Advancements in leukemia management: Bridging diagnosis, prognosis and nanotechnology (Review)

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Abstract. Leukemia is a cancer that starts in blood stem cells in the bone marrow. Today, the proper diagnosis and prognosis of leukemia are essential in mitigating the morbidity and mortality associated with this malignancy. The advent of novel biomarkers, particularly those related to minimal residual disease, has paved the way for personalized therapeutic strategies and enables the quantitative assessment of patient responses to treatment regimens. Novel diagnostic and targeted drug delivery may be helpful for the improved management of leukemia. Genetic clinical parameters, such as chromosomal abnormalities, are crucial in diagnosing and guiding treatment decisions. These genetic markers also provide valuable prognostic information, helping to predict patient outcomes and tailor personalized treatment plans. In the present review, the studies on the diagnostic and prognostic parameters of leukemia were analyzed. The prognosis of leukemia was investigated in most of the studies, and the remaining were performed on diagnosis. The clinical and laboratory prognostic parameters were the most common, followed by diagnostic hematological parameters, diagnostic blood parameter studies, and diagnostic immunological parameters. Clinical and laboratory prognostic and hematologic parameters were the most extensively studied. The methods used to diagnose and prognose the leukemia cases in these studies were predominantly clinical hematology. Numerous surface proteins and receptors, including CD45, CD27, CD29, CD38, CD27, CD123, CD56 and CD25, react similarly in various kinds of leukemia, which are ideal for targeted drug delivery. Drug delivery to leukemia cells encounters several significant obstacles, including heterogeneity, that hinder the effectiveness of treatment. Nanocarriers play a critical role in targeted drug delivery for leukemia by enhancing the precision of treatments directed at surface proteins and receptors. Additionally, they can be functionalized with targeting drugs and antibodies to target specific tissues and cells.

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Contents

1. Introduction

Leukemia refers to a category of blood malignancies characterized by the excessive production of abnormal blood cells and an underlying propensity for the bone marrow to serve as cancer's site of origin (1). Malignant cells use up healthy bone marrow cells to grow, leaving the body without enough platelets and normal white blood cells (WBCs) to prevent bleeding and fight infection (2-4). It is a somatic mutation-driven hematological clonal neoplasm (5-7). Neoplastic cells can self-replicate, develop, and feed progenitor cells such as hematopoietic cells, exhibiting similar behaviour (8-10). Leukemic unipotent stem cells can differentiate into phenotypes of adult blood cells (11,12). Certain forms of leukemia progress more slowly than others, while others begin in myeloid cells rather than lymphocytes. Leukemia types vary in prognoses and available diagnostics (13-15).

Leukemia is among the most prevalent cancers worldwide (16,17). Because of premature mortality, leukemia has a significant impact on developing nations. However, leukemia may be controlled in developed countries (18). The disease rapidly proliferates and can be classified as acute or chronic, myeloid or lymphoid (19). The most prevalent forms of leukemia are those that impact the myeloid and lymphoid systems, including acute myeloid leukemia (AML), chronic

myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) (20).

What causes most leukemia subtypes remains unknown, even though various genetic and environmental risk factors have been identified. It is common practice to perform a bone marrow biopsy to get a final diagnosis, with the results informing various treatments, from chemotherapy to stem cell transplantation. Different types of leukemia have other outcomes (21). The fact that isolated leukemia stem cells differ from normal blood stem cells is a favorable indication for discovering a specific drug targeting them (22).

The present review highlighted methods and studies associated with leukemia diagnosis and prognosis. A systematic review was conducted to learn about the numerous types of leukemia and their associated gene signatures. The parameters used in diagnosis have the potential to enhance treatment efficacy and protect infected patients from developing chronic conditions. An assessment can inform the timing and course of treatment of prognostic factors at the time of diagnosis. Over time, researchers have examined several potential prognostic indicators. The present study discussed current leukemia diagnosis and prognosis factors, concentrating on the clinical and pathological. In the present review, the AML, ALL, CML and CLL categories to were used classify the numerous forms of leukemia.

2. Methods

Implementing comprehensive search strategies for leukemia research. Although the databases search engines automatically look for different forms of search terms, the terms for British or US English were still used. Whole or parts of the search operators (Boolean), truncation symbols and proximity operators were used to add additional layers of control over our search results. Search operators, also known as 'Boolean' operators (AND, OR, NOT), were used to combine terms in exact ways. Parenthesis was used to specify the order of the search operators. Truncation symbols (*, $, ?) were used to control the plural forms and variant spellings. Lastly, the proximity was used to specify the distance between the terms.

The search carried out was as the following: (((TS=('leukemia' OR 'hematologic malignancy' OR 'haematology malignancy' OR 'leukaemia' OR 'cancer of the blood' OR 'blood cancer')) AND TS=('prognosis' OR 'outcome prediction'))) AND TS=('*nano*'). There were 205 results from Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), Arts and Humanities Citation Index (A&HCI), Conference Proceedings Citation Index - Science (CPCI-S), and Emerging Sources Citation Index (ESCI). One article was published in Chinese and another in Persian. Both articles were excluded from the present study. Only the articles published in English were evaluated. These articles were exported in several formats. Plain text, Endnote desktop, and Excel file formats were exported, and all the features of the Web of Science (https://mjl.clarivate.com/home) were included. Similarly, the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was searched using these credentials: ((('leukemia'[Title/Abstract] OR 'hematologic malignancy'[Title/Abstract] OR 'haematology malignancy'[Title/Abstract] OR 'leukaemia'[Title/Abstract] OR 'cancer of the blood'[Title/Abstract] OR 'blood cancer'[Title/Abstract]) AND (='prognosis'[Title/Abstract] OR 'outcome prediction'[Title/Abstract])) AND (nano*[Title/Abstract]). The search produced a total of 120 articles. The search output was exported as a citation manager, and the file format nbib and Endnote 20.6 were used for further analysis. the PRISMA rules were followed for this systematic review and the PRISMA flow chart is shown in Fig. 1.

Systematic information extraction for consistent data analysis in leukemia studies. A standardized data extraction sheet was employed to ensure uniformity in capturing critical information from each study, including study characteristics, participant details, intervention and comparator specifics, outcome measures and methodology. The most current and comprehensive study was selected in cases where multiple studies provided similar findings. All identified studies underwent rigorous screening, with titles and abstracts first assessed for eligibility. Articles meeting the criteria were then subjected to full-text review, and exclusions were documented with specific reasons. The final data synthesis involved comparing and contrasting the extracted data to identify consistent themes and areas requiring further research.

3. Key findings from leukemia diagnostic and prognostic studies

Analyzing the outcome of the comprehensive search on leukemia. In the first round of analysis, 205 published articles were produced by the Web of Science Core Collection and 120 articles were produced from the PubMed database. A total of two articles were excluded from the WoS database and one from PubMed because they were published in languages other than English. In the end, 203 articles from Web of Sciences and 119 articles from PubMed fulfilled the initial screening requirements and were analyzed for improved understanding.

The second step involved classifying the selected papers according to the diagnostic, prognostic and distinctive clinical aspects, as well as related molecular genetics. The retrieved material covered the study's methodology, conclusions, minimal residual disease (MRD) status, and survival rates for pediatric, adolescent and young adult leukemia patients.

There were numerous criteria for diagnosing leukemia, and the present review shows which ones were more accurate and frequently used. As observed in Table I, these factors showed a variety of leukemia types, with CLL being the least prevalent. Numerous surface proteins and receptors, including CD33, CD45, CD27, CD29, CD38, CD27, CD123, CD56 and CD25, react similarly to parameters in various kinds of leukemia, as demonstrated in Fig. 2.

4. Critical assessment of diagnostic and prognostic developments in leukemia

In this section, the current advancements in leukemia diagnostics and prognostics were critically examined, and the significant strides and existing gaps within the literature were highlighted. The current analysis specifically investigated the recent advancements in molecular genetics and their crucial role in improving leukemia care. Focus was addressed on the

Table I. Detail of all parameters in leukemia.

Sr. no.	First author/year	Leukemia type	Parameters	Age (years)	Value of risk	(Refs.)
1	Kassahun *et al*, 2020	AML	Hematological	15-49	51.60%	(23)
2	Jahic *et al*, 2017	AML	Prognostic	<55	86.40%	(24)
3	Zhang *et al*, 2009	ALL	Clinical prognostic	25-50	20-30%	(25)
4	Kardum-Skelin *et al*, 2008	Chronic leukemic	Clinical and laboratory prognostic	>60	>70%	(26)
5	Othieno-Abinya *et al*, 2016	CML	Hematological	NR	46%	(27)
6	Spiers, 2001	CGL	Hematological	60-65	55%	(28)
7	Sharma *et al*, 1992	Leukemia	Blood	NR	51%	(29)
8	Germing *et al*, 2002	CMML	Prognostic	70	15-24%	(30)
9	Tonorezos *et al*, 2013	Childhood leukemia	Metabolic	18-37	31%	(31)
10	Haferlach *et al*, 2010	CLL	Prognostic genetic	>65	89.50%	(32)
11	Jaeger *et al*, 2012	CML	Bone metabolic	4-17	58%	(33)
12	Letestu *et al*, 2010	CLL	Prognostic	29-87	80%	(34)
13	Negrini *et al*, 2014	CLL	Prognostic	>70	NR	(35)
14	Rigolin *et al*, 2012	CLL	Clinic biological	63-69	21.5-35.7%	(36)
15	Babusikova *et al*, 2009	TLL	Prognostic	20-30	44%	(37)
16	Joncourt *et al*, 1995	Leukemia	Drug resistance	15 and 79	35%	(38)
17	Baumgartner *et al*, 2016	AML	Nutritional	46	57.10%	(39)
18	Sillaber *et al*, 2003	CML	Pathophysiology, diagnostic	≥50	90-95%	(40)
19	Zittoun *et al*, 1984	AML	Prognostic value of cytologic	30-65	2.50%	(41)
20	Piard *et al*, 2006	ALL	Pharmacokinetic	2-16	13.20%	(42)
21	Halonen *et al*, 2003	ALL	Serum Iron	2.6-17	14%	(43)
22	Prabhu *et al*, 1986	CML	Prognostic	12-65	39%	(44)
23	Shinawi *et al*, 2012	CLL	Biological prognostic	NR	35%	(45)
24	Royer-Pokora *et al*, 1978	ALV	Transformation	30	23%-80%	(46)
25	Oliveira *et al*, 2001	CLL	Clinical and laboratory	45-86	>30%	(47)
26	Hofmann-Lehmann *et al*, 1997	FLV	Disease progression	Seven weeks	83%	(48)
27	Chiaretti *et al*, 2014	CLL	Prognostic	60	8.6-15.9%	(49)
28	Li *et al*, 2019	ALL	IVIM	11-70	93%	(50)
29	Dhasmana *et al*, 2016	Leukemia	Hematological	5-80	45.10%	(51)
30	Omer *et al*, 2017	AML	Hematological	3-101	29.30%	(52)
31	Heintel *et al*, 2001	CLL	Prognostic	30-68	30%	(53)
32	Molica *et al*, 2007	CLL	Biological	45-85	NR	(54)
33	Levine *et al*, 1971	CLL and CML	Clinical	27-65	90%	(55)
34	Martell *et al*, 2002	CLL	Hematologic	37-87	28%	(56)
35	Bergmann *et al*, 2007	CLL	Prognostic	30-80	19.50%	(57)
36	Sinisalo *et al*, 2004	CLL	Humoral immunity	48-79	20%	(58)
37	Browman *et al*, 1983	ANLL	Predictive	NR	50%	(59)
38	Wieser *et al*, 2009	AML	Prognostic	89	97-99%	(60)
39	Valet *et al*, 2003	AML	Immunophenotypic, cytogenetic and clinical	>53.8	49.8 and 32.8%	(61)
40	Valent *et al*, 2008	CML	Diagnostic and prognostic	NR	NR	(62)
41	Zimm *et al*, 1986	ALL	Biochemical	20-65	50%	(63)
42	Yang *et al*, 2014	Acute leukemia	Morphological	>75	96.80%	(64)
43	Olson *et al*, 2020	LGLL	Hematological	14-65	30-40%	(65)
44	Srinivasan *et al*, 2020	CLL	Clinico-hematological	43-88	65%	(66)
45	Hallek *et al*, 2019	CLL	Prognostic	>65	Low risk 93.2%, very high 23.3%	(67)

Table I. Continued.

Sr. no.	First author/year	Leukemia type	Parameters	Age (years)	Value of risk	(Refs.)
46	Feller *et al*, 2004	AML	MRD	18-76	97%	(68)
47	Mohammadi *et al*, 2020	APL	Blood	10-82	58.50%	(69)
48	Al-Bayaa *et al*, 2020	AML	Clinical	11-55	53% in females; 47% in males	(70)
49	Khazaal *et al*, 2019	CML	Different blood	18-80	67.8% in males; 32.2% in females	(71)
50	Ogasawara *et al*, 2019	CML	Complete blood count	19-89	95.20%	(72)
51	Michailov *et al*, 2019	AML	Mouse sperm	NR	45%	(73)
52	Virk *et al*, 2019	AML	VCS	12-77	56%	(74)
53	Vigón *et al*, 2020	CML	Immunological	NR	$CD56^+$ <4%; CD3-$CD56^+$ $CD16^+$ >7%;	(75)
54	Mahmood *et al*, 2018	CLL	Clinic hematological	>65	67%	(76)
55	Kim *et al*, 2018	Rat basophilic	Allergic	NR	65%	(77)
56	Schillinger *et al*, 2018	CML	Structural	39-91	90%	(78)
57	Khan, 2018	AML	Clinical and hematological	3-65	59%	(79)
58	Podszywalow-Bartnicka *et al*, 2018	Leukemia	Live	NR	10-12%	(80)
59	Nägele *et al*, 2017	ALL	Clinical laboratory	NR	69%	(81)
60	Lekovic *et al*, 2017	CML	Predictive	18-74	76.70%	(82)
61	Burhan, 2017	Leukemia	Biochemical	15-55	75%	(83)
62	M.Elzubeir *et al*, 2016	CLL	Prognostic	42-90	100%	(84)
63	Wenn *et al*, 2015	CML	Prognostic	24-83	10.50%	(85)
64	Trendowski *et al*, 2015	Leukemia	Cell cycle growth control	NR	20%	(86)
65	Huang *et al*, 2015	ALL	Platelet	18-79	57%	(87)
66	Rabizadeh *et al*, 2014	Acute leukemia	Automated blood count	NR	1.7-18.7%	(88)
67	Koltan *et al*, 2015	ALL	Immunological	13-22	15.9-99.8%	(89)
68	Aljamaan *et al*, 2015	*pALL	Clinical	3-7	80%	(90)
69	Rafiq *et al*, 2014	CLL	Hematological, biochemical	20-45	95%	(91)
70	Pietras *et al*, 2014	Lymphoblastic	Immune system	15-25	80%	(92)
71	Deschler *et al*, 2013	AML	Geriatric	60-87	80%	(93)
72	Mauro *et al*, 2012	CLL	Clinicobiologic	51-61	70% vs. 49%	(94)
73	Dong *et al*, 2011	CLL	Prognostic	>60	80%	(95)
74	Onida *et al*, 2002	CML	Prognostic	20-88	71%	(96)
75	Sagatys *et al*, 2012	CLL	Prognostic	>50	>30%	(97)
76	Jinlong *et al*, 2015	AML	Prognostic	15-55	49%	(98)
77	Marcucci *et al*, 2011	AML	Prognostic	10-60	90%	(99)
78	Labib *et al*, 2017	ALL	Prognostic	<2->10	80%	(100)
79	Neaga *et al*, 2021	AML	Prognostic	NR	65%	(101)

NR, not reported; pALL, pediatric acute lymphoblastic leukemia; ALV, avian leukemia viruses; ANLL, acute non-lymphoblastic leukemia; FLV, feline leukemia virus; IVIM, intravoxel incoherent motion; LGLL, leukemia large granular lymphocyte; MRD, minimal residual disease; VCS, volume, conductance and scatter; APL, acute promyelocytic leukemia; TLL, T-cell acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia.

latest diagnostic markers, treatment modalities and prognostic indicators that have the potential to transform personalized medicine in leukemia management. The key areas where recent technologies and therapeutic approaches have shown

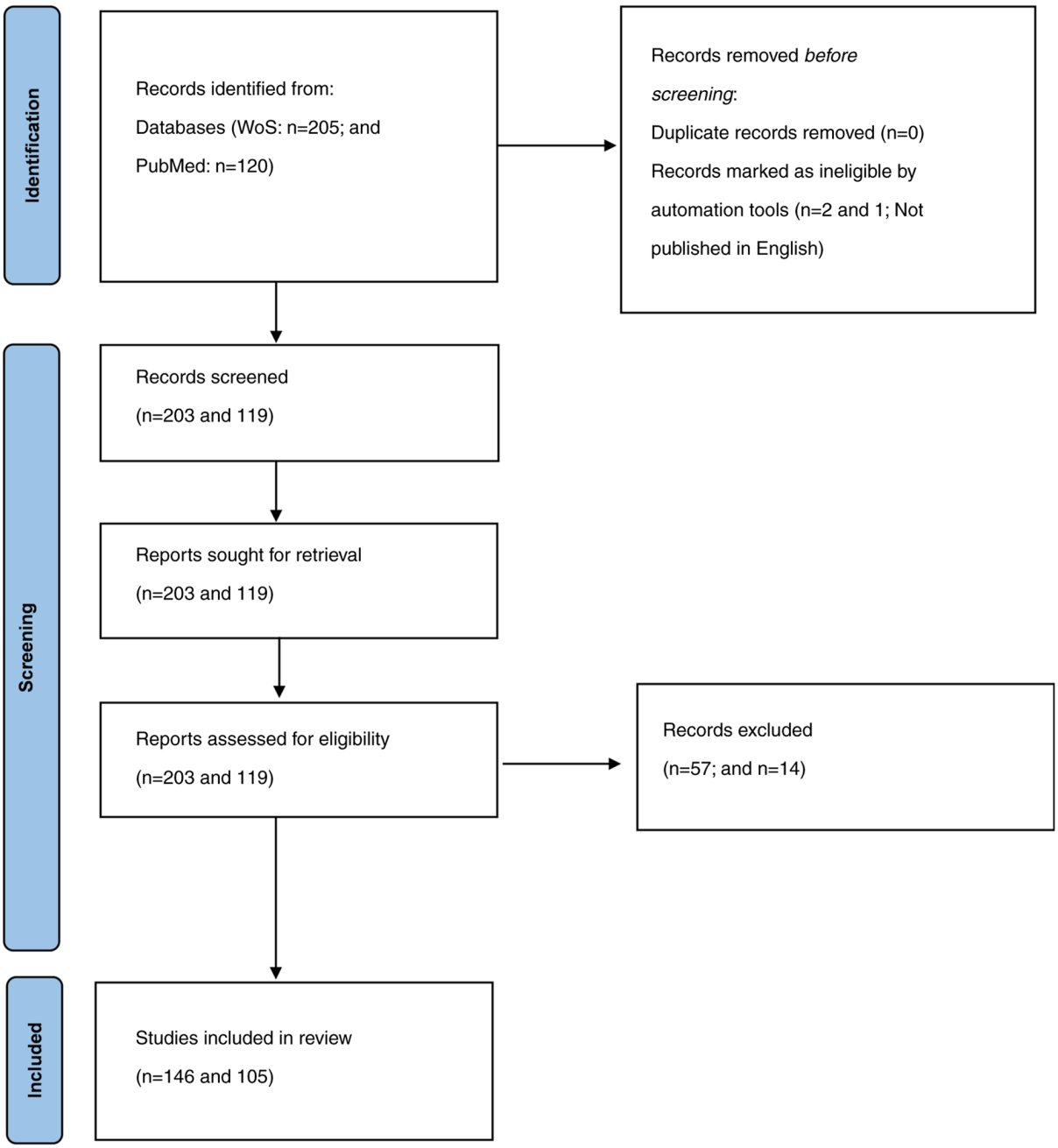

Figure 1. PRISMA flow chart outlines the systematic review process. In the present study, Web of Science Core Collection and PubMed databases were accessed to retrieve the literature. Complete description is provided in the text.

promise were identified, while also pointing out areas where further research is urgently needed to bridge the knowledge gaps. This critical assessment offers a comprehensive overview that can serve as a foundation for future studies, aiming to improve patient outcomes through more tailored and effective treatment strategies.

Baseline characteristics of included studies. Most studies investigated leukemia's prognosis, followed by leukemia diagnosis. The more common studies included clinical and laboratory prognostic parameters, diagnostic hematological parameters, diagnostic blood parameters studies and diagnostic immunological parameters. Clinical and laboratory prognostic and hematological parameters were the most extensively studied, while others have been studied in smaller numbers. The methods used to diagnose and prognose the leukemia cases in these studies were predominantly prediction analysis, such as clinical hematology and drug-related laboratory studies based on gene mutation (Fig. 3).

In the current review, leukemia's diagnostic and prognostic parameters were screened (Table I). The present systematic review provides the most comprehensive results about leukemia diagnosis and prognosis. There were several parameters for leukemia diagnosis and prognosis, but most studies have focused on hematological diagnosis.

As observed in Table I, AML has a progressive increase in the incidence rate from birth to ~50 years of age, a steady increase from ~50-54 years of age, more rapid growth from

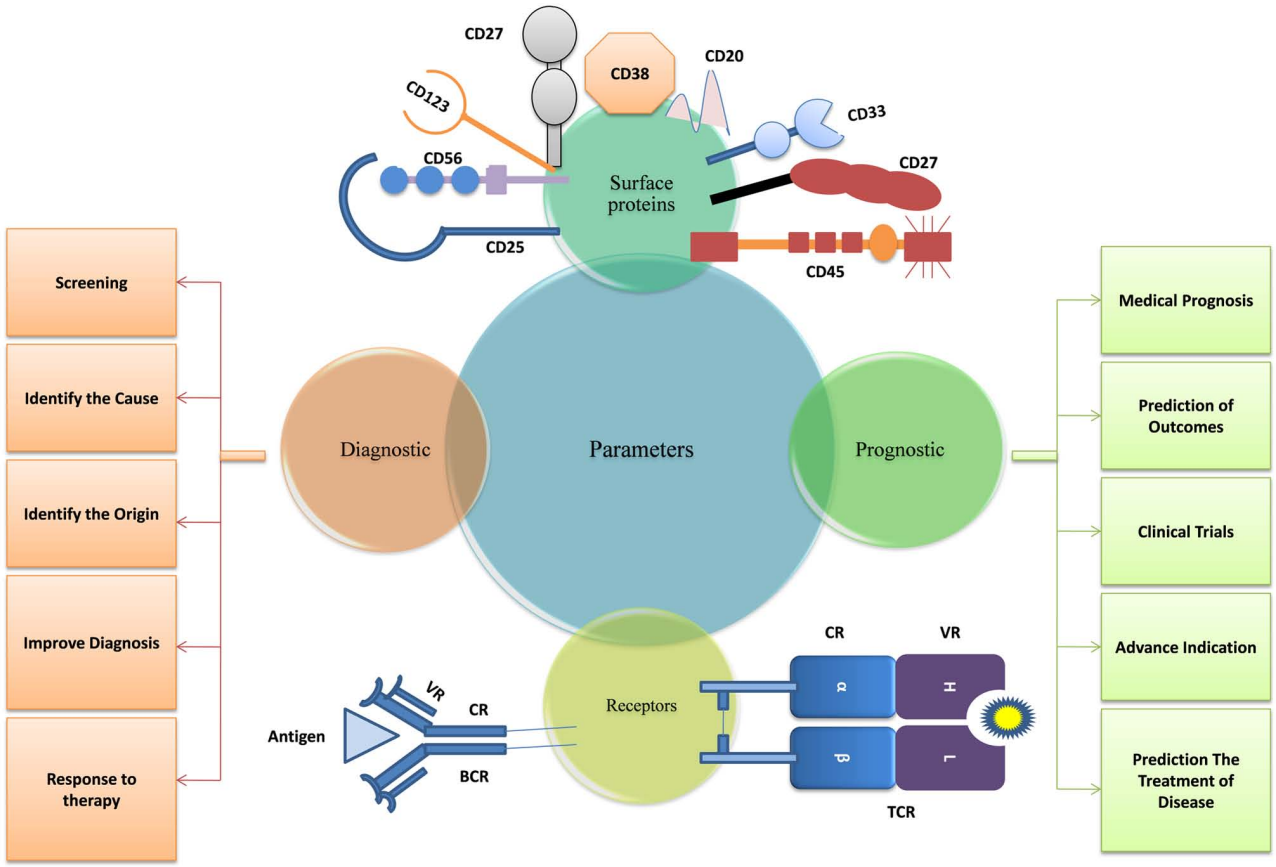

Figure 2. Parameters involved in leukemia diagnosis and prognosis. The sizes and colors of the circles are intended for differentiation and visual clarity. The yellow area surrounded by a dark blue layer represents receptors and their interactions.

~60-64 years of age, and a decline in the incidence rate in the oldest age groups (102,103). In women, the highest rates are observed among those ≥90, whereas in men, those aged 85-89 years-old have the highest rates (41).

Children under the age of five have the highest risk of developing ALL. After reaching its lowest point in the mid-20s, the risk gradually increases again, starting at age 50. In total ~4/10 individuals diagnosed with ALL are adults (50,104).

The rate of developing CML increases gradually from birth but more rapidly between the ages of 65-69. The highest mortality rates are among women aged 85-89 and men ≥90. In most age categories, females and males experience the same rate of occurrence (96,105,106).

CLL is recognized as the second most frequent kind of adult leukemia. The incidence rates of CLL increase with age, peaking between 45-49 years-old for men and gradually for women. The highest mortality rates are among women aged 85-89 and men ≥90. Generally, women have lower incidence rates than men throughout a wide range of ages (107).

5. Exploring diagnostic parameters in leukemia

There are numerous parameters for leukemia diagnosis and prognosis, but clinical diagnosis is more specific and commonly used. Diagnostic parameters are used to screen for leukemia, identify the cause or origin of the disease, and improve leukemia diagnosis to find an improved way to respond to therapy. As observed in Table I, imatinib and the detection of BCR-ABL1 and microRNA (miRNA), which are often performed using polymerase chain reaction (PCR), reverse-transcription quantitative PCR (RT-qPCR), and fluorescence *in situ* hybridization (FISH), are essential for the diagnosis of leukemia (107).

However, modern diagnostic methods such as FISH, flow cytometry (FCM) and RT-qPCR have been incorporated into the effort to define this entity within the context of clinical practice, as revealed in Fig. 4. Algorithms of varying complexity have been created to use these methods to determine leukemia (108,109). Because of its convenience, speed, low cost and overall reliability, FCM has been widely employed to detect overexpression in the diagnostic panel.

Assessing leukemia through blood diagnostics. The first line of defense in detecting leukemia is a blood test. Because normal blood tests are performed for various reasons aside from leukemia, a blood test that includes a complete blood count (CBC) and differentials is a crucial opportunity to screen for leukemia early (110). Some of the hematological parameters studied are hemoglobin <7.4±1.6 g/dl, red blood cells <2.84±0.32 millions/mm^3, erythrocyte sedimentation rate (ESR) <96.5±26.8 mm/1st h, platelets <46.0±70.2x10^3/μl and WBCs <56.1±26.8x10^3/μl (29). The creatinine, total bilirubin, urea and ALT values are higher than the average local population, but the alkaline phosphatase level is markedly lower. Individuals with leukemia have high levels of WBCs, as observed in Fig. 5. The absolute basophil count (111) is

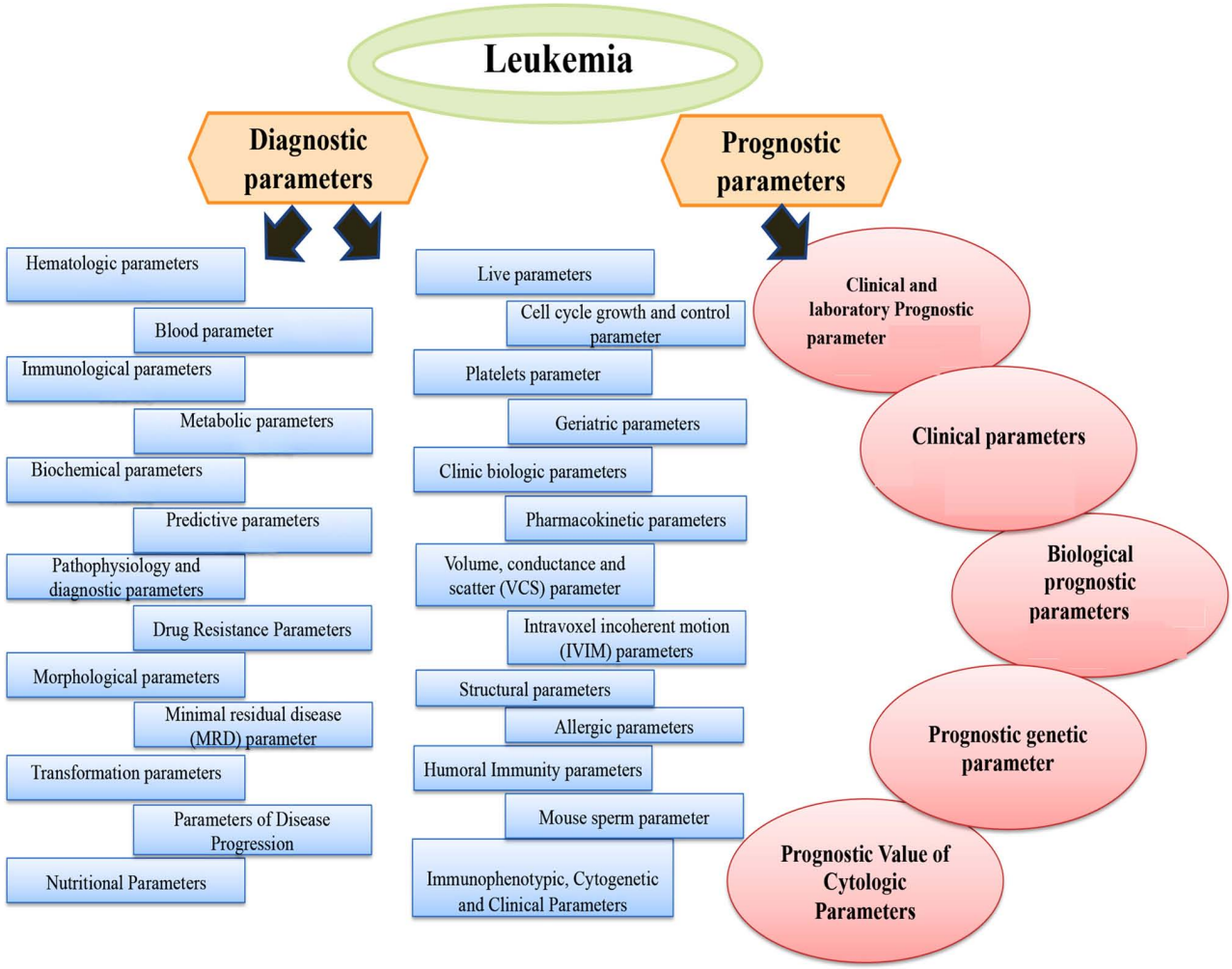

Figure 3. Outlines of the main categories of diagnostic and prognostic parameters for leukemia. Each category is further subdivided into specific factors, emphasizing the convoluted and multidimensional approach necessary for precise diagnosis and prognosis of the leukemia. The arrangement and direction of the arrows advocate a sequence from broad categories to detailed parameters, illustrating the comprehensive evaluation needed to effectively understand and manage leukemia.

a reliable method for detecting CML. An absolute basophil count field is an affordable and simple diagnostic test for the early identification of patients with CML (72).

The platelet-related parameters platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR) and plateletcrit (PCT) are measured using an automated hematology analyzer and whole-blood FCM (112).

Platelet parameter activation and dysfunction are observed in patients with ALL, and glycoprotein expression is used to assess the disease's impact. Future research should look at platelet function from various perspectives, including extending platelet parameters related to illness and investigating platelet parameters with ALL over time. A practical approach for the diagnosis and prediction of disease, and more accessible methods of platelet detection are developed in other studies (112). The mechanism of platelet dysfunction must be unraveled; strategies for the straightforward application of therapeutic, prophylactic platelet transfusions must be developed (87).

Evaluating leukemia via bone marrow diagnostics. A bone marrow biopsy is required to determine the most effective therapy for leukemia. Testing the bone marrow for leukemia cells is crucial since the disease begins in the bone marrow (113). Bone marrow that produces sufficient blood cells can be determined through a bone marrow aspiration or biopsy. These tests identify the cause of fevers and other mysterious illnesses and track the progression of blood and marrow cancers. It is possible to monitor a patient's response to treatment for leukemia by repeating the bone marrow tests utilized after diagnosis (67).

Most individuals with AML, ALL, CML and CLL have an imbalance in their blood, with too few adult red blood cells and platelets and too numerous immature WBCs. Myeloblasts (also known as blasts) are very early blood-forming cells generally not detected in the blood and may take up a large portion of the WBCs. The function of cells differs from fully developed WBCs. Although these results indicate leukemia, a specific diagnosis generally requires analyzing cells from the bone marrow (114).

Molecular-level diagnosis of leukemia. The presence of 20% blast cells in bone marrow or blood is one criterion for diagnosing leukemia; however, molecular genetics changes

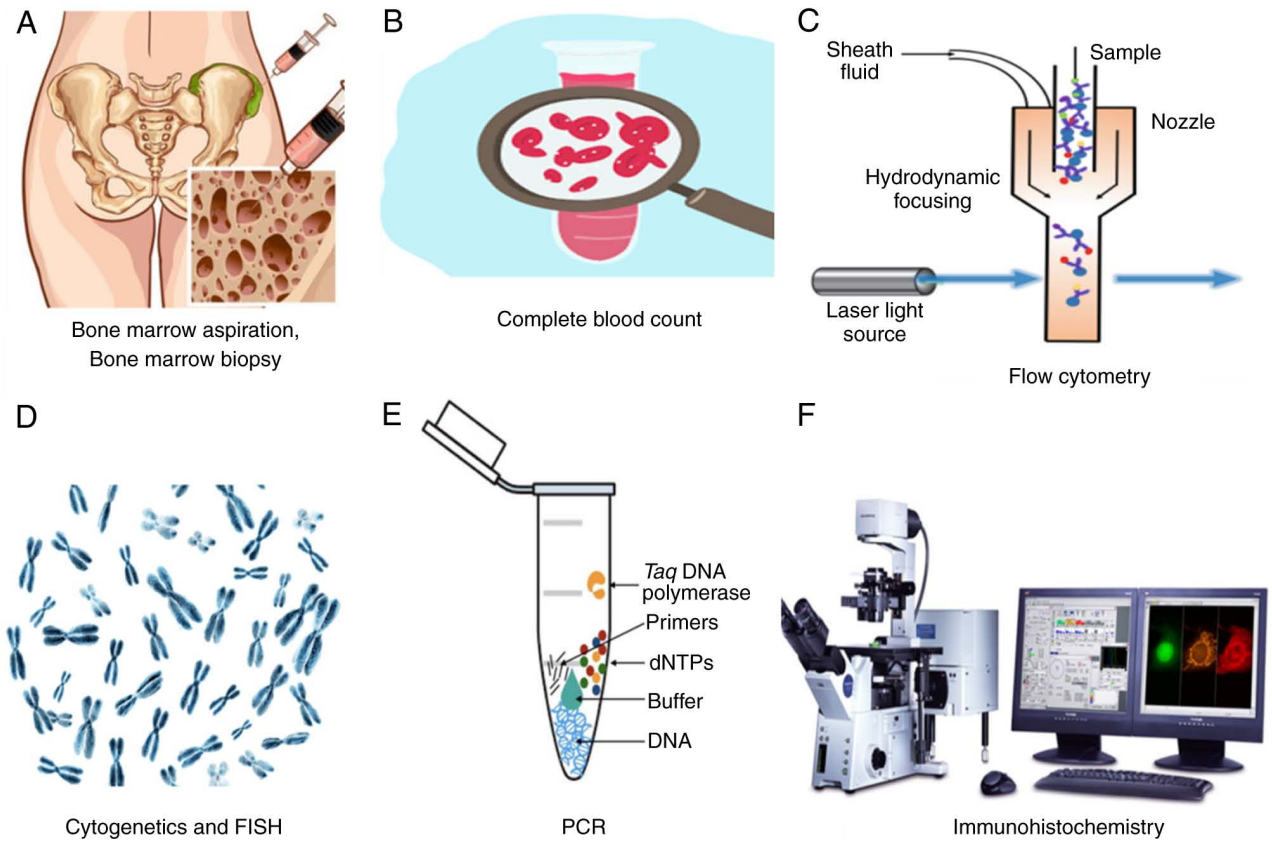

Figure 4. Common diagnostic parameters for leukemia patients. (A) Bone marrow aspiration such as bone marrow biopsy. (B) Complete blood count. (C) Flow cytometry. (D) Cytogenetics and FISH. (E) PCR. (F) Immunohistochemistry. FISH, fluorescence *in situ* hybridization.

should be addressed, which may lower the blast requirement to <20% (115). Both the 2017 European Leukemia Net (ELN) and the 2019 National Comprehensive Cancer Network (NCCN) guidelines advise karyotyping and FISH testing for patients with AML (114,116). Sub-classification of AML, risk stratification and treatment decisions use different tests.

Conducting cytogenetic analysis for leukemia diagnosis. Human chromosomes and chromosomal disorders are the subjects of cytogenetics. Bone marrow samples screen for chromosomal abnormalities such as the Philadelphia (Ph) chromosome. Blood and bone marrow tests show diagnostic results, including a high WBC count and establishing a diagnosis of CML (61).

A human somatic cell contains 23 pairs of chromosomes. There is often a Ph chromosome in the bone marrow of those with CML, around 95% of the total. However, a small subset of the population does not carry the Ph chromosome while having CML-related symptoms. Individuals rarely fail to provide a positive result for the *BCR-ABL1* fusion gene on chromosome 22. Similarly to those with a detectable Philadelphia chromosome, therapy for these patients is compelling.

Regular cytogenetic study is essential to the diagnostic process when dealing with a patient suspected of having acute leukemia. In total, ~50% of adults with AML are found to have chromosomal abnormalities. Recurrent genetic anomalies in AML are categorized by the World Health Organization (WHO) with a list of seven translocations, inversions and variants (114,116). The WHO classifies a patient as having 'AML with myelodysplasia-related features' if they have ≥20% blasts in their blood or marrow and multiple chromosomal abnormalities. Evaluation of at least 20 metaphase cells from bone marrow is advised for diagnosing a normal karyotype, and this number should be doubled to determine an aberrant karyotype. It is possible to diagnose a karyotype abnormality with a simple blood draw (117).

Utilizing karyotyping in leukemia diagnosis and prognosis. Karyotyping is an essential technique for diagnosing and understanding different types of leukemia, as it allows for the detection of chromosomal abnormalities that provide valuable insights into the prognosis and potential treatment options for patients (118,119). The process begins with collecting bone marrow or peripheral blood samples, with bone marrow aspirates commonly used due to their high concentration of dividing cells (120). These samples are then cultured in a laboratory setting to encourage cell division. This is crucial because karyotyping requires cells in the metaphase stage, where chromosomes are most visible under a microscope (117). The cells are fixed onto a microscope slide and stained, which highlights the chromosomal bands, allowing for the detailed analysis and identification of any genetic abnormalities present in the leukemia cells (121).

Karyotyping helps classify AML because it may detect the presence of multiple recurring translocations in AML leukemic cells. Complex chromosomal alterations can also be detected by karyotyping. Variations such as this are used to categorize patients with AML into risk groups. Karyotyping is

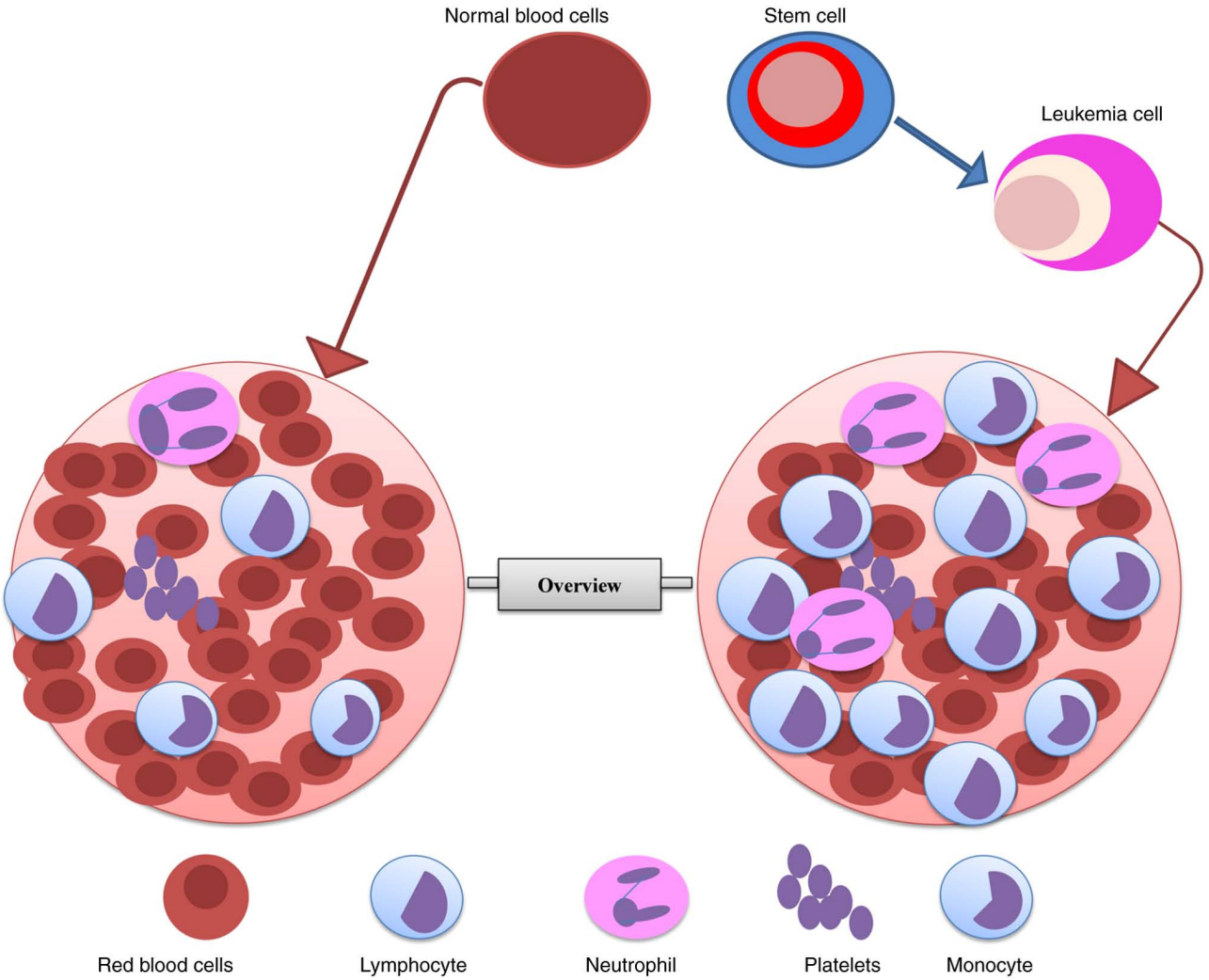

Figure 5. Overview of leukemia and normal red blood cells from stem cells. Schematic overview illustrating the differentiation of stem cells into normal red blood cells and leukemia cells, highlighting the distinct pathways and cellular alterations that distinguish healthy hematopoiesis from the leukemic process. This figure underscores the critical role of stem cells in both normal blood cell formation and the pathological development of leukemia.

currently a beneficial method. However, there are limitations to karyotyping. Before analyzing metaphase chromosomes, the cells in a sample must have proliferated. If the intended cells do not divide, researchers will not be able to examine their metaphase chromosomes (36). This means there is a chance of bias in the karyotyping outcome. A translocation can also involve a section of the chromosome that is too small to be detected by microscopic karyotyping. Cryptic chromosomal alterations are only detectable by an alternative technique such as FISH.

Karyotyping is pivotal in diagnosing and characterizing various types of leukemia. In ALL, karyotyping identifies fundamental translocations such as t(12;21)(p13;q22), which is linked with a favorable prognosis, and t(9;22)(q34;q11), known as the Philadelphia chromosome, which indicates a poorer prognosis (122). For AML, karyotyping detects abnormalities such as translocations t(8;21)(q22;q22) and inversions such as inv(16)(p13q22), which have significant implications for prognosis and treatment strategies (123). In CLL, common findings include deletions on chromosome 13q, which is associated with a relatively improved prognosis, and mutations on chromosome 17p, which are linked to a more aggressive disease course (124). Through these detailed chromosomal analyses, karyotyping not only aids in accurate diagnosis but also helps tailor individualized treatment plans and predict patient outcomes.

Applying FISH techniques in the diagnosis of leukemia. Translocation can be effectively detected using FISH. In FISH assays, specific DNA sequences known as probes are used to target particular regions of chromosomes. A DNA probe is used when its sequence is a perfect match for the DNA sequence in the target region of the genome. Probe DNA typically measures hundreds of kilobases. The conjugated fluorophore can be observed under a fluorescence microscope once bound to the specific region of genomic DNA of interest. As a means of chromosome identification, FISH probes can be designed to target only the centromeres. Locus-specific FISH probes allow for pinpointing a particular gene's location. This means that the FISH assay can detect both overt and cryptic chromosomal translocations. Metaphase and interphase targets are accessible for FISH. A higher degree of sensitivity than karyotyping is typical (94). It is possible to detect the BCR-ABL1 gene with FISH. Genomes are constructed from smaller pieces of

DNA. Using FISH, the BCR and ABL1 genes can be located on chromosomes using colored probes that bind to the DNA.

Prenatal diagnostics have been revolutionized by multiplex ligation-dependent probe amplification (MLPA), a technique from the field of molecular genetics. MLPA uses up to 40 probes specific for a distinct DNA sequence (often exons of a particular gene of interest) to count the number of copies of each DNA sequence. The previous study has demonstrated that MLPA is a reliable approach for identifying genomic aberrations in CLL and has a significant relationship with FISH results in patients with CLL (125). The advantages of MLPA over FISH include speed and cost-effectiveness. While MLPA may simultaneously detect copy number alterations and point mutations in several target locations, FISH can only deliver information for a limited selection of genomic targets at a time.

Faster results from FISH assays are possible. The t(15;17) translocation may be identified with several different FISH techniques, including the dual-color dual-fusion FISH assay. FISH methods are superior to karyotyping for the early detection of acute promyelocytic leukemia (APL) due to their shorter cycle times. Both the 15q24 promyelocytic leukemia (PML) probe and the 17q21 retinoic acid receptor alpha (RARA) probe are used in a dual-color, dual-fusion test. The chromosomal breaks in both areas of study coincide. The assay's carefully thought-out structure allows for detecting PML-RARA. However, in circumstances where the translocation occurs between the RARA gene and other partner genes, the PML gene probe would not help detect the translocation using the dual-color dual-fusion FISH approach. The RARA dual-color break-apart FISH test was created for this reason (126). RARA dual-color break-apart FISH employs two probes for the RARA gene, each fluorescently tagged with a different fluorophore. For example, one RARA probe is labeled with a red fluorophore, whereas the other is marked with a green fluorophore. The binding sites of the two probes are the RARA gene center and the breakpoint (127). Such a generally applicable test design makes identifying RARA translocation with any number of partner genes possible. The FISH test has been widely used to diagnose APL. Fast results mean you can get a diagnosis and start treatment sooner, improving your chances of a positive outcome. However, the FISH assay has its limitations as well. While FISH can detect some cryptic translocations, it cannot identify the extremely rare cryptic RARA translocation. Due to this, RT-qPCR techniques have been developed to detect PML-RARA translocation. The RT-qPCR process can detect RARA translocations that are otherwise undetectable.

Cryptic chromosomal alterations can significantly impact the diagnosis and prognosis of leukemia. For instance, in CML, the Philadelphia chromosome, characterized by a translocation between chromosomes 9 and 22, may not always be detected by conventional karyotyping, especially if the rearrangement is complex or involves minor cryptic alterations. FISH techniques using probes specific to the *BCR-ABL1* fusion gene can reliably identify these cryptic rearrangements, enhancing diagnostic accuracy and improving treatment planning (128). Similarly, in AML, small deletions or subtle translocations, such as those involving the *FLT3* gene, may be missed by traditional karyotyping but are detectable through FISH analysis (128). Applying FISH and other advanced molecular techniques ensures that such cryptic alterations are not overlooked, providing a more comprehensive understanding of the genetic landscape of leukemia.

The clinical and prognostic significance of t(8;21) translocation in AML. T(8;21)-translocated AML produces a chimeric gene on the 8q-derived chromosome capable of transcription. Typically, karyotyping will be able to detect such a translocation. However, if cells containing the translocation do not multiply throughout the experiment or if the translocation is cryptic, a false-negative result may be obtained using karyotyping. To address this issue, the FISH assay has been adapted to be performed on cells in the interphase phase. Some hidden translocation can be discovered by interrogating interphase cells using the dual-color FISH test. This makes it a more sensitive method than karyotyping. A high total remission rate and a favorable prognosis are typical for the AML subtype identified by the t(8;21) translocation tests. Blasts in this kind of AML are not always >20%. However, the presence of t(8;21) should not rule out a diagnosis of AML, even if there are <20% blasts (129).

The role of FCM and immunohistochemistry in leukemia diagnostics. In FCM and immunohistochemistry, cell samples are treated with antibodies, which only bind to particular proteins on cells (130,131). Cells are viewed under a microscope in immunohistochemistry to determine whether or not the antibodies are attached to them (indicating the presence of the corresponding proteins). At the same time, FCM requires a specialized machine.

More straightforward techniques for platelet detection were created as a successful strategy for illness diagnosis and prediction (132,133). Quantitative measurements of PLT, MPV, PDW, platelet-large cell ratio (P-LCR) and platelet count (PCT) were taken using an automated hematology analyzer and whole blood FCM. Unraveling the cause of platelet failure and developing simple methods for administering therapeutic, prophylactic platelet transfusions are necessary (87). Future studies should examine platelet function from various angles, including extending the relationship between platelet parameters and disease and looking at platelet metrics over time.

Understanding immunophenotyping in acute leukemia. Acute leukemia's lineage involvement can be determined using immunophenotyping employing multiparameter FCM. No one appears to agree on what percentage of individuals with acute leukemia should be considered favorable for a marker. For most indicators, the expression threshold is set at ≥20% of leukemic cells, while field has applied for other features (17). Quantifying expression patterns of various surfaces and intracellular antigens is necessary for lineage assignment, diagnosing mixed phenotype acute leukemia (MPAL), and identifying aberrant immunophenotypes to determine MRD. A blast count determined by FCM should not replace morphologic assessment.

MPAL is a potential immunophenotypic presentation of acute leukemia that is positive for BCR-ABL1. These cases of leukemia should be treated as ALL with the addition of an ABL1 tyrosine kinase inhibitor (*TKI*) to the standard

chemotherapy. CML blast crisis is considered as a possible secondary diagnosis in *BCR-ABL1*-positive leukemias (134). Identifiable immunophenotypic characteristics are linked to certain AMLs with recurring genetic abnormalities (41).

The T-cell antigen receptor (TCR) and the B-cell antigen receptor (BCR) are the main actors in the adaptive immune system. The TCR and the BCR identify pathogenic signals, activating the cells and allowing them to perform their protective role. The plasma membrane is the site of TCR and BCR signaling initiation, which later spreads into the cell to activate the cells and trigger an immune defense. However, improper T- and B-cell activation can harm the host and cause autoimmune diseases, immunodeficiency and malignancy (135).

Antibodies to T-cell antigens (CD2, CD3, CD4, CD5 and CD7) bind more cells than those to B-cell antigens (CD19, CD20, CD21, CD22, CD23 and CD24), which together make up 20-40% of the leukocytes in peripheral blood and have a 70:30 ratio. The CD4/CD8 ratio is lower in advanced CLL, and this simple immune function parameter appears to have a significant predictive value for patients, predicting the disease's clinical fate (136).

However, specific antibodies (CD9, CD11b, CD13, CD14, CD15, CD16, CD56, CD57, CD79a, CD95 and CD154) are sporadically negative for frozen cells, indicating either poor binding affinity or the loss of these surface antigens due to shedding or epitope degradation (137). Morphology, immunophenotype, cytochemistry and karyotype are combined to diagnose leukemias and lymphomas. The antigens CD19, CD20, CD21, CD22, CD23, CD24, CD25 and CD37 show the best differentiation between CLL and regular peripheral blood leukocytes (138). The results from the microarray for the expression of 48 CD antigens agree with those from FCM. The microarray allows for thorough immunophenotyping and soluble, fluorescently labeled antibodies can further define the whole cells caught on antibody dots (139).

A low CD4/CD8 ratio is characterized by advanced CLL. The clinical presentation of CLL may be predicted with a high degree of accuracy using this relatively uncomplicated indicator of immunological function.

PCR for leukemia detection and monitoring. This is a highly sensitive assay, capable of detecting even trace amounts of leukemia cells. The process is effective because it raises the DNA concentration in the sample, making it easier to observe. This analysis can detect subtle chromosomal alterations that other methods miss.

A PCR or a DNA test can detect the BCR-ABL fusion gene and other molecular abnormalities. In addition, PCR diagnostics could be utilized to check the efficacy of treatment. This test, for instance, is so sensitive that it can identify even a single abnormal cell among a million normal ones. Both blood and bone marrow cells can be used in this analysis. The PCR can confirm a diagnosis of CML. Testing for the presence of *BCR-ABL* gene copies after treatment is also helpful. When this gene is duplicated, leukemia remains even if no visible cancer cells are current (72).

A follow-up evaluation may be able to quantify disease-specific chromosomal or molecular abnormalities. However, most documented aberrations lack an examination of the link between the disease load in CR and the clinical prognosis. A further drawback of PCR for MRD detection is the lack of standardization and test variability (71).

In this study, genetic abnormalities are analyzed by using multiplex ligation-dependent probe amplification. In a single experiment, the polymerase chain reaction-based method known as MLPA can concurrently identify point mutations, copy number alterations, and perform DNA methylation in up to 50 genomic DNA sequences (140).

RT-qPCR. RT-qPCR is the most precise and sensitive approach for determining how much BCR-ABL1 gene is present in blood and bone marrow samples. Even if the Ph chromosome is absent in blood or bone marrow cells, a single CML cell can be recognized among 100,000-1,000,000 normal cells using cytogenetic testing. Assuming the patient's CML responds to treatment, qPCR testing is advised every three months or two years.

Routine molecular diagnostics testing requires a bone marrow (and blood) sample. The ideal situation would involve the extraction of DNA and RNA and the storage of live cells. However, if cell numbers were restricted, RNA extraction would be prioritized since it lends itself to molecular screening for leukemia-associated mutations. Del 17p was highly prevalent among patients with CLL who visited the Armed Forces Institute of Pathology (Rawalpindi, Pakistan). Not all of these individuals, however, required treatment. However, introducing promising new medications may enhance their overall survival periods (76).

The RT-PCR can assist in diagnosing common gene fusions, including *RUNX1-RUNX1T1, CBFB-MYH11, MLLT3-MLL* and *DEK-NUP214*. RT-PCR can identify these rearrangements if chromosomal morphology is weak or marrow morphology is typical. Still, there are no cytogenetic abnormalities (109). The *NPM1, FLT3, CEBPA* and *MLL* (myeloid/lymphoid or mixed-lineage leukemia, Drosophila) (141), *NRAS* (neuroblastoma *RAS* viral (v) oncogene homolog) (142), and *WT1, KIT* (v) Hardy-Zuckerman 4 oncogenes (143) of feline sarcoma mutations in these genes vary considerably amongst cytogenetic groupings. Alleles of AML produced by *NPM1* or *CEBPA* mutations are considered active entities. Patients with cytogenetically normal AML (CN-AML) who receive other than low-dose chemotherapy or optimal supportive care should have these three mutations examined, even though testing is not necessary outside clinical trials (144).

A molecular diagnosis using RT-PCR can be helpful for common gene fusions like *RUNX1-RUNX1T1, CBFB-MYH11, MLLT3-MLL* and *DEK-NUP214*. If the quality of the chromosomal morphology is low or the marrow morphology is typical, RT-PCR can detect these rearrangements (145). The rate at which these mutations occur varies greatly amongst cytogenetic groupings - the WHO now recognizes AML alleles produced by mutations in *NPM1* or *CEBPA* as distinct entities. In addition to *FLT3* mutations, screening patients for these two signs in treatment trials might be helpful. Although testing for these three mutations is not presently deemed necessary outside clinical trials, the panel recommends doing so for patients with cytogenetically normal AML (CN-AML) who receive treatment other than low-dose chemotherapy or appropriate supportive care.

6. Identifying key prognostic parameters in leukemia

Prognosis is all patient characteristics used to estimate recovery or a disease's chance of recurrence. A prognostic parameter is the patient's malignancy or a trait to be considered while determining the prognosis. A prognostic or predictive factor influences the outcome of a particular cancer treatment. The decision on a course of treatment and a prediction is often made in conjunction with predictive and prognostic criteria. Leukemia is prognosed using factors such as age, WBC count and genetic abnormalities, among others. Age, WBC count, Immunoglobulin Variable Heavy Chain gene (IgVH) status, TP53 deletion and miRNA studies are the established factors with the most established prognostic importance. Almost every subtype of leukemia has prognostic cytogenetic variables, particularly acquired mutations that, if identified, allow for the definition of the optimal therapy for a particular patient.

Although several pre-treatment features have been shown to have predictive value in leukemia, only a few risk factors have surfaced in ongoing leukemia studies with intensive chemotherapy regimens. These include the patient's age, starting WBC count, karyotype and immunophenotype. Variables used to assess the likelihood of a patient surviving a malignant tumor are associated with clinical outcomes in either a positive or negative way. Predictive factors determine the effectiveness of a treatment in a particular medical setting. They may favor one therapy over another prognostic factor. The prognostic impact can be fine-tuned, and therapy selections can be made with the help of scoring systems that combine numerous prognostic indicators with an independent relationship with outcome criteria.

The impact of age on leukemia prognosis and treatment outcomes. The patient's diagnosis age is the most important prognostic factor in leukemia. Patients with AML <30 years-old performed worse than those aged ≥50. In seniors with AML, comorbidities and probable side effects against rigorous cytotoxic treatment are more prevalent, as are impaired performance status, secondary AML following antecedent myelodysplastic syndrome or cytotoxic therapy, and unfavorable chromosomal abnormalities (98). The patients' age remains a significant independent prognostic factor, even once these other characteristics are considered (146). Even with aggressive chemotherapy, the outlook is poor for patients aged ≥65, regardless of cytogenetic risk. Various aspects of the disease's biology have been implicated in the differential prognoses between adults and children. The ability to tolerate a specific treatment varies from one individual to the next. The chemotherapy drugs are metabolized differently depending on age. Methotrexate polyglutamate accumulates at far lower levels in adult B-lineage blasts with ALL than in children with the same condition (100). Most individuals with CML are adults. A total of 1/1000.000 children >10 years of age will contract leukemia. In total, ~1/100.000 adults will have this condition. All types of AML, CML and CLL, as well as their parameters and risk values are summarized in Table I.

Research shows that leukemia can develop at any age. In general, adults ≥60 years-old have a higher risk of developing leukemia. Both AML and CLL are more common in adults. Every year, ~3,811 children are diagnosed with leukemia. When it comes to childhood cancers, ALL is by far the most common type. Any element that raises an individual's probability of acquiring leukemia is considered a 'risk factor'. In most cases of leukemia, the causes or risk factors are unknown (147). For specific patients' most accurate risk categorization, research is needed to determine how to combine variables showing more significant angiogenesis with other prognostic markers, such as cytogenetic anomalies (148).

The importance of WBC count and bone marrow histology in leukemia prognosis. The WBC count tests are pivotal in diagnosing and characterizing different types of leukemia, with distinct patterns observed across the various forms (149-151). In ALL, there is typically an elevated WBC count due to an increase in lymphoblasts, although some cases may present with normal or decreased counts. At the same time, bone marrow histology typically shows a high number of lymphoblasts, immature lymphoid cells that dominate the marrow and replace normal hematopoietic elements (152), while lymphoblasts are generally small to medium-sized, with scant cytoplasm and prominent nuclei. Similarly, AML often shows a high WBC count with a significant number of myeloblasts, yet normal or low counts are also possible in some patients (114). The marrow is infiltrated by myeloblasts and immature myeloid cells featuring a high nucleus-to-cytoplasm ratio, and may include Auer rods and needle-like cytoplasmic inclusions. By contrast, CLL is generally characterized by a very high WBC count due to the accumulation of small, mature-appearing lymphocytes in the bone marrow (124). With their condensed chromatin and uniform appearance, these cells crowd out normal hematopoietic cells, often resulting in hypercellularity.

For CML, an elevated WBC count is observed, along with an increase in granulocytes such as neutrophils, eosinophils and basophils; this condition is also marked by the presence of the Philadelphia chromosome or the *BCR-ABL* fusion gene (153). In CML, bone marrow histology shows marked hypercellularity with an increased number of granulocytic precursors at all stages of maturation, as well as dysplastic megakaryocytes. However, the overall architecture of the bone marrow is preserved. Hairy Cell Leukemia (HCL) can result in a low WBC count, as the abnormal cells accumulate in the bone marrow and disrupt normal blood cell production (154). The bone marrow typically appears fibrotic or presents a 'dry tap' in HCL due to the infiltration of hairy cells. These cells are recognized by their irregular cytoplasmic projections and are often positive for tartrate-resistant acid phosphatase enzyme, leading to pancytopenia as a result of marrow failure.

The white blood cell count at diagnosis is the strongest predictor of treatment effectiveness in leukemia. In total, 50-60% of all patients have a leukocyte count >$10x10^9$/l at diagnosis, and 10% have a leukocyte count >$100x10^9$/l. Leukocyte levels <$10x10^9$/l are also present in another 30-40%. Neutrophil absolute numbers are commonly relatively low. Complete remission and the length of time spent in remission strongly correlate with leukocyte counts. The importance of this pretreatment step cannot be understated in AML and ALL since long-term remissions are unusual in patients presenting with high WBC counts using standard chemotherapy regimens (56).

However, some evidence suggests that, with the help of modern chemotherapy, a high WBC count at diagnosis in ALL is not always indicative of a poor prognosis. Also associated with a poor prognosis is having more than 80% of blasts in circulation. There is a correlation between leukocyte counts and the fraction of circulating blasts, although this has been demonstrated to have an independent prognostic value (155).

Evaluating clinical and laboratory prognostic parameters across leukemia types. All blood samples were routinely examined under a microscope for years, allowing the medical community to amass substantial knowledge about how cells change in various disease states. Laboratory workloads and economic demands have risen over the past few decades. The use of morphologic data for diagnosis has been rapidly declining along with the development of automated cell counters that can report a CBC. Only a small percentage of blood samples are examined under a microscope today (156).

A poor prognosis was observed in patients with either an elevated lactate dehydrogenase (LDH) serum level or a high peripheral blood leucocyte count at diagnosis. The hemoglobin value, PCT, and fibrinogen plasma level at diagnosis affected different outcome indicators. Serum β2-microglobulin elevation was a poor prognostic indicator in patients with AML >65 years-old. However, compared with age and cytogenetic risk, these characteristics have a relatively minor impact on prognosis (157).

Plasma, fibrinogen, bleeding time and LDH activity increased, while hemoglobin, hematocrit and PCT decreased (158). Despite having lower hemoglobin, hematocrit and PCT, patients with AML had increased plasma viscosity, ESR, fibrinogen, leukocyte count, bleeding time and LDH activity. Increased bleeding times were observed in patients with CML with high levels of fibrinogen, plasma viscosity and ESR. The filtering ability of the red cells was consistent across all conditions. The absolute basophil count is an effective screening tool for chronic myeloid leukemia. The total basophil count is a simple, low-cost and rapid diagnostic tool for the early detection of patients with CML (72).

Careful clinical assessment, together with clinical stages according to Rai and Binet, CLLPD subtype and progression variables, is a crucial link for creating a tailored therapy approach and an essential step in determining the degree and course of the disease. In contrast to 'researcher-dependent' parameters, laboratory parameters (hematologic and biochemical) obtained by straightforward venipuncture are objective quantitative measures that improve the use of these indicators as risk factors in CLL (26).

The role of miRNA patterns in determining clinical prognostic parameters for leukemia. The miRNAs have been shown to provide therapeutic guidance for patients with pediatric leukemia who need risk-adapted treatment (25). Oncogenic miRNAs are associated with a poor prognosis (159), whereas tumor suppressor miRNAs, which prevent proto-oncogene expression, are associated with a favorable prognosis (160). The miRNA expression could be a more effective marker for determining tumor origin and prognosis than traditional protein-coding gene arrays. A miRNA profiling study focusing on pancreatic tissues revealed that the expression of just 25 miRNAs can differentiate between pancreatic adenocarcinoma, chronic pancreatitis and normal pancreatic tissue (160,161). MiRNAs are being considered as treatment options for several malignancies, including AML, and biomarkers for diagnosis and prognosis. Experimental, pre-clinical techniques and synergistic activity with chemotherapeutic drugs demonstrated viable therapeutic targets, as revealed in Fig. 6. The development and eventual clinical application of new medicines with mimic or suppressive effects gave way to the therapeutic targeting of miRNAs in the future (101).

Recent research has demonstrated the role of aberrantly expressed miRNAs in the development and spread of cancer. The classification, diagnosis, prognosis, treatments and biomarkers of human cancers can all be based on their expression profiles. Microarray-based technology for miRNA expression profiling has revealed new information about the biology of some adult CLL and AML cases (162). Calin *et al* (163) found that $CD5^+$ and CLL cells express miR-15a and miR-16-1 differentially. MiR-15a and miR-16-1 were linked to disease progression and prognostic factors and triggered apoptosis by targeting BCL2 (163). Mi *et al* (164) found 27 differentially expressed miRNAs between ALL and AML. MiRNAs can diagnose acute leukemia in children (164). Microarrays are a successful way to research miRNA profiling, which helps uncover oncogenes or tumor suppressors involved in disease development.

Notably, several miRNAs change in expression across pediatric and adult instances, indicating the existence of a miRNA pattern unique to children. In adult patients with AML, miR-146 is downregulated, whereas miR-146a is upregulated (162). Contrary to a prior study that found miR-335 downregulated in 27 adult patients with AML, it was upregulated in pediatric M1 patients (165). According to Li *et al* (166), adult patients with APL had elevated miR-100.

Strong evidence came from the high-throughput sequencing data's comparatively high accuracy. Because of the relatively limited sample size, a more thorough examination should be conducted before applying them to the risk stratification of patients with CN-AML. These findings provided insightful investigation into the role and clinical utility of miR-188-5p and let-7a-2-3p. Their prognostic significance and related aberrantly expressed genes and miRNAs can further our knowledge of the internal workings of leukemogenesis and develop new CN-AML therapies (98).

Most patients with ALL are now treated with systemic therapy and protocol-based treatments. However, 20-30% of patients still pass away following treatment. Early relapses are primarily to blame for dismal survival rates of patients with ALL. Such patients continue to receive inadequate care. Therefore, research into the molecular pathophysiology of leukemia in relapsed patients may offer new information about their prognosis (25). It was concluded from the aforementioned study that children who experienced ALL relapse displayed a unique miRNA expression pattern, which included high levels of miRNA expression. Testicular relapse has all but disappeared thanks to improved ALL treatment. However, pediatric ALL therapy is still hampered by controlling central nervous system (CNS) recurrence (167). Therefore, it was concluded that miRNA profiles are responsible for the CNS relapse of juvenile ALL. The miRNA cascade may be employed as a biomarker to identify early pediatric ALL regressions in the

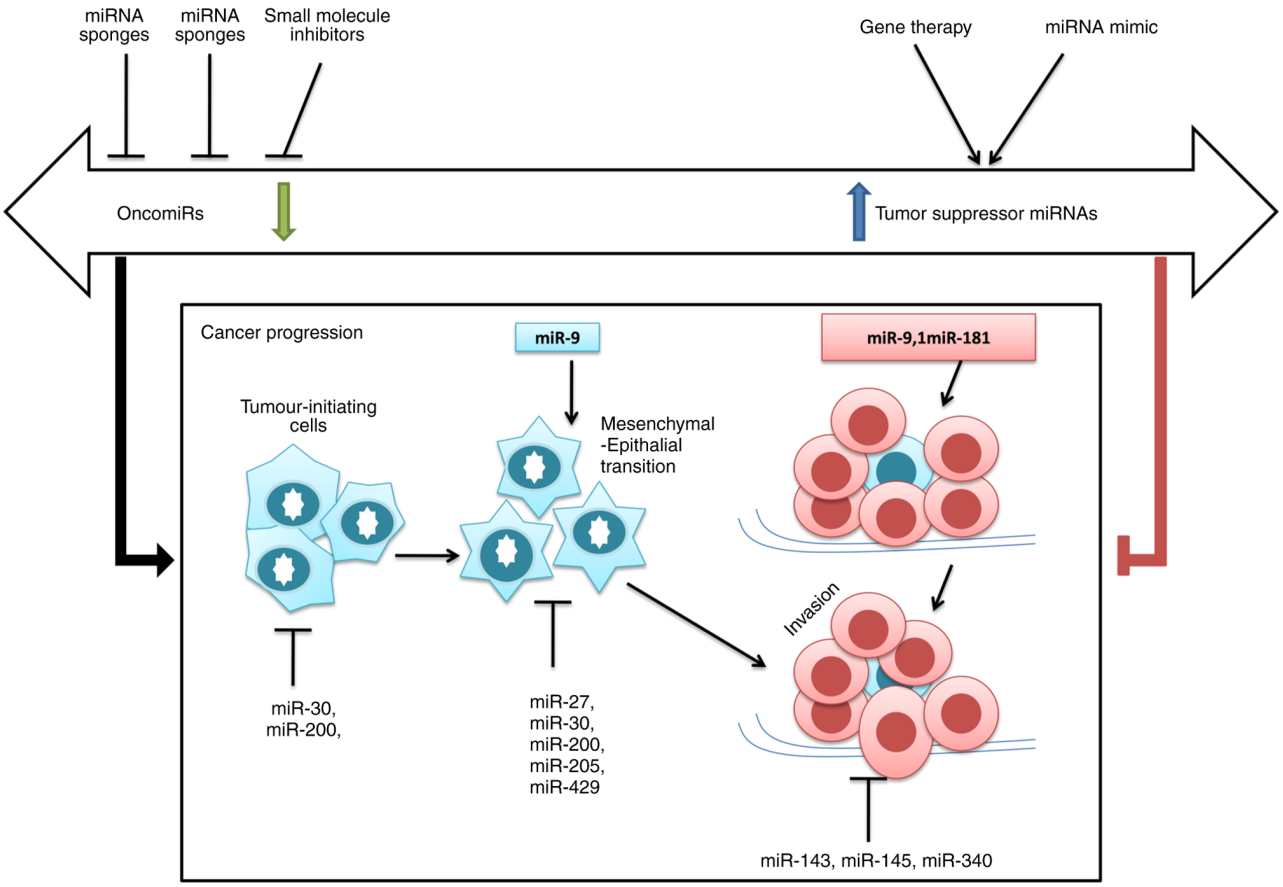

Figure 6. Regulation of leukemia progression at different stages by miRNAs. miR-296, miR-134, miR-470 and the miR-34 family regulate stem-cell pluripotency and activity. MiRNAs regulate leukemia progression at different stages. MiRNAs have the power to suppress oncoprotein-coding mRNAs or cause cancer (black arrowed line). Proposed approaches for modifying the biological activity of cancer-related miRNAs in therapy methods. These molecular therapies aim to either upregulate/imitate tumor suppressor miRNAs or downregulate onco-miRNAs (green arrow). miRNAs or miRs, microRNAs.

CNS. According to miRNA target prediction, some irregularly generated miRNAs may target genes essential in neuron function and neurotransmitter synthesis.

MiRNAs inhibit translation and promote mRNA breakdown to regulate gene expression post-transcriptionally. They modulate biological processes and regulate hematopoietic differentiation. MiRNA expression correlates with AML prognosis (101). Post-transcriptional gene expression regulators regulate development, cell differentiation and cell signaling. MiRNAs modulate gene expression patterns in numerous illnesses, decreasing carcinogenic genes in leukemia. In Figs. 7 and S1 it is depicted how various enzyme complexes act at the transcriptional and post-transcriptional stages to produce mature miRNAs.

Exploring the role of cytogenetics in leukemia prognosis. The clinical outcomes for individuals with AML following intensive treatment are most strongly associated with cytogenetic risk, as was first shown in 1982. A favorable risk aberration is a balanced translocation involving the core binding factor (CBF) transcription factor complexes, such as t(8;21), inversion (16), or t(16;16) (98). The definition of high-risk abnormalities is more contentious. A total of >5,800 patients with AML were included in the most extensive study to examine cytogenetic abnormalities and their association with prognosis. High-risk cytogenetic aberrations discovered in that study include abn(3q) (excluding t(3;5)(q25;q34)), inv(3)(q21q26)/t(3;3)(q21;q26), abn(5q)/del(5q), -5, -7, abn(7q)/del(7q), t(11q23) (excluding t(9;11)(p21-22;q23), and t(11;19)(q23;p13)), t(9;22)(q34;q11), -17, abn(17p). Patients with at least three abnormalities (not counting benign variants) are considered to have a complex-aberrant karyotype, which is the most commonly accepted definition (66). Excluding cytogenetic characteristics from the model reduced negative outcomes and blurred the line between favorable and intermediate survival (168).

While peripheral blood samples are suitable for cytogenetic analysis, this technique is not always effective because CLL clones do not reliably divide in culture. The prevalence of common abnormalities has been reduced thanks to FISH research. FISH panels evaluate the most common cytogenetic aberrations in CLL, and these include del(17p13) [p53], del(11q23) [ATM], trisomy 12, and del(5q) (13q14). To rule out large-cell lymphoma, another CD5-positive B-cell malignancy, some panels evaluate for t(11;14) (49).

TP53, a tumor suppressor gene lost or mutated in numerous cancers, is deleted due to the 17p13 deletion. The standard protein p53, which is the product of *TP53*, binds to the p21 gene and increases p21 production. The p21 subsequently binds to CDK, the protein responsible for signaling cell division, inhibiting CDC's ability to do so and thus stopping the cell cycle from progressing. When p53 is absent, cells divide without control. Patients with CLL who have had their *TP53*

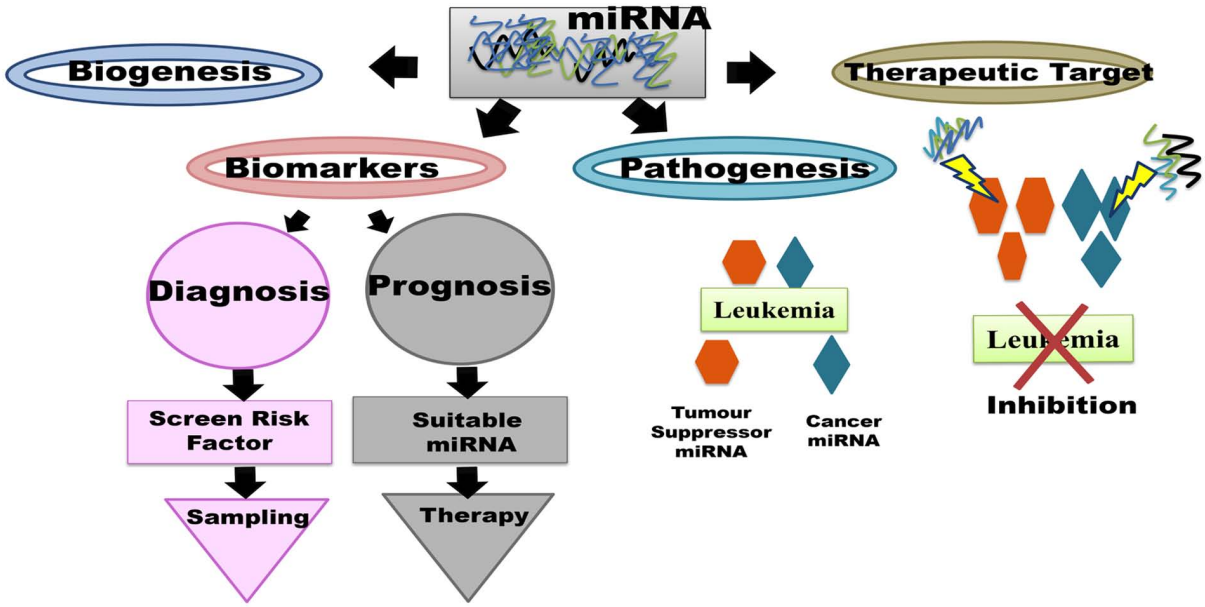

Figure 7. MiRNA's role as a biomarker in leukemia. Because they are implicated in pathogenesis, miRNAs are therapeutic targets in leukemia. Leukemia is caused by the overexpression of onco-miRNA and the downregulation of tumor suppressor miR, which are called pathogenesis. However, lightning denotes targeting and restoration in therapies, while the Red ross denotes leukemia inhibition (111). miRNAs or miRs, microRNAs.

deleted tend to have a poor prognosis and a shorter overall survival than those who have not. Among 50 B-CLL clones without mutated *IgVH*, there are genetic abnormalities such as del (17p13) (134).

Utilizing Rai and Binet staging systems for leukemia prognosis. Rai *et al* (169) and Binet *et al* (170) established the gold standard CLL staging systems. These methods cannot detect stable or progressing sickness (171). The Rai and Binet staging systems affect survival of patients with CLL. The Rai CLL classification system is commonly used in North America. Lymphocytosis >15x10^9/l for four weeks, anemia with hemoglobin level <110 g/l, and thrombocytopenia of <100x10^9/l platelets define the five stages (from 0 to 4). This is on top of whether or not there is splenomegaly or lymphadenopathy. Stage 0 is the lowest possible danger in the Rai system, whereas stages 1 and 2 represent a moderate risk, and stages 3 and 4 mean extreme risk. CLL is classified into stages A, B and C according to the Binet staging approach, which is more often used in Europe. In addition to CLL in several sites (for example, head and neck, axilla, groin, palpable spleen, or clinically enlarged liver), the number of regions implicated and peripheral blood hemoglobin level and PCT are essential.

Reliable prognostic markers for individuals with chronic CLL have become increasingly important as treatment options have expanded in recent years. A prognostic score was constructed using multivariate analysis of 339 patients who were already in stage A at the time of diagnosis and had been thoroughly analyzed for traditional and contemporary predictive markers (34). Finally, a simple, rapid and cost-effective technique was suggested for reliable prognostication in stage A patients, who account for >80% of the CLL population. This enables clinicians to tailor follow-up individually.

Assessing leukemia prognosis through IgVH mutation analysis. The IgVH mutations are analyzed by comparing the DNA sequences of CLL B-cells to germline sequences, a process accomplished using PCR. DNA is considered to be mutated if its sequence deviates from the germline sequence by >2%. Patients with CLL with mutant IgVH have an improved prognosis because they are more likely to go without treatment for longer. Patients with the VH3-21 gene segment are an anomaly; they are members of the mutant group but act like unmutated clones with increased p53 dysfunction and shorter lifespans. CD38 expression, ZAP70 positivity, and del(17p) and del(11q) are associated with unmutated IgVH (11q23). The constraints of PCR testing are also present in IgVH mutation analysis, including the need for freshly collected samples (58). CD38 expression is associated with complex aberrant karyotypes, such as 13q deletions with mutated IgVH and TP53 deletions with unmutated IgVH. The parameters identify overlapping leukemia subgroups (172).

Understanding the prognostic implications of FLT3 mutations in leukemia. In total, ~30% of cases of AML have been linked to *FLT3* mutations. While the *FLT3* mutation is considered a driver mutation, it is not used to classify a specific form of AML. A poor prognosis is typically associated with *FLT3* mutations. AML can now be treated with *FLT3* inhibitors. Common *FLT3* mutations include tyrosine kinase domain point mutations and *FLT3* internal tandem duplicate (*FLT3-ITD*) repetitions (*FLT3-TKD*). The biological effects of these two sorts of mutations may be different. More evidence has emerged connecting *FLT3-ITD* with a poor prognosis. Guidelines for diagnosing and treating AML now consider the proportion of patients with the *FLT3-ITD* mutation compared with those with the *FLT3* wild type (173). *FLT3* mutations are discovered using several methods. One method amplifies *FLT3* and then analyzes the PCR products using capillary

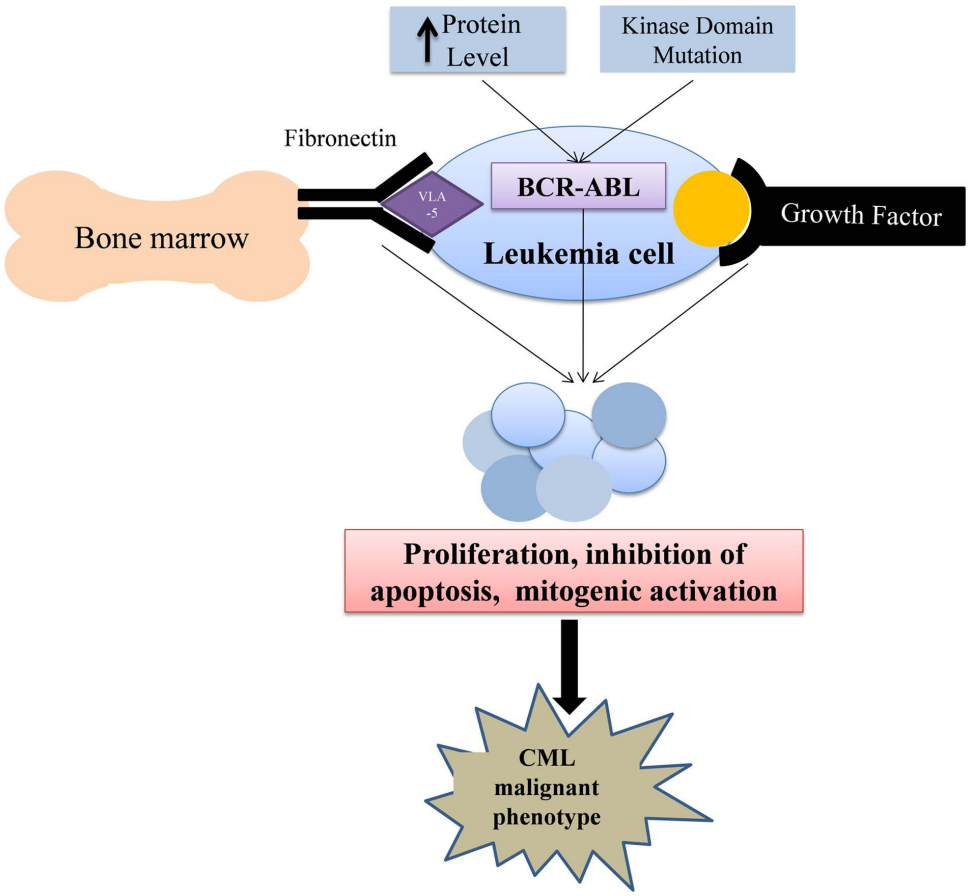

Figure 8. Mechanism of the pathogenesis of CML due to Imatinib inhibition. Modest resistance to imatinib is conferred via kinase domain mutations. Leukemic cells may have very high BCR-ABL levels. Even if BCR-ABL kinase activity is entirely blocked, growth factor signaling or fibronectin signals may still be necessary to sustain survival. VLA-5 denotes late activation antigen-5. These all-trigger cell division, apoptosis inhibition and mitogenic activation resulting in CML's malignant phenotype. CML, chronic myeloid leukemia.

gel fragments. The *FLT3-ITD* PCR products are longer than the wild-type. The peak's height inversely reflects the amount of mutant or wild-type PCR product. Peak height is used to determine the ratio of *FLT3-ITD* mutants to the wild type. This approach may be tweaked to find *FLT3-TKD* (174). The most common *FLT3-TKD* mutation is D835. Wild-type D835 has an EcoRV cutting site. EcoRV may quickly degrade *FLT3* in its natural condition.

Meanwhile, a change to *FLT3* D835 can render the protein inaccessible to EcoRV digestion. Using fragment analysis, the difference after the EcoRV digestion can be distinguished (175). To detect both *FLT3-ITD* and *FLT3-TKD*, a multiplex PCR and fragment analysis method was developed.

Evaluating MRD as a prognostic marker in leukemia. The timing of therapy initiation affects CLL progression since it depends on discovering prognostic variables at the diagnosis and the toxicity of different treatment regimens. However, permanent remission should be the ultimate objective. Determining MRD is becoming increasingly critical. High-precision, cutting-edge methods for detecting MRD include FCM and qPCR (176).

Patients whose therapeutic approaches have monoclonal antibodies may benefit most from the most recent test version. Patients with limited residual illness have a more reserved prognosis and a higher chance of relapse, but the use of additional targeted therapy remians debatable. MRD, defined as one CLL cell per 10,000 leukocytes, is a prognostic marker (177). The capacity to detect AML-MRD has been recognized as crucial for risk stratification and prognosis. MRD can be evaluated with either FCM or molecular testing. In this context, molecular diagnostics take center stage (178).

Mutations in a wide variety of genes have been linked to AML. Not all of these mutations can be used in MRD tests, however. *NPM1* mutations and the fusion genes *RUNX1-RUNX1T1*, CBFB-*MYH11* and *PML-RARA* can be MRD indicators. However, *DNMT3A, ASXL1*, and *TET2* mutations are age-related and are not predictive of outcomes. That being said, it is possible that these mutations are not reliable molecular markers for AML MRD tests (178). Due to the clonal evolution of AML, mutations in genes are constantly being lost and gained during relapses (179).

The results of several molecular assays can be obtained depending on which molecular markers are of interest. A conventional qPCR is a valuable tool when a mutation in a single gene is chosen as an MRD marker. The *NPM1* mutation can be checked with qPCR as an example. The quantitative analysis of *NPM1* can now be performed using the cutting-edge field of digital PCR assay (180).

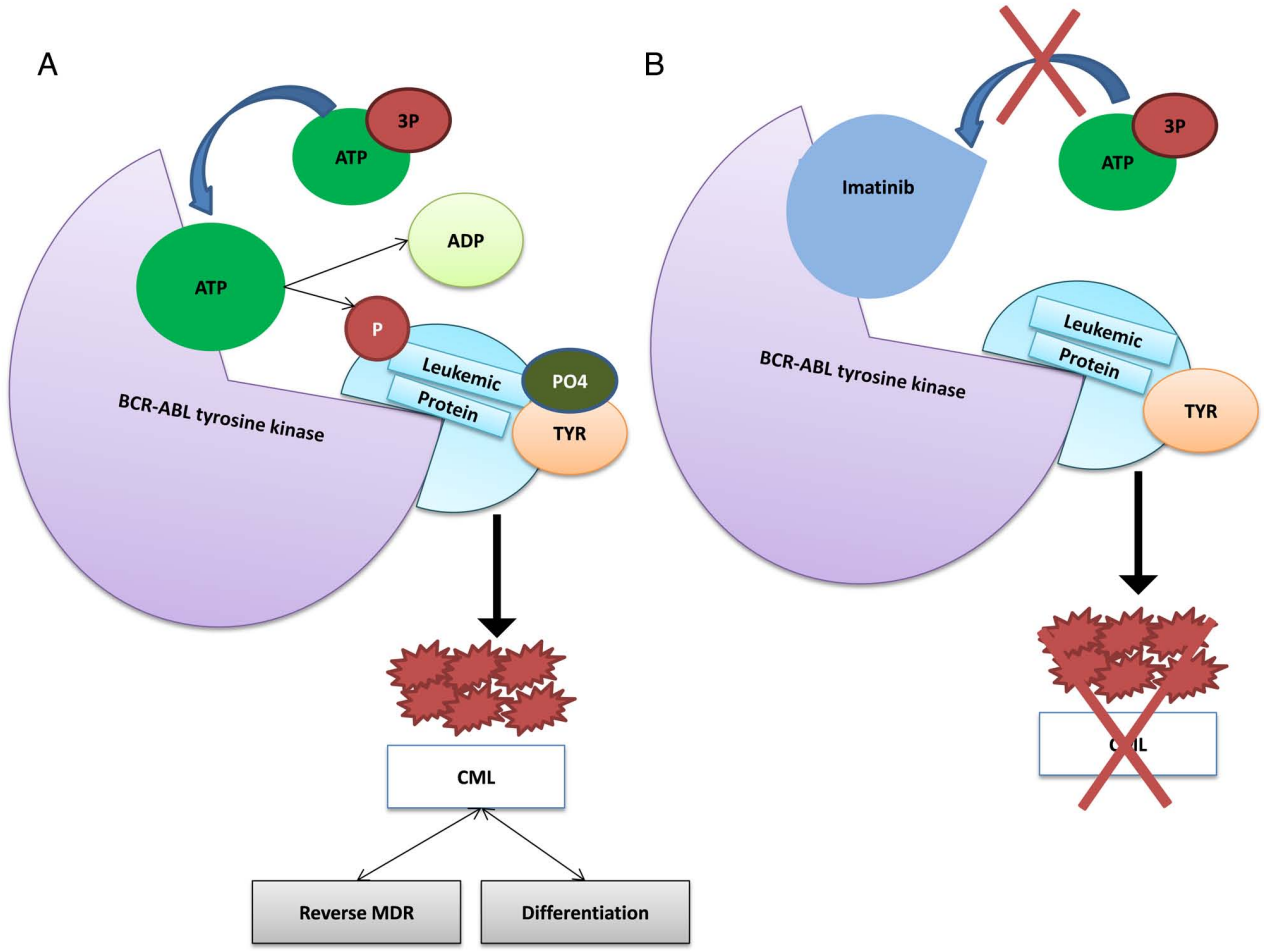

Figure 9. Mode of action of imatinib. (A) CML mechanism. (B) Mechanism of imatinib. Constitutively active BCR-ABL tyrosine kinase transfers phosphate from ATP to leukemic protein tyrosine residues. This results in the excessive myeloid cell proliferation that characterizes CML and induces reverse MDR and differentiation. Imatinib prevents BCR-ABL from binding ATP, which stops the protein from phosphorylating. Substrates necessary for BCR-ABL function cannot be phosphorylated without tyrosine kinase activity, which prevents following cellular events. Imatinib may unbalance the regulation of bone remodeling. According to data, decreased bone resorption is not as crucial as hindered bone production. It is necessary to routinely monitor skeletal activities while receiving long-term imatinib treatment for pediatric CML (139). CML, chronic myeloid leukemia; MDR, multidrug resistance.

Balatzenko *et al* (181) evaluated the effects of BCR-ABL fusion types and the *MDR1* gene on the hematological parameters of patients with CML. P-glycoprotein, expressed by the human MDR1 gene, exports molecules from cells and membranes to the extracellular environment. It protects cells from toxic metabolites. Only individuals with *MDR1* over-expression showed a link between b3a2 and PCT (182). The scientists ascribed this rise to a signaling cascade between 3a2 and *MDR1*.

Role of imatinib in the prognosis of leukemia treatment. Numerous efforts have been made over the past 20 years to pinpoint molecular alterations that could cause cancer cells to resist cytotoxic medications (183). Multiple resistance mechanisms have been discovered in model systems or cell lines. Examples are the reduction in drug uptake or activation, enhancement of drug efflux or inactivation, alteration in target enzyme levels, enhanced repair of DNA damage, altered nucleotide pools, and avoidance of some drugs' inhibitory effects via salvage pathways. It is known that several pathways contribute to drug resistance *in vitro*, but their role *in vivo* is less understood (Fig. 8). There appears to be a variety of factors that contribute to drug resistance *in vivo*. To demonstrate this point, it is considered that drug resistance can develop despite using numerous drugs with different mechanisms of action. More in-depth studies are required on the molecular processes and phenotypic drug resistance patterns in tumor cells to improve tailor chemotherapy to individual patients. Imatinib is an effective medication for the first treatment of CML (82). It was designed to target BCR-ABL specifically. In addition to ABL, however, numerous off-target kinases are also impacted by the medication at plasma concentrations (184). The medication binds to the BCR-ABL protein tyrosine kinase ATP pocket, preventing ATP from binding and blocking the phosphorylation of substrates, as shown in Fig. 9 (185). The sustained response rates to drugs are comparable to the outstanding results reported for adults (186).

In all studies, children and adolescents were administered imatinib (IM) with few complications, including cerebral edema (187), miscarriages and birth abnormalities (188). IM treatment is linked to toxicity, with most side effects appearing within the first two years and potentially improving with continued treatment. Generally, toxicity is mild to moderate

and manageable. Symptoms can include lethargy, rashes, weight gain due to fluid retention (notably infraorbital edema), and occassionally bone pain. Abnormal liver chemistry is possible and can rarely lead to liver failure. Some patients in CP starting at 400 mg/day may experience cytopenias within the first year, often leading to neutropenia, thrombocytopenia and occasionally anemia (188). Other cardiac adverse effects, including tachycardia, pericarditis, hypotension, and hypertension, collectively have an incidence of <1.0%. However, it is unknown how long CML responses will last and whether this medication may have long-term adverse effects in pediatric patients, particularly in different age groups (189).

7. Conclusion

Early diagnosis is critical for effective leukemia treatment, though the most specific and commonly used diagnostic parameters remain unclear. While recent discoveries of recurrent gene mutations have expanded our understanding, their prognostic significance is still being determined. A combination of advanced diagnostic techniques, including cytogenetics, immunophenotyping and molecular genetics such as qPCR, is essential for accurate diagnosis and disease management. The complexity of leukemia requires specialized laboratory assessments. Prognosis in leukemia is influenced by multiple factors, including patient performance status, genetic profiles and treatment response. Emerging studies on miRNAs and molecular responses to treatments such as imatinib highlight their potential prognostic value. However, most existing risk indicators remain prognostic rather than predictive, and the development of universally applicable scoring systems remains a challenge. As deep sequencing and targeted treatments evolve, the complexity of prognostic assessments will likely increase, necessitating the continuous refinement of therapeutic strategies.

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Availability of data and materials

Not applicable.

Authors' contributions

JL, YW and LL conceived and designed the study. JL, CD and YW collected data. JL, CD, LL analyzed and interpreted data. JL, YW and CD wrote the draft of the manuscript. LL critically revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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