1 Introduction

1.1 Cancer

Cancer is the most precarious disease characterized by uncontrolled proliferation of cells without any physiological demands of the organism. Cancer may be defined as unnecessary tissue growth that results from an imbalance between cell division and apoptosis, (programmed cell death); due to various genetic and epigenetic alterations. The specific cause of cancer is elusive, which may be possibly attributed to viral, genetic, chemical, radiation, environmental or immunological factors. Cancer is a genetic disorder arising from the progressive accumulation of many gene alterations. The disease remains challenging despite of mammoth research efforts across the world(1).

Cancer incidence rate is generally expressed as age standardized incidence rate (ASR) per 100,000 persons. As per 2011 statistic, the ASR was reported to be 300/225 (male/female) and 160/138 (male/female) in more developed and less developed areas, respectively(2).

There are varieties of cancer which are classified based on the organ or system affected. For example, it includes brain cancer, breast cancer, cervical cancer, colon cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, prostate cancer, skin cancer, testicular cancer, thyroid cancer and uterine cancer. Hodgkin disease and leukemia are the examples of where system is usually involved. Cancer cells are also divided in to benign and metastatic.

Leukemia is the blood cancer in which the white blood cells becomes cancerous and spreads across the body.
1.2 Leukemia

Leukemia comes from a Greek word meaning “white blood” and is often referred to as cancer of the blood. The term refers to a group of closely related malignant condition affecting the immature blood-forming cells from the bone marrow appearing in the circulation. Leukemias are neoplasms of hematopoietic cells that proliferate initially in the bone, spleen, lymph nodes and later in other tissues. The ASR of leukemia was reported to be 9/6 (male/female) and 4.5/3.6 (male/female) in more developed and less developed areas, respectively (2).

Leukemia is classified broadly related to the cell origin (lymphoid or myeloid) as well as to the rapidity of the clinical course (acute or chronic), but modern categorization have identified specific leukemias on the basis of biologic, antigenic and molecular characterization as given in Figure 1-1(3).

![Figure 1-1: Classification of Leukemia](image)

CMML- Chronic Myelocytic leukemia, CML- Chronic Myelogenous Leukemia
1.2.1 Acute Lymphoblastic Leukemia (ALL)

ALL is the clonal proliferation of immature haemopoietic cells, arising by malignant transformation of a single haemopoietic progenitor. The cellular replication and expansion of the transformed clone follows. The leukemia cells start accumulating in the bone marrow, hampering the normal hematopoiesis and resulting in the replacement of normal WBCs. Leukemic cells finally infiltrate into other tissues such as lymph nodes, liver, spleen, skin, gums, viscera and the central nervous system.

ALL is commonly seen in children, though a considerable number of cases occur in adults. Prior to 1948 the median survival for children with ALL was two months after the diagnosis. Today, the cure rate in children has reached 50 to 60%, however the mortality remains high in adults. ALL in adults differs from pediatrics on account of prognostic factors, response to therapy and patterns of relapse(4).

Acute myeloid leukemia (AML), chronic lymphoblastic leukemia (CLL) and hairy cell leukemia in adults are observed to increase in incidence with age, especially in the sixties and seventies.

1.2.1.1 Signs, symptoms and morbidity of ALL:

Early signs of ALL may be similar to those of flu or other common diseases, such as a prolonged and persistent fever, tiredness, bones or joints ache, or swollen lymph nodes.

The distinct signs, symptoms and morbidity are anemia, fever (neutropenia), weakness and fatigue, frequent infections, loss of appetite and/or weight, swollen or tender lymph nodes, liver or spleen, easy bleeding or bruising (thrombocytopenic purpura), tiny red spots (called petechiae) under the skin, swollen or bleeding gums (5).
1.2.1.2 Diagnostic features of leukemia:

The normal WBC count is 4500-11000/µL, whereas in leukemia, the count is of the order 100000-1000000/µL. In AML the WBCs are immature to the stage of myeloblast or promyelocyte level and in ALL the lymphoblast stage is prominent.

The diagnosis of leukemia is often confirmed by high immature WBC (blast) count in blood and biopsy of lymph node and bone marrow for blast cells. The significant abnormality observed in ALL is increase in lymphoblasts (45%± 30%) (5).

1.2.1.3 Treatment methods for ALL:

The “first phase of treatment” of ALL is the “induction of remission”. L-asparaginase in combination with other chemotherapeutic agents is the primary treatment. During remission, leukemic cells are no longer identifiable in the marrow and normal marrow function is reestablished, as evidenced by morphologic examination of the marrow and peripheral blood. After remission is achieved; treatment is usually directed towards the central nervous system to eliminate occult CNS disease. The third phase of treatment, maintenance, involves the use of prolonged systemic therapy to maintain complete remission for as long as possible. In patients with the poor prognosis, the addition of an intensive period of pre induction and reconsolidation early during maintenance therapy may prolong remission and improved disease free survival (6).

Depending on the staging and type of cancer the treatment strategy may be total cell kill by radiation, bone marrow transplantation, and chemotherapeutic agents viz., 2’-Deoxy-2-Chloroadenosine”; vincristine, cyclophosphamide, cytosine arabinoside, melphalan etc., with L-asparaginase (see Table 1-1).
Table 1-1: List of treatment regimen for ALL

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Remission induction</td>
<td>Vincristine + prednisone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vincristine + prednisone + <strong>L-asparaginase</strong></td>
</tr>
<tr>
<td>2.</td>
<td>Central nervous system therapy</td>
<td>Intrathecal methotrexate</td>
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<tr>
<td></td>
<td></td>
<td>Intrathecal methotrexate + 24-gy irradiation of the cranium</td>
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<tr>
<td>3.</td>
<td>Maintenance therapy</td>
<td>Daily oral 6-mercaptopurin and weekly oral methotrexate, with intermittent short pulses of vincristine and prednisone (induction)</td>
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<tr>
<td>4.</td>
<td>High risk patient to prevent</td>
<td>Vincristine, prednisone, daunorubicin and <strong>L-asparaginase</strong> (intensive consolidation) cylophosphamide, 6-Mercaptopurine, cytarabine, intrathecal methotrexate with whole brain radiation therapy(18gy)</td>
</tr>
<tr>
<td></td>
<td>marrow relapse</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Another intensive regimen</td>
<td>Cyclophosphamide, vincristine, prednisone, daunorubicin and intrathecal methotrexate followed by an early, five-drug intensification (cytaraine, 6-thioguanine, BCNU (carmustine), <strong>L-asparaginase</strong> and intrathecal methotrexate, in addition to radiation therapy.</td>
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1.3 Therapeutic enzymes

Therapeutic application of enzymes involves as anticancer agents, anticoagulants (thrombolytics), etc. However, the enzymes are also used as diagnostics and replacement therapeutics. L-asparaginase is a typical example of anticancer enzyme. The two major classes of therapeutic enzymes includes those acting on small molecules which are nutrients for cancer cells and those acting on macromolecules by degrading biomolecules like membrane structure, functional proteins and nucleic acids.

Therapeutic enzymes are natural products widely distributed in biosphere. The rich resource of therapeutic enzymes includes plant, animal and microorganisms including bacteria, yeast and fungi. The therapeutic use of enzymes of microbial origin is limited because of their immunological issues. The economic feasibility and easy production with cDNA technology has helped to overcome the limitations and are presently dependable resources of therapeutic enzymes. In addition, microbial enzymes are advantageous due to biochemical diversity, simple and fast production and ease of extraction. The use of cheap raw materials, inexpensive media, and high yields of enzymes makes the manufacture of enzymes cost effective. There is an advantage of better yield without any seasonal variation as the technology is based on optimized genetically engineered microorganism.
1.4 Conclusion

Cancer has emerged as one of the major cause for human suffering with unprecedented morbidity and mortality figures. Change in the lifestyle, environment and its interaction on gene expression are gross etiological factors. The diversity of the cancer manifestation and non-availability of safe and effective anticancer agents make it a threatening disease to treat. The anticancer agents may be broadly classified as cytotoxic agents and non-cytotoxic agents. Cytotoxic agents are known for their severe ADRs like cancer, organ damage and emesis. The non-cytotoxic agents are mild in their action; however they cannot be used as a single treatment for cancer. But when given with cytotoxic agents, they are found to be useful in reducing toxicity and increasing efficacy. L-asparaginase is a good example of therapeutic enzyme for cancer treatment. L-asparaginase is highly suitable for treatment of blood cancer as cancer cells are distributed throughout the body along with the blood. L-asparaginase is known to act by hydrolyzing the asparagine and causing deficiency of the amino acid for cancer cells, whereby it limits the growth of cancerous cell. L-asparaginase is non-cytotoxic anticancer agent used with other chemotherapeutic agent as mentioned in Table 1-1.

L-asparaginase used in current therapeutics is from bacterial origin, which has limitations like hypersensitivity and anaphylactic reactions. It is well established that the fungal origin proteins are likely to be less severe in hypersensitivity and anaphylaxis. There is an enormous scope to discover novel fungal resources alternative to the L-asparaginase of bacterial origin.
Exploration of soil and marine sources for microbes producing asparaginase