<td>Floxuridine, fluorouracil</td>
</tr>
<tr>
<td>Purine analogs</td>
<td>Mercaptopurine, thioguanine,</td>
</tr>
<tr>
<td></td>
<td>fludarabine, cladribine</td>
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</tbody>
</table>
C. Natural products

Mitotic inhibitor

Microtubule polymer stabilizer

Topoisomerase I inhibitors

Topoisomerase II inhibitor

Antibiotics

Enzyme

D. Hormone & hormone antagonists

Androgen

Corticosteroid

Estrogen

Progestin

Estrogen antagonest

Aromatase inhibitor

Androgen antagonist

Lutenizing hormone releasing agents

Vinblastine, Vincristine, Vindesine

Docetaxel, Paclitaxel.

Irinotecan, topotecan

Etoposide, teniposide

Bleomycin, dactinomycin, epirubicin,
mitomycin, doxorubicin

Asparaginase

Fluoxymesterone

Prednisone

Diethylstilbestrol

Megastrol acetate

Tamoxifen, tormifene.

Aminogluthethimide,
anastrozole, letrozole

Flutamide, nilutamide;
Bicalutamide

Leuprolide, Goserelin
Thyroid hormones
Levothyroxine, Liothyronine

**E. Miscellaneous agents**

Substituted urea
Hydroxyurca

Methyl hydrazine derivative
Procarbazine

Adrenocortical suppressant
Mitotane

Substituted melamine
Altretamine

Acridine dye
Amsacrine

Biophosphonates
Pamidronate

Photosensitizing agents
Profimer

Cytoprotector
Amifostine, mesna

Platelet reducing agent
Anagrelide

Somatostatin analogs
Octreotide.

**Side effects of chemotherapy**

The alkylating agents used in cancer therapy are potentially cytotoxic, mutagenic and carcinogenic. One of the chemotherapeutic drug melphalan routinely causes myelosuppression, with equal suppression of granulocyte and platelet production, nausea and vomiting are infrequent with this agent. Alopecia is common during extended courses of treatment. Melphlan appears to be more carcinogenic than cyclophosphamide. Another chemotherapeutic agents busulfan can cause pulmonary fibrosis resembling that seen after pulmonary irradiation, and it
may also cause testicular atrophy and hepatocellular dysfunction. In high-dose transplantation regimens; busulfan can cause hepatic venoocclusive disease; mucositis; hemorrhagic cystitis, seizures, rash, pneumonitis and diarrhea. Cyclophosphamide produces significant leukopenia and immunosuppression, but only mild thrombocytopenia. Nausea, vomiting, and alopecia are common side effects and are more common with high dose intravenous therapy (119). Immunosuppression is the major draw back associated with cyclophosphamide administration (2). One of the major side effects of cyclophosphamide administration is urotoxicity (85). Another chemotherapeutic agent ifosfamide, causes less myelosuppression but dose limiting cystitis.

Carcinogenicity of Chemotherapy

The carcinogenic effect of some chemotherapeutic agents in humans has been well documented and supported by laboratory animal studies (2). Carcinogenic chemotherapeutic agents generally exhibit target organ specificity. The bone marrow, lymphatic system and urinary bladder are most commonly affected. As with other carcinogens, genotoxic agents generally act by forming promutagenic DNA adducts and crosslinking (118) although chromosomal abbreviation also occurs (119). P450 metabolic activation is not necessarily required for DNA adduct formation. Nitrogen mustard type compounds have highly reactive electrophilic centres that react with DNA to form adducts without metabolic activation. Cyclophosphamide, in contrast, is a nitrogen mustard that undergoes cytochrome P450 metabolism in the liver, where it is converted to acrolein and 4-hydroxy cyclophosphamide. This latter compound breaks down spontaneously to phosphoramide mustard, which then reacts with DNA.
nations can significantly raise the risk of secondary tumours, especially nonlymphocytic leukemias.

**Radiotherapy**

One of the important techniques of radiation therapy that solved in the early part of this century was the application of continuous radiation by interstitial or intracavitary application. The clinical use of radioactive isotopes, especially by implantation techniques developed separately from external beam radiation therapy. Isotopes such as Cs$^{137}$, Ir$^{192}$, Co$^{60}$ are used in radiation treatment. Iridium and Caesium have a lower energy and thus are much easier to shield. Au$^{198}$ and I$^{125}$ are also used as radiation sources for the removable implants (2).

**Principles of Radiation therapy**

Ionizing radiation is the energy that during absorption, causes the ejection of an orbital electron. A large amount of energy is associated with ionization. Ionizing radiation can be electromagnetic or particulate and electromagnetic radiation can be considered both as a wave and as a packet of energy. Examples of particulate radiation are the subatomic particles, electrons, protons, alpha particles, neutrons, negative pimesons and atomic nuclei.

**Radiation techniques**

Two general types of radiation techniques are used:- clinical brachy therapy and teletherapy. In brachy therapy, the radiation device is placed either within or close to the target
volume. Teletherapy is used as a device quite removed from the patient, as is the case in most orthovoltage or supervoltage machines.

**Adverse effects of Radiation**

Some biologic considerations of localized radiation may decrease the likelihood for tumour control. First and most discussed is the effect of radiation in the immune response. High dose, whole body irradiation has a well known and profound effect on the immune responses. However, this generalized treatment rarely is used in clinical therapy, except as preparations for bone marrow transplantation. After whole body irradiation, there is a prompt fall in the lymphocyte count. The following conclusion concerning the effect of radiation on the immune response can be made (120).

1. Blymphocytes are radiosensitive and undergo interphase and mitotic death after irradiation.
2. All functional T-cell subpopulations have sensitive precursor cells. Suppressor T cell precursors may undergo interphase death.
3. The homing potential of cell is affected by radiation.
4. Resting cells are more sensitive to interphase death than are the same cells when stimulated to divide before irradiation.
5. The effects of wholebody irradiations are qualitatively and quantitatively differ from those caused by localized or regional irradiation.
Localized radiation, despite producing a chronic lymphopenia of T and B cells, does not affect the immune response to bacterial or viral agents because treated patients do not seem to be more susceptible. Radiation induced mutagenesis is of concern for germline and somatic cells.

Exposure to whole body irradiation may induce cancer by a number of different mechanisms. Free radicals have been directly associated with biological effects of ionizing radiations such as lethality, physiological mutation and carcinogenesis. Endothelial dysfunction and pulmonary fibrosis in rats after a single dose of radiation exposure have been reported (21). Ionizing radiation either directly act through the breakage of bonds of DNA or indirectly through ions and free radicals bring about chromosomal changes (22). Micronuclei, an indicator of chromosomal damage caused by exposure to genotoxic or carcinogenic agents can predict the sensitivity to radiotherapy as well (23).

**Photodynamic therapy (PDT)**

PDT, is a relatively new cancer treatment modality that depends on the concerted action of three component system - sensitizer, light and oxygen. PDT is an oxygen dependent photochemical oxidative process. PDT may have more promise as a primary cancer treatment modality than the laser techniques, mainly because PDT offers some tumour selectivity due to selective sensitizer retention compare with sensitizer concentrations in normal tissue. The differential cytotoxicity separates PDT from other chemically based forms of cancer treatment (131).
Tumour necrosis factor is released in a dose dependent fashion from PDT stimulated peritoneal macrophages, and it is possible to potentiate this TNF release with pretreatment with interferon. Indirect mechanism of tumour killing by PDT could be through cytokine stimulation and effect of cytokine on tumour vascular to cause haemorrhagic necrosis due to endothelial cell destruction. Shumaker described an increase in T cell lymphocytes, plasma cells in bladders of patients undergoing bladder PDT (15) and Nseyo documented cytokine production in the urine of patients receiving bladder PDT (86).

Clinical studies show that PDT was used in patients with cutaneous and subcutaneous malignancies. Malignancies involving the skin treated with PDT include basal and squamous cell carcinomas, malignant melanomas, mycosis, fungoides, recurrent metastatic breast carcinoma and kaposis sarcomas (15). PDT should prove to be a useful addition to head and neck tumours. PDT was found to be effective in the treatment of lung neoplasm, ocular tumours, esophageal malignancies, gastrointestinal and genitourinary malignancies (2).

**Endocrine therapy**

It is a treatment modality of cancer in which hormones are mainly used. Endocrine therapy was found to be useful in the treatment of breast cancer. Mechanism of this response may be due to a hormone called oestrogen (130). Human breast cancer cell line MCF-7 has been shown to secrete growth factors such as TGF. The secretion of growth factors appears to be controlled by oestrogen mediated receptors. These growth factors may stimulate the cells through
autocrine or paracrine stimulation. In addition, breast cancer is responsive to endocrine therapies such as progestins, androgens and corticosteroids, that do not involve oestrogens. Treatment with Tamoxifen, progestins, aminogluthimide oestrogens, Oophorectomy, adrenalectomy and hypophysectomy have been shown to be equivalent in many randomized trials.

Endocrine therapies are effective and the choice of a particular one is based on the toxicity. Tamoxifen is being associated with least toxicity and adrenalectomy or hypophysectomy with the greatest (128).

### Toxicities associated with endocrine therapy

<table>
<thead>
<tr>
<th>Endocrine therapy</th>
<th>Toxicities</th>
</tr>
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<tbody>
<tr>
<td>Tamoxifen</td>
<td>Nausea, hot flashes, flare, hypercalcemia,</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia.</td>
</tr>
<tr>
<td>Oopherectomy</td>
<td>Weight gain, fluid retention,</td>
</tr>
<tr>
<td>Progestins</td>
<td>Nausea, Vaginal bleeding, hot flashes</td>
</tr>
<tr>
<td>Aminogluthimide</td>
<td>Lethargy, dizziness, ataxia rash,</td>
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<tr>
<td></td>
<td>Cushinogoid symptoms, nausea.</td>
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<tr>
<td>LHRH analogues</td>
<td>Hot flashes, nausea, head ache</td>
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<tr>
<td>Estrogens</td>
<td>Nausea and vomiting, fluid retention,</td>
</tr>
<tr>
<td></td>
<td>incontinence, Vaginal bleeding, flare,</td>
</tr>
<tr>
<td></td>
<td>hypercalcemia.</td>
</tr>
<tr>
<td>Procedure</td>
<td>Effects/Complications</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Androgens</td>
<td>Masculinization, nausea, weight gain, Flare hypercalcemia</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>Surgical mortality, Addisonian crises, Whingoid symptoms</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>Surgical mortality, Addisonian crises, Diabetes insipidus, anosmia</td>
</tr>
</tbody>
</table>

**Thermotherapy**

Thermotherapy is a treatment modality of cancer in which heat is mainly used. Most importantly, blood circulation is a mechanism for transporting heat, thereby affecting the temperature produced in tissues with heating by any means (137). The amount of blood flow and the temperature dependence of the flow itself influence the delivery of nutrients to the cells and the metabolic status and pH (138). Tumour vessels resemble leaky capillaries and venous sinusoids without the vascular smooth muscle that allows normal vasoactivity such as thermally induced vasodilation (139). Application of hyperthermia in deep body sites for locoregionally advanced disease in which there is a possibility of cure remains a high priority that has required extensive development of thermometry systems and techniques. Hyperthermia treatment was found to stimulate the immune system (15).
Immunotherapy in Cancer

The term Immunotherapy describes a variety of agents and therapeutic approaches that continue to evolve from increased understanding of the biology of tumour cells and their relationship to the surrounding environment. It involves, the recognition of the cells and antigens in antitumour mechanisms, the isolation and production in pharmacological amounts of cytokines that regulate the differentiation, proliferation and activity of immune cells. More recently, treatment approaches based on preventing development of tumour-associated vasculature and approaches actually targeting this vasculature have emerged as promising new therapeutic strategies to affect the tumour environment.

A  Cytokines as therapeutic agents

Cytokines are a series of regulatory proteins which are used in cancer therapy. These proteins bind to specific cell surface receptors differentially expressed on cells of the haematopoietic and lymphoid systems and are responsible for controlling the growth, development and functional activity of these cells (129). The range of biologic effects of these cytokines suggests wide possible therapeutic applications but has also complicated their clinical use when administered in pharmacologic qualities as drugs.

1. Interleukin - 1 (IL-1)

IL-1 has immunostimulatory properties that help to activate T lymphocytes and induce the production of other cytokines. IL-1 is both a chemoprotector and a radio protector, protect-
ing animals against lethal myelosuppressive doses of cytotoxic agents. IL-1 is used as an adjuvant in vaccine trials, in association with chemotherapy and irradiation.

2. **Interleukin - 2 (IL-2)**

The protein stimulates T cell proliferation and activates NK cells. Because of its powerful immune stimulatory properties, it is used as an antitumour agent. For a given IL-2 treatment schedule antitumour activity can be enhanced by the addition of other cytokines, such as interferon - α (IFN-α) and tumour necrosis factor - α (TNF-α) or by the concomitant use of activated antitumour lymphocytes or monoclonal antibodies directed against the tumour.

3. **Interleukin -3 (IL-3)**

IL-3 is also known as multi Colony Stimulating Factor. Initial Clinical trials using IL-3 after administration of GM-CSF have been approved for use after cytotoxic induction and maintenance chemotherapy for acute leukemia.

4. **Interleukin -4 (IL-4)**

IL-4 stimulates B-cells and together with IL-2, acts as a growth factor for cytotoxic T cells. This wide range of immunostimulatory properties has led to clinical trials but not to a defined role in cancer therapy.

5. **Interleukin-6 (IL-6)**

IL-6 has a wide spread biologic effects. In addition to playing a central role in the
induction of the acute phase response, it is important in B-cell growth and differentiation and may serve as an autocrine growth factor in myeloma as well as contributing factor to cancer related cachexia.

6. **Interleukin-11 (IL-11)**  
IL-11 activates haematopoietic cells. It is used clinically to accelerate the recovery of platelets after cytotoxic chemotherapy.

7. **Interleukin-12 (IL-12)**  
IL-12 is important in T-cell activation and is being studied in clinical trials for its antitumour activity.

8. **Interferon (IFN)**  
IFN-α has been described initially for their antiviral properties, IFN-α is an immunomodulator and its effect includes activation of NK cells, modulation of antibody production by B lymphocytes.

9. **Interferon-γ**  
This interferon has weaker antiviral and wider range of immunobiologic properties. It activates monocytes, macrophages and enhances phagocytosis. It is effective in chronic granulomatous disease, in which its prophylactic use decreases the incidence and severity of infection.
10. **Tumour necrosis factor**

TNF-α is the product of activated macrophages which is originally named for its antitumour effects in animal models. Immunomodulatory effects include the induction of surface MHC antigens, interaction with other cytokines such as IL-2. TNF has been inactive systemically in cancer therapy in humans, perhaps because of its toxicity. Hypotension has limited the doses that can be administered systemically. It has been used more successfully in the treatment of recurrent melanoma and soft tissue sarcomas.

B **Haematopoietic growth factors in cancer therapy**

1. **Erythropoietin**

Erythropoietin promotes the proliferation and differentiation of committed erythroid precursors. In addition to its nonmalignant indications, use of this factor results in decreased transfusion requirement during chemotherapy (130).

2. **Granulocyte colony-stimulating factor (G-CSF)**

G-CSF is a growth factor with proliferative activity for bonemarrow progenitors committed to the neutrophil line. G-CSF is widely used in the settings of cytotoxic chemotherapy for solid tumours and leukemia to accelerate recovery of neutrophils and lessen the risk of bacterial infection.
3. **Granulocyte - Macrophage colony stimulating factor (GM-CSF)**

GM-CSF exhibits its predominant proliferative effect on multipotential stem cells. GM-CSF has been used after cytotoxic induction and maintenance chemotherapy for acute leukemia.

4. **Macrophage colony stimulating factor (M-CSF)**

M-CSF is also known as CSF-1 is responsible for the proliferation and activation of monocytes. Clinical trials have not yet defined a role for this protein.

5. **Transforming growth factors**

TGF-α is related to epidermal growth factor (EGF) and binds to the EGF receptor. TGF-β enhances wound healing. It is being studied clinically for their use and for possible prevention or treatment of chemotherapy associated mucositis as well as other nonmalignant indications.

6. **Thrombopoietin**

This haematopoietic growth factor enhances megakaryocyte development and it is used in the treatment of thrombocytopenia associated with cytotoxic chemotherapy.
Toxicity of biologic therapy

In general the predictable toxicities of biologic agents are dose and schedule related. Administration of IFNs on a daily basis is associated with systemic symptoms, including fever, fatigue, and myalgia (121).

High dose IL-2 regimens are associated with significant cardiovascular complications including hypotension and the development of a full blown capillary leak syndrome. Clinical manifestations include decreased serum albumin, weight gain and development of peripheral oedema is the setting of fluid support for blood pressure. Other cardiac complications of high dose IL-2 therapy include arrhythmias, principally supra ventricular and myocardial infarction (121). Patients on IL-2 develop a characteristic erythematous rash that may progress to desquamation and decrease in appetite and may develop nausea and occasional diarrhea. The development of hypothyroidism has been associated with IL-2 therapy and rarely in IFN therapy. Development of neuropsychiatric changes such as confusion, during IL-2 therapy is a reason to halt therapy. Corticosteroids prevent or alternate most IL-2 related side effect.

C. Monoclonal antibodies (MAbs)

Antibodies that bind to tumour associated cell surface antigens can result in the destruction of tumour cells through a number of possible mechanisms; including activation of complement and antibody dependent cell mediated cytotoxicity. They are useful as means, of targeting
cytotoxic radioisotopes, toxins, drugs to tumours, enhances their delivery to tumour while mini-
mizing system exposure.

1. **Murine monoclonal antibodies**

The first MABs used *in vivo* diagnostically and therapeutically used were murine. They are weak activators of the human immune system if when used alone against T or B cell lymphoid malignacies they have generally exhibited only transients antitumour reactivity. A problem with the repeated use of murine MAb has been the development of human antimouse antibodies.

2. **Chimeric, human and humanized monoclonal antibodies**

Although human antibodies have the theoretical advantage of less immunogenicity, longer half-lives and greater immunologic activity, they are difficult to generate in pharmacologic quantities (122). More recently herceptin, a humanized mAb reactive with the new antigen overexpressed on some breast carcinoma cells, has been shown both to have single agent clinical activity and in combination to add to the antitumour activity of paclitaxel or doxorubicin plus cyclophosphamide in the treatment of metastatic breast carcinoma.

**Imaging studies with Radiolabelled antibodies.**

The suggestion that therapy or localization of tumour might be carried out with radiolabeled antibodies was made shortly after the initial demonstrations of antibody to human
tumours at the turn of this century. Chemically coupled antibodies maintain specific binding to antigen (123) supported this possibility. In 1948, David Pressman and his colleagues began innovative studies that demonstrated the localization of radiolabeled antibodies in normal tumours, including lymphosarcomas and osteogenic sarcoma (122).

Studies were also performed using antibodies to myelin. The first attempt at using radiolabeled Carcino Embryonic Antigen (CEA), involving a rabbit polyclonal immunoglobulin preparation (124). Radioimmunoscintrigraphy or the detections of lesions using antibodies coupled to radionuclides, has been studied for sometime. $^{131}$I is readily detectable externally and it is widely available. $^{131}$I requires specialized chelating agents for its use. Technetium -99m (99MTC) is employed in limited studies because of the problem of coupling it to antibody.

Toxicity associated with the infusion of murine monoclonal antibodies has been tolerable. Fever, chills, pruritus, chest tightness, dyspnea, rash, arthralgia, myalgia and hypotension are, the toxicities associated with monoclonal antibody therapy. MAb administration has been associated with hypotension and shortness of breath. The risk of anaphylaxis, which is greater, with repeated dosing has led to routine administration of an intravenous test dose with anaphylactic precaution on hand. The toxicity of the immuno conjugates are largely related to those of the linked cytotoxic moiety. Clinical experience with transtuzumab (Herceptin) has suggested that combination therapy with drug plus doxorubicin and cyclophosphamide not only may result in greater antitumour activity but also may be associated with increased cardiotoxicity (125).
D. Targeted therapy

Tumour associated structures, either tumour associated antigens or receptors can be used for targeted therapy with cytotoxic toxins, chemotherapeutic agents or radionucleides (126).

1. Immunotoxins

Conjugates of plant toxins, such as ricin or bacterial toxins such as pseudomonas species exotoxins to MAbs have been used in therapeutic approaches with responses noted in non-Hodgkins lymphoma and chronic lymphocytic leukemia (126).

2. Chimeric toxins

Fusion genes composed of the cytotoxic portions of bacterial genes, diptheria toxins or pseudomonas species and targetting ligands can be used to produce cytotoxic chimeric protein that target specifically cells expressing the respective high affinity receptor (126).

E. Adoptive cellular therapy, gene therapy and cancer vaccines

This treatment strategy involves the transfer of antitumour effective cells to the tumour bearing host. These cells have principally been either lymphokine activated killer cells. Cells generated by in vitro activation of peripheral blood lymphocytes with IL-2. In some cases, these tumour infiltrating lymphocytes can be shown to exhibit specific cytotoxicity agents and
the autologous tumour gene transfer techniques with molecules such as GM-CSF have been used to increase the immunogenicity of autologous tumour cells for use in vaccination strategies.

**TUMOUR IMMUNOLOGY**

Tumour immunology encompasses the study of specific immune responses to tumours; the antigens on tumour cells that induce immune responses; immunologic effector mechanisms that kill tumour cells and immunological approaches to detecting; diagnosing and treating cancers. Immunosurveillance for tumours is often ineffective, as indicated by the fact that lethal cancers arise in immunocompetent individuals. It is therefore likely that immune responses to tumours are often weak (17).

**Tumour antigens**

Tumour cells might express antigens called tumour antigens. These antigens can stimulate immune responses in the host. It has become apparent that patterns of expression of these antigen differ among different tumour and between tumours and normal tissues. Tumour antigens are often classified into different groups based largely on these patterns of expression.

1. **Tumour-specific antigens (TsAgs):** They are the antigens expressed on tumour cells but not on normal cells (16). These are the antigens most likely to evoke immune responses in the host because they are perceived as foreign. Unique tumour antigens are TsAgs that are
expressed on only one or few clonal tumours, reflecting peculiar mutations that are characteristics of those tumours alone.

2. **Tumour-associated antigens:** They are the antigens expressed concurrently on normal cells in the host, and expression may or may not be restricted to the type of tissue from which the tumour originated.

3. **Tumour Antigens recognized by T lymphocytes:** The major targets of protective antitumour immunity in experimental animals and in humans are the tumour antigens recognized by T lymphocytes.

**Tumour Escape mechanisms**

The tumour cells have developed specific immune mechanisms to escape from the host's immune reactions. The growth of a tumour would seem to be the outcome of an equilibrium of those forces which either favour or inhibit the growth of a tumour. The major mechanisms involved in the tumour cell escape include (1) Sneaking through; (2) Modulation of tumour antigens (3) Masking of tumour antigens (4) Induction of immune tolerance and (5) Production and blocking of antibodies.
1. **Sneaking through**

It is the simplest way of tumour cell escape from host immune surveillance (16). The simplest way to avoid death at enemy hand is to avoid capture. One way cancer cells can avoid immune cytolysis is by sneaking through enemy lines until they are strong enough to resist attack. Some tumours are only weakly immunogenic, so in small numbers that they do not elicit an immune response. But when their numbers increase enough to provoke an immune, the tumour load may be too great for the hosts immune system to mount an effective response. But tumour that have been shown to be strongly immunogenic may interact with the hosts immune system and suppresses the immune system's cytotoxic response. Perhaps the most critical factor that allows tumours to grow is a breakdown in the host immunoregulatory circuit, rather than allowing tumour cells to passively sneak through.

2. **Antigenic modulation**

Certain tumour cells have also demonstrated clever evasive tactics by circumventing immune activation. One such strategy is the modulation of tumour cell surface antigens (15). Certain tumour cells can transfer antigens from their surface to the interior cytoplasm making themselves immunologically invisible. Alternatively tumour cells might stop expressing certain surface antigens. Tumour cells might alter their expression of cell adhesion molecules (e.g. ICAM-1 or LFA-1). By decreasing the expression of these substances, tumour cells can reduce the formation of stable contacts with cytolytic cells. Antigenic modulation can also be
affected by redistributing the antigens within the cell membranes in such a way as to prevent
immune reaction. Moreover, tumour Ags can be removed from the surface of the cancer cell by
"shedding". The glycocalyx (sugar coating) of all nucleated mammalian cell is constantly being
shed and resynthesized. This loss of tumour antigens desensitizes the cancer cell populations
and protects them against subsequent cytolysis in the immune host. Shedding of antigens are
often followed by internalization of antigens and the modulation of surface antigens.

3. **Masking of surface antigens**

Another strategy is to devise a cloaking mechanism that renders one invisible. This
tool is realized in certain cancer by production of a mucoprotein called sialomucin that coats
and masks surface tumour antigens. Tumour cells often produce copious amounts of sialomucin.
This molecule binds to the surface of tumour cells, providing a protective shield against immune
attack. Since sialomucin is normal (self) component, the immune system cannot see through the
surface slime layer to the tumour antigens below. Thus the cancer goes undetected by natural
defenses.

4. **Induction of Immune tolerance**

The observation that certain types of tumours can synthesize various
immunosuppressants has led to speculation that some tumours might be able to actively sup-
press the immune response. By taking the offensive, cancer cells could activate specific T<sub>S</sub> cell
populations, thereby crippling effector T and B cell clones. This would induce a state of immune tolerance to the cancer, which could then be free to take over the antibody (14).

5. Production of blocking antibodies

Cancer cells can also use weapons of their own against the immune system. They do this by somehow invoking the immune system to produce blocking antibodies against the tumour antigens. If specific antibodies that can not fix complement are made in response to antigenic challenge by cancer cells, their subsequent binding to tumour cells will be disastrous for the host. Blocking antibodies cannot activate complement, so lysis of the cell is not possible (17). This also means that no C3a or C3b is formed, thus neutrophil-and-macrophage mediated inflammatory reactions are never elicited. Blocking antibodies also cover the surface cancer cells, preventing Tc cells from binding to the hidden receptors. In this way, killing of tumour cells by complement, phagocytosis, and Tc cell is blocked. Production of those blocking Abs has actually been demonstrated to enhance tumourigenesis by hampering immune attacks on all levels.

Tumours can suppress immune responses of the host, thereby impairing the inflammatory response, phagocytosis, and the complement cascade. Certain of these factors seem to be nonspecific and lead to a generalized decline of immunity. Other factors provide specific protection for the tumour cells expressing them. It is well known that cancer patients show a progressive decline in immune responsiveness to all foreign antigens as the disease progresses.
Effector mechanisms resulting in tumour cell destruction

Both humoral and cell mediated immune responses to tumour antigens have been demonstrated \textit{in vivo} and many immunologic effector mechanisms have been shown to kill tumour cell \textit{in vitro}. The challenge for tumour immunologists is to determine which of these effector mechanisms contribute to protective immune responses against human tumours and to enhance these effector mechanisms in ways that are relatively tumour specific.

a. Antibodies

Antibodies are probably less important than T cells in mediating effective antitumour immune responses. Tumour bearing hosts do produce antibodies against various tumour antigens. In some instances, these antibody responses are specific for viral antigens. For example patients with Epstein Barr Virus - associated lymphomas have serum antibodies against EBV-encoded antigens expressed on the surface of their tumour cells. In other cases, human cancer patients produce antibodies against their own tumours that can be used for \textit{in vitro} “autologous typing” to identify tumour antigens. Hybridomas have been prepared from the B cells of tumour patients that produce monoclonal antibodies reactive with antigens on the patients tumours. Again, these antibodies are not specific for antigens expressed exclusively on tumour cells. The potential for antibody - mediated destruction of tumour cells has largely been demonstrated \textit{in vitro} and is attributable to complement activation or antibody dependent cell mediated cytotoxicity in which Fc receptor-bearing macrophages or NK cell mediate the killing (15).
b. Cytotoxic T lymphocytes (CTL)

CTLs provide effective anti-tumour immunity *in vivo*. CTL mediated rejection of transplanted tumour is the only established example of completely effective specific antitumour immunity *in vivo*. In these cases, the effector cells are predominatly CD8\(^+\) CTLs which are phenotypically and functionally identical to the CTLs responsible for killing virus infected or allogenic cells. CTLs may perform a surveillance function by recognizing and killing potentially malignant cells that express peptides which are derived from mutant cellular or oncogenic viral proteins and which are presented in association with class I MHC molecules. Mononuclear cells derived from the infiltrate in human solid tumours called tumour infiltrating lymphocytes (TILs) also include CTLs with the capacity to lyse the tumour from which they are derived (19).

c. Natural killer cells (NK)

NK cells may be the effector cells of both innate and specific immune response to tumours. NK cells can be activated by direct recognition of tumours, or as a consequence of cytokines produced by tumour - specific T lymphocytes. They use the same lytic mechanisms as CTLs to kill cells but they do not express T cell antigen receptors, and they have a broad range of specificities. NK cells can lyse both virally infected cells and certain tumour cell lines, especially haematopoietic tumours, *in vitro*. IL-2 activated NK cells play an important role in tumour killing. These cells are called lymphokine activated killer (LAK) cells. LAK cells ex-
hibit a markedly enhanced and nonspecific capacity to lyse other cells, including tumour cells. NK cells may play a role in immuno surveillance against developing tumours, especially those expressing viral antigens.

d. Macrophages

Macrophages are potentially important cellular mediators of antitumour immunity. Activated macrophages can preferentially lyse tumour cells and not normal cells, *in vitro* (16). Like NK cells macrophages express Fcγ receptors and they can be targeted to tumour cells coated with antibody. The mechanisms of macrophage killing of tumour target cells include the release of lysosomal enzymes, reactive oxygen metabolites and in mice by nitric oxide. Activated macrophages also produce the cytokine TNF-α - which can kill tumours but not normal cells. TNF kill tumours by direct toxic effects and indirectly by effects on tumour vasculature. Direct toxicity depends on binding of TNF to high-affinity cell surface receptors on to tumour cells. The toxicity is in part due to activation of a cell death pathway similar to that induced by Fas ligand binding to Fas. The toxicity may also be a result of the production of free radicals. Direct toxic effects of TNF may also involve disruption of cytoskeleton proteins or interference with gap junction formation. Some tumour derived angiogenic factors, such as vascular endothelial growth factor potentiate endothelial cell response to TNF.
Immunomodulators

Immunomodulators are agents that may augment the immune responses or restore the effector mechanism of host leading to the establishment of a balance between therapeutically desirable and undesirable host reactions. Immunomodulators have a biphasic effect, either by stimulating or inhibiting the immune response (1). Use of immunomodulators in cancer therapy is gaining great momentum in recent years. They include microorganisms, their products, synthetic compounds, plant products, physiological mediators and cytokines. Immunomodulators of microbial origin include methanol extractable residue of BCG, glucose from sacharomyces, lentinan from Basidiomyces, krestins and endotoxins. Immunomodulators which are synthetically originated involve levamisole, polyribonucleotides, maleic anhydride, vinyl ether and tuftsin (16). Microorganisms belonging to immunomodulators include mixed bacterial vaccines, OK-432 and viruses. Immunomodulators of cytokine origin include interferons, interleukins, TNF and GM-CSF - (15). Immunomodulators physiologically originated include perforins, antibodies, complement etc. Immunorestorative agents possess no direct cytotoxic effects. They restore or stimulate the depressed antigen response and cellular immune functions e.g. are thymopoietin, thymosin etc. Immunosuppressive agents include cyclosporine A, Tacrolimus (FK506), Adrenocortical steroids, cytotoxic drugs and antibody reagents (19).

Immunomodulators of plant origin

Plants and plant products are used as adjuvants in cancer therapy. Polysaccharides,
steroids, terpenes, alkaloids, proteins and amino acids present in plants can stimulate the immune system \(31\). They can reduce the severe toxic side effects of both chemo and radio therapy. For example in the case of *Viscum album* the active component, a peptide is responsible for macrophage activation, antibody production, natural killer cell activity and antibody dependent cellular cytotoxicity \(5\). Similarly an extract from the plant *Picrorhiza kurroa* could enhance phagocytosis, lymphocyte proliferation complement and neutrophil activation. \(30\). Polysaccharides and saponin from *Panax ginseng* is responsible for antibody production, increase in serum complement content and IgG level \(8\). Promising plants of immunomodulators include *Asparagus racemosus, Azadirachta indica, Ocimum sanctum, Tinospora cordifolia* \(31\). Curcumin from the plant *Curcuma longa* has been shown to be a good immunostimulant in normal as well as tumour bearing mice \(20\).

Ayurveda, the traditional system of medicine \(1\) lays emphasis on the promotion of health, a concept to strengthening host defense against different diseases. Several plants are labeled as rasayanas such as *Tinospora cordifolia, Asparagus racemosus, Piper longum and Withania somnifera* etc. *Glycyrrhiza glabra* is another plant used as Rasayana in Ayurveda and it is used to sweeten decoctions, to mitigate the action of drastic drugs and to relieve pain caused by muscle contraction \(34\).

*Withania somnifera* is one of the most important component of Ashwagandha Rasayana used in cancer therapy. It is also used for improving health and ojas and used as an immunostimulant \(21\). Roots of *Asparagus racemosus* also known as Shatavari, is used in the
treatment of cancer, dysentery, inflammation and diseases of the eye (34). *Tinospora cordifolia* and Ashwagndha Rasayana have been found to stimulate macrophages as evidenced by an increase in phagocytosis (6). It was also found to produce leucocytosis with a predominant neutrophilia and prevented varying degrees of leucopenia induced by cyclophosphamide (6).

*Curcuma longa* has been found to stimulate the immune system as evidenced by the increase in total WBC count, bone marrow cellularity, natural killer cell activity and it was also used as an antitumour agent (31). *Piper longum* and *Zingiber officinalis* have been found to be effective against gastric ulceration (34). Oral administration of Brahma rasayana was found to enhance total WBC count, bone marrow cellularity and α-esterase positive cells as well as cytokines such as IFN-γ, IL-2 and GM-CSF in normal and radiation treated mice (11). It is used as an immunostimulant in Ayurvedic formulations (10).

The ginseng plant belongs to the Araliaceae family and is one of the most popular tonics used in oriental countries for thousands of years. Traditional Chinese medicine regards Ginseng a medicinal material that can replenish vital energy and improved body strength (8). Among the major chemical constituents of ginseng, the saponins are the pharmacologically active components and have been the targets of research for many years. Ginseng has an immunomodulatory effect and that administration of ginsenosides not only increase the non-specific resistance of the organism but also modulate the specific immune responses. Effect of ginseng saponins on cellular or humoral immunity has been investigated. *In vitro* administra-
tion of ginsenosides to mice significantly enhanced the lymphocyte transformation reaction in response to stimulation with T/B cell antigens. Other investigators have shown that ginseng can enhance specific antibody production in guinea pigs immunized with influenza vaccines and in rats immunized with diphtheria toxoid (8).

**Combination therapy**

It has shown that combinations of biologic agents have greater therapeutic effects than single agents. Clinical trials have studied the effects of immunostimulatory cytokines such as IL-2 administered together with monoclonal antibodies or with activated antitumour lymphocytes such as lymphokine activated killer cells (LAK) or cells generated from the tumour (18). Preclinical studies suggest that certain combinations of biologic and cytotoxic agents may be synergistic. TNF, for example, is both a radiosensitizer and an enhancer of the antitumour activity of cisplatin and fluorouracil (5Fu) and these combinations have been studied extensively in clinical trials.

1. **Radioimmunotherapy**

The selective targeting of radioisotopes to tumour present many theoretical advantages over external beam irradiation with regard to their therapeutic index. Conjugates using isotopes such as Yttrium - 90 linked with MAbs or small oligopeptide ligands such as Octreotide are under development, together with haematopoietic growth factors and these conjugates may permit delivery of therapeutic doses of irradiations to solid tumours (87). The haematopoietic growth
factors such as IL-3 and GM-CSF are being used in radioimmunotherapy. They are found to be effective in disorders such as myelodysplasia and aplastic anaemia.

2. Chemoimmunotherapy

This potential therapeutic strategy has been hindered by a lack of good conjugation technology and appropriate chemotherapeutic agents. Doxorubicin - antibody constructs have been studied clinically, with little objective antitumour activity in solid tumours and with toxicity apparently related to specific antigen binding. Other constructs, including antitumour antibiotics of greater specific activity, such as calicheamicin are under development.

Levamisole is a synthetic phenyl imidazolethiazole, became available for therapeutic trial as an immune modulating agent and it showed a promise, as an adjunct to chemotherapy. IFN-α is an adjuvant in the treatment of patients with advanced melanoma. It is a conceivable mat, because levamisole is an immune modulating agent. One should expect results more in line with those seen with other biologic response modifiers. The success of biologic response modifiers in affecting favourable disease outcomes has generally been related inversely to disease volume. Therefore, we may expect that the use of levamisole in patients with non-measurable, metastatic, intra-abdominal diseases would be more likely to follow the same model, in that these patients treated in a strictly adjuvant setting after undergoing surgery of curative rather than palliative intent. The combination of 5-Fu and leucovorin has emerged as a combination that offers superior results compared with 5-Fluoro uracil alone with respect to both response rates and survival of patients with carcinoma (88).
3. **Chemotherapy - endocrine therapy combinations**

The hypothesis that chemotherapy and endocrine (chemoendocrine) therapy are synergistic depends on the assumption that there are at least two distinctly different clones of cells, one clone responsive to chemotherapy but resistant to hormone therapy and the other resistant to chemotherapy but responsive to hormone therapy.

Patients were randomized to receive either tamoxifen alone, combination chemotherapy with AC (doxorubicin plus CTX) or a combination of tamoxifen plus AC had a subsequent response to tamoxifen. In chemoendocrine therapy tamoxifen is given along with aminoglutathallimade and danazol (90). Several studies of chemoendocrine therapy have stratified patients by receptor status. Combinations of chemotherapy and endocrine therapy are not recommended for the treatment of metastatic breast cancer.

4. **Surgery and chemotherapy**

Several post operative (adjuvant) chemotherapeutic strategies have employed to help decrease the rate of tumour recurrence after resection in patients with carcinoma. Neoadjuvant therapy in patients with locally advanced carcinoma, a carefully designed phase III study comparing neoadjuvant chemotherapy versus primary resection is highly warranted. The British Medical Research Council (MAGIC) trial using epirubicin and cisplatin in combination with continous infusion 5-fluorouracil and the European organization for Research and Treatment of
Cancer trial using a combination of 5-fluorouracil with leucovorin and cisplatin provide definitive answers concerning the potential benefit of the therapy (89).

5. Surgery and radiation

Surgery and radiation can be combined in many different ways. Radiation rarely fails at the periphery of tumours, where cells are small in number and well vascularized. When radiation fails, it usually does so in the center of the tumour where there are large volume of tumour cells often under hypoxic condition (92).

Radiation can be given before or after surgery. It is not clear how often this really results in a cure, because it may only changes gross tumour to microscopic tumour and still result in tumour recurrence. An additional technique for combining surgery with radiation is limited for the surgical removal of the gross tumour. Because the gross tumour limits the radiotherapeutic treatment, new interest has been raised in using surgery as the boost technique.

6. Radiation and chemotherapy

The principles of combination radiation and chemotherapy increases the therapeutic index. Chemotherapeutic agents directly modify the radiation survival course. A good e.g. of this is the use of actinomycin D in the treatment of childhood rhabdomyo-sarcoma or Wilm’s tumour. A second way to increase the therapeutic index is to use drugs that specially affect
tumour response to radiation. A third mechanism is the combination of drugs and Roentgen rays with independent action or additivity. Enhanced local control is obtained when radiotherapy is followed by adjuvant chemotherapy in locally advanced breast cancer (91).

The major advantage of chemotherapy is that it is distributed widely throughout the body, the combination of radiation and chemotherapy may improve the therapeutic index.

7. Combination of chemotherapy and hyperthermia

From time to time, various substances and modalities have been advocated as potentiators of the lethal effects of heat on cancer cells. The recent clinical interest in the use of hyperthermia together with selected anticancer agents is predominantly the result of the laboratory work of Hahn, who showed that many of the anticancer drugs now used clinically demonstrated significantly increased cell killing at elevated temperatures (93). Giovanella et al observed that hyperthermia in combination with L-erythro-α, β dihydroxy butraldehyde as 100-fold more effective against L1210 mouse leukemia cells than either treatment applied separately.

The mechanism responsible for these temperature effects on cell killing by anticancer drugs are not entirely understood. It is not known, for instance, whether the net increase in DNA damage which is thought to underlie the interaction between hyperthermia and many anticancer drugs is due to an increase in drug uptake, an alteration in intracellular distribution of drug, an alteration in drug metabolism, an increase in drug reaction rates with DNA, or heat-induced
inhibition of DNA repair (94). It is possible that more than one of these mechanisms may be operating for any particular drug at elevated temperature.

8. **Thermoradiotherapy**

Heat is known to sensitize tissues to ionizing radiation. Although hypoxic cells are at least as sensitive to hyperthermia as oxygenated cells (95). The primary effect of hyperthermia may be to inhibit the ability of cells to recover from sublethal radiation injury. Many studies employing hyperthermia at 41°-43°C with low dose radiation have achieved remarkable regressions when compared with hyperthermia alone (96). The timing of the application of hyperthermia has a great influence on the outcome of therapy. For the greatest combined effect, the administration of both modalities should be simultaneous. However, the simultaneous therapy may increase the toxic effect on normal tissues. For example one study found that simultaneous and sequential heat application yielded similar rates of tumour control, but that a 4h delay between radiotherapy and hyperthermia resulted in less normal tissue toxicity (97). In practice, heat is usually applied 3-4h after radiation at intervals of not less than 48-72h.

**Anticancer activity of plant extracts and derived compounds**

Studies from our laboratory had shown that *Viscum album* extract could significantly inhibit the proliferation of tumour cells in culture (101). Iscador, an extract from the plant...
*Viscum album* was found to inhibit 20-methyl cholangrene induced carcinogenesis in mice (111). Iscador was also found to inhibit the lung metastasis induced by B16 F10 melanoma cells (112).

Curcumin, the active constituent of *Curcuma longa* possesses antitumour (102), anticarcinogenic (140), antimetastatic (141) and immunomodulatory (9) activities. A dehydrochalacone, isolated from *Pityrogramma calomelanos* was found to be cytotoxic and tumour reducing (103). Treatment with Brahma rasayanas could significantly enhance the survival of ascites tumour bearing animals and reduced the solid tumour volume induced by Dalton's lymphoma Ascites cells (106). It has also shown to inhibit metastasis of B16F-10 melanoma cells in mice (142). Administration of septilin significantly increased the survival of animals bearing Ehrlich ascites tumour (107). Treatment with *Picrorrhiza kurroa* extract could significantly increase the life span of tumour bearing animals and reduced the solid tumour volume (108) and was found to inhibit hepatocarcinogenesis (143). Extract of some spices such as ginger and pippali were found to be cytotoxic (109) towards Dalton's lymphoma ascites cells. Oral administration of extract of black pepper, asafoetida, pippali and garlic could increase the percentage of life span in the mice induced by Ehrlich ascites tumour (110).

Many of the currently used antineoplastic agents are natural products initially isolated from plants. The vinca alkaloids are present in minute quantities in *Catharanthus roseus*. Although a number of these compounds have been investigated clinically, only vincristine and vinblastine have been approved currently for clinical use in the United States. Vincristine is an essential part of combination chemotherapy regimens for pediatric and adult lymphocytic leukemia
Vinblastine has been an integral component of curative chemotherapy regimens for germ cell cancers of the testis and advanced Hodgkins disease and other diseases.

Extracts of the mandrake plant (*Podophyllum peltatum*) have been used for medicinal purposes for centuries as cathartics or as treatment for parasites or veneral warts (99). It is found to be effective against non Hodgkins lymphomas, germ cell malignancies, leukemias and small cell lung carcinoma. Another important compound taxol is derived from a plant *Taxus brevifolia* was shown to have broad antitumour activity and has been recognized as a new chemotherapeutic agent (100). Camptothecin is obtained from *Camptotheca acuminate*. Camptothecin and its derivative can target eukaryotic DNA topoisomerase I. In clinical trial, camptothecin revealed marginal effects on maliganant melanoma, Hodgkins lymphoma, T lymphoplastic lymphoma. Homoharringtonine (HHT) is present in the bark of several species of cephalotaxes, first isolated and characterised by Powel and his colleagues (195). HHT belongs to a class of cephalotoxin esters which include harringtonine, isoharringtonine, and deoxyharringtonine. It acts on the ribosome to inhibit protein synthesis.

*Withania somnifera*

*Withania somnifera*, Dunal commonly known as Ashwagandha belonging to family Solanaceae is a well known plant which is used in several indigenous drug preparations. In Ayurveda, the roots of Ashwagandha are attributed with the properties of health maintenance and restoration. The similarity between the properties of Ashwagandha roots and the restorative properties of ginseng roots has led to it being called Indian ginseng (136).
The major components present in *Withania somnifera* has been found to be an essential oil ipurinol, a crystalline alchol withanolil, hentria contane , phytosteroids, fatty acids and alkaloids (31). The pharmacological activity is attributed to the presence of several alkaloids - Withanolides (37). Withaferin is the most important of the Withanolides isolated so far (35). Withaferin A, a steroidal lactone isolated from the leaves of Withania, was found to possess antibacterial and antifungal, antitumour and antiinflammatory activities (134). Withaferin A could inhibit Ehrlisch ascites tumour growth in Swiss albino mice (39). Withaferin A, isolated from the roots of *Withania somnifera* reduced the survival of chinese hamster V79 cells in a dose dependent manner (35). The granuloma tissue formation inhibiting activity of Withaferin A has been reported (38). The alcholic extract of the dried roots and the active component Withaferin A isolated from the extract showed significant antitumour and radiosensitizing effects on experimental tumours *in vivo* without any noticeable systemic toxicity (35).

The immunomodulatory activity of an Indian Ayurvedic medicinal preparation, Ashwagandha was studied in mice with myelosuppression induced by azathioprine or prednisolone(25). Treatment with Ashwagandha showed a significant increase in hemolytic antibody responses towards human erythrocytes demonstrating an immunostimulatory effect (26). *Withania somnifera* is reported to have an important role in the regulation of circulating thyroid hormone concentration in female mice. *Withania somnifera* could enhance only serum T (sub4) concentration (136). The active principles of *Withania somnifera* (equimolar concentrations of sitonoindosides VII - X and Withaferin A) were investigated for their effects on rat brain frontal cortical end striatal concentrations of superoxide dismutase, catalases and glutathione peroxidases.
The antioxidant effects of the active principles of *Withania somnifera* may explain, at least in part, the antistress, immunomodulatory cognition facilitating, antiinflammatory and antiaging effects reported in experimental animals and in clinical situations (135). These findings are consistent with the therapeutic use of *Withania somnifera* as an Ayurvedic rasayana and Medhya rasayana (35). *Withania* can be applied externally and were rarely used as narcotics, sedatives and aesthetics (28). It is used as an antiepileptic agent and used as a remedy for female sterility (29). Ashwagandha used as a remedy for Alzheimer’s disease (24). *Withania* could significantly reduce the IgE mediated anaphylaxis as evidenced by the reduction of ovalbumin induced paw oedema (27). It was also found to possess adaptogenic properties (36). Ashwagandha was tested for antistress activity assessed in mice by a swimming endurance test and was found to possess anabolic activity (32). The analgesic activity of *Withania somnifera* was potentiated significantly by cyproheptadine (32). Antitumour and radiosensitizing properties of Ashwagandha were also reported (39). The extract also increased hepatic glucose - 6 phosphatase activity and antiperoxidative effects as mediated either by a decrease in hepatic lipid peroxidation (33) or by increasing the activity of antioxidant enzymes. It was found to be effective in cases of leukoderma, bronchitis, asthma, marasmus etc (35) and was found to possess hypotensive (24); anti-spasmodic (35) activities.

The major drawback in cancer therapeutic methods is the suppression of immune system (66). Hence the use of immunomodulators that can circumvent the immunosuppression are highly relevant in the treatment of cancer. This study was aimed to analyse the immunomodulatory activity of *Withania somnifera* in detail and its implication in the treatment of cancer.
Figure

Withania somnifera