

UNDERSTANDING LEUKAEMIA- AN INTEGRATIVE REVIEW OF PATHOGENESIS, DIAGNOSIS, AND EMERGING THERAPIES

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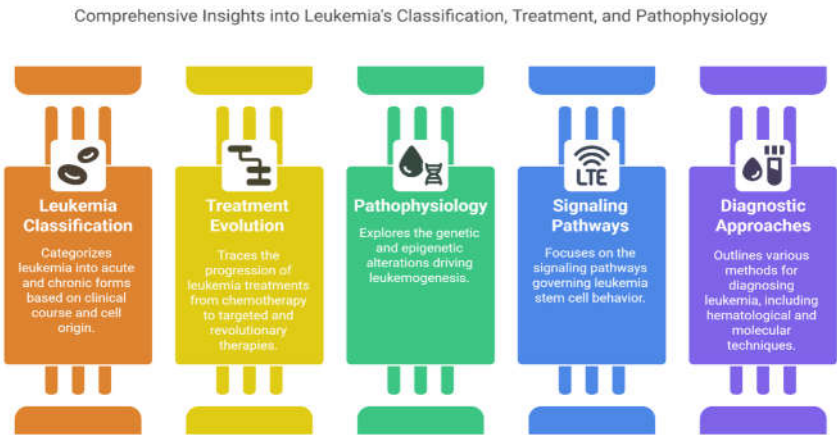
ABSTRACT:

This review article provides a panoramic description of Leukaemia, where unusual WBC growth is observed. It outlines the classification of leukaemia into acute and chronic types (AML, ALL, CML, CLL) based on clinical course and cell origin, and the molecular mechanisms underlying its aetiology, such as chromosomal changes, genetic predisposition, and environmental factors. The article follows the history of development of leukaemia treatments, from initial chemotherapy protocols to recent advances such as TYK inhibitors (e.g., Imatinib) for CML, monoclonal antibodies (e.g., Rituximab) for B-cell malignancies, and CAR-T cell therapy.

It also covers the histopathology and pathophysiology of leukaemia, outlining the multistep leukemogenesis process driven by genetic and epigenetic lesions, clonal evolution, and microenvironment pressures. Some genetic mutations, e.g., the BCR-ABL1 fusion gene in CML, and drug resistance mechanisms are discussed. The review also points out the role of signaling pathways like Notch, Wnt, and JAK/STAT in leukaemia stem cell maintenance and drug resistance. It also touches upon developments in diagnostic techniques, e.g., blood tests, bone marrow examination, and molecular tests like karyotyping and FISH.

Key-words: Leukaemia, Leukemogenesis, Chemotherapy, Targeted Therapy, Immunotherapy, Monoclonal Antibodies, CAR-T Cell Therapy, Leukaemia Stem Cells (LSCs), Signaling Pathways, Genetic Mutations, Molecular Diagnostics, Nanotechnology.

Graphical Abstract:



1. INTRODUCTION

A class of deadly malignant diseases that impact hematopoietic tissues and blood is known as leukaemia. However, the word "leukaemia," which translates to "white blood" from Greek, describes the malignant growth of Leukocytes, or WBC. The pathologic characteristic of leukaemia is likely caused by a variety of reasons, including chromosomal abnormalities, radiation, chemicals, viruses, immunodeficiency, and genetic susceptibility, which are distinguished by both the cell of genesis and the disease's clinical progression. There is also an unusual form of atypical leukaemia with unique clinical features. Leukaemia patients can receive a variety of treatments, such as hematopoietic stem cell transplantation, chemotherapy, radiation, and monoclonal antibodies³. Following the onset of the disease, several therapeutic approaches are chosen based on bone marrow and blood cell specimens' morphologic assessment, the examination of the proliferation of cytoplasmic markers on the cell surface, the detection of chromosomal abnormalities, or molecular/cytogenetic marker screening. Thus, understanding the molecular causes and involvement of pathways in the onset and progression of Leukaemia is essential to further therapeutic study and therapy (Figure1).¹

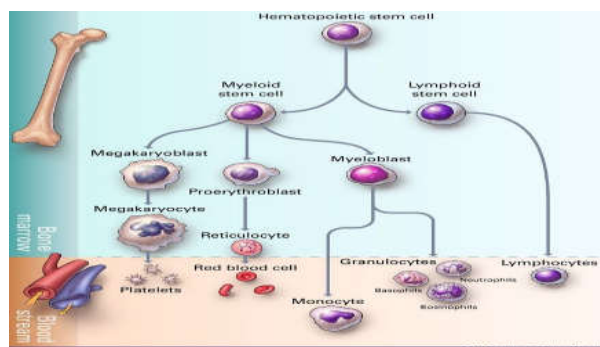


Fig. 1: Leukaemia is usually categorized by the type of stem cell that has developed into a malignant state, either myeloid or lymphoid.

1.1 Biostatistics:

According to statistics, worldwide, 474,519 cases were reported, of which 67,784 were from North America. 3.2% of the death rate, which is approximately 11 per 100,000. The age distributions of ALL and AML, two significant paediatric and adult illnesses, are bimodal, with the elderly CML and CLL primarily affecting cancers. The disease's mortality rate has decreased by 1.5% yearly on average since 2006, while its incidence has increased by 0.6% annually on average.²

1.2 Significance of leukaemia research in advancing oncology

Over the past forty years, for AML, the recommended level treatment has mostly remained constant. Treatment includes integration after remission using stem cell transplantation or chemotherapy based on cytarabine after extensive induction therapy, usually with a combination of cytarabine and an anthracycline. However, approximately 40% to 50% of patients will attain long-term disease-free life because between 50% and 60% of patients relapse.

Due to their disease's inherent resistance to the present conventional cytotoxic therapies along with comparatively low levels of susceptibility to these substances, elderly individuals with AML—typically aged 60 to 65 in most publications—have a much worse prognosis. Crucially, the majority of AML patients belong to this group.

Numerous genes that regulate predictive somatic and genomic pathways and developments in the molecular characterisation of the pathogenic pathways of leukemogenesis have led to the discovery of mutations with potential for therapy.³

We, the authors of this article, present the latest and emerging therapies for leukaemia, emphasizing how insights into its pathophysiology have shaped the evolution of targeted, personalized, and immunotherapeutic treatment strategies.

2. HISTOPATHOLOGY

Acute Leukaemia:

In acute leukemia patients, over 20% of blasts are found in the bone marrow or peripheral blood. Bone marrow biopsies usually exhibit increased proliferation, including erythroid precursors, blasts, and varying numbers of granulocytic or monocytic cells. The following conventional markers are part of the assessment: HLA-DR, CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, and CD117. Additionally, a bone marrow aspirate or peripheral smear is submitted for a gene mutation panel of several genes, including ASXL1, CEBPA, DNMT3A, FLT3, IDH1, IDH2, NPM1, RUNX1, and TP53, which have consequences for prognosis and treatment.

Additionally, B and T lymphoblasts, which make up the bone marrow, have a higher cellularity in ALL. Small nucleoli, dispersed chromatin, and irregular, cleft nuclei with undetectable cytoplasm are characteristics of these cells. Commonly utilized T-cell lymphoid immunostains include TdT, CD2, CD3, CD5, and CD7. CD10, CD19, CD22, HLA-DR, PAX5, CD20, and

CD79a are common B-cell lymphoid immunostains. To validate the diagnosis of the pure lymphoid lineage, myeloid characteristics such myeloperoxidase (MPO) should not be present. A rare condition known as mixed phenotype acute leukaemia (MPAL) combines myeloid and lymphoid characteristics. The inclusion of newer therapeutic medications in treatment algorithms has made cytogenetics screening for Ph chromosome status and Ph-like translocation crucial. ⁴

Chronic Leukemia :

White blood cell counts are often high in chronic leukaemia, and a smear may show a noticeable left shift or granulocyte predominance, commonly seen during the acute illness phase, and persists after several lab tests, considered as CML. It is possible to identify the translocation t(9;22) in CML by applying fluorescent in-situ hybridization (FISH) to peripheral blood. A bone marrow biopsy typically reveals 100% cellular marrow with raised granulocyte precursors, basophils, eosinophils, and occasional monocytes.

In CLL, white blood cells—mainly CD5+ and CD23+ B-lymphocytes—are more common. The clonal lymphocyte population needs to be greater than 5,000/mcL to be diagnosed. If there are fewer than 5,000 clonal cells per milliliter, the situation is called monoclonal B cell lymphocytosis of uncertain significance. For diagnostics, flow cytometry is commonly employed. Patients would need to have their trisomy 12, del(11q), del(13q), immunoglobulin heavy chain variable region (IGHV) gene mutation status, del(17p), and TP53 mutation status assessed to determine the optimal treatment regimens. ⁵

2.1 Pathophysiology

➤ Introduction - Leukemogenesis

It is believed that leukemogenesis (Figure 2), the process by which leukaemia arises, is an evolutionary event that starts in cells located in the primordial cell fraction and hematopoietic stem. Mirroring broader carcinogenesis models, leukaemia's development is not a singular event but rather a multistep process involving the accumulation of multiple independent genetic and epigenetic alterations. A prominent model, particularly relevant to leukaemias arising after alkylating drug therapy or benzene exposure, describes a progression from initial dysplastic changes with distinct clonal cytogenetic abnormalities to the condition of acute myelogenous leukaemia (AML) formation. Characterizing these specific chromosomal aberrations and observing alterations in genes that promote cell growth offer a valuable framework for

investigating chemical leukemogenesis and gaining a comprehensive understanding of leukaemia's origins and progression in general. ⁶

➤ **The Continuum of MDS and AML (Preleukaemia)**

The link between Leukaemia, a condition called myelodysplastic syndrome (MDS), and preleukaemia is crucial to understanding chemical leukemogenesis. It is commonly acknowledged that Protooncogenes and other growth-promoting genes can be affected in their regulation and function by chromosomal abnormalities or deletions. Leukaemia caused by an alkylating medication or occupational exposure frequently follows a natural history in which growing dysplastic or "preleukemic" changes precede AML, and a particular pattern of clonal chromosomal aberrations is also present. Among the blood dyscrasias that characterize preleukaemia are cytopenias and dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis is now primarily referred to as myelodysplastic syndromes (MDS). MDS progresses to overt AML in the same way that solid tissue malignancies grow from metaplasia and dysplasia to carcinoma. This discovery, along with the fact that secondary MDS often progresses to frank AML, makes a compelling case for treating MDS and AML as one disease continuum, particularly when considering chemical leukemogenesis. ⁷

➤ **Protooncogenes and Tumor Suppressor Genes in AML: Multiple Pathways to Malignancy**

Many protooncogenes (like ras) and tumor suppressor genes (like p53, Rb) have shown structural or functional changes in AML at different frequencies, along with mutations in genes on chromosome 5. Nevertheless, the genesis of AML has not been conclusively linked to a single or continuous pattern of protooncogene participation, indicating that several genes may interact through various routes during the disease's evolution. For instance, whereas approximately 25% of de novo AML cases exhibit activation of the ras protooncogene, specifically N-RAS, this frequently happens later in the course of the disease, following the appearance of clonal chromosomal abnormalities. When found, ras mutations are typically found in leukemic cell subclones only. Although it happens infrequently in MDS/AML, inactivation of the p53 gene is linked to aggressive illness and a poor prognosis. Similarly, a poor response to treatment is associated with decreased expression of the retinoblastoma (Rb) protein in certain AML cases. ⁸

2.1.4 Models of Leukemogenesis: Clonal Evolution and Microenvironmental Influence

To explain leukemogenesis, two main theories have been put forth: one focuses on clonal abnormalities in hematopoietic stem and progenitor cells, while the other suggests that stromal cells like fibroblasts, endothelial cells, and macrophages play a major role in altered growth factor production. There is strong evidence that the majority of AML and MDS cases originate from myeloid-restricted HPC. The frequent finding of numerous peripheral cytopenias and dysplasias in s-AML and the frequent presence of aberrant cytokine production in MDS/AML cases support the idea that the origin is stromal.^{7,9}

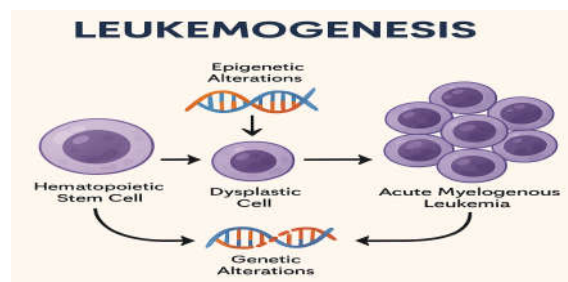


Fig. 2: Process of Leukomogenesis

2.2. Understanding Genetic Mutations in Leukaemia through CML Mutation

Chronic myeloid leukaemia (CML), characterized by the BCR-ABL1 fusion gene originating from the Philadelphia chromosome (Ph), has long been believed to be caused by a single oncogene. An oncokine protein that is constitutively active and causes unchecked cell proliferation is encoded by this fusion gene. the creation of tyrosine kinase inhibitors (TKIs) that specifically target the oncoprotein BCR-ABL saved the lives of many patients. Patients may even qualify for treatment-free remission (TFR) in specific circumstances if they have maintained deep molecular remission. In CML management, reaching TFR has emerged as a new priority.

Nonetheless, the variation in clinical results after TKI therapy implies that BCR-ABL1-independent pathways have a role in the etiology of CML, as mutations in the Abl-kinase domain (Abl-KD) due to acquired secondary resistance (about 60% of cases), many resistance mechanisms, especially primary ones, are still unknown. Primary (5–10%) or secondary (20–30%) resistance to TKIs is present in certain people. Additionally, roughly 5% of CML patients experience poor outcomes as they go between the aggressive blast phase (BP) and the chronic phase (CP). Leukemic stem cells (LSC) may have survival routes other than BCR-ABL1 signalling, as they are also linked to recurrence following TKI withdrawal.

Additional genetic events have been identified as contributing to the pathogenesis of CML as a result of advancements in sequencing technologies. The identification of BCR-ABL1 in healthy people raises the possibility that other genetic changes may be required for complete leukemic transformation. Point mutations and chromosomal abnormalities are among the other genetic aberrations that may result from the genomic instability brought on by BCR-ABL1 itself [8, 28–30]. These mutations may result from the suppression of DNA repair systems and increased DNA damage caused by ROS. The occurrence of CML has also been connected to the accumulation of genetic abnormalities in recognized cancer genes (Figure 3). While genetic data is integrated into the management of acute leukaemia, its incorporation in CML management has been lacking, with risk stratification primarily relying on clinical scoring systems and BCR-ABL1 level monitoring. This highlights the need for systematic studies investigating the mutational landscape across different CML phases.⁸⁻¹⁰

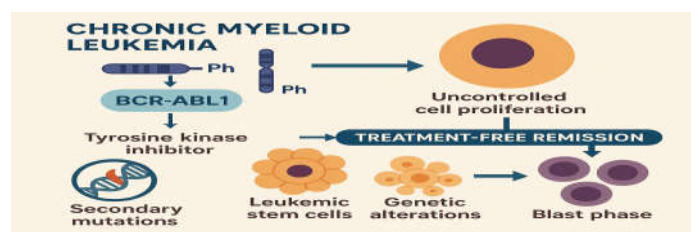


Fig. 3: Genetic Mutations in CML

3. SIGNALING PATHWAYS OF LEUKAEMIA

3.1. Molecular Properties and Origin of Leukaemia Stem Cells

First discovered in AML in 1994, LSCs have now been found in a variety of malignancies. Known as leukaemia-initiating cells, or LSCs, Dick and associates demonstrated that hematopoietic malignancy could only be caused by leukemic cells that exhibited CD34⁺/CD38[−] signals, such as normal adult stem cells. Research on the origin of LSCs has grown significantly. The normal stem cells may become cancer stem cells (CSCs) as a result of random mutations that occur during DNA replication. Additionally, according to certain research, mature cells can give rise to CSCs, including LSCs, by processes such as gene transfer, genomic instability, and microenvironment modification. Another process that can start and spread cancer is cell fusion. Tumor cell phenotypic and genotypic diversity may be influenced by the fusion of tumor cells with lymphocytes (Figure 4). Interestingly, compared to normal cells and cancer cells, LSCs have unique metabolic flexibility. As seen in Fig., LSCs can alternate between glycolysis and oxidative phosphorylation (OXPHOS), which are activities that cancer and healthy cells typically conduct, in order to preserve homeostasis and encourage

tumor growth. A population known as LSCs can also be produced by more differentiated and modified typical stem cells. Self-renewal, high proliferation capability, NF-kappa B pathway activation, and transplant migration are some of the unique features of LSCs. Certain cell surface indicators that can be targeted for cancer treatment are expressed by LSCs. For example, LSCs are frequently CD34⁺/CD38⁺/CD123⁺ in AML. A cell surface glycoprotein called CD34 is connected to the success of therapy, but CD38 is linked to the prognosis of LSC. Bone marrow LSCs can enter the bloodstream more easily if CD123 is upregulated. The CD33 antigen is a promising therapeutic target because it is only found in LSCs and is strongly expressed in AML blasts. Unlike AML, B-ALL has an overview of leukaemia-initiating cells, with LSCs that can be CD34⁺/CD38⁺/CD19⁺. Transformation of primitive hemopoietic cells can result in B-cell precursor ALL, CML, and AML. The resulting LSCs have limited differentiation capacity but can self-renew, which may accelerate the course of the disease. The expression of other antigens, including as CD25, CD26, and IL-1RAP, varies in CML-LSCs. STAT5 activity modulates CD25, and high expression can inhibit the growth of CML-LSCs. Through the NF- κ B and AKT pathways, IL-1RAP binding to CD25 can stimulate CML-LSC proliferation. The SDF1/CXCR4 axis can be cleaved by the multifunctional glycoprotein CD26, releasing bone marrow CML-LSCs into the bloodstream.¹¹

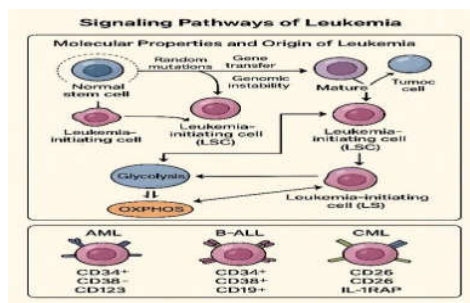


Fig. 4: Signalling Pathways of Leukaemia

3.2. Epigenetic Pathways Involved in Leukaemia Stem Cells

The DNA damage response (DDR) is a complicated DNA repair system that helps to maintain genomic integrity. DDR deficiencies lead to cancer development and can be used to target malignant LSCs while causing minimum harm to healthy cells. DNA repair elements' stability, location, activity, and interactions with DDR factors can all be impacted by abnormal control, hastening leukaemia progression. Thus, discovering unregulated DNA repair molecules has promise for tailored leukaemia therapy. DNA repair gene mutations or polymorphisms, whether inherited or acquired, can increase a person's risk of developing leukaemia. Epigenetics refers to heritable phenotype modifications that occur outside of the DNA

sequence, such as DNA and histone modifications (epigenetic marks) mediated by various enzymes, as illustrated in the figure. Changes in the epigenome contribute to chromosomal instability, give LSCs a survival advantage, and promote cancer initiation and progression. Although normal development requires epigenetic modulators, LSCs are characterized by dysregulated DNA modification patterns. DNA methyltransferase enzymes (DNMTs) mediate cytosine methylation in CpG patterns, it is linked to hematological disorders and is most likely important in LSCs. DNMT deficiency has been associated with Leukemogenesis and LSC growth. Many mutations including TET2, which is linked to a number of hematological illnesses, have been found using genomic profiling. By increasing multipotent and myeloid progenitors, TET2 inhibition can promote HSC self-renewal and lead to the buildup of pre-LSCs. Major histone modifications include lysine (K) residue methylation and acetylation. According to cancer genomics studies, Aberrant EZH2 expression is involved in tumor initiation in a variety of leukaemia, with CML-LSCs showing high expression, which is critical for their survival and maintenance. Efforts are being made to develop drugs that can reverse specific histone methylation marks, such as lysine-specific demethylase 1 (LSD1) inhibitors, which are considered novel epigenetic targets due to LSD1's influence on both DNA and histone methylation. MicroRNAs (miRNAs), or small non-coding RNAs (ncRNAs), are another layer of epigenetic control in leukemogenesis. MiRNAs and DNA methylation can work together to alter the ratio of LSC self-renewal to differentiation. For instance, it has been demonstrated that upregulating miR-130 b and miR-181 in LSCs increases their capacity for self-renewal and carcinogenesis. Because epigenetic signatures are reversible, epigenetic genes make appealing therapeutic targets, and epigenetic modifications are early events in LSC formation (Figure 5). In animal models of CML, targeting EZH2 has demonstrated notable inhibitory effects on LSCs and increased survival. Leukaemia progression is also influenced by other ncRNAs, which target genetic and epigenetic factors and may provide therapeutic opportunities.¹²

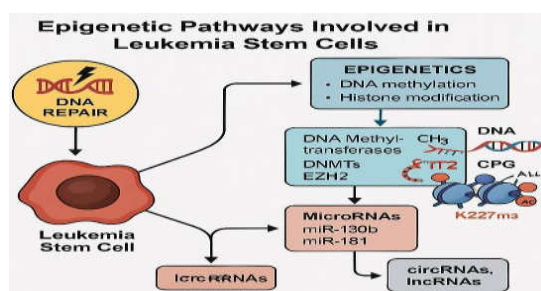


Fig. 5: Epigenetic Pathways Involved in Leukaemia Stem Cells

➤ Hedgehog Pathway

Although it is debatable whether the Hedgehog pathway (Figure 6) is essential for sustaining AML-LSCs, the route is abnormally activated in LSCs. According to certain research, survival of AML-LSCs are influenced by the Hedgehog pathway and its constituents, Smoothened (Smo) and GLI1, which make AML-LSCs more sensitive to chemotherapy. It has been demonstrated that combining hedgehog signaling antagonists with chemotherapy reduces LSC dormancy and encourages differentiation. The maintenance of blast crisis (BC) and the frequency of LSC are regulated by this signaling pathway. Since LSC persistence contributes to AML relapse, blocking Hedgehog can push LSCs into the cell cycle and increase their chemosensitivity. smo knockdown might contribute to a reduction in CML-LSC pathogenesis. Combining the tyrosine kinase inhibitor nilotinib with the Smo antagonist LDE225 may be a unique strategy for the eradication of CML-LSC. Furthermore, mesoporous silica nanoparticles carrying siRNAGLI1 and siRNASMO have shown promise in inducing leukaemia cell death and may be employed as a combination of chemotherapeutic drugs to treat leukaemia.¹³

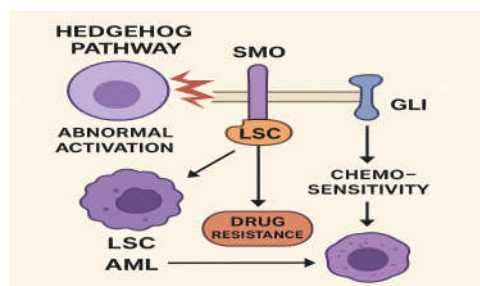


Fig. 6: Hedgehog Pathway

➤ The pathway of Wnt/ β -catenin

The Wnt/ β -catenin signaling pathway (Figure 7) has a major impact on LSC formation. While activating β -catenin can increase MLL-LSCs, preventing it can return LSCs to a pre-LSC-like state and significantly reduce LSC features such as cell proliferation, self-renewal, leukaemia development, and response to GSK9 antagonist therapy. Studying how β -catenin functions in MLL-LSCs suggests the targeted eradication of AML-LSCs. It has been shown that in fully developed leukaemia caused by MLL-AF9, suppressing COX, an abrogator of β -catenin, lowers both β -catenin levels and LSC frequency, indicating that certain AML-LSC subtypes rely on the Wnt pathway for self-renewal. Although its effects in vivo may be limited, WNT974, an inhibitor of Wnt ligand palmitoylation in vitro. Combining WNT974 with other

treatments in the AML microenvironment to preserve Wnt targeting may lead to the elimination of AML-LSC. There is a window of opportunity for therapeutic targeting of LSCs because blocking this receptor has been shown to diminish the leukaemia-initiating ability of LSCs by triggering differentiation without causing damage to normal stem cells¹⁴.

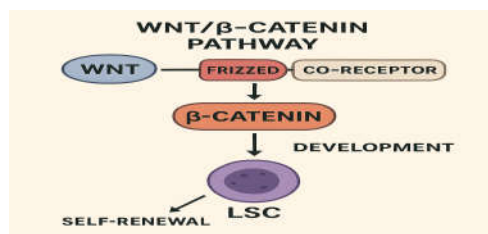


Fig. 7: Wnt/β-catenin Pathway

➤ JAK/STAT Pathway

In relation to disease-initiating stem cells, high STAT3 expression is linked to a lower survival rate and worse clinical outcomes. In AML/MDS stem cells, STAT3 inhibitor AZD9150 has been shown to decrease viability and in vivo leukemic growth, as well as induce hematopoietic differentiation and LSC death. Therefore, abnormal STAT3 pathway activation may be a potential strategy to reduce AML and MDS stem cells, regulated by antisense oligonucleotides. LSC self-renewal has also been linked to STAT5, underscoring the necessity of suppressing several pathways in order to treat aggressive leukaemia. LSCs frequently overexpress the Janus kinase (JAK) pathway alongside STAT signals, and it has been shown that AML-LSC development is inhibited by JAK2 inhibition. The JAK/STAT pathway (Figure 8) targets several growth factor receptors, which is crucial for AML-LSCs.¹⁵

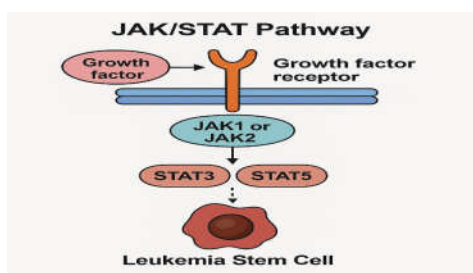


Fig. 8: JAK/STAT Pathway

4. DIAGNOSTIC APPROACHES FOR LEUKAEMIA:

Accurate diagnosis of leukaemia is crucial for determining the most effective treatment strategy. A combination of techniques is typically employed, encompassing hematological and morphological assessments, as well as molecular diagnostic methods.

4.1 Hematological and Morphological Assessments

- **Bone Marrow Examination & Biopsy:** These procedures involve extracting bone marrow fluid and a small tissue sample from the bone marrow, typically from the hip bone.¹ Examination of the bone marrow provides crucial information about the type, number, and maturity of blood cells being produced, confirming the diagnosis of leukaemia and determining its specific subtype.

Key Indications:

Cellularity: The percentage of bone marrow space occupied by cells. In leukaemia, it's often increased.

Blast Count: The number of immature white blood cells (blasts) present.

Cell Morphology: Detailed examination of the appearance of various blood cells within the bone marrow.¹⁶

4.2 Molecular Diagnostics for Leukaemia:

- **G-band Metaphase Chromosome Analysis ('Karyotyping')**

Recently identified leukaemia patients require the culture of bone marrow material for the detection of mitotically active dividing cells, as per traditional cytogenetic examination. To detect structural and numerical flaws as well as individual chromosomes, each chromosome in the "metaphase spread" are prepared to ensure Giemsa (or Leishman) staining produces a characteristic banding pattern. The existence of at least 2 cells with the same abnormality, or 3 cells in the case of chromosomal loss, is defined as a clonal anomaly. The discovery of a clonal chromosomal flaw can serve as proof of a malignant process even in cases where the aberration is not recurring in leukaemia.

Metaphase cytogenetic examination of bone marrow. Helps to detect translocations and associated alterations, like inversions and aneuploidies, which are frequently seen in AML, ALL, and CML. One advantage of cytogenetic analysis is that it provides a low-resolution whole-genome scan and can detect balancing modification, that are relatively prevalent in leukaemia. It is important to remember that only a percentage of leukaemia (about 55% of AML, for example) exhibits extensive chromosomal abnormalities. . When labelled malignancies are investigated using metaphase cytogenetic study, many chromosomal abnormalities will be overlooked.¹⁷.

Important prognostic information may also be provided by chromosomal abnormalities. The most crucial factor in determining prognosis in AML is cytogenetic results. Based on the diagnostic karyotype, three general prognostic categories (good, intermediate, and poor) are established, which can assist in identifying individuals who can benefit from stem cell transplantation. Additional karyotypic anomalies, such as a second Ph chromosome, trisomy 8, isochromosome 17q, or trisomy 19, may indicate that the leukaemia has already advanced to an accelerated phase or blast crisis and may have a detrimental effect on survival in CML patients.¹⁸

- **Fluorescence *in situ* Hybridization**

FISH is a helpful supplement to cytogenetic analysis. Its primary benefit is that there might be carried out on cells that are in interphase because it does not require dividing cells. If marrow is not accessible, blood can be utilized, and since cells do not need to be grown, findings can be acquired more rapidly. Additionally, FISH is more sensitive (0.5–1%) and has a greater resolution (100 kb, depending on the probe) than traditional cytogenetic analysis. However, depending on the probe type, care must be taken to prevent false-positive and -negative results because of signal colocalization or drop-out. To define clear-cut positive results, laboratories should determine cut-off values for every probe set. Furthermore, compared to conventional cytogenetic analysis, FISH has a higher resolution (100 kb, depending on the probe) and a higher sensitivity (0.5–1%).¹⁹

FISH is used to diagnose leukaemia with cryptic cytogenetic abnormalities. Molecular methods or FISH can be used to identify the translocation. Using a disease-specific panel of probes, interphase FISH can detect genomic abnormalities in around 80% of CLL cases. It is especially helpful in detecting deletions of TP53 (17p13) and ATM (11q23), both of which are linked to a poor prognosis. In CLL, interphase FISH analysis is typically regarded as being better than metaphase cytogenetic analysis for the following reasons: the fact that copy-number alterations account for the majority of clinically significant abnormalities in CLL.²⁰

5. THERAPIES IN CANCER

5.1. Chemotherapy

➤ Current Standing of Cancer Chemotherapy

Nowadays, chemotherapy works by stopping the growth and division of cancer cells. Compared to normal cells, cancer cells usually divide significantly more quickly and

experience higher levels of endogenous stress, which makes them more vulnerable to the lethal effects of chemotherapeutic medications. Chemotherapy is still a commonly used treatment option, especially for advanced-stage cancers where radiation therapy or surgical excision are not practical, even with the advent of targeted medicines and immunotherapy. Chemotherapeutic drugs have been the first line of treatment in many of these situations for decades.

The kind and stage of the cancer are the main factors that influence the choice of chemotherapy medications or a combination of them. These medications' main objectives are to destroy malignant cells and reduce the stress brought on by tumor growth. It is acknowledged, although, that the drugs are frequently used in extremely high dosages, which regrettably results in several negative consequences and damage to other healthy cells.

One major problem with chemotherapy is illness recurrence, which frequently occurs when cancer cells become resistant to the medications after extended use. Despite its many uses, a large portion of traditional chemotherapy is regarded as palliative, meaning that treatment aims to ease and prolong a patient's survival rather than offer a permanent cure. These medications can have serious negative consequences on a patient's physical and mental well-being, making it challenging to stick with treatment. Fatigue, nausea, vomiting, low appetite, and hair loss are typical adverse effects. These occur because many anticancer medications directly disrupt the pathways that lead to cell division, impacting not only cancer cells but also quickly dividing normal cells such as those found in the skin, hair follicles, and intestinal linings. Alopecia (hair loss), skin rashes, and mucositis (inflammation of mucous membranes) might result from this. These adverse effects can also be made worse by radiation therapy. Another common problem is fatigue, which is frequently brought on by conditions including anemia, depression, and excessive radiation therapy dosages. Furthermore, radiation and chemotherapy may directly or indirectly alter cognitive functioning, a condition known as "chemo brain," which affects behavior, memory, and cognition. A deterioration of vision may also result from injury to the optic and ocular motor neurons in certain patients. Although individual susceptibility can vary due to factors like metabolism, genetics, other drugs, and environmental influences, the severity of these adverse effects is frequently dose-dependent.²¹

Oncologists frequently administer chemotherapy with prearranged time intervals to allow non-cancerous cells to recuperate in order to lessen these difficulties. The majority of the time, a multidisciplinary group of experts, such as radiologists, dietitians, psychologists, and the main

consultant, together decide on the treatment regimens, which include medication combinations, dosage, cycle lengths, and additional therapies.

There are several ways to give chemotherapy medications. Intravenous (IV) infusion is the most popular technique, in which medications are injected directly into the patient's veins over the course of minutes to hours. Certain drugs can also be administered orally as pills or liquids on a regular basis or at predetermined intervals. To target certain tumor areas, further administration techniques include intramuscular injections and intraabdominal administration. Pressurized intraperitoneal aerosol chemotherapy (PIPAC), a more recent method, has been created to effectively administer chemotherapy to patients who have end-stage peritoneal metastases. Drugs are applied directly to the skin's surface during topical therapy. Additionally, to lower the chance of cancer recurrence, adjuvant chemotherapy is given following initial treatments like radiation or surgery.^{22, 23}

Chemotherapeutic drugs can be categorized according to their molecular target, chemical makeup, source, mode of action, or efficacy against different types of cancer. Alkylating agents, one of the main types of modern medications, damage DNA and stop cell division by interfering with DNA synthesis and linking. Altretamine, chlorambucil, cyclophosphamide, and platinum medications (cisplatin, carboplatin, and oxaliplatin) are a few examples. These are frequently employed as first-line treatments for a variety of malignancies, especially those that grow slowly. Antineoplastic antimetabolites are another important family that can obstruct vital metabolic functions necessary for cell proliferation and development. Azathioprine, gemcitabine, cladribine, clofarabine, fludarabine, and mercaptopurine are a few examples. Antimetabolites generated from pyrimidines, such as fluorouracil, gemcitabine, decitabine, capecitabine, cytarabine, and others, are also very important. Vinca alkaloids (vinblastine, vincristine, vindesine, and vinorelbine), hydroxyurea, paclitaxel, bleomycin, and arsenic trioxide are further antineoplastic medicines that may fall under one or more pharmacological classes and have different modes of action.²⁴

➤ **Future Directions of Cancer Chemotherapy**

The future of cancer chemotherapy is increasingly focused on more systematic and targeted approaches. Several key areas of advancement are shaping this evolution:

- **Targeted Drug Delivery:** Traditional chemotherapy's non-specificity, which damages healthy tissues, is one of its main drawbacks. In order to minimize damage to healthy cells, future research will focus on creating ways to deliver medications only to cancer

cells. This covers a number of tactics, including the use of particular medications, such as Herceptin, in HER2-positive breast cancer, targeted antibodies, aptamer functionalization, and delivery methods based on nanoparticles. These methods have the potential to decrease systemic toxicity and deliver medications locally to the tumor bulk.²⁵

- **Personalized Medicine:** Personalized medicine attempts to customize treatment plans according to the unique biological features of each patient's cancer, acknowledging the heterogeneity of malignancies within and between people. To pinpoint the precise molecular changes causing the cancer, this entails a thorough examination of "omics" data, such as transcriptomics, proteomics, metabolomics, and genomes. These clinically actionable targets are thus the focus of treatment designs. Examples include medications such as trastuzumab, which targets HER2-mutated cells, and dabrafenib and vemurafenib, which target the BRAF gene mutation in melanoma. Choosing the best medications and dosages to enhance patient outcomes is the aim.²⁶
- **Liquid Biopsies:** The development of liquid biopsies, which check blood samples for altered proteins, RNAs, DNAs, and other genetic markers, has the potential to improve cancer diagnosis and track the effectiveness of treatment. Early identification of carcinogenic alterations and quicker modifications to treatment plans may be possible with this non-invasive method.
- **Addressing Toxicity:** In order to greatly enhance the quality of life for cancer patients, future chemotherapy development must prioritize identifying and reducing treatment adverse effects. It is crucial to do research to comprehend the mechanisms underlying chemotherapy-induced toxicities and create management and preventative plans.²⁷

5.2. Emerging Therapies in Leukaemia Treatment

New promising approaches for overcoming challenges such as resistance and relapse in the management of leukaemia treatment are currently in development. Next-generation targeted agents, epigenetic therapies, nanotechnology, and even application of AI and ML for research purposes have been reviewed herein. Innovative treatments can better address outcomes and significantly minimize adverse effects. Patients suffering relapsed or refractory leukaemia may present the highest possibility for cures.²⁷

Nanotechnology & Advanced Drug Delivery Systems

Nanotechnology is going to revolutionize the treatment of leukaemia by delivering medicines precisely to leukaemia cells. This might improve the effectiveness of medications and lead to fewer adverse effects.

• Mechanism of Action:

There is two mode of action for therapeutical Nano-particles

A. Passive Targeting: The "enhanced permeability and retention (EPR) effect" is the foundation of passive targeting (Figure 9). One hallmark of cancerous tissues is that they frequently have poor lymphatic drainage and more permeable (leaky) blood vessels than normal tissues. As a result, macromolecules, including nanoparticles, can enter the tumor tissue through the blood arteries and concentrate there.

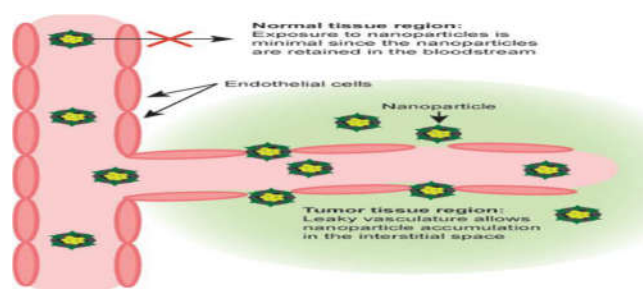


Fig. 9: Mechanism of Nanoparticles against cancer cells via Passive Targeting

B. Active Targeting: Active targeting employs designed nanoparticles to precisely identify and bind to cancer cells, in contrast to passive targeting, which depends on tumor features. Active targeting (Figure 10) improves medication delivery and accumulation while reducing harm to healthy organs by functionalizing nanoparticles contains ligands that attach specifically to cancer cell-overexpressed receptors²⁸.

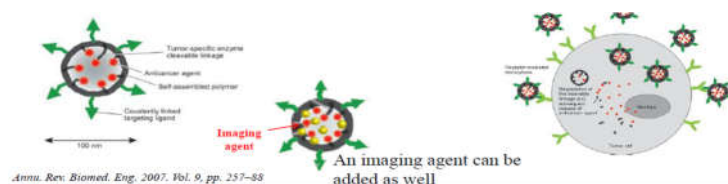


Fig. 10: Mechanism of Nanoparticles against cancer cells via Active Targeting

Key Advantages of Nanotechnology:

- A. Enhanced Drug Delivery: NPs can be engineered to preferentially accumulate in tumour tissues due to their size and the leaky nature of tumour blood vessels (Figure 11). This targeted approach enhances therapeutic efficacy and minimizes damage to healthy tissues.
- B. Improved Imaging and Diagnosis: NPs can be used as imaging agents due to their unique physicochemical properties. For example, iron oxide NPs are utilized in magnetic resonance imaging (MRI) to enable concurrent diagnosis and treatment.
- C. High Drug Loading Capacity: Nanoparticles have a high surface area, allowing for significant drug loading and efficient delivery to tumour sites.
- D. Resistance typically arises when cancer cells start to express a protein called p-glycoprotein, which has the ability to pump anti-cancer medications out of a cell as fast as they pass through the outer membrane of the cell.

According to recent studies, anti-cancer medications may enter cells through nanoparticle delivery without triggering the p-glycoprotein pump ²⁹.

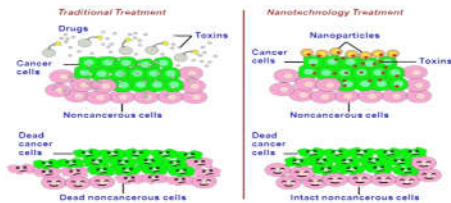


Fig. 11: Advantages of Nanotechnology Treatment

6. MARKETED THERAPIES FOR LEUKAEMIA: ³⁰⁻³³

Table 1: Marketed Pharmacological Treatments for Leukemia: Chemotherapy and Targeted Approaches

Therapy Name (Brand Name)	Type of Therapy	Leukaemia Type(s) Treated
Busulfan (Busulfex, Myleran)	Chemotherapy	Chronic myelogenous leukaemia
Cyclophosphamide	Chemotherapy	CLL, pediatric ALL (as lymphodepleting chemotherapy before KYMRIA ^H)
Cytarabine	Chemotherapy	ALL, CML, AML (in combination therapies)
Cladribine	Chemotherapy	Hairy cell leukaemia
Lomustine	Chemotherapy	Brain, blood cancers
Melphalan	Chemotherapy	Blood, ovary, breast, skin, lung, bladder, pancreas, crevice cancers
Ixabepilone (Ixempra)	Chemotherapy	Breast, skin, lung, blood, prostate, kidney cancers
Vinblastine	Chemotherapy	Breast, blood, testicle, bone cancers

Vindesine or Eldisine	Chemotherapy	Blood cancer
Vinorelbine	Chemotherapy	Lung, blood, ovary, breast cancers
Imatinib (Gleevec, Imkeldi)	Targeted Therapy	CML, ALL (with Philadelphia chromosome), gastrointestinal stromal tumor, systemic mastocytosis
Dasatinib (Sprycel)	Targeted Therapy	CML, ALL (with Philadelphia chromosome)
Idelalisib (Zydelig)	Targeted Therapy	CLL/SLL
Revumenib (Revuforj)	Targeted Therapy	Acute leukaemia with a lysine methyltransferase 2A (KMT2A) gene translocation
Avapritinib (Ayvakit)	Targeted Therapy	Systemic mastocytosis, gastrointestinal stromal tumor
Pemigatinib (Pemazyre)	Targeted Therapy	Liver and bile duct cancer, lymphoma
Quizartinib Dihydrochloride (Vanflyta)	Targeted Therapy	AML with FLT3-ITD mutations
Recombinant Interferon Alfa-2b (Intron A)	Immunotherapy/Interferon	Hairy cell leukaemia
Buparlisib	Targeted Therapy (in development)	Relapsed or refractory DLBCL, mantle-cell lymphoma (MCL), or follicular lymphoma (studied model system)

ACKNOWLEDGMENTS:

The authors gratefully acknowledge Sarojini Naidu Vanita Pharmacy Maha Vidyalaya for providing guidance and necessary support throughout the study.

CONFLICTS OF INTERESTS:

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

CONCLUSION:

In conclusion, the comprehensive overview of leukaemia provided in the sources underscores its significance as a group of malignant hematological diseases with diverse etiologies, classifications, and clinical courses. The review traces the evolution of leukaemia treatment from early, less specific chemotherapy regimens to the current era of targeted and immunotherapies, highlighting pivotal developments such as the introduction of cytarabine and daunorubicin, the breakthrough with imatinib for CML, the advent of monoclonal antibodies like rituximab, and the revolutionary impact of CAR-T cell therapy.

This article describes the intricate stages of leukemogenesis, including genetic and epigenetic changes, clonal evolution, and the impact of the microenvironment, and highlights the crucial role that molecular discoveries have in comprehending the pathophysiology of leukaemia. The specific genetic mutations in CML, particularly the BCR-ABL1 fusion gene, and the signaling

pathways governing leukaemia stem cell (LSC) behavior are extensively discussed, revealing potential therapeutic targets.

Diagnostic approaches for leukaemia have advanced significantly, integrating hematological and morphological assessments with sophisticated molecular diagnostic methods like karyotyping, FISH, and PCR-based techniques, which are crucial for accurate classification, prognostication, and treatment decisions.

The sources also explore emerging therapeutic strategies, including nanotechnology-based drug delivery systems designed to enhance efficacy and minimize side effects. The detailed list of marketed therapies for leukaemia further illustrates the current clinical landscape, encompassing a wide range of chemotherapy agents, targeted inhibitors, and immunotherapeutic modalities.

Ultimately, this review emphasizes that continued research bridging molecular discoveries with therapeutic innovations is essential for further improving outcomes for leukaemia patients. The ongoing exploration of LSC biology, signaling pathways, genetic mutations, and novel therapeutic approaches holds the promise of more personalized, effective, and potentially curative treatments for this complex group of diseases.

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Figure Legends:

1. Fig. 1: Leukaemia is usually categorized by the type of stem cell that has developed into a malignant state, either myeloid or lymphoid.
2. Fig. 2: Process of Leukemogenesis
3. Fig. 3: Genetic Mutations in CML
4. Fig. 4: Signalling Pathways of Leukaemia
5. Fig. 5: Epigenetic Pathways Involved in Leukaemia Stem Cells
6. Fig. 6: Hedgehog Pathway
7. Fig. 7: Wnt/ β -catenin Pathway
8. Fig. 8: JAK/STAT Pathway
9. Fig. 9: Mechanism of Nanoparticles against cancer cells via Passive Targeting
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11. Fig. 11: Advantages of Nanotechnology Treatment

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