Hemato-Oncology across Borders: Scientific Progress and Educational Collaboration

Scientific Supervisor
Olga Aniołek

IN VIA SCIENTIAE

Lazarski University

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Introduction

The monograph "Hemato-Oncology across Borders: Scientific Progress and Educational Collaboration", part of the Lazarski University Publishing Series *In Via Scientiae*, is designed as a unique platform combining scientific advancement with international educational cooperation. This publication is intended for medical students and young researchers, offering them an opportunity to engage with state-of-the-art knowledge while simultaneously developing essential competencies in research methodology and academic writing. Its deliberately diverse chapter structure reflects the multidimensional nature of modern hemato-oncology—ranging from educational and reporting perspectives to advanced clinical standards.

The structure of the work is built upon three complementary pillars:

Educational and interdisciplinary perspective the reporting chapters document the development of soft skills and the experience of working within interprofessional teams. A central element is the account of the Erasmus+ Blended Intensive Program (BIP), Cancer and Exercise: An Interprofessional Approach to Active Learning, conducted in Madrid, Spain, in collaboration with Universidad Europea de Madrid. During this program, students participated in clinical simulations at the university's Simulated Hospital and visited leading medical centers, including the pediatric oncology department at Hospital Niño Jesús and the VENCE exercise oncology unit.

Synthesis of basic and review knowledge the theoretical section systematizes academic knowledge in the fields of cancer biology, the physiology of hematopoiesis, and foundational diagnostic methods. Readers

will find a substantive foundation for understanding the role of reticulocytes in the differential diagnosis of anemia and classical cytogenetic methods, such as karyotype analysis and G-banding.

Clinical standards and precision diagnostics chapters designed as clinical mini-manuals integrate student knowledge with current international guidelines, including the 5th edition of the WHO classification (WHO-HAEM5) and the European LeukemiaNet (ELN) 2022 recommendations. The publication provides detailed discussions of modern targeted therapies (e.g., BTK and BCL-2 inhibitors), transplantation procedures (HCT/HSCT) based on EBMT and ASTCT standards, and methods for monitoring measurable residual disease (MRD), all of which define contemporary standards of patient care.

By integrating molecular, physiological, and clinical perspectives, the monograph effectively bridges the gap between theoretical foundations and their application in real-world medical contexts, serving as a valuable didactic and scientific resource.

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Chapter 1

Every scientific breakthrough in cancer research begins with a simple question: can we better understand what we still cannot fully explain?

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Each year, linear regression models are used to project cancer mortality based on recent epidemiological trends. In 2025, it was estimated that 1,280,000 cancer-related deaths would occur across the European Union, corresponding to 120.9 per 100,000 men and 79.1 per 100,000 women, representing a 3.5% decrease in men and a 1.2% decrease in women compared with 2020. Notably, breast cancer mortality has

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continued to decline, with reductions of 9.8% among European women aged 50–69 and 12.4% among those aged 70–79 [1].

Similarly, the Global Burden of Disease 2021 and Global Cancer Observatory 2022 reports provide a comprehensive evaluation of the prevalence, incidence, mortality, and disability-adjusted life years (DALYs) associated with hematologic malignancies worldwide. Between 1990 and 2022, the number of hematologic cancer cases steadily increased; however, age-standardized mortality remained stable or declined, particularly for acute lymphoblastic leukemia [2].

Yet cancer is not defined solely by its mortality statistics. Beyond survival, individuals living with or beyond cancer often experience profound and long-lasting effects on their quality of life. Common treatment- or disease-related sequelae include fatigue, neuropathic pain, nausea, vomiting, and gastrointestinal dysfunction, among other symptoms. These symptoms may arise from the malignancy itself or as consequences of chemotherapy, radiotherapy, hormone therapy, or surgical interventions. Addressing these multidimensional challenges requires a multidisciplinary, patient-centered, and evidence-informed approach that integrates perspectives from nursing, medicine, physiotherapy, and sports science.

Considering these multidimensional demands, fostering interprofessional learning environments is essential to improving cancer care and survivorship outcomes.

The Erasmus Blended Intensive Program (BIP) on Cancer and Exercise: An Interprofessional Approach to Active Learning represents an important step forward in strengthening international collaboration, advancing contemporary pedagogical models, and promoting evidence-based practice in oncology and rehabilitation sciences. Hosted at the Universidad Europea de Madrid, this initiative brought together faculty and students from multiple institutions, including Lazarski University, Croix-Rouge Française, the European University of Cyprus, the Amsterdam University of Applied Sciences, and the Lithuanian Sports University. A total of 45 students and 10 teachers worked together with a shared interest in the study of cancer, cancer treatment, and survivor outcomes.

The program was conceived with a clear academic purpose: to integrate diverse perspectives and methodological approaches to enrich the understanding of how exercise influences biological, functional, and psychosocial variables in individuals living with or beyond cancer. A fundamental component of this initiative was its multidisciplinary framework, bringing together students and faculty from nursing, medicine, physiotherapy, and sports science. This structure enabled participants to approach cancer care from complementary clinical and scientific viewpoints.

The BIP program was structured in two complementary phases designed to ensure a rigorous, coherent, and multidisciplinary learning experience. The online phase introduced students to essential foundational concepts related to cancer biology, epidemiology, exercise, and treatment. The virtual component ensured that all participants, regardless of their academic background in nursing, medicine, physiotherapy, or sports science, shared baseline knowledge. In addition, an online multidisciplinary seminar fostered early collaborative exchanges, allowing students to reflect on the relevance of integrated care and the complementary role of each profession in the management of these patients.

The on-site week in Madrid expanded on this foundation through a series of advanced academic, clinical, and experimental activities. Students attended discipline-specific lectures delivered by experts in medicine, nursing, physiotherapy, and sports science, aimed at providing a deeper understanding of different types of cancer and their implications. A central element of the program was the simulation-based training conducted in the university's Simulated Hospital. These sessions involved teams composed of one student from each discipline working together to manage realistic clinical scenarios performed by trained actors. The simulations required participants to navigate complex decision-making processes, conduct patient assessments, and address challenging situations such as a patient's refusal of beneficial treatment in culturally or religiously sensitive cases. Each simulation concluded with a structured debriefing session, during which students analyzed their performance, emotional responses, communication strategies, and ethical considerations, promoting reflective practice and professional growth.

The program also offered students the opportunity to visit VENCE, an emblematic initiative of the Universidad Europea de Madrid. VENCE provides individualized and group-based exercise interventions for cancer survivors, illustrating the real-world clinical impact of combining scientific evidence with practical implementation. The visit allowed participants to observe how exercise prescription is integrated into patient care and to appreciate its benefits for functional capacity, symptom management, and quality of life.

Additionally, participants visited Hospital Niño Jesús, a leading pediatric oncology center. The visit provided insights into the hospital environment where children, many of them with leukemia, receive oncological treatment. Special emphasis was placed on understanding infection-control measures, hygiene protocols, and psychosocial considerations essential to the care of immunocompromised pediatric patients. This experience emphasized the complexity of multidisciplinary coordination in such settings.

The organization and academic delivery of the Erasmus BIP Program were made possible through the coordinated efforts of an exceptional multidisciplinary faculty team. The program was led and supported by Beatriz Martínez Pascual, Director of the International Relations Department, together with Carolina Ramírez de Cepeda, BIP Project Coordinator, who ensured the seamless design, planning, and execution of all activities. Academic guidance and curricular integration were strengthened by Cristina González de Ramos, Academic Director of the Faculty of Medicine, Health, and Sport. The teaching component brought together experts from multiple health disciplines: Juan Luis González Pascual, senior lecturer at Universidad Europea and specialist in Nursing and Interprofessional Education; Montserrat González Pascual, professor in the Nursing Department with extensive clinical experience in oncology care; Isabel Mínguez Esteban, lecturer in Physiotherapy and specialist in pelvic floor rehabilitation; Elena Santana Sosa, professor of Sports Sciences and director of pediatric oncology exercise units across several hospitals in Madrid; and Esther San José Martínez, professor in the Faculty of Medicine with recognized

expertise in immunological therapies for oncology patients. Together, their combined leadership, scientific knowledge, and commitment to interprofessional education shaped a comprehensive and rigorous learning experience for all participants.

In addition, participants also benefited from the involvement of medical specialists whose clinical expertise enhanced the educational experience. Among them was Dr. Javier Cornago Navascués, a hematologist currently working at Hospital Ruber Internacional. His participation provided students with a clinically grounded perspective on hematologic cancers and their treatment. The academic experience was further strengthened by the participation of Dr. José Luis López Lorenzo, attending hematologist and Coordinator of Hematopoietic Stem Cell Transplantation at *Hospital Universitario Fundación Jiménez Díaz*, who is a recognized expert in transplantation procedures and hematologic malignancies.

Finally, the program's academic relevance was further enhanced by the participation of Professor Alejandro Lucía, one of the leading international researchers in the field of exercise and cancer. His contributions provided students with cutting-edge insights into current evidence, methodological rigor, and future directions in exercise oncology research.

As demonstrated by this program, interdisciplinary learning contributes to more informed, innovative, and human-centered cancer care.

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Chapter 2

Genetic diagnostics in acute leukemia

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1. Introduction

1.1 Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL)

Leukemias are a group of neoplasms affecting the hematopoietic cell lineages in the bone marrow. The etiology of the disease remains to be fully elucidated; however, a number of environmental factors have been implicated, and a strong genetic component is evident, as leukemias are more common in identical twins and individuals with

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inherited genetic disorders, such as Down and Bloom syndromes [1,2]. A slight sex-related difference is observed, with a higher incidence in male patients, which may be attributed to hormonal differences [3]. The disease prevalence is highest among individuals over the age of 40, except for acute lymphoblastic leukemia (ALL), which primarily affects children between 2 and 5 years of age [4]. The earliest step in leukemogenesis is frequently Clonal Hematopoiesis of Indeterminate Potential (CHIP), in which an expansion of certain mutated hematopoietic cells is observed in the bone marrow. As the disease progresses and the proportion of healthy cells diminishes, physiological hematopoiesis becomes impaired, leading to common symptoms such as fatigue, mucosal bleeding, fever, and recurrent infections. In most cases, leukocytosis predominates in the peripheral blood; however, some patients present with pancytopenia, and a minority may exhibit extremely high leukocyte counts, which are associated with a poor prognosis and an increased risk of central nervous system (CNS) involvement [5]. Consequently, accurate diagnosis and prognosis rely heavily on histopathological and genetic examination of the bone marrow. ALL represents approximately 12% of all adult leukemias but as many as 80% of pediatric cases [6]. Despite this, most deaths related to ALL occur in adults, as treatment outcomes in younger patients are far more favorable. Most ALL cases originate from the B-cell (BC) lineage, while 15–20 % arise from the T-cell (TC) lineage of lymphoblasts [7]. In rare instances, leukemic transformation may also occur in stem cells of other lineages. Bone marrow smears in ALL typically reveal numerous immature cell forms at the periphery, known as blasts, with an absence of intermediate populations. Genetically, ALL is frequently associated with fusion oncoproteins such as ETV6::RUNX1 or BCR:ABL. The ETV6::RUNX1 fusion is the most common genetic abnormality in pediatric ALL and can be found in up to 25% of neoplasms in this population. The fusion protein is created as a result of a mutation linking the *ETV6* proto-oncogene (also known as *TEL*) to another transcription factor, *RUNX1*, thereby affecting cellular signaling and gene expression [8, Figure 1].

In contrast to ALL, acute myeloid leukemia (AML) typically occurs in adults, with a median age at onset of 68 years. Most AML cases arise *de novo*, representing the primary model of disease development, although some cases, known as secondary AML (sAML), are preceded by hematologic conditions such as myelodysplastic syndrome (MDS) or driven by early preleukemic states such as CHIP. Historically, acute leukemias were categorized using the morphological FAB (French-American-British) classification, which divided AML into subtypes M0 through M7 based on the degree of cellular differentiation. A classic example from this system is the M3 subtype, acute promyelocytic leukemia (APL). APL is caused by a t(15;17) translocation resulting in the *PML::RARA* fusion gene. The increased affinity of the PML::RARA oncoprotein for DNA impairs transcription and halts granulocytic differentiation [9]. However, targeted treatment with all-trans retinoic acid (ATRA) overcomes this block, inducing the differentiation of promyelocytes into mature granulocytes and frequently leading to disease remission [10].

While the FAB system laid the historical foundation for understanding leukemias and remains useful for initial morphological assessment, modern clinical practice has evolved far beyond cellular appearance. Today, contemporary diagnostic and classification practices for AML and other myeloid neoplasms rely predominantly on genetic and molecular aberrations. The WHO 5th edition (WHO-HAEM5) classification [11] and the European LeukemiaNet (ELN) 2022 guidelines [12] unequivocally shift the paradigm toward entities defined by specific genetic changes. The current diagnostic framework is based on molecular risk assessment and the strict integration of cytogenetic data with mutational profiling, moving away from morphology alone as the primary classification tool.

The ETV6::RUNX1 (TEL::AML1) fusion protein is generated by the balanced chromosomal translocation t(12;21) (p13;q22), which joins the *ETV6* gene, located on chromosome 12, with *RUNX1*, located on chromosome 21. *ETV6* (ETS translocation variant gene 6) is a dose-sensitive gene encoding a transcription factor responsible for both hematopoiesis regulation and tumor suppression. *ETV6* loss of function and disruption

of normal *RUNX1*-mediated transcription by the ETV6::RUNX1 fusion protein can cause leukemogenesis. The resolution of conventional karyotyping methods does not allow for reliable detection of t(12;21)(p13;q22); therefore, sequence-specific methods such as FISH and RT-PCR are used to screen for this abnormality. The presence of the ETV6::RUNX1 fusion is an important factor in patient risk stratification and is associated with a favorable prognosis, occurring in as many as 25% of pediatric B-ALL cases but only 1% of adult cases.

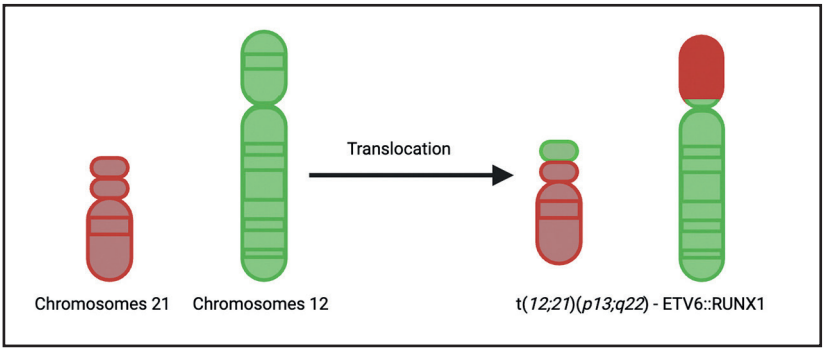


Figure 1. ETV6::RUNX1 t(12;21)(p13;q22)

Figure created using BioRender app.

1.2 The role of chromosomal aberrations in hematologic neoplasms

Hematopoietic neoplasms are characterized by a particularly high incidence of clonal chromosomal aberrations (ChA), which play a key role in their molecular pathogenesis. These aberrations are broadly divided into two classes—numerical and structural aberrations. Numerical aberrations involve an altered number of otherwise normal chromosomes, whereas structural aberrations occur when the chromosomal count is normal but specific structural changes, such as translocations, deletions, or inversions, are present. Such chromosomal rearrangements are considered, in many cases, initiating events in malignant transformation [13].

The resulting genetic alterations can lead either to the formation of chimeric fusion genes with novel or deregulated activity or to abnormal gene expression through position effects, even without disrupting the gene's structural integrity. Although the precise causes of most ChA remain largely unknown, epidemiological studies of various forms of leukemia suggest potential associations with environmental and occupational exposures, while no single initiating agent has been definitively identified [14]. Additionally, the etiology of certain hematologic malignancies has been linked to infectious agents, such as Epstein-Barr virus (EBV) [15] or influenza virus, and to exposure to cytostatic drugs.

1.3 The role of genetic diagnostics in modern hematology

Advances in genetic diagnostics have profoundly reshaped diagnostic, prognostic, and therapeutic approaches in modern hematology. The identification of specific chromosomal abnormalities is essential for disease classification and guides treatment strategies. For example, the detection of the t(9;22)(q34;q11) translocation, resulting in the *BCR::ABL1* fusion gene, has defined chronic myeloid leukemia (CML) as a unique molecular entity and enabled the development of targeted therapy with tyrosine kinase inhibitors (TKIs). However, a significant proportion of patients with acute myeloid leukemia (AML) do not exhibit classic chromosomal abnormalities in their karyotype. In these patients, the pathogenesis is driven by sequence-level alterations, specifically single-nucleotide variants (SNVs) and insertions/deletions (indels). Consequently, mutational changes in genes such as *NPM1*, *FLT3*, *CEBPA*, *TP53*, *IDH1*/*IDH2*, *RUNX1*, *ASXL1*, and various splicing genes have fundamental diagnostic, prognostic, and therapeutic significance. These recurrent mutations are now critical for contemporary disease classification, accurate risk stratification, and the implementation of targeted management. The integration of cytogenetic, molecular, and next-generation sequencing (NGS) analyses into routine clinical practice has enhanced diagnostic precision, enabled measurable residual disease monitoring, and led to the discovery of novel therapeutic targets. Despite all the

progress in the rapidly developing field of genetic diagnostics, there is still a dire need for improvement in the clinical implementation of the data obtained to achieve the long-standing goal of fully personalized medicine.

2. Classical diagnostic methods

2.1 Karyotype analysis

Chromosomes can be observed as distinct structures only during mitotic or meiotic division, when chromatin condenses to ensure the equal distribution of genetic material between the two daughter cells. The highest degree of chromatin condensation occurs during metaphase, at which point chromosomes are most easily visualized. Each chromosome consists of two parallel chromatids joined at a specific region known as the centromere, which divides the chromosome into a short (p) arm and a long (q) arm. Based on centromere location, human chromosomes can be classified as metacentric, submetacentric, or acrocentric. Cytogenetic analysis enables the identification of chromosomal abnormalities, including changes in chromosome number, deletions, inversions, amplifications, or translocations of DNA segments. These alterations are fundamental to the diagnosis of many hematologic disorders, and karyotype analysis represents an important diagnostic tool in their evaluation.

Karyotype analysis is based on differential chromosome staining, which produces a characteristic banding pattern for each chromosome. To obtain suitable genetic material, peripheral blood containing leukocytes can be collected, whereas in cases requiring a high proportion of actively dividing cells, a bone marrow aspirate is preferred. Cytogenetic examination requires the isolation of cells during mitosis, when chromosome condensation occurs, allowing their identification. To generate a high number of metaphase cells, the mitotic inhibitor Colcemid is applied, preventing tubulin polymerization and thus arresting the examined leukocytes at metaphase [16]. First introduced in the 1970s, G-banding is one of the most commonly used techniques for karyotype analysis and involves

the sequential treatment of chromosomes with the proteolytic enzyme trypsin and Giemsa stain. Other, less commonly used techniques include R-banding (the reverse of G-banding), C-banding (centromere staining), and Q-banding (which utilizes fluorescent staining). The resolution and number of visible bands depend on the local degree of chromatin condensation. Each chromosome pair displays a distinct and reproducible banding pattern, allowing precise identification of individual homologs and facilitating the detection of structural or numerical abnormalities. To facilitate karyotype analysis, chromosomes are arranged into 22 pairs of autosomes and two sex chromosomes. Generally, each subsequent pair of autosomes is smaller than the previous one, except for the last two pairs, where autosome 22 is actually larger than autosome 21. Despite its diagnostic value, karyotyping has certain limitations, as its resolution is relatively low and only large structural alterations can be visualized. Problems related to chromosome morphology can also affect the detection of some specific rearrangements, such as inversions. However, gene mutation analyses alone are also prone to overlooking key structural changes that can be detected through karyotype analysis; therefore, both assessments are considered complementary rather than substitutive. Classical karyotyping allows for the detection of large-scale chromosomal abnormalities exceeding 5–10 Mb, including aneuploidies, polyploidies, deletions, duplications, inversions, or translocations. Although karyotyping can be performed on solid tumors, it is mainly associated with hematologic malignancies, where the analysis of chromosomal instability facilitates disease diagnosis and risk stratification.

2.2 Fluorescence in situ hybridization (FISH)

Fluorescence *in situ* hybridization (FISH) is a cytogenetic technique that employs DNA probes labeled with fluorochromes to identify the location of specific genes or DNA sequences directly within their native chromosomal context. The use of multiple fluorochromes, either individually or in combination, together with appropriate fluorescence filter systems, enables the generation of multicolored images that can be digitally analyzed using appropriate software. Since the 1990s, FISH has been

widely applied in the study of quantitative DNA alterations, including gene amplifications, deletions, and duplications. In addition, it is an essential method for detecting structural rearrangements, such as translocations and deletions, that underlie many hematologic malignancies. Some key examples include the identification of the *BCR::ABL1* fusion in CML and *KMT2A* (previously known as Mixed Lineage Leukemia – *MLL*) rearrangements in ALL [17]. By precisely mapping chromosomal breakpoints, FISH provides critical information for both diagnosis and prognostic evaluation. In clinical genetics and genetic counseling, this technique facilitates the assessment of the potential impact of identified chromosomal abnormalities. Its ability to detect changes not only in metaphase but also in interphase cells makes it a valuable tool for the rapid identification of prognostically significant genetic alterations, thereby supporting timely therapeutic decisions and personalized patient management.

3. Modern molecular methods

While classical cytogenetic methods, such as karyotyping and FISH, are invaluable for detecting large structural and numerical chromosomal aberrations, they lack the resolution to identify sequence-level alterations. As previously noted, a significant proportion of patients with AML have a normal karyotype, making classical methods insufficient for complete diagnostic profiling. To detect the critical single-nucleotide variants and indels that define modern diagnostic and prognostic frameworks according to WHO and ELN guidelines, high-resolution molecular methods are strictly required.

3.1 qPCR and RT-qPCR (Quantitative PCR and Quantitative Reverse-Transcription PCR)

The groundbreaking invention of the polymerase chain reaction (PCR) by Kary Mullis in 1983 revolutionized the fields of molecular biology and biotechnology. In the following years, advances in genetic amplification and detection methods allowed for the introduction of real-time analysis

systems. Unlike conventional PCR, which requires post-amplification gel electrophoresis for product visualization, the qPCR (quantitative PCR) approach utilizes fluorescent dyes or sequence-specific probes to monitor the accumulation of the amplification product during each cycle, providing quantitative results without the need for post-PCR processing. Currently, qPCR is a standard diagnostic tool enabling both the detection of gene expression levels and the identification of specific point mutations. In hematologic malignancies, RT-qPCR (Reverse Transcription PCR) is routinely employed to detect recurrent oncoprotein fusion transcripts such as *BCR*::*ABL1*, *PML*::*RARA*, and *RUNX1*::*RUNX1T1*, which are characteristic of specific leukemia subtypes [16]. Moreover, the high sensitivity of PCR makes it an indispensable tool for monitoring measurable residual disease (MRD), allowing the detection of leukemic cells at levels undetectable by conventional cytogenetic methods. The ability to track molecular remission or early relapse provides valuable prognostic information and supports individualized treatment decisions.

3.2 Next generation sequencing (NGS)

Decades after the discovery of the first genetic sequencing methods, such as Sanger sequencing, a new era dawned with the introduction of next-generation sequencing (NGS). Currently, several different approaches are used to sequence millions of DNA fragments in parallel, providing comprehensive genomic data [18,19]. Some of the most widely used platforms include Illumina and Oxford Nanopore sequencing. The two methods differ not only in their approach to sequencing but also in the read lengths they generate and the data outputs they require. Illumina utilizes a method known as sequencing by synthesis (SBS), in which fluorescently labeled nucleotides enable detection at specific wavelengths upon incorporation. To this end, a multistep process is required, including DNA fragmentation, library preparation, sequencing by synthesis, and bioinformatic analysis. Illumina systems are known for their high accuracy, short read lengths, and high sequencing throughput. In contrast, Oxford Nanopore is based on the analysis of changes in ionic current resulting from the passage

of a read through a membrane pore. Unlike other NGS platforms, Nanopore sequencing can generate ultra-long reads and is especially useful for the detection of structural and copy number variants (CNVs).

NGS can be performed in several configurations, including targeted gene panels focusing only on clinically relevant genes, whole-exome sequencing (WES) covering all coding regions, or whole-genome sequencing (WGS) for complete genomic characterization. NGS enables the detection of both simple and complex genetic aberrations, such as single-nucleotide variants (SNVs), insertions, deletions, and CNVs. However, despite its refined resolution, certain gene fusions that are clearly detectable with classical methods remain undetected by WGS because of suboptimal sequencing depth, as noted by Lazarevic and Johansson [19]. Moreover, NGS does not retain spatial information, which is often crucial in genomic data analysis. Currently, to address the limitations of this method, novel approaches, such as *in situ* sequencing, are being developed. Recently, single-cell NGS (scNGS) technology has been introduced to address intratumor heterogeneity (ITH) [20]. scRNA-Seq enables the creation of transcriptome libraries to compare differences among cancer cell populations within a single neoplasm and helps address ITH. To enable even finer transcriptomic resolution, Holo-Seq methods are used, which can detect small RNAs such as microRNAs [21]. In acute myeloid leukemia and acute lymphoblastic leukemia, NGS is used to identify mutations in key driver genes, e.g., *FLT3*, *NPM1*, *CEBPA*, and *TP53*. Clinically, NGS plays a significant role in refining disease classification, determining prognosis, and guiding targeted therapeutic strategies. By providing a comprehensive molecular profile, NGS facilitates precision medicine approaches that optimize treatment selection and improve patient outcomes.

4. Clinical significance of genetic testing

Advances in molecular genetics have significantly improved the diagnosis, risk stratification, and treatment of acute leukemias. In AML, tyrosine

kinase inhibitors (TKIs) such as midostaurin and gilteritinib are employed in patients harboring *FLT3* mutations. In ALL, *BCR::ABL1*-positive disease benefits from TKI therapy with imatinib or ponatinib, and ongoing research into *MLL*-rearranged ALL has led to the development of menin inhibitors [22]. Beyond therapeutic implications, molecular profiling has become an essential component of the diagnostic workup. The detection of specific mutations or translocations, such as *PML::RARA* in APL or *RUNX1::RUNX1T1* in AML, confirms the diagnosis and allows for the early initiation of appropriate targeted therapy. Furthermore, genetic abnormalities serve as independent prognostic markers, correlating with treatment response, relapse risk, and overall survival.

4.1 Most common genetic alterations in AML and ALL

In AML, the most common ChA include t(8;21)(q22;q22), inv(16), and t(15;17) [23]. Key mutations found in AML can be divided into seven groups, including the signaling genes, splicing factor genes, *NPM1* gene mutation, tumor suppressor genes, DNA methylation genes, chromatin modifier genes, and cohesin complex genes. The most common mutation in AML occurs in the *FLT3* tyrosine kinase gene (signaling genes group), leading to constitutive activation of the protein [24]. TKIs such as midostaurin and gilteritinib are two FDA- and EMA-approved drugs for the treatment of patients harboring this mutation. Unfortunately, some SNPs, such as F691L and D835, are associated with acquired resistance to tyrosine kinase inhibitors. Another common mutation, found in up to 20% of *de novo* AML cases, affects one of the Krebs cycle enzymes, known as isocitrate dehydrogenase (*IDH*). This enzyme is responsible for the oxidative decarboxylation of isocitrate to α-ketoglutarate and occurs in two isoforms, *IDH1* and *IDH2*, which are mutated in 8% and 12% of cases, respectively [25]. As a result of these genetic alterations, increased levels of the oncometabolite 2-hydroxyglutarate are generated, and subsequent histone demethylation disrupts appropriate hematopoietic differentiation. Different drugs have been developed to target the two

isoforms, with the *IDH1* inhibitor ivosidenib approved in the EU and the *IDH2* inhibitor enasidenib lacking EU marketing authorization.

Genetic alterations in patients with ALL vary greatly across age groups. High hyperdiploidy (HHD), a state in which a nonrandom pattern of additional chromosomes is observed, can be found in up to 30% of pediatric patients. However, this positive prognostic factor is observed in only 1% of adult cases [26]. Other recurrent genetic alterations in ALL comprise t(12;21)(p13;q22), resulting in the *ETV6::RUNX1* fusion, and t(9;22)(q34;q11), generating the BCR::ABL1 fusion protein. *ETV6::RUNX1* is associated with a favorable prognosis, whereas *BCR::ABL1* is associated with a poorer outcome [27]. Moreover, the current WHO-HAEM5 classification describes similar disease subtypes lacking a specific fusion protein, introducing B-ALL/LBL with *BCR::ABL1*-like features and B-ALL/LBL with *ETV6::RUNX1*-like features. Other critical abnormalities include *IKZF1* and *CRLF2* gene rearrangements, both associated with poor outcomes, as well as a high-risk *KMT2A* mutation. Alterations in this transcriptional regulator are especially common in pediatric ALL but are also present in other types of leukemia. The *KMT2A* gene encodes a histone methyltransferase that regulates hematopoietic homeobox genes, such as *HOXA9* and *MEIS1*, in myeloid progenitor stem cells [28]. To regulate expression levels, *KMT2A* requires direct interaction with the menin protein via the menin-binding domains (MBDs), which enables appropriate binding to chromatin. Thus, a novel and highly specific class of molecules, known as menin inhibitors, is being developed to treat patients with this mutation.

4.2 Diagnosis and prognosis

Leukemias are subdivided into distinct classes based on the cytogenetic and molecular abnormalities that underlie their pathogenesis. Genetic characterization not only facilitates accurate subtype identification but also provides the basis for risk stratification into favorable, intermediate, or adverse prognostic categories. For instance, t(8;21) and inv(16) are considered favorable abnormalities in AML, as they are associated with a better prognosis. Other mutations, such as a germline

*DDX*41 mutation, are thought not only to be favorable prognostic factors but also to be linked to a set of specific characteristics, such as late onset, male sex, and a low WBC count [29]. In contrast, inv(3) or *FLT3* internal tandem duplication (*FLT3*-ITD) usually indicates worse treatment outcomes [30]. According to the 2022 European LeukemiaNet (ELN) guidelines, *FLT3*-ITD mutations are classified as intermediate-risk, irrespective of allelic ratios or the co-occurrence of *NPM1* mutations.

In ALL, favorable prognostic markers include the presence of the *ETV6::RUNX1* fusion and HHD [31]. These are associated with higher remission rates and long-term survival, particularly in pediatric patients. Intermediate-risk cases often involve normal karyotypes or nonrecurrent abnormalities without clear prognostic implications. Conversely, adverse-risk features include rearrangements such as *BCR::ABL1* [t(9;22)], *KMT2A* (*MLL*) gene rearrangements, hypodiploidy, or iAMP21 (intrachromosomal amplification of chromosome 21), which predict inferior outcomes and treatment resistance.

4.3 Targeted therapy selection

The chemotherapy regimen for hematologic malignancies consists of several phases, namely induction, consolidation, and maintenance. The induction phase aims to achieve remission and restore normal hematopoiesis. Standard induction therapy for ALL lasts 4 to 6 weeks and includes the administration of vincristine, an anthracycline (commonly doxorubicin), and prednisone. This protocol is often accompanied by intrathecal methotrexate administration to prevent CNS involvement. The consolidation phase employs the modified nucleoside cytarabine, often in combination with blinatumomab, a bispecific T-cell engager (BiTE) antibody that targets CD19 and the T-cell receptor CD3, promoting selective cytotoxicity. The use of this specific antibody is, however, limited to B-ALL, as it relies on CD19 expression. To address the less common type of ALL, T-cell ALL (T-ALL), drugs such as nelarabine have been developed [32]. The subsequent maintenance phase relies on the prolonged administration of 6-mercaptopurine. Chemotherapy

protocols for AML include different medications with some overlap; i.e., the induction phase consists of idarubicin, cytarabine, and etoposide (ICE) [33]. Historically, allogeneic stem cell transplantation (allo-HSCT) was considered the only curative measure to reduce the risk of relapse; however, recent advances in the field of chimeric antigen receptor T-cell therapies (CAR-T) have demonstrated a novel and highly potent treatment option with even better outcomes.

In recent years, novel therapeutic protocols have increasingly incorporated cytogenetic and molecular data to enable precise risk stratification and facilitate the implementation of targeted therapies for patient groups most likely to benefit from this highly targeted treatment. For example, the Philadelphia chromosome (Ph+) [t(9;22)(q34;q11)], a karyotypic hallmark of CML, is also present in other forms of acute leukemia, in which the translocation between chromosomes 9 and 22 creates a constitutively active kinase known as BCR::ABL. Consequently, all patients harboring this specific mutation may significantly benefit from treatment with TKIs such as imatinib or newer-generation agents such as ponatinib. Drugs such as midostaurin, gilteritinib, and quizartinib are used in the treatment of *FLT3*-mutated AML, while IDH inhibitors, including ivosidenib (IDH1 inhibitor) or enasidenib (IDH2 inhibitor), are administered to patients with IDH1 or IDH2 mutations, respectively. Combination regimens incorporating hypomethylating agents, e.g., azacitidine, have demonstrated markedly improved response rates when used alongside venetoclax, especially in older patients [34]. According to Kayser et al. [32], patients with *IDH1* mutations benefit from ivosidenib in combination with azacitidine, whereas those with *IDH2* mutations respond favorably to venetoclax plus azacitidine.

Novel targeted drug regimens incorporated into the treatment protocols of patients with leukemia utilize a class of drugs known as immune checkpoint inhibitors, which are gaining immense popularity in the field of oncology. These agents target immune regulatory pathways such as programmed cell death protein 1 (PD-1) and its ligands PD-L1 and PD-L2, or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Other developing therapies include agents targeting the anti-apoptotic protein

MCL-1, a member of the BCL-2 family, and the second-generation XPO1 inhibitor eltanexor, which inhibits nuclear export [34]. However, it remains unclear which AML subtypes might demonstrate the most favorable response to these drugs.

5. Summary and conclusions

The remarkable progress in the field of genetics over the past several decades has substantially accelerated the development of technologies that enhance the diagnosis and treatment of patients with cancer. Advances in cytogenetics have equipped medical professionals with tools to detect genetic and chromosomal abnormalities, enabling not only more refined disease classification but also the development of a new class of drugs that directly target the underlying molecular mechanisms. Methods such as G-banding and fluorescence *in situ* hybridization (FISH) now have a well-established role in the diagnosis of leukemia, and in recent years, technological progress has led to the development of even more advanced molecular techniques, such as quantitative reverse transcription PCR (RT-qPCR) and next-generation sequencing (NGS). These methods allow for highly precise detection of genetic alterations and enable clinicians to diagnose and monitor disease progression with unprecedented accuracy. Scientists continue to push the boundaries of leukemia treatment and diagnostics. Future directions in leukemia research are rapidly moving toward the integration of emerging technologies such as single-cell sequencing, epigenetic analysis, and artificial intelligence (AI).

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Chapter 3

Hematopoietic cell transplantation (HCT/HSCT): indications, procedure, and prognosis

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1. Introduction

Hematopoietic cell transplantation (HCT), also called hematopoietic stem cell transplantation (HSCT), is a therapeutic procedure in which hematopoietic stem cells from bone marrow, peripheral blood, or umbilical cord blood are administered to restore or replace hematopoiesis. Although the term bone marrow transplantation is still

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used, it is too narrow for contemporary practice because bone marrow is only one possible graft source [1].

Over the past two decades, HCT has advanced through improvements in donor selection, high-resolution HLA typing, reduced-toxicity conditioning, antimicrobial support, post-transplant cyclophosphamide (PTCy), and the integration of measurable residual disease (MRD) into transplant decision-making. Contemporary HCT practice therefore integrates disease indications, procedural steps, immunological principles, complications, and prognostic assessment in accordance with current EBMT and ASTCT recommendations [1,2].

2. Hematopoietic cell transplantation—theoretical and medical foundations

2.1 Bone marrow structure and function

Bone marrow is a parenchymal organ that fills the marrow cavities of bones and serves as the primary site of hematopoiesis. In the adult human body, active red bone marrow is found primarily in flat bones, such as the sternum, ribs, and pelvic bones, as well as in the proximal ends of long bones. Its microenvironment, called the hematopoietic niche, is a highly organized network of stromal cells, extracellular matrix, blood vessels, and nerves, whose components collectively regulate the self-renewal, proliferation, and differentiation of hematopoietic stem cells (HSCs) [4].

HSCs are multipotent cells capable of differentiating into all blood cell lineages: erythrocytes, leukocytes, and platelets. This process is tightly controlled by cytokines and growth factors, including erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), and stem cell factor (SCF). In addition to its hematopoietic function, bone marrow contains mesenchymal stromal cells (MSCs), which can differentiate along osteogenic, chondrogenic, and adipogenic lineages and help maintain the hematopoietic niche and modulate immune responses [5].

2.2 History and development of hematopoietic cell transplantation

The history of HCT dates back to the mid-20th century, when animal studies showed that marrow-derived cells could restore hematopoiesis after myeloablative injury. Clinical advances followed through the work of E. Donnall Thomas, whose early studies of bone marrow transplantation in leukemia and subsequent development of allogeneic transplantation helped establish HCT as a potentially curative therapy. Thomas received the Nobel Prize in Physiology or Medicine in 1990 for this work [6].

The identification of the major histocompatibility complex (MHC), known in humans as the HLA system, enabled donor selection based on immunological compatibility. The development of unrelated donor registries, high-resolution molecular HLA typing, and modern GVHD prophylaxis later expanded access to allogeneic HCT, including matched unrelated, mismatched unrelated, haploidentical, and cord blood transplantation [7,8].

2.3 Types of hematopoietic cell transplantation

Clinically, HCT is classified mainly by donor-recipient relationship and graft source. By donor type, it may be autologous, syngeneic, or allogeneic. Allogeneic HCT may use a matched sibling donor, a matched or mismatched unrelated donor, or a haploidentical related donor. By graft source, hematopoietic cells may come from bone marrow, mobilized peripheral blood, or cord blood [1,3].

Autologous HCT (auto-HCT) involves collection, cryopreservation, and later reinfusion of the patient's own hematopoietic stem cells. The patient's stem cells are typically mobilized with granulocyte colony-stimulating factor (G-CSF) alone, G-CSF plus plerixafor, chemotherapy plus G-CSF, or disease-specific regimens, followed by leukapheresis. The purpose of auto-HCT is to permit high-dose therapy in chemosensitive diseases, particularly multiple myeloma and selected lymphomas. Auto-HCT avoids graft rejection and graft-versus-host

disease (GVHD), but it lacks a graft-versus-leukemia/tumor effect and may carry a risk of reinfusing malignant cells [1,3,9].

Allogeneic HCT (allo-HCT) uses hematopoietic cells from another person. The graft can be bone marrow, peripheral blood stem cells, or umbilical cord blood. In healthy related or unrelated donors, peripheral blood stem cells are generally mobilized with G-CSF alone, whereas bone marrow is harvested under anesthesia. Chemotherapy-based mobilization is not standard for healthy allogeneic donors; chemomobilization is used mainly for autologous transplantation and in selected patient-directed settings. The therapeutic effect of allo-HCT combines conditioning with immunological graft-versus-leukemia/tumor activity, but allogeneic immune interactions can also cause GVHD and contribute to graft failure [1,3,8,9].

2.4 Immunological basis of transplantation

The success of allo-HCT depends on the interaction between recipient tissues and donor immune cells. The genes encoding HLA molecules are located within the MHC region on chromosome 6. HLA class I molecules (HLA-A, HLA-B, HLA-C) are expressed on almost all nucleated cells and present peptides to CD8+ T cells. HLA class II molecules (HLA-DR, HLA-DQ, and HLA-DP) are expressed mainly on antigen-presenting cells and present peptides to CD4+ T cells. Contemporary donor selection includes both class I and class II HLA loci. A conventional 10/10 matched unrelated donor requires allele-level matching at HLA-A, -B, -C, -DRB1, and -DQB1; HLA-DPB1 permissiveness and donor-specific anti-HLA antibodies may also influence donor choice [7,8].

HLA disparity is a major driver of T-cell alloreactivity. T cells may recognize intact mismatched HLA-peptide complexes directly; in indirect allorecognition, peptides derived from mismatched HLA proteins are presented by self-HLA. This alloreactivity mediates both the toxic graft-versus-host reaction and the beneficial graft-versus-leukemia effect. Even in HLA-identical transplantation, minor histocompatibility antigens may trigger clinically relevant immune responses. Residual

recipient immunity and donor-specific anti-HLA antibodies can contribute to graft rejection, emphasizing the need to integrate donor selection with immunosuppressive planning [7,8].

3. Indications and procedure for transplantation

3.1 Current indications for HCT

Current indications cannot be defined by diagnosis alone. EBMT and ASTCT recommendations classify indications according to diagnosis, disease phase, remission status, MRD, biological risk, patient fitness, donor availability, and access to non-transplant options such as targeted therapy or CAR T cells [1,2]. The following table provides an educational synthesis and does not replace disease-specific guidelines or assessment by a multidisciplinary transplant team.

Table 1. HCT indications, transplant strategies, and selection criteria by disease

Disease / clinical context	Preferred transplant approach	Main qualification considerations
Acute myeloid leukemia (AML)	Allo-HCT in CR1 for adverse-risk AML and most intermediate-risk AML; it may also be considered for relapsed or refractory disease when adequate disease control can be achieved and estimated non-relapse mortality is acceptable.	MRD, cytogenetic and molecular risk, age, HCT-CI, DRI, donor type, and conditioning intensity must be considered together. Favorable-risk AML is generally not treated with allo-HCT in CR1 unless MRD persists [1].
Acute lymphoblastic leukemia (ALL)	Allo-HCT for high-risk disease, MRD-positive remission, Ph+ or Ph-like disease in selected clinical contexts, and relapsed or refractory disease after adequate disease control has been achieved.	MRD is a major prognostic factor and an important consideration in treatment decisions. In B-ALL, blinatumomab, inotuzumab, and CART-cell therapy may alter the timing or need for allo-HCT [1,2].

Disease / clinical context	Preferred transplant approach	Main qualification considerations
Myelodysplastic syndromes / myeloproliferative neoplasms	Allo-HCT for higher-risk MDS and selected cases of myelofibrosis or transformed MPN in transplant-eligible patients.	Disease scores, transfusion burden, blasts, cytogenetics, mutations, age, comorbidities, and donor availability shape the indication [1].
Chronic myeloid leukemia (CML)	Allo-HCT is now reserved for selected patients with TKI-resistant or TKI-intolerant disease, advanced-phase disease, or failure of modern systemic treatment options.	CML is no longer a routine indication for allo-HCT in the TKI era [1,2].
Multiple myeloma	Auto-HCT remains a standard consolidation strategy for eligible patients; allo-HCT is not routine and is limited to selected high-risk settings or clinical trials.	Outcomes depend on induction, depth of response, MRD, cytogenetic risk, maintenance therapy, and relapse biology [1,2].
Hodgkin lymphoma and non-Hodgkin lymphomas	Auto-HCT for chemosensitive relapsed disease in selected settings; allo-HCT, CAR T-cell therapy, or other cellular therapies may be used for selected relapsed or refractory cases depending on subtype and prior therapies.	PET response, line of therapy, CAR T-cell therapy availability, prior auto-HCT, comorbidities, and disease subtype guide treatment selection [1,2].
Severe aplastic anemia, inherited bone marrow failure syndromes, immunodeficiencies, hemoglobinopathies, and selected metabolic diseases	Allo-HCT can be curative in carefully selected patients.	Donor type, age, organ damage, infection status, transfusion history, and disease-specific risk inform candidacy and prognosis [1,2].
Solid tumors	HCT is not a general oncologic indication; its use is restricted to selected tumor types and disease stages or to investigational settings.	Routine use outside clearly defined indications is not supported [1,2].

3.2 Stages of the transplant procedure

The transplant procedure is a coordinated, multistage process involving specialists in hematology, transplant immunology, apheresis, transfusion medicine, infectious diseases, pharmacy, nursing, nutrition, rehabilitation, and psychosocial care.

Pretransplant assessment should confirm the diagnosis and disease risk and evaluate remission status, MRD when validated for the disease, performance status, organ function, infectious risk, fertility counseling, psychosocial status, HCT-CI, and, for malignant diseases, DRI, as well as donor and graft-source options. HLA typing of the recipient and donor should include HLA-A, -B, -C, -DRB1, and -DQB1. HLA-DPB1, donor-specific anti-HLA antibodies, CMV serostatus, ABO compatibility, donor-recipient sex mismatch, donor age, and graft source are assessed according to donor type and center policy [1,7,8,12–14].

Mobilization and collection differ between autologous and allogeneic procedures. In auto-HCT, the patient's stem cells are mobilized using G-CSF alone or in combination with chemotherapy, plerixafor, or both, and are collected by leukapheresis before cryopreservation. In allo-HCT, related or unrelated donors of peripheral blood stem cells are usually mobilized with G-CSF alone; alternatively, bone marrow is harvested under anesthesia, or a cord blood unit is selected from a bank. For auto-HCT, a generally accepted minimum collection target is approximately 2 × 10^6 CD34+ cells/kg. Allogeneic peripheral blood graft targets are commonly higher, with the exact target depending on graft source, donor type, disease, graft processing, and institutional protocol [3,9].

Conditioning prepares the recipient for engraftment, provides antitumor cytoreduction when relevant, and suppresses recipient immunity to reduce the risk of graft rejection. Regimens are broadly grouped into myeloablative conditioning (MAC), reduced-intensity conditioning (RIC), and non-myeloablative conditioning (NMA). Selection should not be based on age alone; it is individualized according to disease biology, MRD, remission status, HCT-CI, DRI, organ function, donor and graft

source, infection risk, and expected relapse versus non-relapse mortality. In general, MAC may reduce relapse risk but may increase toxicity, whereas RIC and NMA can reduce early toxicity and expand transplant access to older patients and those with comorbidities [1,10,12–14].

3.3 Post-transplant care and prevention of complications

Post-transplant care spans early, intermediate, and long-term phases. It includes hematologic support, infection surveillance and prophylaxis, prevention and treatment of GVHD, monitoring for relapse or MRD recurrence, graft function assessment, vaccination, endocrine and fertility follow-up, and survivorship care [3,11,15,18].

Irradiated cellular blood components are used until immune reconstitution is adequate and institutional stopping criteria are met to reduce the risk of transfusion-associated GVHD. Leukoreduced and CMV-safe products, antimicrobial prophylaxis, nutritional support, and rehabilitation are adapted to conditioning intensity, donor type, GVHD, neutropenia duration, and local epidemiology [3].

Infection prevention after HCT is risk-adapted. It commonly includes antibacterial prophylaxis during profound neutropenia, antiviral prophylaxis against HSV and VZV, CMV monitoring and prophylaxis or preemptive therapy in at-risk patients, antifungal prophylaxis according to the risk of mold infection, and Pneumocystis jirovecii prophylaxis after engraftment. Specific regimens are selected according to individual risk and institutional protocols [3].

GVHD prophylaxis depends on donor type, HLA match, graft source, conditioning intensity, and center protocol. Conventional approaches include a calcineurin inhibitor with short-course methotrexate after MAC and cyclosporine or tacrolimus with or without mycophenolate mofetil after RIC. ATG or alemtuzumab may be used in selected settings. PTCy, usually followed by a calcineurin inhibitor plus mycophenolate mofetil, is a standard platform in haploidentical HCT and is increasingly used in unrelated and matched donor transplantation, especially after RIC and with peripheral blood grafts. Systemic corticosteroids remain

the first-line treatment for acute GVHD; ruxolitinib is established for glucocorticoid-refractory acute GVHD [11,15,16].

4. Prognosis and treatment outcomes after HCT

4.1 Factors influencing prognosis

Prognosis after HCT is context-dependent. It is shaped by disease entity, disease phase, remission status, MRD, cytogenetic and molecular risk, prior therapies, patient age and fitness, HCT-CI, DRI, organ function, infection history, donor type, HLA match, graft source, conditioning intensity, GVHD prophylaxis, and post-transplant complications. For this reason, survival should not be expressed as a single overall percentage without specifying the population to which the estimate applies [1,12–14].

Donor selection influences both toxicity and relapse risk. HLA-identical sibling donors and fully matched unrelated donors remain preferred when available, but haploidentical and mismatched unrelated donors have become viable alternatives because of PTCy-based platforms and improved supportive care. Donor age, CMV serostatus, graft source, ABO mismatch, sex mismatch, and donor-specific anti-HLA antibodies may modify risk and should be interpreted in the clinical context rather than in isolation [7,8,11].

4.2 Post-transplant complications

HCT complications may occur early, late, or very late. Early complications include mucositis, cytopenias, bloodstream infections, invasive fungal disease, viral reactivation, sinusoidal obstruction syndrome/veno-occlusive disease, endothelial injury syndromes, graft failure, acute GVHD, and early relapse. Late complications include chronic GVHD, immunodeficiency, recurrent infections, endocrine disorders, infertility, bone loss, secondary malignancies, pulmonary disease, cardiovascular complications, and psychosocial morbidity [3,15,18].

GVHD remains one of the major complications of allo-HCT. Acute GVHD most often affects the skin, gastrointestinal tract, and liver. Chronic GVHD can resemble autoimmune and fibrotic disorders and may involve the skin, eyes, mouth, lungs, liver, fascia, genital tract, and other organs. GVHD prophylaxis lowers risk but does not eliminate it, prevention and treatment strategies must balance GVHD control against preservation of the graft-versus-leukemia effect and relapse prevention [7,11,15].

4.3 Survival and quality of life analysis

Outcomes vary substantially by disease status. For example, AML in MRD-negative CR1, AML with persistent MRD, relapsed or refractory AML, multiple myeloma after induction, and chemosensitive relapsed lymphoma represent distinct prognostic populations and are not directly comparable. HCT prognosis is determined by the interaction among disease risk, MRD and remission status, patient fitness, donor and graft platform, conditioning intensity, GVHD, infection, relapse, and the quality of post-transplant care [1,12–14].

Quality of life after HCT is a separate clinical endpoint. Many survivors experience chronic GVHD, fatigue, sexual dysfunction and fertility problems, neuropathy, endocrine dysfunction, cognitive symptoms, return-to-work difficulties, and psychosocial distress. Long-term follow-up should therefore include structured survivorship care, revaccination, screening for late effects, rehabilitation, psychological support, and management of chronic GVHD [18].

4.4 Development directions in HCT

HCT is evolving toward risk-adapted, biology-informed transplantation. Areas of progress include haploidentical and mismatched unrelated donor platforms, PTCy-based GVHD prophylaxis, MRD-guided timing of HCT, preemptive post-transplant therapy, serial chimerism monitoring, and more precise prediction of relapse and non-relapse mortality [1,7,11,14].

Individualized transplantation includes selecting conditioning intensity and GVHD prophylaxis according to disease biology, MRD, HCT-CI, DRI, donor and graft source, and comorbidities. CAR T-cell and other cellular therapies increasingly interact with HCT as alternatives, bridges to transplantation, post-HCT salvage, or consolidation in selected high-risk settings. Their roles continue to evolve and remain indication-specific; however, they do not uniformly replace HCT [1,2,17].

5. Conclusion

HCT/HSCT remains a potentially curative or disease-controlling treatment for selected hematologic malignancies, bone marrow failure syndromes, inherited immune or metabolic diseases, hemoglobinopathies, and other selected nonmalignant conditions. HCT or HSCT is the appropriate umbrella term; bone marrow transplantation refers specifically to procedures in which bone marrow is the graft source.

Transplant candidacy and prognosis require individualized assessment. Contemporary practice integrates diagnosis and disease phase, MRD and remission status, HLA matching, comorbidities, donor and graft source, conditioning intensity, GVHD prophylaxis, infection and relapse risk, as well as long-term care. Mobilization strategies also differ fundamentally between autologous recipients and healthy allogeneic donors. These factors must be considered together when selecting an HCT strategy and counseling patients.

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Chapter 4

Targeted treatment in chronic lymphocytic leukemia

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1. Introduction

The most frequent type of leukemia among adults in high-income populations is chronic lymphocytic leukemia (CLL). The treatment of CLL has evolved substantially in recent years, with targeted therapies representing one of the most important advances in this field. These therapies are more precise, less destructive, and less toxic than traditional chemotherapy. In the treatment of chronic lymphocytic leukemia, they are gradually replacing traditional chemotherapy. The main reason for choosing this topic is

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the need to gain a better understanding of the function and efficacy of targeted treatment for chronic lymphocytic leukemia [1].

2. Definition of CLL

According to the National Cancer Institute, chronic lymphocytic leukemia is a slowly developing lymphoproliferative malignancy characterized by the accumulation of mature, clonal B lymphocytes with a distinctive immunophenotype in the peripheral blood, bone marrow, and lymphoid tissues. While CLL manifests predominantly in the bloodstream and bone marrow, small lymphocytic lymphoma (SLL) is considered its extramedullary counterpart. Both entities share identical biological and immunophenotypic characteristics; however, SLL is clinically distinguished by its localization to the lymph nodes and spleen, accompanied by a minimal presence of neoplastic cells in the peripheral circulation [1].

3. Epidemiology

The most common form of leukemia diagnosed is CLL [1]. According to the latest data from the Surveillance, Epidemiology, and End Results (SEER) database, CLL has an age-adjusted incidence rate of 4.6 cases per 100,000 people annually. CLL mostly occurs in older adults, with a median age at diagnosis of approximately 70 years. Fewer than one in ten cases are diagnosed in patients under 45 years of age. CLL is extremely rare in children. The incidence of CLL increases markedly with age. Regarding sex-based disparities, men are approximately twice as likely as women to develop CLL. However, research suggests that women may have a greater likelihood of aggressive disease progression [2]. It is estimated that approximately 0.6% of people may receive a CLL diagnosis at some point in their lives. In 2024, 20,700 new cases of CLL were reported in the United States, accounting for 1% of all new cancer

diagnoses that year. Patient survival has improved over the years. In 1975, the 5-year relative survival rate was 65.1%, whereas more recently it has increased to 88.5% [2].

4. Clinical presentation and symptoms of chronic lymphocytic leukemia

The clinical presentation of CLL is highly variable. Patients with CLL frequently present without symptoms at diagnosis, as shown in Table 1. Most patients are diagnosed based on routine complete blood counts showing elevated lymphocyte counts. According to Mukkamalla et al. [3], only one in ten individuals exhibits symptoms at diagnosis, such as fever >38°C, night sweats without any other signs of infection, exhaustion, and unanticipated weight loss exceeding 10% within six months. Similar findings are shown in Table 1. Skin examination is a key component of the physical assessment in patients diagnosed with CLL because skin cancers are commonly observed as a complication in patients with chronic lymphocytic leukemia. Apart from lymphoid organs, the skin is the most commonly affected non-lymphoid organ in patients with CLL. Leukemia cutis typically appears on the face and may present as a papule, macule, plaque, ulcer, blister, or nodule. A skin biopsy is necessary to establish a diagnosis of CLL involvement. Patients with CLL are likely to have more pronounced reactions to insect bites. Additionally, some may develop secondary skin changes due to bleeding, vasculitis, or infections [3]. On physical examination, lymphadenopathy, which can be localized or widespread, is present in 50–90% of patients with CLL. The most commonly affected lymph nodes are cervical, supraclavicular, and axillary. Affected lymph nodes are typically firm, painless, rounded, and movable on palpation. Splenomegaly, or splenic enlargement, is the second most frequent examination finding, occurring in 25–55% of patients. Hepatomegaly, or liver enlargement, is palpable 2–6 cm below the right costal border in approximately 15–25% of patients [3].

Table 1. Symptoms of adult chronic leukemia before diagnosis

Symptom	Cases, n (%) n = 2877	Controls, n (%) n = 111	Likelihood ratio (95% CI)	OR in multivariable analysis (95% CI)
Infection	608 (21)	1887 (15)	1.4 (1.3 to 1.6)	1.5 (1.3 to 1.6)
Cough	415 (14)	1394 (11)	1.3 (1.2 to 1.5)	1.2 (1.1 to 1.4)
Hypertension	403 (14)	1619 (13)	1.1 (1.0 to 1.2)	1.2 (1.1 to 1.4)
Shortness of breath	214 (7)	666 (5)	1.4 (1.2 to 1.7)	1.3 (1.1 to 1.5)
Fatigue	196 (7)	409 (3)	2.1 (1.8 to 2.5)	2.1 (1.8 to 2.6)
Diarrhea	138 (5)	381 (3)	1.6 (1.3 to 2.0)	1.4 (1.1 to 1.7)
Lymph-adenopathy	90 (3)	18 (0.1)	22 (13 to 37)	22 (13 to 36)
Malaise	70 (2)	170 (1)	1.8 (1.4 to 2.4)	1.7 (1.3 to 2.3)
Weight loss	65 (2)	93 (1)	3.1 (2.3 to 4.3)	3.0 (2.1 to 4.2)
Bruising	58 (2)	115 (1)	2.3 (1.6 to 3.1)	2.3 (1.6 to 3.2)

Source: [4].

The relatively low frequency of individual symptoms presented in Table 1 suggests that most patients with CLL are asymptomatic at diagnosis. These results are consistent with the clinical nature of CLL, which is more often detected during routine blood tests than through the presence of symptoms.

5. Diagnostic criteria of CLL

During the diagnostic process, it is important to confirm that the patient has CLL rather than another lymphoproliferative disorder. To establish the diagnosis, it is necessary to evaluate a blood smear, the immunophenotype, and, in selected cases, the genetic features of circulating lymphoid

cells. The peripheral blood must contain at least 5×10^9/L clonal B lymphocytes for at least three months to meet the diagnostic criteria for CLL. Conversely, small lymphocytic lymphoma (SLL) is diagnosed when peripheral clonal B-cell counts are below the 5×10^9/L threshold and the disease is confirmed histopathologically. Flow cytometry is used in the diagnostic process to verify B-cell clonality by detecting immunoglobulin light chains. CLL cells found in the blood typically appear as small, mature lymphocytes. They have a dense nuclear structure in which the chromatin is partially aggregated, and the nucleoli are indistinct. Morphologic features associated with CLL include Gumprecht nuclear shadows and cellular debris. Typical CLL may be accompanied by a small fraction of larger, less mature cells—prolymphocytes. The finding of >55% prolymphocytes shifts the diagnosis toward prolymphocytic leukemia. A moderate increase in prolymphocytes (>10%) in patients with CLL is clinically significant and suggests more aggressive clinical behavior associated with high-risk features such as *NOTCH1* or *TP53* aberrations [5].

6. Immunophenotype

The diagnosis of CLL requires immunophenotypic analysis because leukemic B cells are characterized by the co-expression of the T-cell-associated marker CD5 with the typical B-cell antigens CD19, CD20, and CD23. Additionally, the leukemic clone must exhibit monoclonality, showing restricted expression of either κ or λ immunoglobulin light chains, although CD5 expression may also occur in other lymphoid malignancies. A core set of markers, including CD19, CD5, CD20, CD23, κ, and λ, is generally sufficient to confirm the diagnosis. For atypical presentations, supplemental markers such as CD43, CD79b, CD81, CD200, CD10, or ROR1 can be used to clarify the classification [5]. To confirm a single clone, the leukemic cells must exhibit light-chain restriction, meaning that they express either the κ or λ light chain. However, an uncommon form of CLL, termed biclonal CLL, is characterized by cells expressing both κ and λ light chains [3].

Marrow examination

A marrow aspirate and biopsy are usually not required to establish the diagnosis of CLL; however, these procedures can be valuable in determining whether cytopenias are caused by leukemic involvement of the marrow or by other factors. In patients with chronic lymphocytic leukemia (CLL), bone marrow aspirates typically show that more than 30% of nucleated cells are mature. The infiltration pattern, either diffuse or nodular, helps assess the overall extent of leukemic involvement. Before initiating cytotoxic therapy, a bone marrow biopsy may provide useful baseline information. Repeating the biopsy is also recommended in patients with persistent post-treatment cytopenias to distinguish between disease-related and therapy-induced causes. Moreover, bone marrow biopsy is essential to confirm complete remission [5].

7. Risk stratification

In CLL, there are two accepted clinical staging systems—the Rai staging system and the Binet staging system. Both staging systems are easy to use, reasonably priced, and based on physical examination and laboratory testing, and both describe distinct clinical outcomes across three prognostic groups [2]. The Rai system stratifies patients into three risk groups based on the presence of lymphocytosis, organ or lymph node enlargement, and disease-related cytopenias. Low-risk disease is defined solely by lymphocytosis (leukemia cells in the blood and/or marrow, with lymphoid cells >30%); intermediate-risk disease includes patients with lymphocytosis and enlarged lymph nodes at any site and/or splenomegaly and/or hepatomegaly; and high-risk disease is indicated by the presence of disease-related anemia (hemoglobin [Hb] <11 g/dL) or thrombocytopenia (platelet count $<100 \times 10^9$/L) [2]. The Binet staging system is based on the number of involved lymph node areas and/or organomegaly and the presence of anemia or thrombocytopenia. Five areas of involvement are considered (defined by enlarged lymph nodes >1 cm or organomegaly): 1—head and neck, 2—axillae, 3—groins, 4—palpable

spleen, and 5—palpable liver. Stage A is characterized by preserved hematologic function (Hb>10 g/dL and platelets ⩾100 × 10^9/L) and involvement of up to two (0, 1, or 2) of the five specified nodal or organ areas. Stage B is characterized by preserved hematologic function (Hb>10 g/dL and platelets ⩾100 × 10^9/L) but with involvement of three or more of the five nodal or organ areas. Stage C is defined by the presence of anemia (Hb <10 g/dL) and/or thrombocytopenia (platelet count <100 × 10^9/L), regardless of the number of involved areas [2]. The Rai or Binet staging system helps stratify patients according to disease risk; however, biomarkers provide additional predictive information. The presence of *TP53* abnormalities, such as del(17p) and/or *TP53* gene mutations, serum β2-microglobulin levels, and IGHV mutation status are among the most significant prognostic indicators. *TP53*-related genetic changes are typically present, at least in part, in high-risk CLL [5].

8. Classical treatment methods

Chemoimmunotherapy

The combination of fludarabine, cyclophosphamide, and rituximab (FCR) may still be considered in selected young and healthy individuals with CLL who do not have a 17p deletion or *TP53* mutation and have *IGHV* gene mutations. Due to the increased risk of infections, toxicity, and persistent immunosuppression, FCR treatment is not advised for older patients [3]. Rituximab (RTX) activates the classical pathway of the complement system and triggers antibody-dependent cell-mediated cytotoxicity. The majority of B-cell malignancies are treated with rituximab [6]. It has been reported that RTX can trigger apoptosis in malignant B cells, as evidenced by higher rates of tumor cell death in patients with CLL receiving RTX compared with those who were untreated [7]. Bendamustine plus rituximab (BR) may be considered in selected patients over 65 years of age without a 17p deletion or *TP53* mutation but with *IGHV*-mutated CLL. Ibrutinib-targeted therapy is preferred over BR in patients with *IGHV*-unmutated chronic lymphocytic leukemia. Although

targeted therapies currently represent the preferred standard of care in CLL, chemoimmunotherapy may remain an option for selected patients, particularly when access to targeted therapies is limited. Chemoimmunotherapy often causes side effects, particularly in older patients, because the risk of adverse effects increases with age [8].

9. Targeted therapies in CLL

Modern management of CLL has fundamentally shifted toward targeted agents, with Bruton's tyrosine kinase (BTK) inhibitors and venetoclax-based regimens now established as the frontline standard of care. Therapeutic strategies are strictly tailored to the biological profile of the disease, prioritizing fixed-duration targeted therapy or continuous kinase inhibition. This therapeutic evolution is also reflected in contemporary Polish clinical practice. Current recommendations of the Polish Adult Leukemia Group (PALG) and the Polish Society of Haematologists and Transfusiologists (PTHiT) prioritize targeted therapies, including BTK inhibitors and venetoclax-based regimens, as the preferred treatment approach for both frontline and relapsed/refractory CLL. Thus, traditional chemoimmunotherapy is no longer used in routine clinical practice, as targeted therapies have become the standard treatment for CLL [9].

BCL2 Inhibitors

The anti-apoptotic protein BCL-2 is essential in the treatment of CLL because it prevents programmed cell death. BCL-2 inhibitors represent a significant advancement in the management of CLL, particularly for patients with high-risk disease or those who are intolerant to chemoimmunotherapy. The BCL-2 family consists of pro-apoptotic and anti-apoptotic members. The anti-apoptotic subfamily includes the proteins BCL-2, BCL-XL, BCL-w, Bfl-1/A1, MCL-1, and BCLB/Boo. Pro-apoptotic proteins are divided into multidomain effectors (BAK and BAX) and BH3-only proteins (BAD, BID, BIK, BIM, BMF, HRK, PUMA,

and NOXA). Anti-apoptotic BCL-2 family members inhibit apoptosis by binding and neutralizing active BAK and BAX, preventing mitochondrial outer membrane permeabilization (MOMP), a key event in the intrinsic apoptotic pathway. MOMP enables the release of apoptotic proteins, including caspases, into the cytosol, triggering cell death. In addition, sequestration of BH3-only proteins by anti-apoptotic BCL-2 members is critical, as BH3-only proteins can either inhibit anti-apoptotic members or directly activate pro-apoptotic effectors to initiate apoptosis [10]. Venetoclax is a once-daily oral medication that is metabolized by the liver. It reaches peak concentrations 5–8 hours after dosing and has an elimination half-life of 14–18 hours. Because food intake enhances venetoclax bioavailability and absorption depends on the fat content of the meal, it is recommended that the medication be taken with food. Maximum concentrations were observed approximately two hours after venetoclax was administered with meals and were higher than when it was administered in the fasting state [11].

In a phase 1 clinical study involving 116 patients with relapsed or refractory chronic lymphocytic leukemia, the drug dose was determined to be 400 mg per day. The first dosing attempt was unsafe and unsuccessful—an aggressive dose of 200 mg, with a 72-hour washout period, was administered before starting daily treatment, which led to the development of life-threatening tumor lysis syndrome (TLS) in all three subjects. This finding indicated that a ramp-up period was needed. Patients in the expansion cohort received weekly escalating doses (20, 50, 100, 200, and then 400 mg). This strategy, combined with tumor lysis syndrome prophylaxis such as allopurinol and hydration, achieved its goal: no patients developed clinical TLS [12,13].

Toxicity of therapy

Tumor lysis syndrome (TLS) is the most severe and potentially fatal side effect observed in patients receiving venetoclax. In early trials, the risk was especially notable. However, the incidence of clinical TLS has been considerably reduced by using a 5-week dose ramp-up period in combination with prophylaxis (hydration and allopurinol) [13,14]. Neutropenia

(grades 3–4) is the most common hematologic toxicity, occurring in 40% of patients receiving venetoclax monotherapy [14,15] and in up to 60% of patients when venetoclax is combined with an anti-CD20 monoclonal antibody [16,17]. During treatment, anemia (25%) and thrombocytopenia (20%) may also be observed [16]. Nausea, diarrhea, and fatigue are the most frequently reported nonhematologic side effects. These symptoms are usually mild and rarely necessitate permanent treatment discontinuation.

Measurable residual disease (MRD) as a marker of treatment response

Patients with chronic lymphocytic leukemia who achieve high rates of undetectable measurable residual disease (uMRD), which are strongly correlated with longer progression-free survival and overall treatment success, experience profound remission following venetoclax-based regimens. This makes MRD an important prognostic measure in the treatment of CLL. MRD refers to the small number of leukemic cells that persist after treatment and are frequently not detected by standard clinical diagnostic methods [17].

Murano study

The MURANO trial was a landmark phase 3 study aimed at evaluating the efficacy and safety of fixed-duration therapy combining venetoclax and rituximab (VenR) in patients with relapsed or refractory chronic lymphocytic leukemia (CLL). A total of 389 participants were randomly assigned to receive venetoclax with rituximab or the standard chemoimmunotherapy regimen of bendamustine plus rituximab. The group of patients treated with VenR received six cycles of venetoclax with rituximab, followed by venetoclax alone for up to 2 years. The BR group received six cycles of bendamustine with rituximab. At the four-year follow-up, patients treated with VenR showed a clear advantage in progression-free survival (PFS) compared with those treated with BR. A total of 57% of patients treated with VenR remained free of disease progression, whereas only 5% of patients in the BR group did.

Monitoring of MRD was a key component of the study. Patients who achieved undetectable MRD (uMRD), defined as no detectable CLL cells in the peripheral blood using highly sensitive assays (<1 CLL cell per 10,000 leukocytes), showed significantly longer progression-free survival. In contrast, patients with high-risk genetic alterations (such as *TP53*, *NOTCH1*, and *BIRC3*) were less likely to achieve uMRD and showed faster relapse. Venetoclax combined with rituximab was generally well tolerated, with manageable adverse events. These data indicate that fixed-duration BCL-2-targeted therapy can yield strong and durable responses in patients with relapsed or refractory CLL [18,19].

Bruton's tyrosine kinase (BTK) inhibitors

An important class of targeted treatments in CLL is BTK inhibitors. B-cell receptor signaling is mediated in large part by BTK. There are two categories of BTK inhibitors: irreversible inhibitors and reversible inhibitors [20]. The first irreversible BTK inhibitor, ibrutinib, was approved by the U.S. Food and Drug Administration (FDA) in 2013, representing a major therapeutic advance in CLL. Irreversible, second-generation BTK inhibitors—acalabrutinib and zanubrutinib—were developed to reduce the adverse effects associated with ibrutinib [20].

Mechanism of action

B-cell receptor (BCR) signaling is critical for the development and survival of both normal and malignant B cells [21]. In CLL-like mouse models, the absence of BTK significantly delayed disease progression and limited leukemic infiltration, although lymphoid structures continued to develop. *In vivo*, BTK inhibition abolished tumor development, whereas BTK overexpression increased cancer incidence and overall mortality [22]. BTK—Bruton's tyrosine kinase—is one of the most important components of the BCR signaling pathway. Antigen interactions initiate this pathway, causing Src family kinases (Lyn) to phosphorylate the CD79A/CD79B heterodimer, which in turn activates BTK [23]. In cancer cells, such as those in CLL, the BCR pathway is often constitutively activated. As a result of this continuous signaling, important downstream components such

as Syk, PLC-γ2, and PI3K undergo a series of phosphorylation events that ultimately lead to NF-κB transcriptional activity [24,25]. A targeted therapeutic approach using BTK inhibitors blocks BTK kinase activity, disrupting this critical pathway. Most BTK inhibitors (BTKis), especially first-generation agents, act as irreversible inhibitors by targeting the ATP-binding site in the BTK kinase domain. The formation of a strong covalent bond with the cysteine residue at position 481 of BTK is necessary for this mechanism to work. This persistent covalent bond results in the irreversible loss of enzyme activity. Blocking the phosphorylation cascade completely stops BCR signal transduction, thereby achieving the therapeutic effect [26].

Clinical experience has demonstrated a profile of side effects associated with ibrutinib, the first-generation BTK inhibitor that has revolutionized the therapeutic approach to B-cell malignancies. Although patients experienced side effects such as bleeding and atrial fibrillation, ibrutinib was largely free of infectious and bone marrow toxicities. Since congenital BTK deficiency is not associated with hemorrhage or cardiovascular symptoms, these side effects were unexpected. Other side effects, such as late-onset hypertension and, rarely, fatal ventricular arrhythmias, have been documented with greater clinical exposure.

Studies have shown that BTK is essential for platelet activation and cardiac signaling. The observed bleeding and cardiovascular side effects are "on-target," resulting from the inhibition of BTK and related TEC family kinases outside B cells. Unlike congenital BTK deficiency, in which other kinases can compensate, drug therapy blocks multiple kinases simultaneously, disrupting platelet and cardiac pathways and leading to these adverse events [26].

Second-generation BTK inhibitors in CLL

Second-generation covalent BTK inhibitors were developed to improve the efficacy and tolerability of first-generation agents. These drugs maintain irreversible binding to the cysteine 481 (C481) residue of BTK but are engineered to have higher selectivity for BTK and reduced off-target kinase inhibition. For example, acalabrutinib shows markedly

less inhibition of kinases such as EGFR, ITK, or TEC compared with first-generation inhibitors. Mechanistically, this higher specificity is associated with lower incidences of atrial fibrillation, major bleeding, and hypertension, as shown in head-to-head and meta-analyses of randomized trials (e.g., the ELEVATE-RR and ALPINE studies), demonstrating hazard ratios for atrial fibrillation of approximately 0.28 when comparing second-generation with first-generation agents. Clinical trials have confirmed these advantages: in the ELEVATE-RR study, acalabrutinib demonstrated noninferior efficacy compared with ibrutinib, with significantly reduced cardiac toxicity [26]. Similarly, the ALPINE trial showed that zanubrutinib achieved higher overall response rates and progression-free survival, along with a lower incidence of atrial fibrillation [26,27]. Second-generation BTK inhibitors such as acalabrutinib and zanubrutinib have become preferred therapeutic options in CLL treatment due to comparable efficacy and superior safety profiles compared with first-generation agents. Current research is directed toward developing noncovalent (reversible) BTK inhibitors, such as pirtobrutinib, intended to overcome resistance mediated by C481 mutations [26].

Mechanisms of resistance to BTK inhibitors

Resistance to covalent BTK inhibitors most frequently results from mutations within the BTK binding site, particularly at C481 [28]. The canonical C481S substitution, first described in 2014 [29,30], accounts for more than 90% of resistance cases in patients treated with ibrutinib. This mutation prevents covalent bond formation between the drug and BTK, leading to loss of inhibitory activity while preserving kinase function. Less common genetic variants, such as C481F, C481Y, and L528W, have also been reported. The C481S mutation remains predominant in acalabrutinib-treated patients, although additional mutations, such as T474I, may occur either alone or in combination with C481S [31]. In patients treated with zanubrutinib, a notable frequency of kinase-impairing mutations such as L528W has been reported [32]. Mutations in downstream signaling molecules such as PLCγ2 have also been recognized, but they are not considered independent mechanisms

of resistance and coexist with BTK mutations [33]. The emergence of C481S and related mutations has spurred the development of reversible, noncovalent BTK inhibitors that maintain efficacy against both wild-type and C481-mutated BTK [26]. The identification of such resistance mechanisms has accelerated the development of next-generation, reversible, noncovalent BTK inhibitors such as pirtobrutinib, which are designed to overcome C481 mutations and restore BTK inhibition [34].

BTK protein degraders: a new therapeutic class

The development of a new class of targeted drugs—BTK protein degraders—was accelerated by the emergence of BTK-mutant clones under selective pressure. By leveraging the ubiquitin-proteasome pathway, these agents facilitate the targeted ubiquitination and subsequent destruction of the BTK protein inside the cell [28]. The broad activity of BTK protein degraders across both wild-type and mutant BTK, including C481S, L528W, and T474I, was demonstrated in preclinical data [35]. Clinical results are promising. The first-in-human study of NX-2127 showed not only rapid BTK protein degradation but also a reduction in nodal disease, notably in patients previously identified as having BTK mutations [36]. Furthermore, data concerning other degraders, such as BGB-16673, highlight a favorable safety profile and substantial clinical activity, with an objective response rate (ORR) of 70% in patients with CLL [37]. If clinical development continues successfully, BTK degraders could provide a promising approach to overcoming resistance in patients presenting with multiple BTK-mutant clones acquired during treatment with earlier-generation BTK inhibitors.

Anti-CD20 monoclonal antibodies

Anti-CD20 monoclonal antibodies are approved for the treatment of B-cell non-Hodgkin lymphomas and chronic lymphocytic leukemia (CLL), typically in combination with chemotherapy. The first monoclonal antibody approved for therapeutic use was rituximab, a chimeric monoclonal antibody targeting the pan-B-cell marker CD20 [38]. The hydrophobic transmembrane protein CD20 (human B-lymphocyte-restricted differentiation

antigen, Bp35) has a molecular weight of approximately 35 kDa [39]. It is expressed on pre-B and mature B lymphocytes and is extensively expressed in most B-cell non-Hodgkin lymphomas and chronic lymphocytic leukemia (CLL). CD20's potential as a therapeutic target is supported by its widespread presence on B cells [39].

Mechanism of CD20 mAbs (monoclonal antibodies)

Current research indicates that CD20 monoclonal antibodies mediate tumor cell death through the following mechanisms: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct cytotoxic effects mediated by antibody binding to CD20 molecules [40]. ADCC is a mechanism in which the Fc portion of an antibody bound to CD20 on the B-cell surface is recognized by Fcγ receptors on effector cells, such as natural killer (NK) cells. This interaction activates NK cells to release cytotoxic granules, inducing apoptosis of the target B cell [40]. The other mechanism, CDC, is initiated when anti-CD20 monoclonal antibodies attach to the B-cell surface, thereby activating the complement cascade. This activation leads to the formation of the membrane attack complex (MAC), which creates pores in the cell membrane and causes lysis of malignant B cells [41]. Certain anti-CD20 mAbs can directly induce cytotoxic effects. The binding of these antibodies induces clustering of CD20 molecules, thereby activating an intracellular signaling pathway that leads to apoptosis without requiring immune effector cells or complement activation [41]. In addition, anti-CD20 monoclonal antibodies can mediate antibody-dependent cellular phagocytosis (ADCP), in which macrophages recognize the Fc portion of the bound antibody and contribute to the elimination of B cells [41].

Rituximab

The first mAb authorized for use in cancer treatment was rituximab. This chimeric mouse/human IgG1 monoclonal antibody targets the CD20 epitope on a large extracellular loop [42]. Rituximab, a type I monoclonal antibody, exerts its therapeutic effects through CDC, ADCC, and ADCP [43]. A wide range of B-cell malignancies, including diffuse large

B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), mantle cell lymphoma (MCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), hairy cell leukemia (HCL), and chronic lymphocytic leukemia (CLL), are treated with rituximab, which has been widely used in the management of these diseases [44]. Current clinical initiatives focus on improving combination and delivery methods for rituximab. A new formulation of rituximab with hyaluronidase has been approved for subcutaneous use in FL, CLL, and DLBCL [45]. Furthermore, the phase 3 AUGMENT study, which is moving toward chemotherapy-free regimens, demonstrated the effectiveness of combining rituximab with immunomodulatory medications such as lenalidomide for relapsed/refractory (r/r) disease [46]. Despite the success of rituximab, relapse occurs after treatment in some patients due to various resistance mechanisms. These mechanisms include trogocytosis, complement exhaustion, and CD20 internalization [47,48].

Obinutuzumab – the second-generation anti-CD20 mAb

Obinutuzumab (GA101) is recognized as a second-generation CD20-targeting antibody engineered to enhance cytotoxic activity against B-cell malignancies. Obinutuzumab is a humanized, glycoengineered IgG1 type II monoclonal antibody that targets an epitope on the large extracellular loop of CD20 [42]. Obinutuzumab's superior activity stems from Fc region glycoengineering, which was specifically designed to increase its affinity for FcγR receptors found on immune effector cells. Compared with rituximab and ofatumumab in preclinical studies, obinutuzumab shows reduced fucosylation of oligosaccharides linked to Asp297 in its Fc region, which improves FcγRIII binding [49] and results in greater ADCC [50]. CDC is decreased with obinutuzumab, a type II monoclonal antibody [51,52]. However, it may induce direct cell death (DCD) through a lysosome-mediated, non-apoptotic mechanism [53,54]. Obinutuzumab has consistently demonstrated greater effectiveness than equivalent doses of rituximab [51,55,56], although the exact reasons are not entirely clear. Furthermore, in a phase 3 trial, obinutuzumab demonstrated better progression-free survival (PFS) and overall survival (OS) when used in conjunction with chemotherapy

compared with the same chemotherapy regimen combined with rituximab as first-line treatment for patients with CLL [57].

Obinutuzumab + venetoclax

The CLL14 trial was designed to determine whether venetoclax-obinutuzumab was superior to chlorambucil-obinutuzumab in patients with previously untreated chronic lymphocytic leukemia (CLL) and comorbidities [58]. The duration of treatment in both groups consisted of 12 cycles, each lasting 28 days. Crossover was not allowed [59]. Obinutuzumab was administered intravenously for a total of six cycles. During cycle 1, patients received 100 mg on day 1 and 900 mg on day 2 (or, alternatively, 1000 mg on day 1), followed by 1000 mg on days 8 and 15. In cycles 2 through 6, obinutuzumab was administered at a dose of 1000 mg on day 1 of each cycle [58]. Chlorambucil was administered orally at a dose of 0.5 mg per kilogram of body weight on days 1 and 15 of each cycle for 12 cycles [58]. Therapy with venetoclax was initiated on day 22 of cycle 1 with a 5-week dose ramp-up schedule consisting of 20 mg per day during the first week, 50 mg per day during the second week, 100 mg per day during the third week, 200 mg per day during the fourth week, and 400 mg per day during the fifth week. After the ramp-up phase, venetoclax was administered at a fixed dose of 400 mg daily until the end of cycle 12 [58]. The risk of TLS was evaluated using the patient's absolute lymphocyte count and lymph node size to guide prophylactic measures [58]. In patients with previously untreated CLL and comorbidities, fixed-duration targeted treatment with venetoclax-obinutuzumab was effective and associated with prolonged progression-free survival compared with standard treatment with chlorambucil-obinutuzumab. Three months after the end of treatment, measurable residual disease (MRD) negativity rates were significantly higher in the venetoclax-obinutuzumab group: in peripheral blood, 75.5% were MRD-negative, compared with 35.2% in the chlorambucil-obinutuzumab group ($P<0.001$), and in bone marrow, 56.7% were MRD-negative, compared with 17.1% in the chlorambucil-obinutuzumab group ($P<0.001$) [58]. Moreover, half of

the patients in the venetoclax-obinutuzumab group achieved a complete response, suggesting greater efficacy than that of other therapies typically administered to older patients with CLL [59].

Phosphoinositide 3-kinase inhibitors—PI3K inhibitors

PI3K kinases are divided into three classes. Class I is the most relevant in the context of CLL pathogenesis and lymphocyte biology because it phosphorylates PIP2 to PIP3 by adding a phosphate group to the inositol group, which already has phosphate groups attached to the 4'- and 5'-hydroxyl groups of inositol trisphosphate. The accumulation of PIP3 acts as a critical secondary messenger, driving survival and proliferative signals that are essential for the malignant phenotype of CLL cells [60]. Class I PI3Ks include four catalytic isoforms (α, β, δ, and γ). Each paralog is a heterodimer composed of a regulatory subunit and a catalytic subunit [61]. PI3Kα and PI3Kβ are ubiquitously expressed across tissues, whereas PI3Kγ is expressed in T lymphocytes and PI3Kδ in B lymphocytes and their precursors. PI3Kα and PI3Kδ are crucial for the formation of healthy B cells [60]. In B cells, transmembrane proteins, including CD19, and cytosolic adaptors, including BCAP and TC21, are involved in pathways that regulate PI3K signaling. PI3K is a therapeutic target because it is activated downstream of the B-cell receptor (BCR), and B- and T-cell malignancies are associated with dysregulation or overactivation of this pathway [60]. Three PI3K inhibitors have been approved for the treatment of CLL and/or other lymphoid malignancies. The first-in-class PI3Kδ inhibitor registered for the treatment of relapsed/refractory (R/R) CLL was idelalisib [62]. Idelalisib therapy led to CLL cell death by inducing caspase-dependent apoptosis, regardless of cytogenetic factors such as 17p13 deletion or 11q22 deletion [62,63]. Idelalisib has lower cytotoxic effects on non-pathological B lymphocytes and did not show any cytotoxic effects on T lymphocytes or NK cells [64]; importantly, it preserves ADCC function mediated by NK cells [65,66]. Idelalisib demonstrates multiple effects in CLL by directly inhibiting malignant B cells and disrupting the supportive microenvironment. It reduces the production of key cytokines (BAFF, TNF-α, CCL3, CCL4, CXCL13) [65,67] and

blocks CXCR4/CXCR5 signaling [68], thereby disrupting CLL cell adhesion and homing, leading to redistribution into the peripheral blood and apoptosis [68,69]. Importantly, NK cell function remains unchanged. The maximum tolerated dose of idelalisib was established in the initial phase 1 trial [70], which included a total of 54 heavily pretreated patients with R/R CLL. Idelalisib was administered at doses of 50–350 mg daily as a single dose or BID (bis in die) [71]. Idelalisib treatment was well tolerated, with an overall response rate (ORR) of 72%, although none of the patients achieved a complete response (CR). The median duration of response was 16.2 months, while the median progression-free survival (PFS) was 15.8 months. However, the median overall survival was not reached. The 150 mg BID dose was recommended for further clinical testing and achieved a significantly longer median PFS (32 months) compared with other dosing schedules (7 months) [70]. In the phase 3 Study 116, patients with chronic lymphocytic leukemia (CLL) were treated with either idelalisib with rituximab (R-idelalisib) or rituximab (R) monotherapy [72]. The study group included heavily pretreated patients who had relapsed within 24 months after their last treatment. In 95 patients, a 17p13 deletion or *TP53* mutation was noted, and 184 patients had unmutated IGHV status. In the event of progression, patients in the control arm were permitted to cross over to the idelalisib treatment [72]. Study 116 was stopped early due to the demonstrated superiority of the idelalisib-treated arm over the control group. After 18 months of follow-up, PFS was 20.3 months in the R-idelalisib arm compared with only 6.5 months in the rituximab arm [73]. The overall response rate was 85.5% in the R-idelalisib arm, although a complete response (CR) was achieved in only one patient. The results of Studies 116 and 117 showed that the R-idelalisib group had a higher median OS than the R-placebo group (40.6 vs. 34.6 months). Compared with the control group, patients with a p53 mutation showed a significant improvement in OS (28.5 vs. 14.8 months) when treated with R-idelalisib [73]. Hepatotoxicity, diarrhea and colitis, skin changes, and infections are among the many side effects of idelalisib that frequently lead to early treatment withdrawal [74]. In addition, patients face an

increased risk of serious and opportunistic infections. The increased risk of *Pneumocystis jirovecii* (PJP) pneumonia and cytomegalovirus (CMV) infections represents a significant concern; therefore, prophylactic treatment is mandatory during and after idelalisib therapy. Neutropenia is a common adverse effect of idelalisib treatment, occurring in approximately 50% of patients [74,75].

Duvelisib

Duvelisib is a first-in-class, highly selective, and potent dual inhibitor of PI3Kδ and PI3Kγ. PI3Kδ is predominantly expressed in leukocytes, whereas PI3Kγ is mostly found in T cells and myeloid cells. Inhibition of PI3Kγ aims to reduce the production of cytokines that support leukemic cell survival [62]. Duvelisib was approved by the Food and Drug Administration in 2018 and by the European Medicines Agency in 2021 for the treatment of patients with relapsed/refractory CLL/SLL who had received at least two prior therapies. This approval was based on a global phase 3 trial comparing duvelisib with ofatumumab [76]. The study included 319 patients who had experienced disease progression after at least one prior therapy; none had previously been treated with BTK or PI3Kδ inhibitors. Patients meeting these criteria were randomized to receive duvelisib or ofatumumab. Among patients treated with duvelisib, 31% had a *TP53* mutation and 59% had unmutated *IGHV*, whereas among patients treated with ofatumumab, 33% had a *TP53* mutation and 73% had unmutated *IGHV*. Progression-free survival (PFS) was longer in patients treated with duvelisib than in those treated with ofatumumab (13.3 vs. 9.9 months), regardless of del(17p)/*TP53* status. Moreover, the overall response rate (ORR) was higher with duvelisib, at 74 %, compared with 45% in patients treated with ofatumumab [77].

Adverse effects of duvelisib

In patients with CLL, there are adverse effects typical of PI3K inhibitors, including diarrhea, hepatitis, elevated liver enzymes, colitis, pneumonitis, and infectious complications. The most common all-grade toxicities were diarrhea (42%), fatigue (40.5%), and elevated ALT

(38.5%). Patients are at an increased risk of serious infectious complications, such as *Pneumocystis jirovecii* pneumonia and cytomegalovirus infection [62]. PI3K inhibitors such as idelalisib and duvelisib represent an important class of targeted therapies for patients with chronic lymphocytic leukemia. Even though PI3K inhibitors are effective, they are associated with notable toxicities, which limit their use as targeted treatments.

In contemporary CLL management, PI3K inhibitors have been significantly marginalized due to their severe immune-mediated adverse events. Current ESMO recommendations prioritize more favorable alternatives, such as venetoclax-based combinations and novel BTK inhibitors.

10. Future perspectives in CLL treatment

CAR T cells (chimeric antigen receptor T cells)

The first CAR T-cell therapy was approved by the U.S. Food and Drug Administration (FDA) in 2017 for the treatment of children with acute lymphoblastic leukemia (ALL). Since then, CAR T-cell therapy has been expanded to the treatment of other hematologic and lymphoid malignancies [78]. Renier J. Brentjens, M.D., Ph.D., of Roswell Park Comprehensive Cancer Center in Buffalo, NY, a leading expert in the CAR T-cell field, explained that because T cells are crucial in CAR T-cell treatment, "we are giving patients a living drug."

CAR T-cell therapy procedure

This treatment relies on collecting the patient's blood and isolating T cells. In the next step, T cells are genetically engineered in laboratories to express special proteins on their surface called chimeric antigen receptors (CARs). The CARs enable the T cells to recognize and bind to specific antigens present on the surface of malignant cells, thereby enhancing their cytotoxic activity. The modified T cells are then expanded until there are hundreds of millions of them, forming the final

CAR T-cell therapy product. The entire process—from blood collection to the infusion of the cells back into the patient—typically takes about 3 to 5 weeks [78].

Adverse effects of CAR T-cell therapy

Like all cancer treatments, CAR T-cell therapies may cause adverse effects. The most common side effects of CAR T-cell therapy are infections and B-cell aplasia (a massive loss of antibody-producing B cells).

Cytokine release syndrome (CRS)

The most severe toxicity associated with CAR T-cell therapy is a systemic inflammatory reaction known as cytokine release syndrome (CRS) [79,80,81]. Recognition of the tumor antigen initiates activation of CAR T cells, leading to CRS, a severe, body-wide inflammatory reaction. CAR T-cell infusion results in elevated serum cytokine levels, including IFN-γ, IL-6, TNF-α, GM-CSF, IL-2, IL-8, and IL-10, ultimately leading to a cytokine storm. IL-6 synthesis stimulates DCs, macrophages, and monocytes to release additional cytokines. The result is an exacerbated inflammatory response. Secretion of IL-6 leads to more severe CRS symptoms [82]. Moreover, activated endothelial cells can release IL-6 and endothelial permeability factors in the setting of hyperinflammation [83]. This mediates hemodynamic dysfunction, capillary leak, and consumptive coagulation disorders in patients with CRS by destabilizing vascular integrity. The subsequent leakage, tissue edema, and hypotension ultimately lead to organ dysfunction [84,85].

Immune effector cell-associated neurotoxicity syndrome–ICANS

A major, often delayed, complication of CAR T-cell therapy is immune effector cell-associated neurotoxicity syndrome (ICANS). This syndrome affects the central nervous system (CNS) and is considered a form of cytokine-mediated neurotoxicity [86]. It is known that endothelial activation contributes to ICANS pathogenesis [86]. Cytokines released during CRS can damage the blood-brain barrier (BBB) and facilitate the entry of inflammatory cytokines and immune cells into the central

nervous system [87,88,89]. ICANS is associated with symptoms such as headache, tremors, impaired speech, confusion, delirium, and impaired consciousness [90].

11. Conclusion

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in adults and is typically diagnosed in patients over the age of 65. The introduction of targeted therapies has significantly transformed the management of this disease. Targeted therapies have effectively replaced traditional chemoimmunotherapy, which for many years served as the mainstay of CLL treatment. Although chemoimmunotherapy was effective, its use was often associated with considerable toxicity and numerous adverse effects. In recent years, novel agents—predominantly Bruton's tyrosine kinase (BTK) inhibitors and BCL-2 inhibitors, often combined with anti-CD20 monoclonal antibodies—have markedly improved patient outcomes and established a new frontline standard. The introduction of these therapies has made CLL treatment more effective, precise, and better tolerated. Considerable hope is currently placed on CAR T-cell therapy, which offers the possibility of curing patients who do not respond to other treatment modalities. However, further optimization of this therapy and improvement of its safety profile are essential, as complications such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) remain serious challenges.

Table 2. Summary of treatment modalities in CLL

Therapy class	Overall efficacy	Advantages	Limitations
Chemoimmunotherapy	Good efficacy, particularly in younger and fit patients	Induction of long term remission in selected subgroups	Significant toxicity; reduced effectiveness in *TP53*- disrupted CLL

Therapy class	Overall efficacy	Advantages	Limitations
BCL-2 inhibition	Exhibits high efficacy with frequent achievement of MRD negativity	Time-limited treatment; deep and durable responses	Risk of tumor lysis syndrome; frequent neutropenia
Venetoclax combined with anti-CD20 antibodies	Among the most effective regimens in both frontline and relapsed settings	High rates of durable remission and prolonged progression-free survival	Myelosuppression, particularly neutropenia; requires TLS monitoring
First-generation BTK inhibitor	Provides durable disease control across risk groups	Effective in patients with high-risk cytogenetics	Atrial fibrillation, hypertension, and bleeding risk
Second-generation BTK inhibitors	Comparable efficacy to ibrutinib	Increased tolerability and reduced cardiovascular toxicity	Resistance mutations may still arise
PI3K inhibitors	Moderate efficacy, primarily in relapsed/refractory CLL	Useful in heavily pretreated patients	Incidence of immune- mediated toxicity and infections
Anti-CD20 monoclonal antibodies	Limited efficacy as monotherapy; improved efficacy in combination regimens	Potentiates anti-leukemic activity of targeted agents	Infusion-related reactions; potential antigen loss or resistance
CAR-T cell therapy	Highly effective in selected patients with refractory disease	Possibility of inducing long-term remission or functional cure	Cytokine release syndrome and neurotoxicity; high cost

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Chapter 5

Tumor lysis syndrome (TLS) – risk and treatment

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1. Definition

Tumor lysis syndrome (TLS) is a life-threatening metabolic disorder that occurs in oncology patients during chemotherapy and, in some cases, radiotherapy, leading to the rapid breakdown of tumor cells. In this condition, potentially fatal metabolic disturbances result from the release of substantial amounts of intracellular contents into the extracellular space and subsequently into the systemic circulation. TLS occurs in tumors that have a large cellular mass, a high proliferation index, and high chemosensitivity [1].

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2. Pathophysiology

The disorder occurs because of the rapid breakdown of tumor cells, usually during or within seven days after the initiation of chemotherapy. The resulting release of intracellular contents leads to electrolyte imbalances, most notably hyperuricemia, hyperphosphatemia, hypocalcemia, and hyperkalemia, which may cause life-threatening complications [2]. Hyperuricemia, a hallmark of TLS, stems from excessive nucleic acid degradation and accelerated purine catabolism; meanwhile, rapid lysis of malignant cells, which contain high intracellular phosphate levels, leads to hyperphosphatemia and subsequent hypocalcemia [3]. Hyperkalemia arises from the release of intracellular potassium after tumor lysis, as well as disruption of the sodium-potassium ATPase gradient, which promotes potassium efflux into the bloodstream during intensive tumor cell breakdown and heightened metabolic activity, leading to a shortage of ATP needed to maintain the potassium gradient. Electrolyte disturbances occur at specific intervals after cytotoxic treatment. The earliest imbalance is hyperkalemia, which typically occurs 12 to 24 hours after therapy; hyperphosphatemia followed by hypocalcemia is usually seen after 24 to 48 hours; and lastly, hyperuricemia develops 48 to 72 hours after the initiation of chemotherapy [2].

3. Risk factors

The development of tumor lysis syndrome depends on several factors, including patient condition, tumor type, and specific treatment administered. Individuals at increased risk for TLS may have pre-existing renal impairment, oliguria, reduced fluid volume, and hyperuricemia. Kidney impairment is the primary contributor to increased susceptibility to TLS; therefore, careful monitoring and proactive management of hydration status and uric acid levels are essential both before and throughout treatment [4]. Rapidly proliferating hematologic cancers, including acute myeloid leukemia (AML),

acute lymphoblastic leukemia (ALL), and high-grade non-Hodgkin lymphomas (NHL), are most observed among patients with tumor lysis syndrome [5]. The disorder has also been observed, although rarely, in solid tumors such as small cell carcinoma, breast cancer, germ cell tumors, neuroblastoma, and melanoma, as well as in certain indolent or less aggressive hematologic malignancies [5]. Furthermore, patients with these cancers can be classified into three risk categories: high risk, in which more than 5% of patients develop TLS; intermediate risk, with an incidence of 1–5%; and low risk, in which fewer than 1% of patients are affected [4].

Table 1. Risk stratification of patients prone to developing tumor lysis syndrome based on tumor type

High-risk tumors	Intermediate-risk tumors	Low-risk tumors
• AML with WBC ⩾100,000μL • ALL with WBC ⩾100,000μL or LDH > 2× ULN • Diffuse large B-cell lymphoma with a large tumor (>10cm) and LDH > 2× ULN • Advanced Burkitt lymphoma • Early stage leukemia with elevated LDH • Burkitt lymphoma with elevated LDH	• AML with WBC 25,000–100 000/μL • ALL with WBC <100,000/μL and LDH <2×ULN • Diffuse large B-cell lymphoma with LDH >2×ULN and without a large tumor • Early stage leukemia with LDH <2×ULN • Burkitt lymphoma with LDH <2× ULN	• AML with WBC <25,000/μL and LDH <2×ULN • CLL • CML • MM • Indolent lymphomas • Solid cancers

ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia;

CLL – chronic lymphocytic leukemia; CML – chronic myeloid leukemia;

MM – multiple myeloma; WBC – white blood cells;

LDH – lactate dehydrogenase; ULN – upper limit of normal.

Source: [4].

TLS can also develop because of antineoplastic treatment, including chemotherapy, corticosteroids, molecularly targeted agents, immunotherapy, and, in some cases, radiotherapy or chemoembolization [6]. The syndrome has been reported in connection with the use of specific cytotoxic regimens, such as bortezomib, cyclophosphamide, and dexamethasone polytherapy in multiple myeloma, as well as ibrutinib-, fludarabine-, and rituximab-based therapies in chronic lymphocytic leukemia [7]. In addition, several other therapeutic agents have been linked to TLS, including thalidomide, hydroxyurea, paclitaxel, etoposide, venetoclax, obinutuzumab, and chimeric antigen receptor T-cell (CAR-T) therapies used in the treatment of hematologic malignancies [4,8]. Moreover, various therapeutic agents and endogenous substances may further increase the likelihood of developing tumor lysis syndrome by increasing the amount of uric acid in the body. Agents reported to contribute to the development of TLS include commonly used substances such as alcohol, caffeine, and ascorbic acid, as well as therapeutic agents such as cisplatin, thiazide diuretics, diazoxide, methyldopa, nicotinic acid, pyrazinamide, phenothiazines, ethambutol, levodopa, epinephrine, aspirin, and theophylline [9].

4. Diagnosis and classification

Diagnosis relies on clinical suspicion, detailed history and physical examination, laboratory testing, and an electrocardiogram. The diagnosis is based on the Cairo-Bishop classification [10]. The criteria provide both laboratory and clinical information on tumor lysis syndrome, facilitating its assessment and classification.

4.1 Laboratory criteria

Laboratory tumor lysis syndrome (LTLS) is characterized by abnormal biochemical laboratory results. Diagnosis requires presence of at least

two of the following laboratory criteria: hyperuricemia, defined as a uric acid level exceeding the upper limit of normal or a ⩾25% increase from baseline; hyperkalemia, defined as a potassium level above the upper limit of normal or a ⩾25% increase from baseline; hyperphosphatemia, defined as a phosphate level exceeding the upper limit of normal or a ⩾25% increase from baseline; or hypocalcemia, defined as a calcium level below the lower limit of normal or a ⩾25% decrease from baseline [1,11,12].

4.2 Clinical criteria

Clinical tumor lysis syndrome (CTLS) is confirmed when at least one of the following clinical manifestations accompanies the laboratory abnormalities: acute renal failure, defined as a serum creatinine level ⩾1.5 times the upper limit of normal; cardiovascular dysfunction characterized by arrhythmias or sudden cardiac death; or neurological complications such as seizures [1,11,12].

5. Consequences and risks associated with TLS

Tumor lysis syndrome is a life-threatening condition with a variety of critical symptoms caused by electrolyte imbalances. The presentation of TLS varies and may range from an asymptomatic condition characterized only by laboratory abnormalities to a severe symptomatic condition with multiorgan failure leading to death [13]. One of the leading and most problematic consequences of TLS is renal dysfunction, which can result in uric acid nephropathy, nephrocalcinosis, and obstructive nephropathy. Renal dysfunction can also be induced by allopurinol, which is used in the treatment of TLS and may cause xanthine nephropathy [3]. Apart from acute kidney injury, metabolic changes can cause cardiac dysfunction, impaired muscle function, gastrointestinal disturbances, and neurological distress.

Table 2. Key metabolic abnormalities and their clinical manifestations in tumor lysis syndrome

Metabolic abnormality	Associated clinical manifestations
Hyperkalemia	Cardiac conduction disturbances, such as ECG changes and arrhythmias; muscle weakness, cramps, or paresthesia; gastrointestinal symptoms, including nausea, vomiting, and diarrhea
Hyperuricemia	Renal dysfunction due to uric acid crystal deposition within the renal tubules
Hyperphosphatemia	Acute kidney injury secondary to calcium phosphate precipitation; resulting secondary hypocalcemia
Hypocalcemia	Neuromuscular irritability (tetany, muscle spasms, paresthesia); altered mental status; seizures

Source: [12,13].

6. Tumor lysis syndrome treatment

TLS usually involves a multidisciplinary approach. Cooperation among nephrologists, hematologists, and intensive care specialists is often essential for the successful management of this condition [14,15]. Therapeutic strategies should be tailored to each patient, considering their clinical condition, renal function, metabolic abnormalities, the ability to monitor the patient's status, and the specific type of anticancer treatment used for the primary illness. The foundation of TLS management is usually based on early recognition, laboratory testing, and adequate hydration. Fluid intake is a key factor in diluting harmful solutes released during tumor lysis and maintaining sufficient urine output, and it remains a central component of treatment. Alongside hydration, diuretics may be used appropriately, especially to control volume overload, with furosemide typically being the preferred diuretic. Target hydration volumes are approximately 2–3 L/m^2/day, and patients' hydration status is generally assessed approximately every 6 to 8 hours [16]. While managing TLS, it is important to address electrolyte disturbances and exercise

caution when adjusting treatment. Pharmacological management varies depending on the patient's risk profile. Allopurinol is commonly used to prevent hyperuricemia in low- to intermediate-risk patients, whereas rasburicase is recommended for high-risk patients or for patients with significant hyperuricemia if it is not contraindicated. Febuxostat may be considered in patients who show hypersensitivity to allopurinol [16]. The selection and dosage of these agents are adjusted according to the clinical context and current guidelines. In the treatment of hyperkalemia, oral potassium-lowering agents may represent one of the available treatment options, while rapid-acting insulin and glucose infusion are commonly used in cases of inadequate response or symptomatic hyperkalemia. Hyperkalemia management also focuses on ECG monitoring, specifically in patients with high potassium levels (⩾6 mmol/L) and AKI. Hyperphosphatemia is generally managed by eliminating exogenous phosphate intake from IV solutions and using phosphate binders when appropriate [16]. In cases of symptomatic hypocalcemia, calcium gluconate may be considered [1]. Particular caution in treating electrolyte abnormalities is warranted to avoid potential complications such as calcium phosphate precipitation, which may lead to crystal formation, renal tubular damage, cardiac arrhythmias, and exacerbation of hypocalcemia [16]. Urine alkalinization with acetazolamide or sodium bicarbonate may increase uric acid clearance, but its clinical use is limited because of the risk of calcium phosphate precipitation and subsequent renal impairment [3]. If a metabolic disorder persists and remains resistant to previously applied pharmacological treatment, hemodialysis should be considered [1]. Oliguria or anuria and refractory volume overload may also indicate the need for hemodialysis [5]. The intensity of laboratory monitoring and treatment in patients with tumor lysis syndrome is determined by the risk of TLS, renal function, tumor mass, the dynamics of electrolyte abnormalities, and local institutional protocols. In high-risk patients and those receiving treatment for TLS, serial assessment of electrolytes, creatinine, and uric acid levels may be required as frequently as every 4 to 6 hours, as well as continuous cardiac monitoring when indicated. Symptomatic individuals may require management in an acute care setting, such as an intensive care unit [13,15].

7. Prophylaxis of tumor lysis syndrome

It is necessary to identify patients at the highest risk of developing TLS as early as possible. Prophylaxis for tumor lysis syndrome primarily consists of intravenous hydration and the administration of hypouricemic agents such as allopurinol and rasburicase. The choice of prophylactic strategy is guided by the estimated TLS risk, which is determined by tumor type, disease burden, and the planned therapeutic regimen [15]. It is recommended that patients at any level of TLS risk avoid foods high in potassium or phosphate [16]. In patients at risk of TLS who have acute kidney injury or preexisting renal dysfunction, hemodialysis may be applicable, but further investigation is needed [5]. Moreover, because of the risk of acute kidney injury, nephrotoxic drugs should be avoided whenever clinically feasible. Additionally, nephrocalcinosis in the context of TLS has primarily been described in early case reports involving patients with severe hyperphosphatemia during periods when urinary alkalinization was routinely practiced. The contribution of hyperphosphatemia-induced nephrocalcinosis to acute kidney injury in TLS has not been fully characterized in the modern era of preventive management. To minimize the risk of symptomatic calcium-phosphate precipitation, current recommendations emphasize adequate hydration with isotonic saline, while asymptomatic hypocalcemia should generally remain untreated [17]. Continuous monitoring of vital signs, laboratory results, and clinical symptoms is critical to maintaining patient safety and, when necessary, allowing prompt intervention to improve the patient's condition. Furthermore, ongoing education for healthcare professionals, patients, and their caregivers is essential for preventing tumor lysis syndrome, ensuring early detection, timely intervention, and adherence to prophylactic measures.

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Chapter 6

The role of reticulocytes in differentiating aplastic and hemolytic anemia

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1. Introduction

Maintaining homeostasis is one of the most crucial functions of an organism, ensuring the continuation of all essential life processes. Proper blood composition supports effective gas exchange and oxygen delivery to target tissues, thereby enabling cellular respiration and the production of adenosine triphosphate (ATP) required for biochemical reactions and molecular transformations. When the number of red blood cells decreases due

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to their loss, damage, or ineffective hematopoiesis, symptoms of anemia may develop, such as fatigue, pallor, dizziness, and tachycardia. These symptoms are in part attributable to reduced hemoglobin levels, which impair oxygen transport throughout the body. To maintain stable vital functions, the bone marrow begins to compensate for the hemoglobin deficiency through the rapid production of reticulocytes. Elevated levels of these young forms of red blood cells indicate an adequate bone marrow response and enable the body to restore homeostasis [1]. A heightened reticulocyte level is distinctly observed in hemolytic anemia, in which bone marrow function is preserved and red blood cells can be replaced with newly generated ones. However, an increased reticulocyte count should always be correlated with the patient's clinical status and other laboratory findings to exclude chronic blood loss, which may be another cause of increased erythropoiesis [1]. This compensatory response is not observed in all types of anemia, such as aplastic anemia, in which hematopoietic cell function is impaired. Such conditions require specific diagnostic techniques and advanced treatment, including immunotherapy, blood transfusions, and stem cell transplantation, to ensure stable hemoglobin levels that can support the core functions of the human body. The absolute reticulocyte count (ARC) is valuable in distinguishing among different causes of anemia, but other laboratory parameters, such as lactate dehydrogenase, haptoglobin, iron, folate, vitamin B12, and serum creatinine levels, along with a peripheral blood smear, must also be taken into account to identify nutrient deficiencies or renal failure. A thorough patient history is necessary to exclude hemorrhage, which can also affect the ARC and contribute to the development of anemia symptoms. An individualized approach and clinical correlation are necessary in each case of anemia to ensure appropriate treatment strategy [1].

2. Reticulocytes—development and function

Reticulocytes are erythroid cells that develop in the bone marrow and continue to mature after they are released into the peripheral blood [1].

Initially, hematopoietic stem cells (HSCs), which have migrated to the bone marrow during gestation, interact with various growth factors and cytokines, leading to the proliferation of burst-forming unit–erythroid (BFU-E) cells, which then differentiate into colony-forming unit–erythroid (CFU-E) cells. Proerythroblasts undergo terminal differentiation within erythroid islands, involving complex genetic and structural changes that enable their subsequent function [2]. The process begins with chromatin and nuclear condensation, leading to nuclear expulsion, and is followed by the breakdown of various organelles, such as the Golgi apparatus, mitochondria, and endoplasmic reticulum [1]. Enucleation is believed to be facilitated by nuclear polarization, vimentin removal, and actin accumulation, which enable the mobility of the nucleus. The process also involves the differential distribution of cytoskeletal and membrane proteins that are characteristic of newly formed reticulocytes [2]. Hemoglobin can only be synthesized in the bone marrow, and the process consists of globin production and heme synthesis [3]. Alpha- and beta-globin chains are produced through genetic transcription in the cytosol of erythrocyte precursors, whereas heme is synthesized in the cytosol and mitochondria and requires adequate iron levels [3]. The hemoglobinization process is dependent on transferrin receptor (TfR) expression, which is high in early erythroblasts. When hemoglobin synthesis is nearly complete, TfR expression decreases, and at the orthochromatic erythroblast stage, residual transferrin receptors accumulate at the site of enucleation [2]. Reticulocyte hemoglobin content (CHr) is a laboratory parameter that reflects the hemoglobin present in reticulocytes and correlates with the effectiveness of hematopoiesis, which can be impaired by low iron levels due to inflammation or nutrient deficiencies [4,5]. Once the cells have entered the bloodstream, partial ribonucleic acid (RNA) degradation occurs, with the exception of ribosomal RNA, which can be used in further diagnostics [1]. The volume and membrane structure of reticulocytes undergo important changes that improve their functionality. Their biconcave shape ensures an adequate surface area for gas exchange and enables them to enter capillaries in different tissues [6]. The entire process is triggered by elevated

erythropoietin (EPO) levels, which occur in response to hypoxic stress that occurs when the number of erythrocytes is insufficient to supply tissues with oxygen [1,6]. EPO is a glycoprotein produced in the kidneys by interstitial peritubular cells, as well as by perisinusoidal cells of the liver, and remains essential for red blood cell proliferation and differentiation [7]. Its synthesis depends on tissue oxygenation, which regulates the hypoxia-inducible factor system. Under hypoxic stress, EPO expression is triggered by hypoxia-inducible factor (HIF-1), which binds to the *EPO* gene [2]. Aspects of erythroid cell maturation are illustrated in the diagram below (Figure 1). EPO production depends on the organism's demand for red blood cells, and bone marrow responsiveness is an indicator of its function and a differentiating factor in hematologic conditions. If erythroid cell function is preserved, as in hemolytic anemia, the bone marrow response to decreasing hemoglobin levels and red blood cell counts is clearly evident. By contrast, this response is not observed when the function of hematopoietic cells is impaired, as in aplastic anemia. Reticulocytes remain essential for the regulation of homeostasis and are important in the laboratory diagnosis of hematologic diseases.

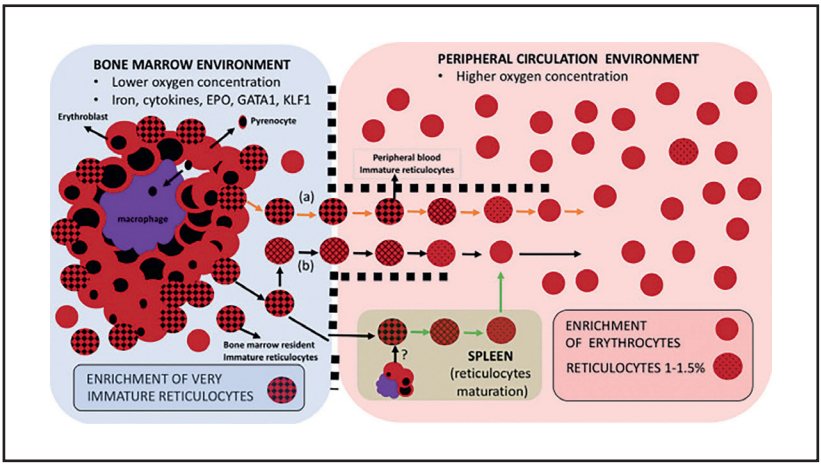


Figure 1. Erythroid cell maturation in the bone marrow and peripheral blood environments

Source: adapted from [8].

3. The difference in aplastic and hemolytic anemia

Aplastic anemia results from bone marrow failure, leading to pancytopenia. Reticulocyte production decreases, and hemoglobin levels become too low to provide the cells with an adequate amount of oxygen. The pathogenesis is complex and involves both acquired and hereditary causes. Bone marrow dysfunction is idiopathic in 65% of cases; however, among hereditary variants, Fanconi anemia is known to be the most common cause [9]. It is usually diagnosed in children, who most commonly present with pancytopenia, hepatitis, and bone defects. Past viral infections and autoimmune disorders may also contribute to the development of aplastic anemia through increased production of cytokines by autoreactive T-helper lymphocytes, such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF), which deplete hematopoietic stem cells by disrupting their intracellular pathways. Natural compensatory mechanisms, including increased erythropoietin production to stimulate erythropoiesis, are impaired, leading to reduced tissue oxygenation. Consequently, therapeutic methods such as blood products transfusions, stem cell transplantation, and immunosuppressive therapy, must be performed to restore homeostasis [9]. The approach is highly individualized and depends on the patient's age, functional status, including kidney and liver efficiency, past function, previous blood transfusions, and disease severity, which may influence bone marrow activity [9]. If left untreated, aplastic anemia may lead to severe complications such as heart failure, an increased risk of hemorrhage, and serious infections, eventually leading to death.

Chronic kidney disease (CKD) is a condition that may lead to inadequate bone marrow activity, ultimately resulting in anemia. This occurs primarily because of decreased EPO production and an insufficient stimulatory effect on the bone marrow due to uremic toxins. In CKD, reduced blood flow disrupts oxygen delivery to the kidneys, adapting them to lower oxygen levels. As a result, hypoxia-inducible factor-mediated signaling becomes impaired, and EPO expression is not adequately increased. Additionally, because of chronic inflammation,

hypoxia-induced EPO production may be suppressed by cytokines such as interleukin 1 (IL-1), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β), which are also thought to contribute to EPO resistance. Increased levels of hepcidin, an acute-phase protein, lead to reduced iron absorption, further contributing to ineffective erythropoiesis [7]. Moreover, the differential diagnosis must include myelodysplastic syndromes, myelophthisis, nutritional deficiencies, and drug- and radiation-induced cytopenias. In myelophthisic syndrome, normal bone marrow is replaced by tumor tissue or fibrosis, which may suppress its erythropoietic function, although it is typically not associated with bone marrow hypocellularity [9].

Folate and vitamin B12 deficiencies lead to megaloblastic anemia, which can mimic the symptoms of bone marrow dysplasia but can be reversed with appropriate supplementation. On the other hand, in hemolytic anemia, in which bone marrow function is preserved, the reticulocyte count increases significantly to compensate for the continuous loss of erythrocytes. However, this response is usually insufficient to fully compensate for reduced hemoglobin levels due to continuous red blood cell damage; therefore, patients may present with symptoms such as fatigue, dyspnea, tachycardia, and hypotension.

A common secondary cause of erythrocyte destruction is the presence of specific antibodies that target and break down red blood cells, with consequent complement activation or phagocytosis resulting in autoimmune hemolytic anemia [10]. Immune system disorders may be caused by previous viral infections or other autoimmune diseases that lead to an increased number of antibodies [11]. Hemolysis may occur intravascularly or, more commonly, extravascularly and may involve both cold and warm antibodies [11]. Other causes of hemolysis that should be taken into account include mechanical trauma, which leads to direct fragmentation of blood elements, or blood transfusions, which activate the immune system and may induce the production of autoantibodies that interact with red blood cells [11].

Therapeutic agents, such as ceftriaxone or piperacillin, may lead to drug-induced immune hemolytic anemia, which can usually be reversed

by discontinuing the administered medication [11]. There are also primary causes of hemolytic anemia, with hereditary spherocytosis being the most prevalent. The cytoskeleton cannot adequately adhere to the erythrocyte lipid bilayer membrane because of genetic mutations that lead to abnormalities in membrane and cytoskeletal proteins such as spectrin or ankyrin. The lipid bilayer and cytoskeletal network are no longer properly supported; thus, the cells lose their biconcave shape [12]. A spherical shape and a weakened membrane reduce plasticity and resistance to osmotic changes, leading to red blood cell degradation. Treatment may include splenectomy because erythrocyte destruction is most pronounced in the spleen [12].

Hemolysis can also occur when the hemoglobin structure is abnormal, as seen in hemoglobinopathies such as sickle cell disease or thalassemia [13]. In sickle cell disease, a mutation in the beta-globin gene leads to the production of abnormal hemoglobin S [14]. Consequently, red blood cells assume a crescent shape and become stiff and sticky, thereby disrupting blood flow and eventually leading to hemolysis with reticulocytosis. In this case, ARC may be used as an indicator of the bone marrow's response to hemolysis as well as a marker of treatment efficacy [15].

In thalassemia, the synthesis of alpha or beta chains is decreased because of autosomal recessive mutations in the alpha-globin or beta-globin genes [16]. Because of the abnormal hemoglobin structure, red blood cells cannot bind and transport oxygen effectively, which leads to their premature destruction through hemolysis and increased erythropoiesis as a compensatory mechanism.

There are also enzymopathies in which decreased levels of enzymes involved in metabolic processes in red blood cells, such as glucose-6-phosphate dehydrogenase (G6PD) or protein kinase D (PKD), lead to hemolysis [13]. The diagnostic process should also include clinical evaluation along with laboratory findings. For instance, patients presenting with hematuria and elevated hemolysis markers, such as an elevated reticulocyte count and increased lactate dehydrogenase levels, may be affected by paroxysmal nocturnal hemoglobinuria (PNH). PNH is

an acquired membranopathy that leads to complement-mediated intravascular hemolysis, thrombosis, and bone marrow failure [9,13]. The disease exhibits characteristics of both hemolytic and aplastic anemia and illustrates the complexity of the diagnostic process. Both hereditary and secondary types of hemolytic anemia most frequently result in a rapid increase in the absolute reticulocyte count, a natural compensatory response that is one of the most important factors in distinguishing hemolytic from aplastic anemia. However, various medical conditions can lead to reticulocytosis; therefore, the diagnostic process must be thorough.

Acute or chronic blood loss is one of the most common causes of anemia and results in increased erythropoiesis as a natural compensatory mechanism; therefore, blood loss should be excluded [17]. The differential diagnosis of hemolytic anemia is complex, and treatment options must focus on managing the underlying conditions and usually include blood transfusions, plasmapheresis, or diuresis.

4. The significance of laboratory diagnostics

Modern laboratory diagnostics enable the differentiation of a wide range of anemia types and, consequently, the administration of appropriate treatment. They are particularly useful for distinguishing between aplastic and hemolytic anemia, which arise from different mechanisms of disease development. A reduced red blood cell count, resulting in a decreased hemoglobin levels is observed in both types of anemia and is responsible for the usual symptoms. A comparison of hemoglobin levels among healthy individuals and patients with aplastic or hemolytic anemia is illustrated in the graph on the following page (Figure 2).

The number of young red blood cells, known as the absolute reticulocyte count (ARC), is a key differentiating factor that indicates bone marrow activity. Along with a complete blood count and peripheral blood smear, ARC provides data necessary for distinguishing various causes of cytopenia [9]. Distinguishing between hemolysis and

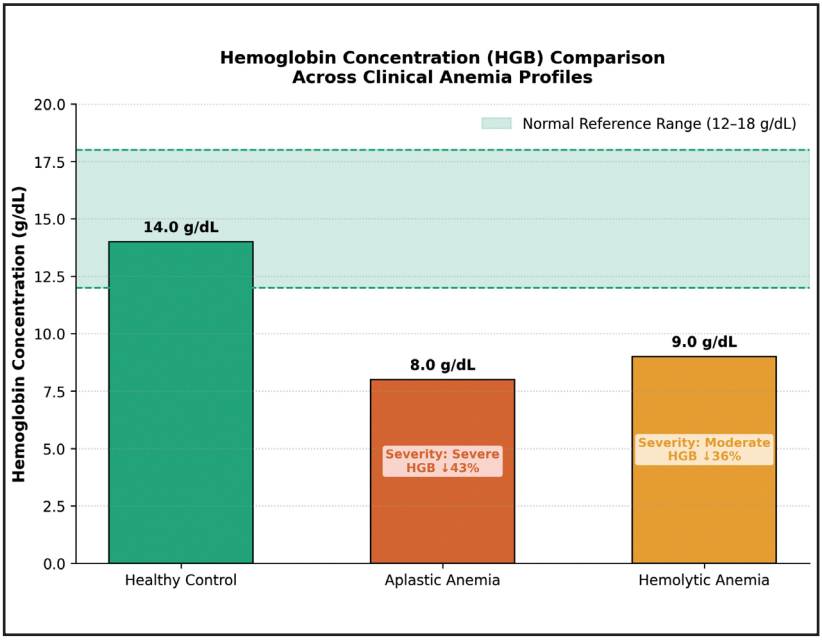


Figure 2. Comparison of hemoglobin levels among healthy individuals and patients with aplastic or hemolytic anemia
Source: based on data from [18–20].

hemorrhage requires consideration of the clinical history, physical examination, and laboratory findings, as both conditions may lead to reticulocytosis. Increased lactate dehydrogenase and indirect bilirubin levels, together with decreased haptoglobin, may support hemolysis as the cause of anemia, while a positive direct antiglobulin test usually indicates an immune-mediated mechanism of hemolysis [11]. In aplastic anemia, compensation for the decreased number of red blood cells is not possible because of impaired hematopoietic cell function. Thus, the number of reticulocytes does not increase, resulting in progressive, uncompensated anemia. The absolute reticulocyte count is used not only to diagnose and differentiate the disease but also to monitor the bone marrow response to various anemia therapies, as

it is a parameter that reflects erythropoietic activity [18]. In contrast, patients with hemolytic anemia present with an elevated absolute reticulocyte count in response to increased red blood cell loss. This response reflects preserved erythropoietic cell function and enables the differential diagnosis of aplastic and hemolytic anemia. Another parameter that may be used to diagnose and monitor anemia is CHr, which can be elevated in some cases of hemolytic anemia [21]. This can be explained by increased reticulocyte production, which consequently increases the overall hemoglobin content of reticulocytes. Additionally, patients with aplastic anemia may present with a slightly elevated mean corpuscular volume (MCV) due to a decrease in stem cell number and ineffective maturation of the remaining erythroblasts triggered by increased levels of EPO and cell-stimulating factors, leading to macrocytic anemia [22]. However, this phenomenon is not broadly observed and should not be considered the main differentiating factor, but rather an additional one. Increased MCV may also be observed in hemolytic anemia because of prolonged hemolysis followed by marked reticulocytosis, leading to folic acid depletion [23]. Patients usually present with other abnormalities in laboratory test results, such as decreased white blood cell and platelet counts in aplastic anemia; increased levels of lactate dehydrogenase and indirect bilirubin, accompanied by decreased levels of haptoglobin, in hemolytic anemia; and decreased hematocrit in both types of anemia. These parameters, combined with ARC, are taken into account during diagnosis and may be helpful in differentiating the diseases. Moreover, the diagnostic evaluation of patients with aplastic anemia must include bone marrow aspiration or trephine biopsy to identify the specific cause of bone marrow failure. Each case must be considered individually and requires consideration of the patient's current medical condition. The differentiation process should also include clinical evaluation together with laboratory diagnostics. A comparison of blood parameters in both types of anemia is presented in the table on the following page (Table 1).

Table 1. Blood parameters in aplastic and hemolytic anemia

Parameter (unit)	Reference range	Aplastic anemia	Hemolytic anemia
Hemoglobin (g/dL)	M: 13–18; F: 12–16	5–8	7–10
Hematocrit (%)	M: 40–54; F: 36–48	15–25	20–30
Absolute reticulocyte count ($\times 10^9$/L)	25–100	<10	>100
Reticulocyte production index (RPI)	1–2	<1	>3
White blood cells (WBC) ($\times 10^9$/L)	4.5–11.0	1.0–3.0	5.0–12.0
Platelets ($\times 10^9$/L)	150–400	10–50	150–500
Mean corpuscular volume (MCV) (fL)	80–100	80–100 or >100	80–100 or >100
Lactate dehydrogenase (LDH) (U/L)	100–220	100–220	300–1000
Indirect bilirubin (mg/dL)	0.2–0.8	0.2–0.8	1–3.5
Haptoglobin (mg/dL)	30–200	30–200	<30
Peripheral smear	—	Pancytopenia, normocytic or macrocytic	Schistocytes, spherocytes

Prepared by the authors based on data from [13,19,20,24–26]. Values are illustrative and may vary depending on the severity of anemia and the patient's comorbidities.

Precise identification of blood cell populations is possible through advanced laboratory techniques. One of the most important is flow cytometry, which enables the distinction of different cells by detecting specific markers on their surface.

Reticulocytes can be detected based on characteristic cell-surface markers, residual RNA, or cytoskeletal structure. Peripheral blood smears can be stained with laboratory dyes such as methylene blue or

brilliant cresyl blue to identify reticulocytes, which appear polychromatic under light microscopy. They are also larger than mature erythroblasts and display the highest concentration of residual intracellular RNA, which is visible as aggregated granules when stained with reticulin. The adhesion markers most commonly found on early-stage reticulocytes are TfR, also known as CD71, glycoprotein IV (CD36), and integrin receptor α4β1 (VLA-4). CD71 is essential for iron-dependent erythroid growth, and CD36 is involved in cell differentiation. Their expression decreases as reticulocytes mature. VLA-4 is an adhesion glycoprotein mostly present on erythroid progenitors, and its synthesis is drastically reduced before reticulocytes enter the bloodstream; it can therefore be found only on the most immature forms. Increased expression of these molecules indicates accelerated erythropoiesis, which may be caused by different types of hemolytic anemia or hemorrhage. Another useful method is flow cytometry, which enables the distinction and enumeration of different cells by detecting specific markers on their surface [15]. The technology uses one or more lasers to measure the light-scattering and fluorescence parameters needed to analyze particles previously suspended in a buffered salt-based solution [27]. Before the procedure, the cells are stained with fluorescent dyes or fluorescently conjugated antibodies to facilitate precise detection. The enumeration of reticulocytes by flow cytometry enables an accurate assessment of erythropoietic activity, which can be helpful in differentiating the causes of anemia. First, the patient's whole blood must be mixed with a thiazole orange solution to stain the reticulocytes [1]. The dye binds to rRNA in reticulocytes, allowing them to be detected by the laser [1]. The collected fluorescence parameters are analyzed to determine the actual number of young blood cells, which is used for further diagnostic evaluation of anemia. Moreover, flow cytometry has proven useful for confirming autoimmune causes of hemolytic anemia. Reticulocyte-bound IgG can be readily detected using this method, even in patients with low autoantibody levels. The conventional direct antiglobulin test, known as the Coombs test, which is used for autoantibody detection, may not be sufficiently sensitive

and can lead to false-negative results; therefore, flow cytometry is increasingly used in advanced diagnostics.

The graph below shows a comparison of blood cell counts among healthy individuals and patients with aplastic or hemolytic anemia based on parameters that can be obtained by flow cytometry (Figure 3).

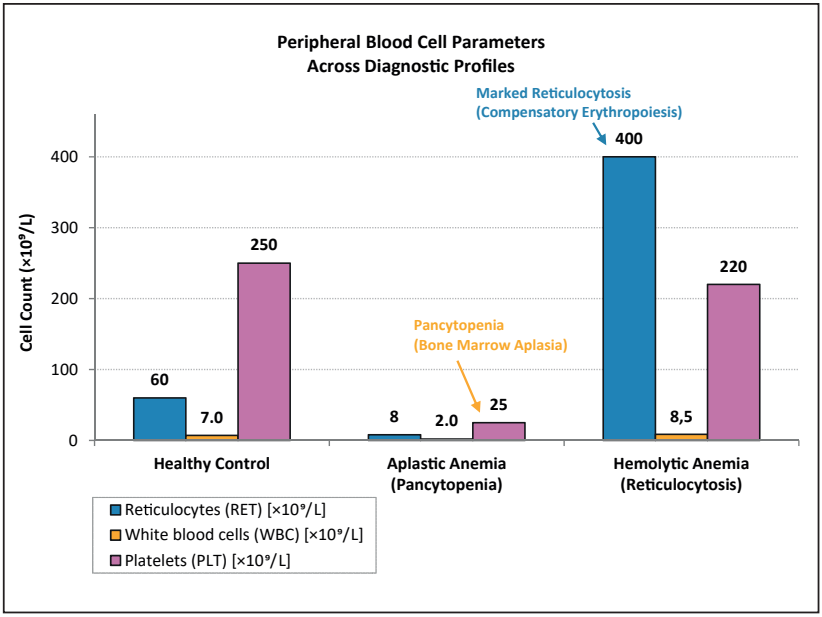


Figure 3. Comparison of selected blood cell populations comparison between healthy individuals and patients with aplastic or hemolytic anemia (The reticulocyte count is markedly increased in hemolytic anemia, indicating increased bone marrow activity. In aplastic anemia, all hematopoietic pathways are impaired, resulting in pancytopenia and a significantly reduced reticulocyte count.)

Source: prepared by the authors based on data from [1,13,19,22,25].

Accurate diagnosis facilitated by advanced laboratory techniques leads to appropriate treatment choices, resulting in therapeutic success and improved clinical outcomes. The treatment of hemolytic anemia,

especially when autoimmune in origin, includes the management of underlying diseases that contribute to immune dysregulation, blood component transfusions, and folic acid supplementation [11]. Patients with aplastic anemia, confirmed by bone marrow biopsy and further evaluated using laboratory, cytogenetic, and molecular tests to determine the most effective and personalized treatment options, usually need to undergo immunosuppressive therapy, blood component transfusions, and ultimately stem cell transplantation, which targets the main problem in aplastic anemia, namely bone marrow failure [9]. Therapeutic interventions must take into account the patient's age, disease severity, and donor availability. Among patients under 40 years of age with severe or very severe aplastic anemia, allogeneic hematopoietic stem cell transplantation (HSCT) is the preferred therapeutic method. Patients over 40 years of age diagnosed with severe aplastic anemia are usually considered for triple immunosuppressive therapy (IST). Patients with concomitant cardiac or renal conditions that may require lower-intensity IST should preferably undergo optimization of treatment for the underlying diseases to become medically fit for full IST. The treatment of moderate aplastic anemia is individualized and depends on the number of RBC transfusions and the patient's quality of life. It usually consists of lower-intensity IST, while HSCT is considered occasionally in younger patients with more complex clinical needs [9]. Each case requires accurate clinical evaluation and laboratory diagnostics and must be followed by an individualized therapeutic approach aimed at achieving a satisfactory treatment outcome.

5. Summary

The differential diagnosis of aplastic and hemolytic anemia requires the integration of various laboratory parameters, with the absolute reticulocyte count remaining a useful indicator of the erythropoietic response. Along with other clinical and laboratory findings, ARC may be helpful in distinguishing among different causes of anemia. The evaluation must include the patient's comorbidities and a detailed clinical assessment.

Key diagnostic parameters, including hemoglobin level, white blood cell count, platelet count, and mean corpuscular volume, are measured as part of a complete blood count, while flow cytometry provides an accurate assessment of reticulocytes and immune-mediated hemolysis. Precise diagnosis enables targeted treatment, improves clinical outcomes, and ensures an individualized approach to patient care. Recognition of laboratory and pathophysiological differences is essential for effective patient management.

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