Leukemia

Weijie Li, MD, PHD
Editor

eXon PUBLICATIONS

Leukemia

Leukemia

Edited by

WEIJIE LI, MD, PHD

Department of Pathology and Laboratory Medicine,
Children's Mercy Hospital, University of Missouri-Kansas City
School of Medicine, Kansas City, USA

eXon
PUBLICATIONS

Leukemia
ISBN: 978-0-6453320-7-0
DOI: https://doi.org/10.36255/exon-publications-leukemia

Edited by
Weijie Li, MD, PHD, Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

Published by
Exon Publications, Brisbane, Australia

First Published in October 2022

CONTENTS

DOI: https://doi.org/10.36255/exon-publications-leukemia.frontmatter

FOREWORD

Leukemia is a hematological malignancy caused by clonal proliferation of abnormal lymphoid or hematopoietic cells in the bone marrow and/or lymphoid system. There are many different types of leukemias. Acute leukemia progresses fast, and patients' condition deteriorates quickly without treatment; while chronic leukemia progresses slowly, and the patients may stay with the disease for a long time even without treatment. Acute leukemia is predominantly the malignancy of lymphoid or hematopoietic precursors (blasts). Based on cell origin, acute leukemia can be classified as acute lymphoblastic leukemia, acute myeloid leukemia, or acute leukemia of ambiguous lineage. Chronic leukemia represents the malignancy of mature or maturing lymphoid or hematopoietic cells, and predominantly affects adults. Leukemias can be further classified into many subtypes with the incorporation of more clinical, phenotypic, and genetic information. The frontline treatment for leukemia is chemotherapy.

Tremendous advances have been made in the leukemia field during the last several decades. Our understanding of the biology of different types of leukemia has been significantly improved through a huge amount of basic and clinical research. The application of advanced diagnostic technologies has led to the identification of many new leukemia subtypes with distinct genetic defects. Some of these defects have the potential for targeted therapy. Technological advances have also allowed for the identification of many new prognostic and predictive biomarkers, and the more sensitive detection of minimal residual disease. These biomarkers have significantly contributed to the success of individualized risk stratification and subsequent risk-adapted therapies. The clinical outcomes of all leukemias have been significantly improved. As the most common malignancy in children, pediatric acute lymphoblast leukemia, a fatal disease in the past, has become a highly curable disease with a curable rate of over 80%.

This *Leukemia* book written by an international team has covered many of these achievements. It provides the most up-to-date information on important topics such as new WHO classification, prognostic and predictive biomarkers, minimal residual disease, pharmacogenomics, diagnostic use of flow cytometry, and gives updates on several interesting leukemia entities such as infant leukemia, hairy cell leukemia, acute myeloid leukemia with myelodysplasia-related changes, secondary acute myeloid leukemia, and myeloid/lymphoid neoplasms with PDGFRA rearrangement, respectively. I am sure this book is of great value to a wide audience seeking information in the leukemia field either for clinical purposes or research purposes. It has been my pleasure to preview this interesting book and help bring it forth to readers. I congratulate all the authors for their great work.

Jignesh Dalal, MD
Professor, Department of Pediatrics, School of Medicine
Member, Developmental Therapeutics Program,
Case Comprehensive Cancer Center
Case Western Reserve University
Cleveland, Ohio, USA
October 2022

DOI: https://doi.org/10.36255/exon-publications-leukemia.foreword

PREFACE

Leukemia is a heterogeneous group of hematological malignancies characterized by the proliferation of abnormal lymphoid or hematopoietic cells in the bone marrow, frequently involving peripheral blood, spleen, and lymph nodes. Leukemia is the 10th most common human malignancy, with over 60,000 new cases diagnosed in the United States every year and approximately 1.5% of people diagnosed at some point during their lifetime. Tremendous advances have been made in the biology and genetics of leukemia during the past several decades, which have translated into significantly improved clinical management and survival outcome. This 10-chapter book provides the most up-to-date information on important topics such as prognostic and predictive biomarkers, new World Health Organization (WHO) classification, pharmacogenomics, and measurable (minimal) residual disease (MRD). Also included in this book are in-depth reviews of several interesting leukemia entities, which update these entities from various aspects including etiology, pathogenesis, histopathology, diagnosis, treatments, and clinical outcomes.

The WHO classification of tumors has provided a unified tumor classification system enabling people across the world to share their experience, knowledge, and research results. Ongoing research work conducted by many researchers and physicians has been leading to our continuously improved understanding of tumors and the continual production of new editions of WHO classification with updates. It is no surprise that the newly released 5th edition of WHO classification of hematolymphoid tumors has numerous revisions, which are succinctly summarized in Chapter 1. All the leukemia entities have been updated or revised with a variable degree of changes, including the addition of new subtypes, deletion of subtypes, terminology or nomenclature revision, name changes, revised diagnostic criteria, revised categorization, etc. No matter whether you need to study or you are interested in studying this 5th edition of WHO classification, you will find that Chapter 1 is very helpful.

Chapters 2 and 3 provide comprehensive overviews of two unique leukemia entities: infant leukemia and hairy cell leukemia. Both entities have distinct genetic alterations, which are detailed in these two chapters. Chapter 3 also gives extensive updates on the current treatments for newly diagnosed and relapsed/refractory hairy cell leukemia. One of the biggest improvements in our journey of understanding and treating leukemia is the application of flow cytometry immunophenotyping in the diagnosis and classification. Chapter 4 provides an overview of the principles and the significant roles of flow cytometry in the diagnosis and classification of leukemias. Flow cytometry is also a useful tool for detecting MRD, which is the strongest independent prognostic factor for leukemia. Patients with undetectable MRD or good MRD responses have significantly lower relapse risk and better survival outcomes compared with similarly treated patients with detectable MRD or adverse MRD responses. MRD testing has become increasingly important in risk stratification and guiding individualized therapy for patients with leukemias, especially acute leukemia. Quantitative PCR is another commonly used method for MRD testing. Recently, innovative technologies such as

next-generation sequencing and digital PCR have also been applied in the clinical studies of MRD assessment and have shown improved sensitivity and accuracy. Chapter 5 gives a comprehensive overview of the methods and the clinical significance of MRD testing in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

Chapters 6 and 7 are dedicated to two types of AML with poor prognosis: AML with myelodysplasia-related changes (AML-MRC) and secondary AML. Chapter 6 gives full coverage of AML-MRC, including epidemiology, pathology, genetic alterations, diagnosis, clinical features, treatments, and prognosis, while Chapter 7 focuses on the pathogenesis and treatments of secondary AML. Chapter 8 covers a rare disease of myeloid/lymphoid neoplasms with platelet-derived growth factor receptor alpha (PDGFRA) rearrangement, with emphasis on the diagnosis and the differential diagnosis.

ALL is the most common childhood cancer. Thanks to the risk stratification and subsequent risk-adapted treatment, ALL has become the most curable malignancy in children, with long-term survival rate close to 90%. Chapters 9 and 10 tell the key elements contributing to the success of risk stratification and risk-adapted therapies in ALL. Chapter 10 provides an up-to-date comprehensive overview of the prognostic and predictive biomarkers in B-ALL, which include over 20 genetic biomarkers, some immunophenotypic biomarkers, and other biomarkers that predict prognosis or therapeutic response. Recent advances in molecular diagnostic technologies have led to a rapid expansion of the list of molecular biomarkers associated with ALL. Most of these recently identified biomarkers are included in Chapter 10. These new genetic biomarkers show promise to improve the accuracy of risk prediction, and eventually achieve better risk-adapted treatment and clinical outcomes. Chapter 9 focuses on the genetic variations that are associated with toxicity and/or resistance to most of the chemotherapy drugs used for ALL, which are also important factors to guide personalized treatment.

I thank all the authors for their outstanding contributions and the time they have devoted to this book. Most of the chapters in this book are aimed primarily at physicians, researchers, and other healthcare professionals, but some areas may also capture the interest of the laypersons. Studies of leukemia have always been at the forefront in applying the findings of basic research to the understanding and treatments of human diseases and have many exciting achievements. I hope this book will encourage readers to dive into this field, embracing the achievements cheerfully and viewing the challenges optimistically.

Weijie Li, MD, PhD, FCAP
Department of Pathology and Laboratory Medicine
Children's Mercy Hospital
University of Missouri-Kansans City School of Medicine
Kansas City, MO, USA
October 2022

DOI: https://doi.org/10.36255/exon-publications-leukemia.preface

LIST OF CONTRIBUTORS

AGNIESZKA JANUS, MD, PHD

Department of General Hematology, Copernicus Memorial Hospital, Lodz, Poland

ALESSANDRO FIORENTINI, MD

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

ATTILIO OLIVIERI, MD

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

DEBORA CAPELLI, MD

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

DEYANIRA ESCALANTE-BAUTISTA, MS

Laboratorio de Hematobiología, ENMyH, Instituto Politécnico Nacional, Mexico City, Mexico; Unidad de Investigación Médica en Genética Humana, UMAE Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social IMSS, Av. Cuauhtémoc 330, Col Doctores, Delegación Cuauhtémoc, 06720, Mexico City, Mexico

DIEGO MENOTTI, MD

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

DORIS CERECEDO, PHD

Laboratorio de Hematobiología, Escuela Nacional de Medicina y Homeopatía, I.P.N., Guillermo Massieu Helguera No. 239, Col. La Escalera Ticomán, 07320, Ciudad de México (CDMX), Mexico

FRANCESCO SARACENI, MD

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

HAYDEÉ ROSAS-VARGAS, PHD

Unidad de Investigación Médica en Genética Humana, UMAE Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social IMSS, Av. Cuauhtémoc 330, Col Doctores, Delegación Cuauhtémoc, 06720, Mexico City, Mexico

LEI ZHANG, PHD

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

MOTOHARU SHIBUSAWA, MD

IMS group Shinmatsudo Central General Hospital, Department of Hematology, Chiba, Japan

PRABHJOT KAUR, MD

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH, USA

SAHIBU SULTAN M. HABEEBU, MD, PHD

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

SEVGI GÖZDAŞOĞLU, MD

Department of Pediatrics, Divisions of Hematology and Oncology, Ankara University Medical School, Ankara, Turkey

TADEUSZ ROBAK, MD, PHD

Department of Hematology, Medical University of Lodz, Lodz, Poland

TETSUYA TANIMOTO, MD

Navitas Clinic Kawasaki, Kanagawa, Japan

WAHAB A. KHAN, PHD

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH, USA

WEIJIE LI, MD, PHD

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

DOI: https://doi.org/10.36255/exon-publications-leukemia.contributors

1

The 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors

Weijie Li

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

Author for correspondence: Weijie Li, Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, USA. Email: wli@cmh.edu

Cite this chapter as: Li W. The 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 15 Sep 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-who-5th-edition-hematolymphoid-tumors

Abstract: The WHO classification of tumors of various organ systems, also known as the WHO Blue Books, has provided a unified tumor classification system enabling people across the world to share their knowledge and research results. Newer editions with updates have been made every five to ten years to reflect our better understanding of these diseases through the ongoing research work conducted by many researchers and physicians. The last edition of the WHO classification of hematolymphoid tumors was the 4th edition released in 2008 and revised in 2017. Recently, the 5th edition of the WHO classification of hematolymphoid tumors was released, with the online version available since August 2022, and the print version expected to be out at the end of 2022. The 5th edition has been completely rewritten with numerous changes and updates, which include revised hierarchical classification structure, addition or deletion of entities or subtypes, changes or revisions of terminology or nomenclature, revisions or changes of

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

diagnostic criteria, and updates of pathogenesis, clinical and genetic features. Stroma-derived tumors of lymphoid tissue and tumor-like lesions have been included for the first time. This chapter provides a brief overview of the 5th edition of the WHO classification of hematolymphoid tumors with a focus on the changes and updates from a reader's perspective.

Keywords: 5th edition of the WHO classification of hematolymphoid tumors; hematolymphoid tumors; hematopoietic neoplasm; lymphoid neoplasm

INTRODUCTION

The World Health Organization (WHO) classification of tumors of various organ systems, also known as the WHO Blue Books, has provided a unified tumor classification system enabling people across the world to share their knowledge and research results. Newer editions with updates have been made every five to ten years to reflect our better understanding of these diseases through the ongoing research work conducted by many researchers and physicians. The last edition of the WHO classification of hematolymphoid tumors was the 4th edition released in 2008 (1), which was revised in 2017 (R4th WHO-Hem) (2). The electronic version of the 5th edition of the WHO classification of hematolymphoid tumors (5th WHO-Hem) was released online in August 2022 (3), and the print version is expected to be out at the end of 2022 (4). The main updates of almost all the entities have been described in two fantastic review articles written by the authors of the 5th WHO-Hem and published in *Leukemia* in July of 2022 (5, 6). With a completely new team of editors and authors, the 5th WHO-Hem has been completely rewritten with significant rearrangement of the contents and numerous changes and updates, which include the addition of some non-hematolymphoid tumors and non-neoplastic lesions for the first time. As a reader, I applaud this great work and appreciate all the authors who have dedicated themselves to this book, and all the researchers whose research findings have contributed significantly to our better understanding of these diseases, although I personally do not think all the changes, especially the change of certain terminologies, are necessary. This chapter provides a brief overview of the 5th WHO-Hem with a focus on the changes and updates from a reader's perspective. Hopefully, it will help other readers in their learning of the 5th WHO-Hem.

REVISED LINEAGE-BASED CLASSIFICATION STRUCTURE AND REARRANGEMENT OF THE CONTENTS

As shown in Figure 1, according to the differentiation of the tumor cells, the majority of hematolymphoid tumors can be classified into one of two general categories: myeloid and lymphoid. The latter is composed of two groups: B-cell and T/NK-cell. Further classification is based on the maturation stage, phenotypic character, histomorphologic features, clinical information, and cytogenetic/molecular genetic findings. To better reflect this lineage-based framework, the 16 chapters of the

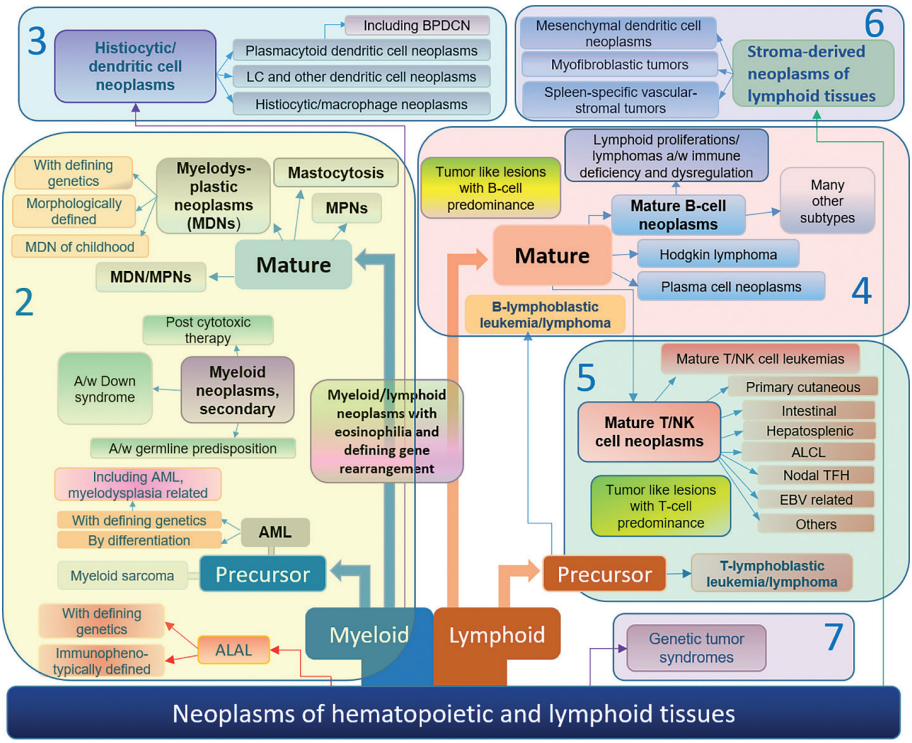

Figure 1. Lineage-based hierarchical classification structure and arrangement of the contents in the 5th edition of WHO classification of hematolymphoid tumors. The numbers represent corresponding chapters: 2, *Myeloid proliferations and neoplasms*; 3, *Histiocytic/Dendritic cell neoplasms*; 4, *B-cell lymphoid proliferations and lymphomas*; 5, *T-cell and NK-cell lymphoid proliferations and lymphomas*; 6, *Stroma-derived neoplasms of lymphoid tissues*; 7, *Genetic tumor syndromes*. ALAL, Acute leukemia of ambiguous lineage; ALCL, anaplastic large cell lymphoma; AML, acute myeloid leukemia; A/w, associated with; BPDCN, blastic plasmacytoid dendritic cell neoplasm; LC, Langerhans cell; MDN, myelodysplastic neoplasm; MPN, myeloproliferative neoplasm; TFH, T follicular helper cell.

R4th WHO-Hem have been combined and rearranged into 4 chapters in the 5th WHO-Hem: *Myeloid proliferations and neoplasms* (chapter 2), *Histiocytic/dendritic neoplasms* (chapter 3), *B-cell lymphoid proliferations and lymphomas* (chapter 4), and *T-cell lymphoid proliferations and lymphomas* (chapter 5). Two new categories, *Stroma-derived neoplasms of lymphoid tissues* (chapter 6) and *Genetic tumor syndromes* (chapter 7), have been added (Figure 1).

To be consistent with other volumes of the 5th edition of WHO Blue Books, each entity in the 5th WHO-Hem has been described under the headings of *Definition*, *ICD-O coding*, *ICD-10 coding* (new), *Related terminology* (replacing *Synonyms* in R4th WHO-Hem), *Subtype(s)*, *Location*, *Clinical features*, *Epidemiology*, *Etiology*, *Pathogenesis* (new), *Macroscopic appearance*, *Histopathology* (replacing *Microscopy*, *Cytochemistry and Immunophenotype* in R4th WHO-Hem), *Cytology* (new), *Diagnostic molecular pathology* (new), *Essential and desirable diagnostic criteria* (new), *Staging*, *Prognosis and prediction*. The *Definition* part is much shorter and

more concise for most of the entities compared with the R4th WHO-Hem. There is a discussion of differential diagnosis at the end of the *Histopathology* section, which is new and overall very useful albeit the variation in length and depth. *Pathogenesis* section describes the evidence or theories of tumorigenesis related to the entity. *Diagnostic molecular pathology* lists the key molecular and/or cytogenetic findings. In the previous editions, well-defined diagnostic criteria had only been given for some myeloid neoplasms and plasma cell neoplasms (1, 2). A big improvement in this edition is the generation of essential diagnostic criteria for all the entities, and desirable diagnostic criteria for some entities.

Myeloid proliferations and neoplasms include 9 sub-categories (families): *Myeloid precursor lesions* (new), *Myeloproliferative neoplasms (MPNs)*, *Mastocytosis*, *Myelodysplastic neoplasms* (*MDNs*, previously known as *myelodysplastic syndrome*, *MDS*), *MDN/MPNs*, *Acute myeloid leukemia (AML)*, *Secondary myeloid neoplasms* (new), *Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangement*, *Acute leukemias of mixed or ambiguous lineage*. Juvenile myelomonocytic leukemia (JMML), a subtype of *MDN/MPN* in the R4th WHO-Hem, is now included in the *MPN* family due to its well-established molecular pathogenesis and lack of key features of MDN (7). MDNs in the 5th WHO-Hem are separated into three groups: *MDNs, with defining genetic abnormalities*; *MDNs, morphologically defined*; and *MDNs of childhood*. Blast count (low or increased blasts) has replaced lineage involvement (single or multiple) as a modifier for MDN entities. AML is separated into two groups: *AML with defining genetic abnormalities* and *AML defined by differentiation*. The former contains 13 entities including 2 newly added entities with defining genetic abnormalities and *AML, myelodysplasia related*, which was named "AML with myelodysplasia related changes" and was a separated group in the R4th WHO-Hem (2). *AML defined by differentiation* replaces "AML NOS" in the R4th WHO-Hem (2). Secondary myeloid neoplasms include *Myeloid neoplasm post cytotoxic therapy*, *Myeloid neoplasms associated with germline predisposition,* and *Myeloid proliferations associated with Down syndrome*. AML cases transformed from MPNs are not included in this family, and they should be called MPN in blastic phase. Secondary AML transformed from MDNs or MDN/MPNs belongs to *AML, myelodysplasia related*. Myeloid neoplasms arising in patients with Fanconi anemia, and RASopathies are discussed in the chapter of *Genetic tumor syndromes*. There are three new subtypes (*FLT3* rearrangement, *ETV6::ABL1* fusion, and other tyrosine kinase fusion genes) added into *Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangements*. The entities in *Acute leukemias of mixed or ambiguous lineage* are grouped under either *Acute leukemia of ambiguous lineage (ALAL) with defining cytogenetic abnormalities* or *ALAL, immunophenotypically defined*. The former includes mixed-phenotype acute leukemia (MPAL) with *BCR::ABL1* fusion, MPAL with *KMT2A* rearrangement, and ALAL with other defined genetic alterations. The latter includes ALAL, B/myeloid; ALAL, T/myeloid; ALAL, rare types; ALAL, NOS; and acute undifferentiated leukemia.

B-cell lymphoid proliferations and lymphomas consist of 5 sub-categories (families): *Tumor-like lesions with B-cell predominance* (new), *Precursor B-cell neoplasms*, *Mature B-cell neoplasms*, *Hodgkin lymphoma*, and *Plasma cell neoplasms (PCNs) and other diseases with paraproteins*. Precursor B-cell neoplasms include 12 entities of *B-lymphoblastic leukemia/lymphoma (B-LBL/L) with defined genetic alterations* and *B-LBL/L, NOS*. Mature B-cell neoplasms consist of 12 sub-families/entities: *Pre-neoplastic and neoplastic small lymphocytic proliferations*, *Splenic B-cell*

lymphomas and leukemias, *Lymphoplasmacytic lymphoma*, *Marginal zone lymphoma (MZL)*, *Follicular lymphoma (FL)*, *Cutaneous follicle center lymphoma*, *Mantle cell lymphoma*, *Transformations of indolent B-cell lymphomas* (new), *Large B-cell lymphomas*, *Burkitt lymphoma*, *KSHV/HHV-8 associated B-cell lymphoid proliferations and lymphomas*, *Lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation*. The last one replaces *Immunodeficiency-associated lymphoproliferative disorders* (a separated chapter) in the R4th WHO-Hem (2) and includes rare T/NK-cell lymphomas. Large B-cell neoplasms include 18 entities: *Diffuse large B-cell lymphoma, NOS; T-cell/histiocyte-rich large B-cell lymphoma; Diffuse large B-cell lymphoma / high grade B-cell lymphoma with MYC and BCL2 rearrangements*; *ALK-positive large B-cell lymphoma*; *Large B-cell lymphoma with IRF4 rearrangement*; *High grade B-cell lymphoma with 11q aberrations*; *Lymphomatoid granulomatosis*; *EBV-positive diffuse large B-cell lymphoma*; *Diffuse large B-cell lymphoma associated with chronic inflammation*; *Fibrin-associated large B-cell lymphoma*; *Fluid overload-associated large B-cell lymphoma*; *Plasmablastic lymphoma*; *Primary large B-cell lymphoma of immune-privileged sites*; *Primary cutaneous diffuse large B-cell lymphoma, leg type*; *Intravascular large B-cell lymphoma*; *Primary mediastinal large B-cell lymphoma*; *Mediastinal grey zone lymphoma*; and *High-grade B-cell lymphoma, NOS*. Hodgkin lymphomas remain the same, but classic Hodgkin lymphomas are described together with subtypes not separated. The family of *PCNs and other diseases with paraproteins* has been reorganized, and it includes 4 groups: *Monoclonal gammopathies (MGs)*, *Diseases with monoclonal immunoglobulin deposition*, *Heavy chain diseases*, and *PCNs*. MGs consist of *Cold agglutinin disease* (new), *IgM and non-IgM MG of undetermined significance (MGUS)*, *and Gammopathy of renal significance* (*MGRS*, new). PCNs include *Plasmacytoma*, *Plasma cell myeloma/multiple myeloma,* and *PCNs with associated paraneoplastic syndrome*.

T-cell and NK-cell lymphoid proliferations and neoplasms include 3 sub-categories (families): *Tumor-like lesions with T-cell predominance* (new), *Precursor T-cell neoplasms*, and *Mature T-cell and NK-cell neoplasms*. Mature T-cell and NK-cell neoplasms consist of 9 sub-families/entities: *Mature T-cell and NK-cell leukemias* (new), *Primary cutaneous T-cell lymphoid proliferations and lymphomas*, *Intestinal T-cell lymphoid proliferations and lymphomas*, *Hepatosplenic T-cell lymphoma*, *Anaplastic large cell lymphoma*, *Nodal T-follicular helper (TFH) cell lymphoma*, *Other peripheral T-cell lymphomas (peripheral T-cell lymphoma, NOS)*, *EBV-positive NK-cell and T-cell lymphomas*, *EBV-positive T-cell and NK-cell lymphoid proliferations and lymphomas of childhood*. Mature T-cell and NK-cell leukemias include 6 entities: *T-prolymphocytic leukemia*, *T-large granular lymphocytic leukemia* (*T-LGLL*), *NK-large granular lymphocytic leukemia*, *Adult T-cell leukemia/lymphoma*, *Sezary syndrome*, and *Aggressive NK-cell leukemia*. Primary cutaneous T-cell lymphoid proliferations and lymphomas include 9 entities: *Primary cutaneous CD4-positive small or medium T-cell lymphoproliferative disorder*, *Primary cutaneous acral CD8-positive T-cell lymphoproliferative disorder*, *Mycosis fungoides*, *Lymphomatoid papulosis*, *Primary cutaneous anaplastic large cell lymphoma*, *Subcutaneous panniculitis-like T-cell lymphoma*, *Primary cutaneous gamma/delta T-cell lymphoma*, *Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma*, and *Primary cutaneous peripheral T-cell lymphoma, NOS*. Intestinal T-cell lymphoid proliferations and lymphomas comprise 5 entities: *Indolent T-cell lymphoma of the gastrointestinal tract*, *Indolent NK-cell lymphoproliferative disorder of the gastrointestinal tract*, *Enteropathy-associated T-cell lymphoma*, *Monomorphic epitheliotropic T-cell*

lymphoma, and *Intestinal T-cell lymphoma, NOS*. Nodal TFH cell lymphoma (nTFHcL) has 3 subtypes: *nTFHcL, angioimmunoblastic-type*; *nTFHcL, follicular-type;* and *nTFHcL, NOS*. EBV-positive T-cell and NK-cell lymphoid proliferations and lymphomas of childhood consist of 4 entities: *Severe mosquito bite allergy*, *Hydro vacciniforme lymphoproliferative disorder*, *Systemic chronic active EBV disease*, and *Systemic EBV-positive T-cell lymphoma of childhood*.

Originated from mesenchymal dendritic cells, *Follicular dendritic cell neoplasms* and *Fibroblastic reticular cell tumor* have been removed from "Histiocytic and dendritic cell neoplasms" to the new category of *Stroma-derived neoplasms of lymphoid tissues*, which also includes *Myofibroblastic tumors* and *Spleen-specific vascular-stroma tumors*. *Genetic tumor syndromes*, another newly added category, includes 4 syndromes: *Fanconi anemia*, *Bloom syndrome*, *Ataxia-telangiectasia syndrome,* and *Rasopathies*.

NEWLY ADDED CATEGORIES, FAMILIES, ENTITIES, AND SUBTYPES

The newly added categories or families are listed in Table 1, and the new entities or subtypes are listed in Table 2 (myeloid) and Table 3 (lymphoid). From these tables, we can see that the 5th WHO-Hem not only identifies or separates many new distinct entities or subtypes, but also expands its territory by including some non-hematolymphoid tumors—stroma-derived neoplasms of lymphoid tissues, as well as some reactive lesions—tumor-like lesions with B-cell or T-cell predominance. Although it is conceptually odd to include these diseases in this WHO Blue Book of hematolymphoid neoplasms, adding them does provide some benefits, such as raising awareness of these lymphoma mimickers and providing convenience for eliminating the need to search for these entities in other books. Besides the follicular dendritic cell neoplasms (categorized in *Histiocytic/dendritic cell neoplasms* in the R4th WHO-Hem), the new category of *Stroma-derived neoplasms of lymphoid tissues* also includes *Intranodal palisaded myofibroblastoma* (a mesenchymal tumor specific to lymph node) and spleen-specific vascular-stromal tumors including *Littoral cell angioma*, *Splenic hamartoma,* and *Sclerosing angiomatoid nodular transformation*. Other stroma-derived tumors located in but not specific to lymph node and spleen are not included here, and they are in the "soft tissue and bone" volume. Tumor-like lesions with B-cell predominance selectively collect 5 entities: *Reactive B-cell rich lymphoid proliferations that mimic lymphoma (RBRLPs)*, *IgG4-related disease* and 3 types of *Castleman disease*. RBRLPs include florid follicular hyperplasia, progressive transformation of germinal center, EBV-associated lymphadenopathy, autoimmune associated lymphadenopathy, indolent B-lymphoblastic proliferation, etc. Although these lesions are commonly encountered or considered during lymphoma workup, actually all the benign lymphadenopathies and other related lesions mimic lymphomas in some way, and their possibilities should always be explored before the final diagnosis. The histological finding of lymph nodes involved by IgG4-related disease is often variable, which makes the diagnosis very challenging and leads to a broad differential diagnosis including some lymphomas (8). Unicentric Castleman disease, idiopathic multicentric Castleman disease and KSHV/HHV8 associated Castleman disease share

TABLE 1 **Newly added categories or families in the 5th edition of WHO classification of hematolymphoid tumors**

Under the category of	Newly added category/family
Chapters	Stroma-derived neoplasms of lymphoid tissues Genetic tumor syndromes
Myeloid neoplasms	Myeloid precursor lesions Secondary myeloid neoplasms
Histiocytic/dendritic cell neoplasms	Plasmacytoid dendritic cell neoplasms
B-cell lymphoid proliferations and neoplasms	Tumor-like lesions with B-cell predominance
Mature B-cell neoplasms	Large B-cell lymphomas
T/NK-cell lymphoid proliferations and neoplasms	Tumor-like lesions with T-cell predominance
Mature T/NK cell neoplasms	Mature T-cell and NK-cell leukemias Primary cutaneous T-cell lymphoproliferations and lymphomas Intestinal T-cell and NK-cell lymphoid proliferations and lymphomas EBV-positive T/NK-cell lymphomas Nodal T-follicular helper (TFH) cell lymphoma
Stroma-derived neoplasms of lymphoid tissues	Mesenchymal dendritic cell neoplasms Myofibroblastic tumors Spleen-specific vascular-stromal tumors

some histologic features, but they have different etiology, pathogenesis, and clinical course, and should be diagnosed distinctively in conjunction with clinical features and the results of the other tests. Tumor-like lesions with T-cell predominance include *Kikuchi-Fujimoto disease*, *Autoimmune lymphoproliferative syndrome (ALPS)*, and *Indolent T-lymphoblastic proliferation (ITLP)*. ITLP shows proliferation of non-clonal T-lymphoblasts in the lymph node with preserved nodal architecture. ITLP can be associated with benign lesions such as Castleman disease or certain malignancies (9). Rare T-lymphoblastic lymphoma cases may have features of ITLP (10); therefore, a comprehensive workup is always required to rule out T-lymphoblasts lymphoma for all ITLP cases. Besides the characteristic coagulative necrosis, Kikuchi-Fujimoto disease can have frequent large immunoblasts and histiocytes, which can mimic high grade lymphomas or histiocytic sarcoma. ALPS usually shows interfollicular proliferation of CD4/CD8 dual negative T cells and a high proliferation index, which can mimic T-cell lymphomas.

In line with other WHO series, the 5thWHO-Hem has also generated a category of *Genetic tumor syndromes* to include genetic syndromes associated with increased risk for hematolymphoid tumors. These genetic syndromes include *Fanconi anemia*, *Ataxia telangiectasia*, *Bloom syndrome,* and *RASopathies*. Some genetic diseases with increased risk for myeloid malignancies, such as *GATA2*-deficiency, Shwachman-Diamond syndrome, dyskeratosis congenita, etc., are not included here. Instead, they are described in *Myeloid neoplasms associated with germline predisposition* in the family of *Secondary myeloid neoplasms*. Myeloid neoplasms with germline

TABLE 2 **Newly added or deleted entities/subtypes in myeloid and mesenchymal neoplasms in the 5th edition of WHO classification of hematolymphoid tumors**

Under the category/family/entity of	Newly added entity/subtype
Myeloid precursor lesions	Clonal hematopoiesis Clonal cytopenia of undetermined significance
Systemic mastocytosis	Bone marrow mastocytosis
MDN with defining genetic abnormalities	MDN with low blasts and *SF3B1* mutation MDN with biallelic *TP53* inactivation
MDN, morphologically defined	MDN with low blasts MDN, hypoplastic
AML with defining genetic abnormalities	AML with *NUP98* rearrangement AML with other defined genetic alterations
AML with other defined genetic alterations	AML with *RUNX1T3*(*CBFA2T3*)*::GLIS2* AML with *KAT6A::CREBBP* AML with *FUS::ERG* AML with *MNX1::ETV6* AML with *NPM1::MLF*
ALAL with defining genetic abnormalities	ALAL with other defined genetic alterations
ALAL with other defined genetic alterations	MPAL with *ZNF384* rearrangement ALAL with *BCL11B* rearrangement
Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangement	Myeloid/lymphoid neoplasm with *FLT3* rearrangement Myeloid/lymphoid neoplasm with *ETV6::ABL1* fusion Myeloid/lymphoid neoplasms with other tyrosine kinase fusion genes
Plasmacytoid dendritic cell neoplasms	Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm
Histiocyte/macrophage neoplasms	Rosai-Dorfman disease ALK-positive histiocytosis
Myofibroblastic tumors	Intranodal palisaded myofibroblastoma
Spleen-specific vascular-stromal tumors	Littoral cell angioma Splenic hamartoma Sclerosing angiomatoid nodular transformation of spleen
	Deleted entity/subtype
Myeloproliferative neoplasms	Accelerated phase of CML
MDS	MDS with single lineage dysplasia MDS with multilineage dysplasia MDS, unclassifiable
AML	AML with mutated RUNX1 Acute panmyelosis with myelofibrosis

ALAL, acute leukemia of ambiguous lineage; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDN, myelodysplastic neoplasm; MDS, myelodysplastic syndrome; MPAL, mixed-phenotype acute leukemia.

TABLE 3 Newly added or deleted entities/subtypes in lymphoid neoplasms in the 5th edition of WHO classification of hematolymphoid tumors

Under the category/family/entity of	Newly added entity/subtype
TL-lesions with B-cell predominance	Reactive B-cell rich lymphoid proliferations that can mimic lymphoma IgG4 related disease Unicentric Castleman disease Idiopathic multicentric Castleman disease KSHV/HHV8-associated multicentric Castleman disease
B-lymphoblastic leukemias/lymphomas (B-LBL/L)	B-LBL/L with *ETV6::RUNX1*-like features B-LBL/L with *TCF3::HLF* fusion B-LBL/L with other defined genetic alterations
Mature B-cell neoplasms	Transformations of indolent B-cell lymphomas
Splenic B-cell lymphomas and leukemias	Splenic B-cell lymphoma/leukemia with prominent nucleoli
Marginal zone lymphoma	Primary cutaneous marginal zone lymphoma
Large B-cell lymphomas	Fluid overload-associated large B-cell lymphoma Primary large B-cell lymphoma of immune-privileged sites
Primary large B-cell lymphoma of immune-privileged sites	Primary large B-cell lymphoma of the vitreoretina Primary large B-cell lymphoma of the testis
LPs and lymphomas associated with ID and dysregulation	Hyperplasias arising in ID/dysregulation Polymorphic lymphoproliferative disorders arising in ID/dysregulation Lymphomas arising in ID/ dysregulation
Follicular lymphoma	Classic follicular lymphoma Follicular large B-cell lymphoma Follicular lymphoma with uncommon features
Monoclonal gammopathies	Cold agglutinin disease Monoclonal gammopathy of renal significance
PCNs with associated paraneoplastic syndrome	AESOP syndrome
TL-lesions with T-cell predominance	Kikuchi-Fujimoto disease Autoimmune lymphoproliferative syndrome Indolent T-lymphoblastic proliferation
Primary cutaneous T-cell lymphoproliferations and lymphomas	Primary cutaneous peripheral T-cell lymphoma, NOS
Intestinal T-cell and NK-cell lymphoid proliferations and lymphomas	Indolent NK-cell lymphoproliferative disorder of the gastrointestinal tract
EBV-positive NK/T-cell lymphomas	EBV-positive nodal T- and NK-cell lymphoma
	Deleted entity/subtype
Precursor lymphoid neoplasms	NK-lymphoblastic leukemia / lymphoma
Mature B-cell neoplasms	B-cell prolymphocytic leukemia

ID, immune deficiency; LP, lymphoid proliferation; NOS, not otherwise specified; PCN, plasma cell neoplasm; TL, tumor-like.

predisposition and potential organ dysfunction include two new genetic diseases: germline *SAMD9* mutations (*MIRAGE syndrome*) and Germline *SAMD9L* mutations (*SAMD9L-related Ataxia Pancytopenia syndrome*). Germline *TP53* mutation (*Li-Fraumeni syndrome*) has been added to *Myeloid neoplasms with germline predisposition without a pre-existing platelet disorder or organ dysfunction*.

Myeloid precursor lesions have been included as a family for the first time in this book under the category of *Myeloid proliferations and neoplasms*. Clonal hematopoiesis (CH) refers to the presence of a population of blood and/or bone marrow cells that share an acquired mutation or chromosome abnormality. The incidence of CH increases with age (11), which is reflected in the term—*Aging-related CH (ARCH)*. There are no well-defined criteria for CH and ARCH. In contrast, *Clonal hematopoiesis of indeterminate potential (CHIP)* is a well-defined term, and it refers to the CH carrying somatic mutations of myeloid malignancy-associated genes with a variant allele fraction (VAF) ≥ 2% (≥4% for X-linked gene mutations in males) and without unexplained cytopenia or a diagnosis of hematologic disease (12). CHIP has an increased risk for myeloid malignancy with a progression rate of 0.5–1% /year, and it also has an increased risk of cardiovascular disease and overall mortality (13). *Clonal cytopenia of undetermined significance (CCUS)* refers to the presence of persistent (> 4 months) cytopenia(s) in the patient meeting the other diagnostic criteria of CHIP. CCUS has an increased risk for myeloid malignancy, which varies according to clone size, number of somatic mutations and the presence of certain gene mutations.

Plasmacytoid dendritic cell neoplasms are a new family in the category of *Histiocytic/dendritic cell neoplasm*. They include *Blastic plasmacytoid dendritic cell neoplasm* and *Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm*. The former was described in a separate chapter in the R4th WHO-Hem (2). The latter is a new entity that refers to a clonal proliferation of plasmacytoid cells with low grade morphology and in association with chronic myelomonocytic leukemia (CMML), AML, MDN or MPN. There are two entities, *Rosai-Dorfman disease (RDD)* and *ALK-positive histiocytosis*, newly added to *Histiocyte/macrophage neoplasms*. The presence of frequent gain-of-function gene mutations in MAPK signaling pathway in RDD indicates that it is a neoplastic disease (14). ALK-positive histiocytosis is characterized by the presence of *ALK* gene translocation (most commonly *KIF5B::ALK*), and it responds to ALK-inhibitor therapy, although it has a broad clinicopathological spectrum (15, 16). The infant form usually involves hematolymphoid tissues and other systems, showing systemic symptoms, and often remitting spontaneously or after chemotherapy. ALK-positive histiocytosis can also occur in other age groups with involvement of multiple organ systems or one organ system. The histomorphology of *ALK-positive histiocytosis* overlaps with that of *Juvenile xanthogranuloma (JXG)*. Therefore, histiocytic proliferations, especially those with the morphology of JXG, should be stained for ALK to rule out ALK-positive histiocytosis.

As shown in Table 2 and Table 3, there are new genetic abnormalities identified as distinct subtypes in AML, ALAL, B-LBL/L, MDN, and myeloid and lymphoid neoplasms with eosinophilia. These new subtypes include *SF3B1* mutation and *TP53* inactivation in MDN, *NUP98* rearrangement in AML, *ZNF384* rearrangement and *BCL11B* rearrangement in ALAL, *TCF3::HLF* fusion, *ETV6::RUNX1*-like features in B-LBL/L, *FLT3* rearrangements, *ETV6::ABL1* fusion, and novel types of *JAK2* rearrangements in *myeloid/lymphoid neoplasms with eosinophilia*

and defining gene rearrangement. A new subtype named "*XXX* (*entity name) with other defined genetic alterations*" is added to all these entities to accommodate the provisional subtypes with distinct genetic features.

As shown in Table 3, there are quite a lot of new entities or subtypes added to *Mature B-cell neoplasms*. Splenic B-cell lymphoma/ leukemia with prominent nucleoli is a new entity that includes the cases of *Hairy cell leukemia variant* and *CD5-negative B-cell prolymphocytic leukemia* of the R4th WHO-Hem (2). Two new subtypes of FL, *Follicular large B-cell lymphoma (FLBL)* and *FL with uncommon features (uFL)* have been recognized besides the *Classic FL (cFL)* that represents the majority (85%) of FL cases, with *BCL2* rearrangement and at least in part a follicular growth pattern. The FLBL subtype mostly represents FL grade 3B in the R4th WHO-Hem, and the uFL subtype includes two subsets with histomorphologic features significantly different from cFL. One subset shows blastoid or large centrocyte cytology, and the other demonstrates a predominantly diffuse growth pattern. The former more frequently shows variant immunophenotypic and genotypic features and is likely associated with an inferior survival outcome (17). *Large B-cell lymphomas (LBCLs)* is a newly added family in the 5th WHO-Hem, which consists of 17 distinct entities except for DLBCL, NOS. *LBCL of immune-privileged sites* is a new entity describing a group of aggressive B-cell lymphomas that share common biological features and arise as primary lymphomas in the central nervous system, the vitreoretinal compartment, or the testes. *Fluid overload-associated LBCL* is also a new entity that is frequently associated with an underlying condition causing fluid overload (18). It is distinct from primary effusion lymphoma by its different immunophenotype and lack of association with KSHV/HHV8. *Transformation of indolent B-cell lymphomas* is a new group of mature B-cell neoplasms including aggressive lymphomas arising in patients with a clonally related indolent B-cell lymphoma. The format for reporting transformed lymphomas is: aggressive lymphoma entity, followed by "transformed from" and the name of the related indolent lymphoma. Monoclonal gammopathy of renal significance (MGRS), cold agglutinin disease, and AESOP syndrome (adenopathy and extensive skin patch overlying a plasmacytoma) have been added to *PCNs and other diseases with paraproteins* in the 5th WHO-Hem. Cold agglutinin disease is autoimmune hemolytic anemia caused by monoclonal cold agglutinins originating from a clonal B-cell proliferation not meeting the criteria for a B-cell lymphoma (19). MGRS refers to a plasma cell or B-cell proliferation not fulfilling the criteria for lymphoma but secreting a monoclonal immunoglobulin resulting in kidney injury (20).

There are 3 new entities added to the family of *Mature T-cell and NK-cell neoplasms*. *Primary cutaneous peripheral T-cell lymphoma, NOS*, represents the primary cutaneous T-cell lymphomas (CTCLs) that don't meet the diagnostic criteria of the established CTCL entities. *Indolent NK-cell lymphoproliferative disorder of the GI tract*, previously known as a reactive process, is included as a new entity in the category of *Intestinal T/NK-cell proliferations and neoplasms*. It has somatic mutations related to JAK-STAT signaling pathway and is negative for EBV infection. *Nodal EBV-positive T and NK-cell lymphoma* is added as a new entity in the group of *EBV-positive NK/T cell lymphomas* besides the relatively common *Extranodal NK/T-cell lymphoma*. EBV-positive nodal T and NK-cell lymphoma occurs mostly in East Asians with poor prognosis and distinct genetic features (21).

DELETED ENTITIES/SUBTYPES

Compared with the many new entities and terminology changes, the number of entities or subtypes completely removed is very limited (see Table 2 and Table 3). With the clinical application of tyrosine kinase inhibitors (TKIs) and careful disease monitoring in chronic myeloid leukemia (CML), the progression to advanced phase disease is uncommon, and the designation of an accelerated phase (AP) is not very relevant to disease deterioration. Therefore, the AP of CML is removed in the 5th WHO-Hem in order to emphasize other risk features related to TKI resistance. With the significantly different nomenclature, the MDN entities based on single- /multilineage dysplasia or ring sideroblasts do not exist anymore. In addition, *MDS, unclassifiable*, as seen in the R4th WHO-Hem, is deleted. *AML with RUNX1 mutations* is removed because of its lack of enough specificity. *B-cell prolymphocytic leukemia* is deleted due to its heterogeneous nature, and the cases now belong to *Splenic B-cell lymphoma/leukemia with prominent nucleoli* (new), *Transformations of indolent B-cell lymphomas* (new), or *Mantle cell lymphoma,* respectively. NK-lymphoblastic leukemia/lymphoma, a provisional entity in the R4th WHO, is not listed in the 5th WHO-Hem due to the lack of clear-cut and reliable diagnostic criteria.

REVISED NOMENCLATURE AND TERMINOLOGY CHANGES

As we can see from Table 4 and Table 5, there are many terminology changes or nomenclature revisions. Most of the changes are to reflect our better understanding of these diseases. In contrast, one type of name change is to harmonize the nomenclature with other fields and the rest of the WHO 5th edition series. For example, the Human Genome Organization Gene Nomenclature Committee has recommended the new designation of gene fusions using double colon marks (::) (22). Hence, double colon marks replace the hyphen to join two fusion gene partners as used in previous editions (1, 2). This format change of gene fusion in myeloid neoplasms is not listed in Table 4. The International Union of Immunological Societies has renamed *primary immunodeficiencies associated with germline mutations* as “inborn errors of immunity”, and the 5th WHO-Hem has adopted this terminology. Another type of name change is purely language editing, e.g., “excess blasts” to “increased blasts”, “classical” to “classic”. Fortunately, there is only a very limited number of this type of name change. We can optimistically call this type of change as “job security type”. The qualifier “unclassifiable” used for some entities in the R4th WHO-Hem (2) sounds paradoxical, and it has been replaced with “NOS” or the whole entity has been removed in the 5th WHO-Hem.

The 5thWHO-Hem uses *Myelodysplastic neoplasm (MDN)* to replace *Myelodysplastic syndrome (MDS)* with the purpose to emphasize their neoplastic nature and being terminologically consistent with *MPN*. MDNs have been reclassified based primarily on the presence of recurrent cytogenetic abnormalities and blast count. Single/multiple lineage dysplasia and ring sideroblasts are not used to classify MDN anymore. Childhood MDNs have different biological

TABLE 4 Revised nomenclature and name changes of myeloid or mesenchymal neoplasms in the 5th edition compared with the revised 4th edition of WHO classification of hematolymphoid tumors

WHO classification, 5th edition	WHO classification, revised 4th edition
Myeloid proliferations and neoplasms	
Chronic myeloid leukemia	Chronic myeloid leukemia, *BCR-ABL1*–positive
Chronic eosinophilic leukemia	Chronic eosinophilic leukemia, not otherwise specified
Myeloproliferative neoplasm, not otherwise specified	Myeloproliferative neoplasm, unclassifiable
Myelodysplastic neoplasms (MDNs) MDN, with defining genetic abnormalities MDN with low blasts and 5q deletion MDN with low blasts and *SF3B1* mutation MDN with biallelic *TP53* inactivation MDN, morphologically defined MDN with low blasts MDN, hypoplastic MDN with increased blasts MDNs of childhood Childhood MDN with low blasts Childhood MDN with increased blasts	Myelodysplastic syndromes (MDSs) MDS with single lineage dysplasia MDS with ring sideroblasts MDS with multilineage dysplasia MDS with excess blasts MDS with excess blasts and erythroid predominance MDS with excess blasts and fibrosis MDS with isolated del(5q) MDS, unclassifiable Childhood MDS Refractory cytopenia of childhood
Myelodysplastic/myeloproliferative neoplasm with neutrophilia	Atypical chronic myeloid leukemia, *BCR-ABL1*–negative
Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis	Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis
Myelodysplastic/myeloproliferative neoplasm, NOS	Myelodysplastic/myeloproliferative neoplasm, unclassifiable
AML with defining genetic abnormalities	AML with recurrent genetic abnormalities
AML with *KMT2A* rearrangement	AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
AML with *MECOM* rearrangement	AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
AML with *CEBPA* mutation	AML with biallelic mutation of *CEBPA*
Acute erythroid leukemia	Pure erythroid leukemia
AML, myelodysplasia-related	AML with myelodysplasia-related changes
Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangement	Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement
Myeloid/lymphoid neoplasm with *JAK2* rearrangement	Myeloid/lymphoid neoplasms with *PCM1-JAK2*
Stroma-derived neoplasms of lymphoid tissues	
EBV-positive inflammatory follicular dendritic cell sarcoma	Inflammatory pseudotumor-like follicular/fibroblastic dendritic cell sarcoma

AML, acute myeloid leukemia.

TABLE 5 **Revised nomenclature and name changes of lymphoid neoplasms in the 5th edition compared with the revised 4th edition of WHO classification of hematolymphoid tumors**

WHO classification, 5th edition	WHO classification, revised 4th edition
B-CELL lymphoid proliferations and lymphomas	
B-LBL/L with **high** hyperdiploidy	B-LBL/L with hyperdiploidy
B-LBL/L with *BCR::ABL1* fusion	B-LBL/L with t(9;22)(q34;q11.2); *BCR-ABL1*
B-LBL/L with *BCR::ABL1*-like features	B-LBL/L, *BCR-ABL1*-like
B-LBL/L with *KMT2A* rearrangement	B-LBL/L with t(v;11q23.3); *KMT2A*-rearranged
B-LBL/L with *ETV6:: RUNX1* fusion	B-LBL/L with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-LBL/L with *TCF3::PBX1* fusion	B-LBL/L with t(1;19)(q23;p13.3); *TCF3-PBX1*
B-LBL/L with *IGH::IL3* fusion	B-LBL/L with t(5;14)(q31.1;q32.1); *IGH/IL3*
In situ follicular B-cell **neoplasm**	In situ follicular **neoplasia**
In situ mantle cell **neoplasm**	In situ mantle cell **neoplasia**
DLBCL/ HGBCL with *MYC* and *BCL2* rearrangements	HGBCL with *MYC* and *BCL2* **and/or *BCL6*** rearrangements
High-grade B-cell lymphoma with 11q aberrations	**Burkitt-like** lymphoma with 11q aberration
EBV-positive diffuse large B-cell lymphoma	EBV-positive diffuse large B-cell lymphoma, **NOS**
Mediastinal grey zone lymphoma	B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL
KSHV/HHV8-positive DLBCL	HHV8-positive DLBCL, **NOS**
KSHV/HHV8-positive germinotropic lymphoproliferative disorder	HHV8-positive germinotropic lymphoproliferative disorder
Lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation	Immunodeficiency-associated lymphoproliferative disorders
Inborn error of immunity-associated lymphoid proliferations and lymphomas	Lymphoproliferative diseases associated with **primary immune disorders**
Plasma cell neoplasms and other diseases with paraproteins (family name)	Plasma cell neoplasms (family name)
Immunoglobulin-related (AL) amyloidosis	Primary amyloidosis
Monoclonal immunoglobulin deposition disease	Light chain and heavy chain deposition disease
T-/ NK-cell lymphoid proliferations and lymphomas	
T-LBL/L, **NOS**	T-LBL/L
Early T-precursor lymphoblastic leukemia / **lymphoma**	Early T-cell precursor lymphoblastic leukemia
T-large granular lymphocytic leukemia	T-cell large granular lymphocytic leukemia

(Continued)

TABLE 5 **Revised nomenclature and name changes of lymphoid neoplasms in the 5th edition compared with the revised 4th edition of WHO classification of hematolymphoid tumors (*Continued*)**

WHO classification, 5th edition	WHO classification, revised 4th edition
NK-large granular lymphocytic leukemia	Chronic lymphoproliferative disorder of NK cells
Primary cutaneous acral CD8-positive **lymphoproliferative disorder**	Primary cutaneous acral CD8-positive T-cell **lymphoma**
Intestinal T-cell and NK-cell lymphoid proliferations and lymphomas (family name)	Intestinal T-cell lymphoma (family name)
Indolent T-cell **lymphoma** of the gastrointestinal tract	Indolent T-cell **lymphoproliferative disorder** of the gastrointestinal tract
Nodal TFH cell lymphoma, angioimmunoblastic-type	Angioimmunoblastic T-cell lymphoma
Nodal TFH cell lymphoma, follicular-type	Follicular T-cell lymphoma
Nodal TFH cell lymphoma, NOS	Nodal peripheral T-cell lymphoma with TFH phenotype
Extranodal NK/T-cell lymphoma	Extranodal NK/T-cell lymphoma, **nasal-type**
Hydroa vacciniforme lymphoproliferative disorder	Hydroa vacciniforme-**like** lymphoproliferative disorder
Systemic chronic active EBV disease	Chronic active EBV infection of T- and NK-cell type, systemic form

CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; HGBCL, high grade B-cell lymphoma; LBL/L, lymphoblastic leukemia/lymphoma.

and genetic features from adult MDNs. *Childhood MDN with low blasts* replaces the term "Refractory cytopenia of childhood" used in the R4th WHO-Hem (2). It includes two subtypes: *Childhood MDN with low blasts, hypocellular*; and *Childhood MDN with low blasts, NOS*. *Childhood MDN with increased blasts* refers to the cases with ≥2% blasts in the peripheral blood and/or ≥5% blasts in the bone marrow.

Because of the presence of multiple translocation partner genes in the entities with a key gene involvement, only the key genes are listed in the names of these entities, e.g., AML with *KMT2A* rearrangement. Since the typical translocation or inversion may not be demonstrated by conventional cytogenetic study, the chromosomal changes are removed from the names of these entities (not listed in Table 4), e.g., AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* changed to AML with *RUNX1::RUNX1T1*. Similar changes are also made for the entities with defining genetic abnormalities of B-LBL/L and myeloid/lymphoid neoplasms with eosinophilia. The revised name of *AML with biallelic mutation of CEBPA* in the R4th WHO-Hem (1, 2) has been changed back to the name without "biallelic" to include single mutations located in the basic leucine zipper region of the gene, which also showed the association with favorable prognosis (23). *AML with myelodysplasia-related changes* is now renamed as "AML, myelodysplasia-related

(AML-MR)". *Pure erythroid leukemia* is renamed as "Acute erythroid leukemia" with the purpose to keep the name more consistent with other subtypes.

There are lots of name changes or nomenclature revisions in mature B-cell neoplasms. The addition of *BCL2* rearrangement besides *MYC* rearrangement is the only requirement for the revised entity *DLBCL/HGBL with MYC and BCL2 rearrangements*. The *BCL6* rearrangement is removed from this entity because the lymphomas with dual *MYC* and *BCL6* rearrangements are more diverse and show gene expression profile and mutational landscape significantly different from lymphomas with dual *MYC/BCL2* rearrangements (24). Those cases with *MYC* and *BCL6* dual rearrangements are now classified as either a subtype of *DLBCL, NOS* or *HGBL, NOS* depending on their cytomorphologic features. *Burkitt-like lymphoma with 11q aberration* in the R4th WHO-Hem is now named as *High-grade B-cell lymphoma with 11q aberration*. *Mediastinal gray zone lymphoma* replaces the old term *B-cell-lymphoma, unclassifiable with features intermediate between DLBCL and classic Hodgkin lymphoma*. The group of immunodeficiency-associated lymphoproliferative disorders has been renamed as " *Lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation*" and included in the family of *Mature B-cell neoplasms* in the 5th WHO-Hem. The reorganization of the lymphoid proliferations and lymphomas in this group reflects the consensus gained from the *Workshop on Immunodeficiency and Dysregulation* organized by the Society of Hematopathology and European Association for Hematopathology in 2015 (25, 26). The new framework recognizes the same histopathologic features shared by the diseases with different underlying causes of immunodeficiency. Three-part nomenclature is recommended for the diagnosis of the disorders in this group, and it includes histological diagnosis, viral association, and immune deficiency/dysregulation setting. Histological subtypes include hyperplasia (specify type), polymorphic lymphoproliferative disorder, mucocutaneous ulcer, and lymphoma (same diagnostic criteria as for immunocompetent patients). The associated viruses are mostly EBV and KSHV/HHV8. Immune deficiency/dysregulation settings include inborn error of immunity (specify type), HIV infection, posttransplant (solid organ or bone marrow), autoimmune disease, iatrogenic/therapy-related (specify) and immune senescence.

There are also quite a few nomenclature revisions and name changes in mature T/NK cell neoplasms. *NK-large granular lymphocytic leukemia* replaces "Chronic lymphoproliferative disorder of NK cells" given recent evidence that it is monoclonal and shares many similarities with T-LGLL. *Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract* in the R4th WHO-Hem has been renamed as "indolent T-cell lymphoma of the gastrointestinal tract" to highlight its clinical features of persistence and poor response to chemotherapy. *nTFHcL angioimmunoblastic-type*, *nFTHcL follicular type* and *nTFHcL NOS* have replaced the previous subtype names "Angioimmunoblastic T-cell lymphoma", "Follicular T-cell lymphoma" and "Peripheral T cell lymphoma with TFH phenotype", respectively. The changes are to recognize their similarities in clinical presentation, immunophenotype and genetic features (27). The qualifier "nasal-type" of *Extranodal NK/T-cell lymphoma* has been removed in the 5th WHO-Hem in order to recognize the presence of this disease at various extranodal sites. *Chronic active EBV infection, systemic form* has been renamed as "Systemic chronic active EBV disease" to emphasize its overall fatal outcome.

REVISED DIAGNOSTIC CRITERIA

In the R4th WHO-Hem and other previous editions, well-defined diagnostic criteria are only provided for some myeloid neoplasms and plasma cell neoplasms. As a big improvement, all the entities in WHO Blue Books of the 5th edition have essential diagnostic criteria, and some entities also have desirable diagnostic criteria. Essential diagnostic criteria list the diagnostic elements required to make the diagnosis, while desirable diagnostic criteria list the finding(s) supporting the diagnosis, but not mandatory. Although the diagnosis is always the best judgment of a pathologist based on all the information he/she has, these diagnostic criteria do assist in the workup of the cases and making the diagnosis, especially for the junior pathologists or young attendings. Of course, not all diagnostic criteria are perfectly formulated, and more refinements or revisions are expected to see in future editions.

There are quite a lot of changes made to the existing diagnostic criteria. Besides the previously defined two criteria (≥20% myeloid blasts and extramedullary proliferation of blasts), the blast phase of CML has one more criterion in the 5th WHO-Hem: presence of bona fide lymphoblasts in the peripheral blood or bone marrow (even if <10%). The diagnostic criteria of chronic eosinophil leukemia (CEL) have been revised, and the changes include: (i) the time interval required to define sustained hypereosinophilia decreased from 6 months to 4 weeks; (ii) requirement of both clonality and abnormal bone marrow morphology; (iii) removal of increased blasts (≥2% in peripheral blood or 5–19% in bone marrow) as an alternative to clonality. The revised criteria lead to a better distinction between CEL and other diseases with hypereosinophilia (28). JMML is now a subtype of MPN, and it also has revised diagnostic criteria. The changes to the diagnostic criteria of JMML include: (i) absence of *KMT2A* rearrangements as one of the required diagnostic criteria; (ii) elimination of monosomy 7 as a cytogenetic criterion; (iii) hypersensitivity to GM-CSF by colony assay and STAT5 hyperphosphorylation combined as one minor criterion; (iv) thrombocytopenia with hypercellular bone marrow added as one minor criterion. Diagnostic criteria of chronic myelomonocytic leukemia (CMML) have been revised and they consist of prerequisite and supporting criteria. The cutoff for absolute monocytosis (the first prerequisite criterion) is lowered from $\geq 1.0 \times 10^9$ /L to $\geq 0.5 \times 10^9$ /L. Other prerequisite criteria include <20% blasts, not meeting the diagnostic criteria for MPNs and myeloid/lymphoid neoplasms with eosinophilia. Supporting criteria include dysplasia, clonality, and abnormally increased fraction of classical monocytes (newly added criterion) (29). If monocytosis is $\geq 1.0 \times 10^9$ /L, prerequisite criteria plus one of the supporting criteria can make the diagnosis. If monocytosis is $< 1.0 \times 10^9$ /L, detection of clonal cytogenetic or molecular abnormality and documentation of dysplasia are required for the diagnosis. Two new subtypes of CMML are introduced based on white blood cell count (WBC): myelodysplastic CMML (WBC $< 13 \times 10^9$ /L) and myeloproliferative CMML (WBC $\geq 13 \times 10^9$ /L).

The cut-off of blast percentage to define AML is arbitrary, and the blast enumeration can vary due to sampling variations and subjective evaluation. In the 5th WHO-Hem, the cutoff value of 20% blasts is not required for the diagnosis of AML if the leukemic blasts harbor *PML::RARA, RUNX1-RUNX1T1, CBFB-MYH11,*

DEK::NUP214, RBM15::MRTFA, KMT2A rearrangement, *MECOM* rearrangement, *NUP98* rearrangement, or *NPM1* mutation; while in the R4th WHO-Hem, only the first three genetic abnormalities listed here had this privilege. The diagnostic criteria for AML-MR have also been revised. Multilineage dysplasia has been removed from the diagnostic criteria, which means that AML-MR includes only two types of AML now: AML with a history of MDN or MDN/MPN, and AML with at least one of the defining genetic abnormalities for AML-MR. AML with only morphologic evidence of multilineage dysplasia is no longer qualified for the diagnosis of AML-MR. The defining cytogenetic criteria for AML-MR have been revised as well, and the changes include the elimination of the balanced cytogenetic abnormalities, and the addition of defining somatic mutations: *ASXL1*, *BCOR*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *EZH2*, *STAG2*.

The diagnostic criteria for systemic mastocytosis have been revised. The major criterion stays the same, but the expression of CD30 has been added to the minor criterion of abnormal phenotype, and the presence of the active *KIT* mutation(s) other than codon 816 has also been accepted as one minor criterion. And for the minor criterion of basal serum tryptase, the tryptase level should be adjusted in patients with hereditary alpha-tryptasaemia (30).

UPDATED CYTOGENETIC/MOLECULAR GENETIC INFORMATION

Besides the above-mentioned changes and updates, there are numerous other updates involving almost every entity, covering from pathogenesis, and pathology to clinical features and prognosis. With tremendous advances in sequencing technology and large-scale integrated data analysis, new findings in genetic studies are abundant. These findings have led to the identification of many new subtypes and altered signaling pathways with potential for targeted therapy. There is no way to mention all the updates here, and the following are just a few examples.

Newly discovered recurrent cytogenetic abnormalities listed in *AML with other defined genetic abnormalities* include *RUNX1T3* (*CBFA2T3*)*::GLIS*, *KAT6A::CREBBP, FUS::ERG*, *MNX1::ETV6*, and *NPM1::MLF1*. Those listed in "B-LBL/L with other defined genetic abnormalities" include *DUX4* rearrangement, *MEF2D* rearrangement, *ZNF384* rearrangement, *NUTM1* rearrangements, *MYC* rearrangement, *PAX5alt* or *PAX5 p.P80R*. With more data accumulated, neoplasms with these new genetic abnormalities will very likely become separated subtypes in the next edition, and there will be more newly discovered genetic alterations listed here. Biallelic *TP53* alterations have been frequently found in MDNs, acute erythroid leukemia, and myeloid neoplasms post cytotoxic therapy, and they usually predict a worse prognosis (31). Gene mutations in the MAPK pathway are commonly seen in histiocytic neoplasms. *CXCR4* mutations are detected in a significant proportion of lymphoplasmacytic lymphoma cases, mostly concurrent with *MYD88* mutations, and the presence of them is associated with resistance to ibrutinib therapy (32). The mutational profiles of extranodal MZL (EMZL) and nodal MZL differ, and there are significant genetic differences among EMZLs arising in different anatomic sites (33). *STAT3* mutation is commonly seen in CD8+ T-LGLL and

gamma/delta T-LGLL, and it is associated with neutropenia and unfavorable overall survival; while *STAT5b* mutation is associated with a poor prognosis only in CD8+ T-LGLL but has no prognostic impact in CD4+ T-LGLL and gamma/delta T-LGLL (34).

CONCLUSION

Numerous changes and updates have been implemented in the 5th WHO-Hem to reflect our better understanding of these diseases through the tremendous amount of research work by many researchers and physicians. These changes and updates include revision of hierarchical classification structures, addition or deletion of categories/entities or subtypes, changes or revisions of nomenclature/terminology, changes or revisions of diagnostic criteria, and updates from many other aspects.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, et al. (Eds.): World Health Organization classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: IARC 2008.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds.): World Health Organization classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. Lyon: IARC; 2017.
3. WHO Classification of Tumours Editorial Board. Hematolymphoid tumors [Internet; beta version ahead of print (in progress)]. Lyon (France): International Agency for Research on Cancer; 2022 [cited 2022 Aug 29]. (WHO classification of tumors series, 5th ed.). Available from: https://tumourclassification.iarc.who.int
4. Cree IA. The WHO Classification of Haematolymphoid Tumours. Leukemia. 2022;36(7):1701–1702. https://doi.org/10.1038/s41375-022-01625-x
5. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720–1748. https://doi.org/10.1038/s41375-022-01620-2
6. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703–1719. https://doi.org/10.1038/s41375-022-01613-1
7. Lasho T, Patnaik MM. Juvenile myelomonocytic leukemia - A bona fide RASopathy syndrome. Best Pract Res Clin Haematol. 2020;33(2):101171. https://doi.org/10.1016/j.beha.2020.101171
8. Cheuk W, Chan JK. Lymphadenopathy of IgG4-related disease: an underdiagnosed and overdiagnosed entity. Semin Diagn Pathol. 2012;29(4):226–34. https://doi.org/10.1053/j.semdp.2012.07.001

9. Ohgami RS, Arber DA, Zehnder JL, Natkunam Y, Warnke RA. Indolent T-lymphoblastic proliferation (iT-LBP): a review of clinical and pathologic features and distinction from malignant T-lymphoblastic lymphoma. Adv Anat Pathol. 2013;20(3):137–40. https://doi.org/10.1097/PAP.0b013e31828d17ec
10. Li W, Kats A, Cooley LD, Farooqi MS, August K. T-lymphoblastic leukemia/lymphoma with interfollicular growth pattern and Castleman-like morphologic features. J Hematopathol 2021; 14: 163–169. https://doi.org/10.1007/s12308-021-00446-8
11. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–98. https://doi.org/10.1056/NEJMoa1408617
12. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16. https://doi.org/10.1182/blood-2015-03-631747
13. Marnell CS, Bick A, Natarajan P. Clonal hematopoiesis of indeterminate potential (CHIP): Linking somatic mutations, hematopoiesis, chronic inflammation and cardiovascular disease. J Mol Cell Cardiol. 2021;161:98–105. https://doi.org/10.1016/j.yjmcc.2021.07.004
14. Jacobsen E, Shanmugam V, Jagannathan J. Rosai-Dorfman disease with activating KRAS mutation - response to Cobimetinib. N Engl J Med. 2017;377:2398–9. https://doi.org/10.1056/NEJMc1713676
15. Kemps PG, Picarsic J, Durham BH, Helias-Rodzewicz Z, Hiemcke-Jiwa L, van den Bos C, et al. ALK-positive histiocytosis: a new clinicopathologic spectrum highlighting neurologic involvement and responses to ALK inhibition. Blood. 2022;139:256–80. https://doi.org/10.1182/blood.2021013338
16. Chang KTE, Tay AZE, Kuick CH, Chen H, Algar E, Taubenheim N, et al. ALK positive histiocytosis: an expanded clinicopathologic spectrum and frequent presence of KIF5B-ALK fusion. Mod Pathol. 2019;32:598–608. https://doi.org/10.1038/s41379-018-0168-6
17. Laurent C, Adélaïde J, Guille A, Tesson B, Gat E, Evrard S, et al. High-grade follicular lymphomas exhibit clinicopathologic, cytogenetic, and molecular diversity extending beyond Grades 3A and 3B. Am J Surg Pathol. 2021;45:1324–36. https://doi.org/10.1097/PAS.0000000000001726
18. Kaji D, Ota Y, Sato Y, Nagafuji K, Ueda Y, Okamoto M, et al. Primary human herpesvirus 8-negative effusion-based lymphoma: a large B-cell lymphoma with favorable prognosis. Blood Adv. 2020;4(18):4442–4450. https://doi.org/10.1182/bloodadvances.2020002293
19. Berentsen S, Barcellini W, D'Sa S, Randen U, Tvedt THA, Fattizzo B, et al. Cold agglutinin disease revisited: a multinational, observational study of 232 patients. Blood 2020;136:480–8. https://doi.org/10.1182/blood.2020005674
20. Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. Nat Rev Nephrol. 2019;15:45–59. https://doi.org/10.1038/s41581-018-0077-4
21. Wai CMM, Chen S, Phyu T, Fan S, Leong SM, Zheng W, et al. Immune pathway upregulation and lower genomic instability distinguish EBV-positive nodal T/NK-cell lymphoma from ENKTL and PTCL-NOS. Haematologica. 2022;107(8):1864–1879. https://doi.org/10.3324/haematol.2021.280003
22. Bruford EA, Antonescu CR, Carroll AJ, Chinnaiyan A, Cree IA, Cross NCP, et al. HUGO Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions. Leukemia. 2021;35:3040–3. https://doi.org/10.1038/s41375-021-01436-6
23. Tarlock K, Lamble AJ, Wang YC, Gerbing RB, Ries RE, Loken MR, et al. CEBPA-bZip mutations are associated with favorable prognosis in de novo AML: a report from the Children's Oncology Group. Blood. 2021;138(13):1137–1147. https://doi.org/10.1182/blood.2020009652
24. Cucco F, Barrans S, Sha C, Clipson A, Crouch S, Dobson R, et al. Distinct genetic changes reveal evolutionary history and heterogeneous molecular grade of DLBCL with MYC/BCL2 double-hit. Leukemia 2020;34:1329–41. https://doi.org/10.1038/s41375-019-0691-6
25. Natkunam Y, Gratzinger D, de Jong D, Chadburn A, Goodlad JR, Chan JK, et al. Immunodeficiency and Dysregulation: Report of the 2015 Workshop of the Society for Hematopathology/European Association for Haematopathology. Am J Clin Pathol. 2017;147:124–8. https://doi.org/10.1093/ajcp/aqw200

26. Natkunam Y, Gratzinger D, Chadburn A, Goodlad JR, Chan JKC, Said J, et al. Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal? Blood 2018;132:1871–8. https://doi.org/10.1182/blood-2018-04-842559
27. Dobay MP, Lemonnier F, Missiaglia E, Bastard C, Vallois D, Jais JP, et al. Integrative clinicopathological and molecular analyses of angioimmunoblastic T-cell lymphoma and other nodal lymphomas of follicular helper T-cell origin. Haematologica 2017;102:e148–e51https://doi.org/10.3324/haematol.2016.158428
28. Fang H, Ketterling RP, Hanson CA, Pardanani A, Kurtin PJ, Chen D, et al. A test utilization approach to the diagnostic workup of isolated eosinophilia in otherwise morphologically unremarkable bone marrow: a single institutional experience. Am J Clin Pathol. 2018;150:421–31. https://doi.org/10.1093/ajcp/aqy064
29. Selimoglu-Buet D, Wagner-Ballon O, Saada V, Bardet V, Itzykson R, Bencheikh L, et al. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. Blood. 2015;125:3618–26. https://doi.org/10.1182/blood-2015-01-620781
30. Valent P, Akin C, Hartmann K, Alvarez-Twose I, Brockow K, Hermine O, et al. Updated diagnostic criteria and classification of mast cell disorders: a consensus proposal. Hemasphere. 2021;5:e646.
31. Bernard E, Nannya Y, Hasserjian RP, Devlin SM, Tuechler H, Medina-Martinez JS, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020;26(10):1549–1556. Erratum in: Nat Med. 2021 Mar;27(3):562. Erratum in: Nat Med. 2021 May;27(5):927. https://doi.org/10.1038/s41591-021-01367-w
32. Treon SP, Xu L, Guerrera ML, Jimenez C, Hunter ZR, Liu X, et al. Genomic landscape of Waldenström macroglobulinemia and its impact on treatment strategies. J Clin Oncol. 2020;38:1198–208. https://doi.org/10.1200/JCO.19.02314
33. Vela V, Juskevicius D, Dirnhofer S, Menter T, Tzankov A. Mutational landscape of marginal zone B-cell lymphomas of various origin: organotypic alterations and diagnostic potential for assignment of organ origin. Virchows Arch. 2022;480:403–13. https://doi.org/10.1007/s00428-021-03186-3
34. Teramo A, Barilà G, Calabretto G, Vicenzetto C, Gasparini VR, Semenzato G, et al. Insights into genetic landscape of large granular lymphocyte leukemia. Front Oncol. 2020;10:152. https://doi.org/10.3389/fonc.2020.00152

2

Infant Leukemia

Sevgi Gözdaşoğlu

Department of Pediatrics, Divisions of Hematology and Oncology, Ankara University Medical School, Ankara, Turkey

Author for correspondence: Sevgi Gözdaşoğlu, Department of Pediatrics, Divisions of Hematology and Oncology, Ankara University Medical School, Ankara, Turkey. E-mail: sgozdasoglu@hotmail.com

Cite this chapter as: Gözdaşoğlu S. Infant Leukemia. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 09 Sep 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-infant-leukemia

Abstract: Infant leukemias are rare entities of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) that are generally diagnosed in infants under the age of one. Patients often present with bulky hepatosplenomegaly, leukemia cutis, central nervous system symptoms, and hyperleukocytosis. Infant leukemia has specific biological, clinical, cytologic, and cytogenetic features. Cytogenetics is an important factor for diagnosis and risk stratification. Infant B-lineage ALL is characterized by a higher white blood cell count, higher incidence of *KMT2A* rearrangements, and negative CD10. Relapse rate is also higher in these cases. *KMT2A* rearrangements are clearly associated with poorer outcome in infants with ALL. *KMT2A* rearrangements in acute myelomonocytic leukemia (FAB M4), acute monocytic leukemia (FAB M5), and acute megakaryoblastic leukemia (FAB M7), are also common in infants, but *KMT2A* rearrangements are not a significant risk factor for infants with AML. As the diagnosis of megakaryoblastic leukemia is challenging, megakaryocytic markers should be investigated in all infant leukemias. It should be noted that transient abnormal myelopoiesis, a condition sharing the same morphology and immunophenotype with acute megakaryoblastic leukemia, frequently occurs in infants with Down syndrome and the somatic mutation of *GATA1* is distinct in this situation.

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

Keywords: clinical characteristics of infant leukemia; infant leukemia; *KMT2A* rearrangements in infant leukemia; leukemia cutis in infant leukemia; prognosis of infant leukemia

INTRODUCTION

Infant leukemias are rare and distinct subgroup of pediatric acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) occurring in infants under the age of one. Approximately 35% of all childhood cancers are acute leukemias, of which 80% are ALL and 15 to 20% are AML. Infant leukemias account for 2 to 5% of pediatric patients with ALL and approximately 10% of pediatric patients with AML (1, 2). The peculiar clinical and biological characteristics of acute leukemia in infants differ markedly from those of older children. These patients usually present with high initial white blood cell counts, massive organomegaly, early central nervous system (CNS) involvement, and leukemia cutis (3–5). Infant leukemia tends to be more aggressive with poor prognosis (2, 4). Congenital leukemia is defined as developing within the first months of life (4, 6, 7). In this chapter, my main objective is to analyze the clinical, cytologic, and cytogenetic characteristics of infant leukemias. Chemotherapy of infant leukemia is excluded in this chapter.

INCIDENCE

Approximately 6500 children and adolescents in the United States develop acute leukemia each year. AML comprises only 15–20% of these cases. The incidence of pediatric AML is estimated to be between 5 and 7 cases per million people per year, with a peak incidence of 11 cases per million people per year at 2 years of age (8). Brown reported that the estimated incidence of infant leukemia is 41 cases per million in the United States. The incidence of ALL in infants is significantly lower than in children aged 1 to 14 years old. On the other hand, the incidence of AML in infants is approximately twice that of older children and adolescents. Females have a higher risk of developing infant leukemia than males (9). Juvenile myelomonocytic leukemia and the related myeloproliferations associated with congenital syndromes such as Down syndrome and Noonan syndrome are the most notable in the literature (6, 10). Infant AML associated with Down syndrome represents a significant component of this distinct subgroup (11).

CLINICAL CHARACTERISTICS

The typical symptoms of leukemia, such as anemia, bleeding, febrile neutropenia, organomegaly, and bone pain, are commonly seen in acute infant leukemia. The patients tend to present more aggressive findings including high initial white blood cell counts, massive organomegaly, early central nervous system involvement

and leukemia cutis (1–4, 11). Developmental stage, fetal hematopoiesis and its different origins might play a role in these different clinical features in infant leukemias (11). Zhang et al retrospectively analyzed 59 patients with congenital leukemia reported between 2001–2016 years and found the following clinical features: leukemia cutis (67.8%), hepatosplenomegaly (47.5%), CNS involvement (blast cells in cerebrospinal fluid 25.4%), and spontaneous remission (11.9%) (12). Hyperleukocytosis was detected in 62.7% of cases with an average white blood cell count of 68.5 x 10^9 /L (normal range 4–10 x 10^9 /L) but anemia was less common at 35.7%. Blast cells were present in most patients' peripheral blood and bone marrow (12). The risk of early death is critically high due to severe bleeding and leukostasis in the patients with hyperleukocytosis (>100,000/µl). Emergency strategies include exchange transfusion and leukapheresis treatment; these should be done as soon as possible (13, 14).

Leukemia cutis

Leukemia cutis is a particular feature of neonatal AML and commonly observed in infants with AML while it is rarely seen in ALL. Cutaneous infiltrates are present in about two-thirds of patients and can occur without any peripheral blood or bone marrow involvement. Leukemia cutis typically presents as multiple papules, nodules, plaques, or subcutaneous myeloid sarcoma (Figure 1). These lesions may be blue, red, brown, or purple. The appearance has been described as a blueberry muffin rash. Leukemia cutis is commonly seen in FAB-M4 and FAB-M5 AML (10, 11, 15, 16). Prognosis in these cases is very poor (10, 11, 15, 16). In the French ELAM02 cohort, skin involvement was significantly more prevalent in infants, occurring in 14.5% of children who were less than two years old compared to 2.6% of older children. One hypothesis is that chemotherapy may not be sufficient to penetrate the skin leading to a greater incidence of relapse in those patients (11). On the other hand, spontaneous remissions are specific of this period, which is associated with a significant shift in hematopoiesis from liver to marrow, reinforcing the importance of stage of development in AML in the absence of known negative prognostic factors (11, 16).

Liver

Liver involvement may be prominent and sometimes dominate clinical presentation. Few cases have been reported in the medical literature with the liver as the primary site of involvement in congenital leukemia. Lewis et al. reported a case of a four week-old infant diagnosed with acute megakaryoblastic leukemia who presented with ascites caused by massive infiltration of hepatic sinusoids by leukemia cells associated with t(1;22)(p13;q13). The bone marrow and the peripheral blood smear did not initially show the presence of blasts. Marrow fibrosis appeared after infiltrative disease in the liver and liver fibrosis. In the absence of bone marrow involvement, it can be difficult to diagnose as leukemia (17). In addition, patients with jaundice, ascites, pleural effusions, cardiopulmonary distress, seizures, and disseminated intravascular coagulation are also reported in the medical literature (17–19).

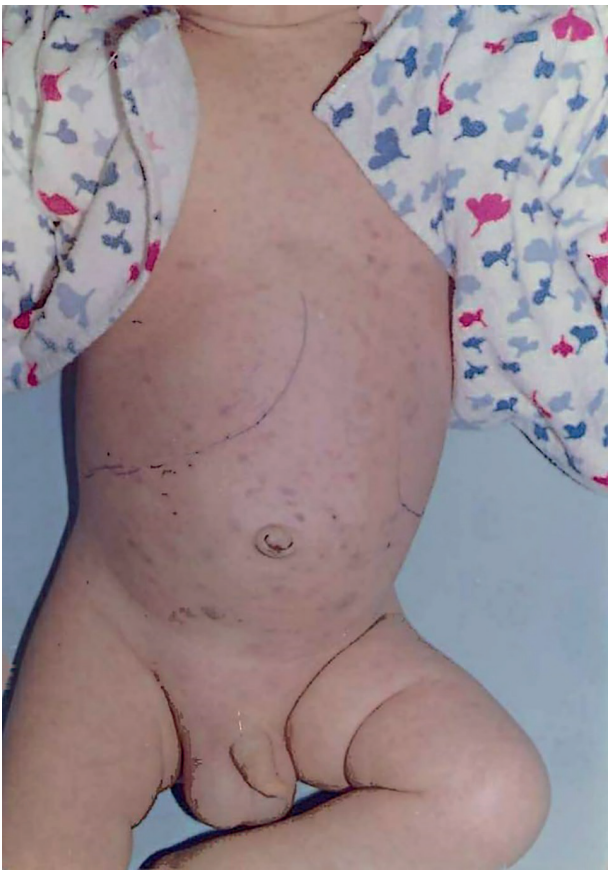

Figure 1. **The picture of a 4-month boy with infant leukemia showing hepatosplenomegaly, leukemia cutis and scrotal swelling.**

Central nervous system

CNS involvement is more common in infant leukemia. Infants less than 2 years of age represented 35–45% of patients with CNS involvement as CNS3 (>5 blasts within the cerebrospinal fluid or central nervous system symptoms at diagnosis) according to Children's Oncology Group (COG) study (18). A bulging fontanelle, papilledema, retinal hemorrhage, reduced level of consciousness and seizures are observed (11, 19–21). Central nervous system disease in infants is also more common as 35%–45% in AML and 14%–41% in ALL (10, 11).

CYTOLOGY

Infant leukemias have specific morphological features and immunophenotypic characteristics. Acute myeloblastic (FAB-M4), acute monoblastic (FAB-M5), and

acute megakaryoblastic (FAB-M7) subgroups are striking features of infant leukemia with AML according to the French-American-British (FAB) classifications (Figure 2) (4, 11, 22–25). AML can be diagnosed if ≥20% blasts with myeloid markers are present in the bone marrow and is associated with the presence of recurrent cytogenetic abnormalities (11). Acute megakaryoblastic leukemia (AMKL) is very frequent in infants. Diagnosis of AMKL is really challenging as the number of megakaryoblasts, which are hard to spot, in peripheral blood is very limited. Bone marrow aspiration is also difficult due to fibrosis. Megakaryoblasts show cytoplasmic blebs, cell clumping and binucleation (very rarely seen) (6, 11). Megakaryoblasts are immunophenotypically positive for CD33, CD36, CD41, CD42b, and CD61 (11). In infants with ALL, L1 subtypes are frequently observed than L2 subtype while L3 subtype is absent. These are CD19 positive, HLA-DR positive, and CD10 negative minimally differentiated early B-cell precursors (10). On the other hand, infant leukemia patients can be of undefined lineage, either due to a mixed phenotype (mixed phenotype acute leukemia) or lack of differentiation markers (acute undifferentiated leukemia) (5).

ETIOPATHOGENESIS OF INFANT LEUKEMIAS

During the past decade, etiopathology of leukemogenesis was researched extensively. Genome sequencing data provided a better understanding of infant leukemia. In the literature, there are many noteworthy publications on this subject. Many infant leukemias show similar genetic variation with either lymphoid or myeloid acute leukemias. It is clear that leukemia in newborns and in young infants is the result of leukemogenic events that occur in utero (2). Indirect evidence to support this hypothesis was provided by studies of monozygotic twins with leukemia. Monozygotic twins essentially share a hematopoietic system in utero through vascular anastomoses and they show a concordance rate approaching 100% for leukemia and the same genetic lesion can be identified in both twins. Dizygotic twins who have entirely separate vascular system do not show this extreme harmony (6). Many infant leukemias show similar genetic alterations with either lymphoid or myeloid differentiation (6, 10). Cytogenetic analysis provides significant information on the diagnosis, prognosis, and follow-up of the patients and to determine most effective treatment protocols associated with minimal side effects.

KMT2A rearrangements

Structural abnormalities involving the long arm of chromosome 11, region 2, band 3 (11q23) are cytogenetic changes consistently seen in hematopoietic malignancies, and they are the most common genetic lesion observed in infant leukemia. A high proportion of infant leukemia cases are characterized by chromosomal translocations involving the histone lysine methyltransferase 2A gene (KMT2A gene previously named as MLL “Mixed Lineage Leukemia gene”) at chromosome 11q23. KMT2A rearrangements (KMT2A-r) result in the fusion of N terminus of the KMT2A gene with the C terminus of a partner gene; about 135 different KMT2A partner genes have been identified (22–25). KMT2A-r, which was first detected in patients with ALL and then in cases with AML, is the most common

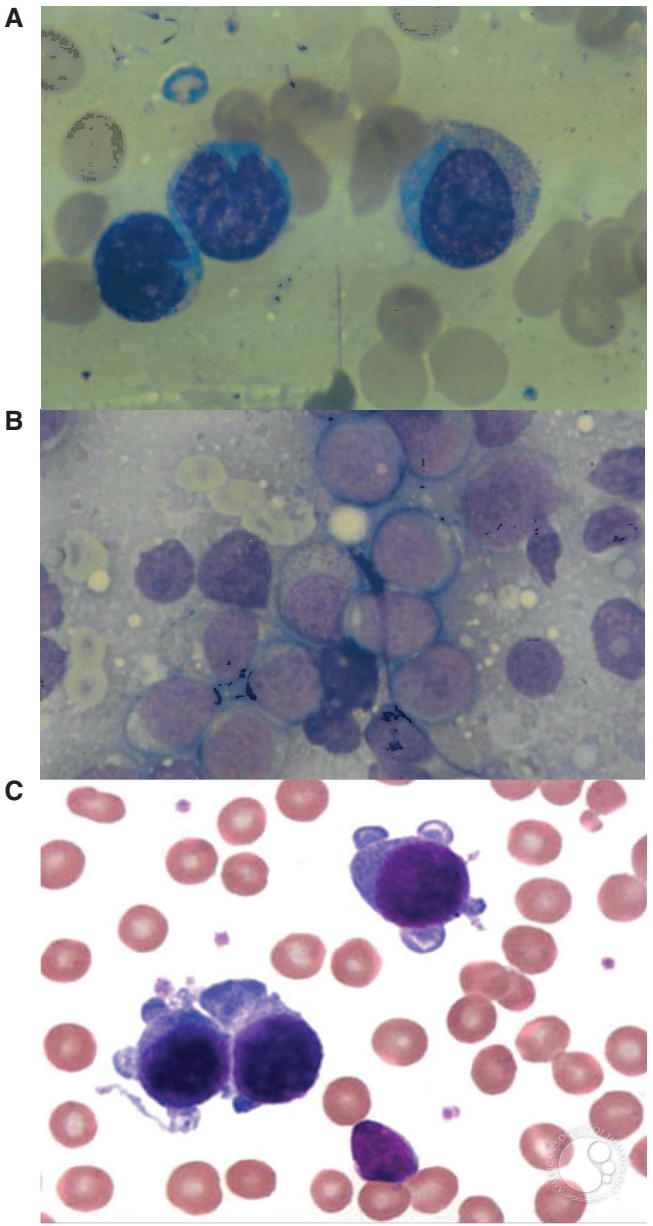

Figure 2. Bone marrow smears. A, Bone marrow smear of acute monoblastic leukemia. May-Grünwald-Gemsa staining, X100. **B,** Bone marrow smear of acute myelomonoblastic leukemia. May-Grünwald-Giemsa staining, X160. **C,** At high magnification, the megakaryoblasts showing cytoplasmic budding suggestive of platelet formation. Figure C is from John Lazarchick, imagebank.hematology.org (Myeloid Neoplasms and acute leukemia (WHO 2016) > Acute Myeloid Leukemia > Myeloid proliferations related to Down syndrome > Myeloid Leukemia associated with Down Syndrome).

cytogenetic abnormality in infant leukemias. KMT2A gene regulates hematopoietic differentiation and plays an important role in leukemogenesis. The t(4;11)(q21;q23) or t(11;19)(q23;p13) occurs in most cases with ALL whereas t(9;11)(q22;p23) is the most common translocation in patients with AML (11, 22–25). KMT2A-r is observed in approximately 40–60% of infants with AML, and rare translocations for this age group including t(1;22) and t(7;12) have also been identified (11).

KMT2A fusion proteins are potent oncoproteins that regulate leukemogenesis in infants with few cooperating mutations; fetal and neonatal hematopoietic progenitors may be more sensitive to KMT2A proteins (11). In infant ALL, four partner genes have been identified in 93% of patients: *AFF1* (49%), *MLLT1* (22%), *MLLT3* (17%), and *MLLT10* (5%). In infant AML, three partner genes have been observed in 66% of patients: *MLLT3* (22%), *MLLT10* (27%) and *ELL* (17%) (5, 6). Most of these KMT2A rearrangements in AML are morphologically classified as FAB-M4 or FAB-M5. KMT2A fusion can also be seen with AMKL (6, 11). KMT2A-r leukemias occur frequently in two different clinical presentations: (i) infants with de novo acute AML or ALL; and (ii) patients with treatment-related AML after exposure to DNA topoisomerase II inhibitors (5, 22–25). The growing fetus is more sensitive to the effects of potential DNA damage during the early stages of pregnancy. Transplacental exposures to DNA-topoisomerase inhibitors may be related to the etiology of infant acute leukemias with KTM2A-r. Topoisomerase inhibitors include chemotherapeutic agents, benzene metabolites such as benzoquinone, isoflavones, flavonoids, lignans, podophyllin resin, quinolone antibiotics and some pesticides (22, 23).

KMT2A has varying prognostic implications in infant ALL less than 12 months of age, depending on the presence or absence of KTM2A-r. Infants with ALL and KMT2A-r do not achieve remission, while the prognosis and survival of ALL infants without KMT2A-r are similar to ALL of older children. Among patients with KMT2A-r ALL, additional independent prognostic factors include age and white blood cell counts at diagnosis—younger infants and those with the higher white blood cell counts having poorer prognosis (5, 26, 27). In infant AML, KMT2A-r is not a significant risk factor (5). KMT2A-r AML are morphologically classified as FAB-M4 or FAB-M5 and KMT2A-r is also frequently associated with megakaryoblastic leukemia in infants (5, 6, 11).

Acute megakaryoblastic leukemia is frequent among infants with AML and is associated with other chromosomal translocations such as t(1;22)(p13; q13) and inv(16)(p13.3, q24.3) (6). The fusion of GLIS2 to CBFA2T3 as a result of inv(16)(p13.3, q24.3) is the most frequently identified chimeric oncogene to date in AMKL patients (6). Patients with CBFA2T3-GLIS2 fusion gene is found only in patients aged <3 years and it is the second most frequent gene in cases aged less than one year. Core binding factor rearrangements are uncommon in infants (11). Infants with Down's syndrome have an increased risk for leukemia, particularly acute megakaryoblastic leukemia, which most often resolves spontaneously, and is called transient neonatal myelopoiesis or transient abnormal myelopoiesis. The underlying genetic lesion is a mutation in the N-terminal region of the erythroid/megakaryocytic transcription factor GATA-1 which is located on the short arm of chromosome X. Mutation of GATA-1 is pathognomonic for transient leukemia and acute megakaryoblastic leukemia in children with Down syndrome (6, 25–27).

HOX gene dysregulation is a common feature of AML. *HOX* genes play a key role in the regulation of hematopoietic development. In leukemia, dysregulated *HOX* gene expression can occur due to chromosomal translocations involving upstream regulators such as KMT2A (6, 28). Translocations of 11q23 are seen in approximately 5–10% of adult AML and specifically one to three years after treatment with chemotherapy regimens containing anthracyclines or other topoisomerase II inhibitors (22).

Transient neonatal myelopoiesis is a rare condition in the neonatal period connected with trisomy of chromosome 21. It is characterized by high blast cells in peripheral blood and bone marrow, and it usually resolves without specific therapy in 1 to 3 months (27). Li-Thiao-Te et al. (29) reported transient leukemia with an isolated pericardial effusion in a phenotypically normal neonate. Trisomy 21 was found on blast cells. Congenital leukemia associated with trisomy 21 on blast cells has a good prognosis (29). Independent adverse prognostic features are age < 1 month, leukemia cutis, initial high white blood cell counts, poor response to induction chemotherapy and presence KMT2A-r (3, 5, 6, 10). Curative results have not been achieved with standard chemotherapy schemes in infant leukemia due to its destructive nature.

Other factors

Maternal alcohol consumption during pregnancy, marijuana use, and paternal smoking one month prior to pregnancy are associated with an increased risk of infant leukemias (30). Ross et al. showed that a high birth weight is a significant risk factor of developing childhood leukemia. Insulin-like growth factor-1 is important in blood formation and regulation and has been shown to stimulate the growth of both myeloid and lymphoid cells in culture. It was postulated that high levels of insulin-like growth factor-1 might produce large babies and may contribute to the development of leukemia (31). Emerenciano et al. reported that there are significant associations between risks for infant leukemias and maternal hormone intake during pregnancy (30).

CONCLUSION

Infant leukemia is an aggressive disease which has specific clinical, biological, cytologic, and cytogenetic characteristics. Skin lesions such as papulonodular erythematous or violaceous lesions should be considered as leukemia cutis in infants. The most common genetic lesion observed in infant leukemia is KMT2A-r which is an important prognostic factor and should be investigated especially in infants with ALL. As the diagnosis of megakaryoblastic leukemia is challenging, megakaryocytic markers should be investigated in all infant leukemias. In consideration with infant metabolism and the characteristics of infant leukemia, new curative, tolerable chemotherapy protocols are required with minimal side effects.

Conflict of Interest: The author declares no potential of interest with respect to research, authorship and/or publication of this chapter.

REFERENCES

1. Silverman LB, Sallan SE. Acute lymphoblastic leukemia. In: Nathan DG, Ginsburg D, Orkin SH, Look AT, editors. Nathan and Oski's hematology of infancy and childhood. 6th ed. Philadelphia: W.B. Saunders Company; 2003; p.1135–66.
2. Bennett C, Hsu K, Look AT. Myeloid leukemia, myelodysplasia and myeloproliferative disease in children. In: Nathan DG, Ginsburg D, Orkin SH, Look AT, editors. Nathan and Oski's hematology of infancy and childhood. 6th ed. Philadelphia: W.B. Saunders Company; 2003; p.1167–1218.
3. Lanzkowsky P. Manual of Pediatric Hematology and Oncology; 3rd ed. San Diego: Academic Press; 1999. 386 p.
4. Gözdaşoğlu S, Çavdar OA, Babacan E, Cin Ş, Ünal E, Özkan AU, et al. Congenital Leukemia. Jour of Ankara Med School. 1991;13(1):69–73.
5. Brown P, Pieters R, Biondi A. How I treat infant leukemia. Blood. 2019;133(3):205–214. https://doi.org/10.1182/blood-2018-04-785980
6. Wertheim G. Infant acute leukemia. Clin Lab Med. 2021;41(3):541–50. https://doi.org/10.1016/j.cll.2021.04.002
7. Eguchi M. Acute leukemia of infants and neonates. Rinsho Ketsueki. 2021;62(8):1308–18.
8. Rubnitz JE, Gibson B, Smith FO. Acute myeloid leukemia. Hematol Oncol Clin North Am. 2010;24(1):35–63. https://doi.org/10.1016/j.hoc.2009.11.008
9. Brown P. Treatment of infant leukemias. Challenge and promise. Author manuscipt. Hematology Am Soc Hematol Educ Program. 2013:596–600. https://doi.org/10.1182/asheducation-2013.1.596
10. Felix CA, Lange BJ. Leukemia in infants. The Oncologist. 1999;4(3):225–40. https://doi.org/10.1634/theoncologist.4-3-225
11. Calvo C, Fenneteau O, Leverage G, Petit A, Baruchel A, Mechinaud F. Infant acute myeloid leukemia: A unique clinical and biological entity. Cancers. 2021;13(4):777–89. https://doi.org/10.3390/cancers13040777
12. Zhang Q, Ren Z, Yang J, Yin A. Analysis of 59 cases of congenital leukemia reported between 2001–2016. J Int Med Res. 2019;47(10):4625–4635. https://doi.org/10.1177/0300060519872899
13. Creutzig U, Rössig C, Dworzak M, Stary J, von Stackelberg A, Wössman W, et al. Exchange transfusions and leukapheresis in pediatric patients with AML with high risk of early detah by bleeding and leukostasis. Pediatr Blood Cancer. 2016;63(4):640–5. https://doi.org/10.1002/pbc.25855
14. Sonta-Jakimczyk D, Szczepanski T, Bubala H, Kulagowska-Timberman E, Kuhny D. Acute lymphoblastik Leukemia in a 3-week-old neonate. Wiad Lek. 2004;57(7–8):395–7.
15. Fender AB, Gust A, Wang N, Scott GA, Mercurio MG. Congenital Leukemia Cutis. Pediatr Dermol. 2008;25(1):34–7. https://doi.org/10.1111/j.1525-1470.2007.00578.x
16. D'Orazio JA, Pullian JF, Moscow JA. Spontaneous resolution of a single lesion of myeloid leukemia cutis in an infant: case report and discussion. Pediatr Hematol Oncol. 2008;25(5):457–68. https://doi.org/10.1080/08880010802104494
17. Lewis MS, Kaicker S, Strauchen JA, Morotti RA. Hepatic involvement in congenital acute megakaryoblastic leukemia: a case report with emphasis on the liver pathology findings. Pediatr Dev Pathol. 2008;11(1):55–8. https://doi.org/10.2350/07-03-0243.1
18. Van Dongen JC, Dalinghaus M, Kroon AA, de Vries AC, Van den Heuvel-Eibrink MM. Successful treatment of congenital a premature infant. J Pediatr Hematol Oncol. 2009;31(11):853–4. https://doi.org/10.1097/MPH.0b013e3181b38b6c

19. Ledet DS, Wheless JW, Rubnitz JE, Brannon Morris E. Levetiractam as monotherapy for seizures in a neonate with acute lymphoblastic leukemia Eur J Paediatr Neurol. 2010;14(1):78–9. https://doi.org/10.1016/j.ejpn.2008.12.007
20. Johnston DL, Alonzo TA, Gerbing RB, Aplenc R, Woods WG, Meshinchi S, Gamis AS. Central nervous system disease in pediatric acute myeloid leukemia: A report from the children's Oncology Group. Pediatr Blood Cancer. 2017;64(12):10.1002 / pbc. 26612. https://doi.org/10.1002/pbc.26612
21. Ito M, Nishimaki S, Nakano Y, Tanaka F, Goto H, Yokota S. A case of fetal leukemia with intracranial hemorrhage and early onset jaundice. Fetal leukemia with intracranial hemorrhage and jaundice. Arch Gyneol Obstet. 2009;279(4):599–601. https://doi.org/10.1007/s00404-008-0784-3
22. Douet-Guilbert N, Morel F, Le Bris MJ, Sassolas B, Giroux JD, DeBraekeleer M. Rearrangement of MLL in a patient with congenital acute monoblastic leukemia and granulocytic sarcoma associated with a t(1;11) (p36;q23) translocation. Leuk Lymphoma. 2005;46(1):143–6. https://doi.org/10.1080/104281904000010783
23. Menghrajani K, Zhang Y, Famulare C, Devlin SM, Talmann MS. Acute myeloid leukemia with 11q23 rearaangements: A study of therapy-related disease and therapeutic outcomes. Leuk Res. 2020 98: 106453. https://doi.org/10.1016/j.leukres.2020.106453
24. Ferguson EC, Talley P, Vora A. Translocation (6;17) (q23;q11.2: a noval cytogenetic abnormality in congenital acute myeloid leukemia? Canver Genet Cytogenet. 2005;163(1):71–3. https://doi.org/10.1016/j.cancergencyto.2005.03.016
25. Manola KN. Cytogenetics of pediatric acute myeloid leukemia. Eur J Haematol. 2009;83(5):391–405. https://doi.org/10.1111/j.1600-0609.2009.01308.x
26. Guest EM, Stam RW. Updates in the biology and therapy for infant acute lymphoblastic leukemia. Curr Opin Pediatr. 2017;29(1):20–6. https://doi.org/10.1097/MOP.0000000000000437
27. Jackowska T, Steczowicz M, Pawelec K, Pacholska J. Congenital leukemia and transient myeloproliferative disorder: diagnostic difficulties: case reports. Med Wieku Rozwo. 2006;10(3):595–601.
28. Rice KL, Licht JD. HOX deregulation in acute myeloid leukemia. J Clin Invest. 2007;117(4):865–8. https://doi.org/10.1172/JCI31861
29. Li-Thiao-Te V, Bourges-Petit E, Capiod JC, Horle B, Micheli J, Morin G, et al. Congenital transient leukemia: a case report. Arch Pediatr. 2008;15(1):33–6. https://doi.org/10.1016/j.arcped.2007.08.023
30. Emerenciano M, Koifman S, Pombo-de-Oliveira MS. Acute leukemia in early childhood. Braz J Med Biol Res. 2007;40(6):749–60. https://doi.org/10.1590/S0100-879X2007000600002
31. Ross JA, Potter JD, Shu XO, Reaman GH, Lampkin B, Robison LL. Evaluating the relationships among maternal reproductive history, birth characteristics, and infant leukemia: a report from the Children's Cancer Group. Ann Epidemiol 1997;7:172–179. https://doi.org/10.1016/S1047-2797(97)00012-4

3

Hairy Cell Leukemia

Agnieszka Janus[1] • Tadeusz Robak[1,2]

[1]Department of General Hematology, Copernicus Memorial Hospital, Lodz, Poland;
[2]Department of Hematology, Medical University of Lodz, Lodz, Poland

Author for correspondence: Tadeusz Robak, Department of Hematology, Medical University of Lodz, Lodz, Poland. E-mail: robaktad@csk.umed.lodz.pl

Cite this chapter as: Janus A, Robak T. Hairy Cell Leukemia. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 22 Jul 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-hairy-cell-leukemia

Abstract: Hairy cell leukemia is a rare, indolent, chronic lymphoid neoplasm originating from a mature B lymphocyte. Diagnosis is based on hairy cell morphology, immunological phenotype by flow cytometry and/or immunohistochemistry in trephine biopsy, and the presence of $BRAF^{V600E}$ somatic mutation. In the classic form of the disease, the purine nucleoside analogues pentostatin and cladribine are recommended for the first-line treatment. These agents induce durable and unmaintained complete response in more than 70% of cases and up to 35% of patients demonstrate overall survival longer than 20 years. When rituximab is combined with cladribine in early relapse, complete response can be achieved in 89–100% of patients, with a three-year risk of relapse of only 7%. More recently, several new drugs have been introduced for the treatment of patients with hairy cell leukemia. Clinical trials have confirmed that the immunotoxin moxetumomab pasudotox, BRAF kinase inhibitors (vemurafenib and dabrafenib), and the Bruton kinase inhibitor ibrutinib are useful agents in the treatment of patients who are refractory to purine analogs.

Keywords: BRAF inhibitors for hairy cell leukemia; clinical presentation of hairy cell leukemia; epidemiology of hairy cell leukemia; pathogenesis of hairy cell leukemia; treatment of hairy cell leukemia

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

INTRODUCTION

Hairy Cell Leukemia (HCL) is a rare, indolent B-cell neoplasm. It is characterized by the progressive infiltration by mature lymphocytes with typical "hairy" projections within the peripheral blood (PB), bone marrow (BM) and spleen, resulting in pancytopenia, splenomegaly, and susceptibility to infections (1, 2). A characteristic molecular feature of the disease, present in ≥95% of cases, is the *BRAF*^{V600E} somatic mutation. It is important to distinguish between classic HCL and HCL-like diseases, including splenic B-cell lymphoma/leukemia, unclassifiable, as well as the variant form of HCL (HCL-v) and the splenic diffuse red pulp lymphoma (SDRPL) (3, 4). Monocytopenia is characteristic of classic HCL (1, 2).

In HCL-v, the neoplastic lymphoid cells are hybrids, with an intermediate morphology between prolymphocytes and hairy cells. In SDRPL, villous lymphoid cells have a polar distribution of the villi and a small or undetectable nucleolus in the PB. HCL-v is characterized by lymphocytosis with lymphoid cells of relatively large size, prominent nucleoli, and cytopenias without monocytopenia. In HCL-v and SDRPL, the neoplastic cells do not express CD25 or CD123 and the *BRAF*^{V600E} mutation is lacking. However, the relationship between HCL-v and SDRPL remains unclear (3, 5). In this chapter we concentrate on the pathogenesis, laboratory and clinical characteristics, and treatment of classic HCL.

EPIDEMIOLOGY

Hairy cell leukemia accounts for less than 2% of all leukemias. Its incidence is 0.3 cases per 100,000 individuals with an average male-to-female ratio of 1.5-2:1 and median age at diagnosis of 58 years (6). The incidence is approximately three times higher in White than in Black populations (7). HCL-v is estimated to be 0.2 cases per 100,000. HCL-v affects mainly elderly patients with a median age of 71 years.

PATHOGENESIS

The pathogenesis of HCL is not fully understood. Exposure to ionizing radiation, pesticides, and farming have been suggested as possible etiologies (8–10), cases of HCL have been reported among family members with the same HLA haplotype (11). In most patients, HCL arises from a late, activated memory B cell that acquires a single somatic point mutation in the DNA sequence of v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), a kinase-encoding proto-oncogene (*BRAF*^{V600E}) (12).

The *BRAF*^{V600E} mutation is detected in up to 80–90% of classic HCL patients (13, 14). The mutation involves a thymine-to-adenine transversion at nucleotide in exon 15 of *BRAF* at position 1799 of the gene-coding sequence located in chromosome 7q34. The replacement produces an amino acid change from valine (V) to glutamate (E) at position 600 (V600E) of the protein sequence, ultimately

leading to aberrant activation of BRAF kinase and the downstream MEK-ERK signaling. Indeed, the *BRAF*^{V600E} mutation has been found to be more or less ubiquitous in studies involving hundreds of classic HCL patients (4, 15, 16). The *BRAF*^{V600E} mutation itself is clonal and heterozygous, although a minority of patients lose the wild-type allele as a result of a concomitant 7q deletion (13, 17). The mutation remains stable over the whole disease course, from the initial diagnosis to relapses, which can occur even many years after initial presentation. The mutation is responsible for continuous BRAF activation, and, in turn, provides continuous signaling to the RAS–RAF–MEK-ERK signaling pathway, whose aberrant activation activates a distinct phenotype and enhances the survival of the HCL cell.

In addition to the *BRAF*^{V600E} mutation, the most common genetic alteration in classical HCL is a loss in copy number for chromosome7q (13). A mutated immunoglobulin heavy chain variable region (*IGHV*) gene profile is detected in 90% of HCL patients. The absence of a BRAF mutation (*BRAF*^{WT}) is associated with the activation of mutations in the mitogen-activated protein kinase kinase 1 (*MAP2K1*) gene by unmutated *IGHV* and *VH4-34* rearrangements (18); this small subset of classic HCL patients has poor prognosis and poor response to nucleoside purine analogs (PNAs). In addition, a whole-exome sequencing study of patients with classic HCL confirmed the presence of various cancer-associated genes, including *EZH2* and *ARID1A*, together with novel inactivating mutations of the cell cycle inhibitor *CDKN1B* (p27) (19). *CDKN1B* is the second most commonly mutated gene in HCL.

CLINICAL PRESENTATION

In most HCL cases, patients are asymptomatic, with pancytopenia incidentally discovered on a routine blood cell count examination (20, 21). If symptomatic, they typically present with symptoms related to worsening pancytopenia (fatigue, bruising, gingival bleeding, epistaxis, menorrhagia), splenomegaly (abdominal fullness, discomfort growing after eating) and recurrent infections. Rare clinical manifestations include polyarteritis nodosa, cutaneous leukocytoclastic vasculitis, bone involvement or central nervous system involvement (22, 23).

Peripheral blood and BM smear usually reveal the presence of typical hairy cells (Figure 1 and Figure 2). These are lymphoid cells, medium-sized, with abundant pale blue cytoplasm, small cytoplasmic projections and a mature-looking nucleus, giving the cell the appearance of a “fried egg”. However, BM is often difficult to aspirate due to extensive fibrosis induced by leukemic hairy cell infiltration (dry tap). Trephine biopsy is indicated for confirming any diagnosis of HCL. The BM biopsy specimen typically reveals massive infiltration by cells characterized by a wide rim of pale-staining cytoplasm that surrounds and separates the monotonous, bland hairy cell nuclei, as well as well-preserved cytoplasmic borders that reinforce the ‘fried egg’ appearance (Figure 3). The characteristic pattern of infiltration which is interstitial, diffuse or patchy, allows differentiation from the other B-cell lymphoid malignancies, where lymphoid cells with closely packed nuclei usually aggregate or form focal nodules (24, 25). The nucleus is round,

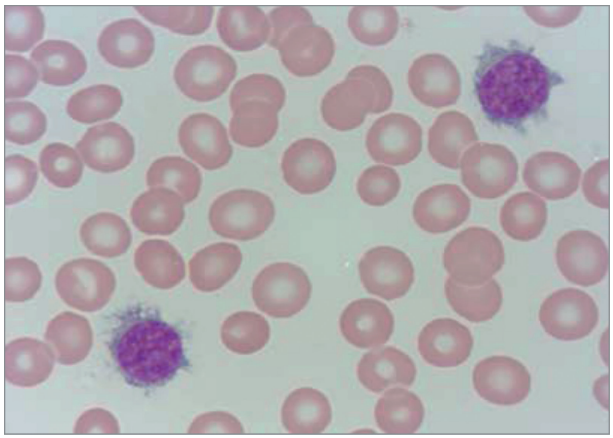

Figure 1. **Morphological features of classic HCL cells with small- to medium-sized cells with oval to indented nucleus and circumferential cytoplasmic projections.**

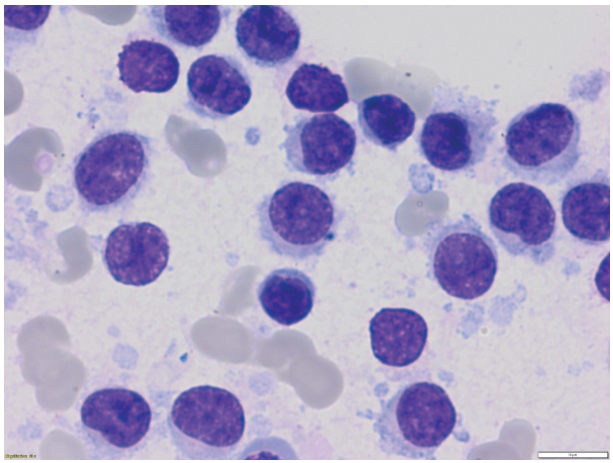

Figure 2. **Bone marrow smear in classic HCL.**

oval or slightly serrated. Ki-67 is very low. Silver stains demonstrate an increase in reticulin fibers. Infiltrating hairy cells often disrupt the microvasculature of the bone marrow, leading to red blood cell extravasation and formation of pseudosinus and blood lakes (26). More precise assessment of the extent of infiltration can be provided by immunohistochemical staining (27). HCL cells are typically positive for B-cell associated antigens like e.g., CD20, but also for annexin-1 and VE1 (a $BRAF^{V600E}$ stain). Cases with hypocellular BM have been also reported (28). In these cases, special caution is recommended in order to avoid a misdiagnosis with aplastic anemia.

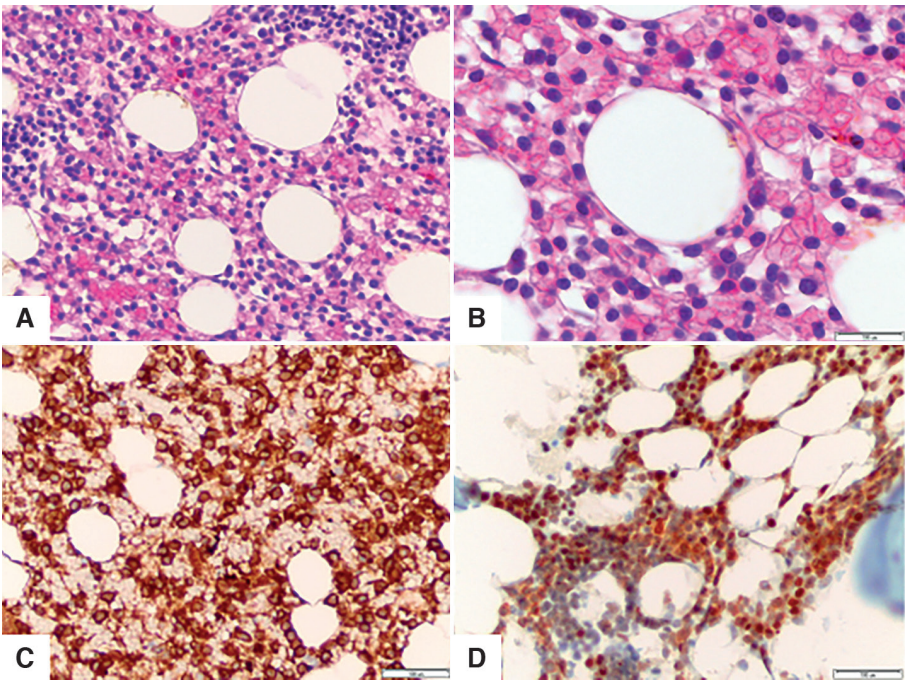

Figure 3. **The histology of bone marrow involved by hairy cell leukemia with diffuse infiltration by regular, small cells (A, B) strongly expressing CD20 (C) and CD25 (D).**

Immunophenotypic analysis is crucial for establishing the diagnosis. HCL cells show a mature B cell phenotype, with immunoglobulins on the surface with restricted light chains. They strongly express pan-B cell antigens including CD19, CD20, and CD22 and the hairy-specific antigens CD11c, CD25, CD103, CD123 (bright) (Figure 4). Staining for CD200 expression is intense (1, 2, 20). A majority (74%) of HCL also expresses annexin A1, which is not expressed in any other type of B cell neoplasm. HCL-v is typically negative for annexin A1, which can be a helpful distinguishing feature. However, since it is also expressed by myeloid cells and by some T cells, annexin A1 staining must be interpreted in conjunction with staining for a B cell antigen (16).

Although 96% of the patients with HCL demonstrate an enlarged spleen, diagnostic or therapeutic splenectomy is rarely performed nowadays due to the evident BM involvement. Histologically, the disease is associated with expansion of red pulp areas and severe atrophy of the white pulp. The normal splenic architecture of cords and sinusoids is destroyed by hairy cell infiltration, resulting in the formation of blood lakes and pseudosinus. The HCL infiltrate is histologically similar to that described in the bone marrow. The lymph nodes are rarely enlarged and therefore, rarely evaluated by pathologists. Nodal involvement is usually confined to retroperitoneal and abdominal nodes. Hairy cells infiltrate the cortex and the medullary cord regions, while the sinuses are typically intact and follicular structures are spared (28, 29).

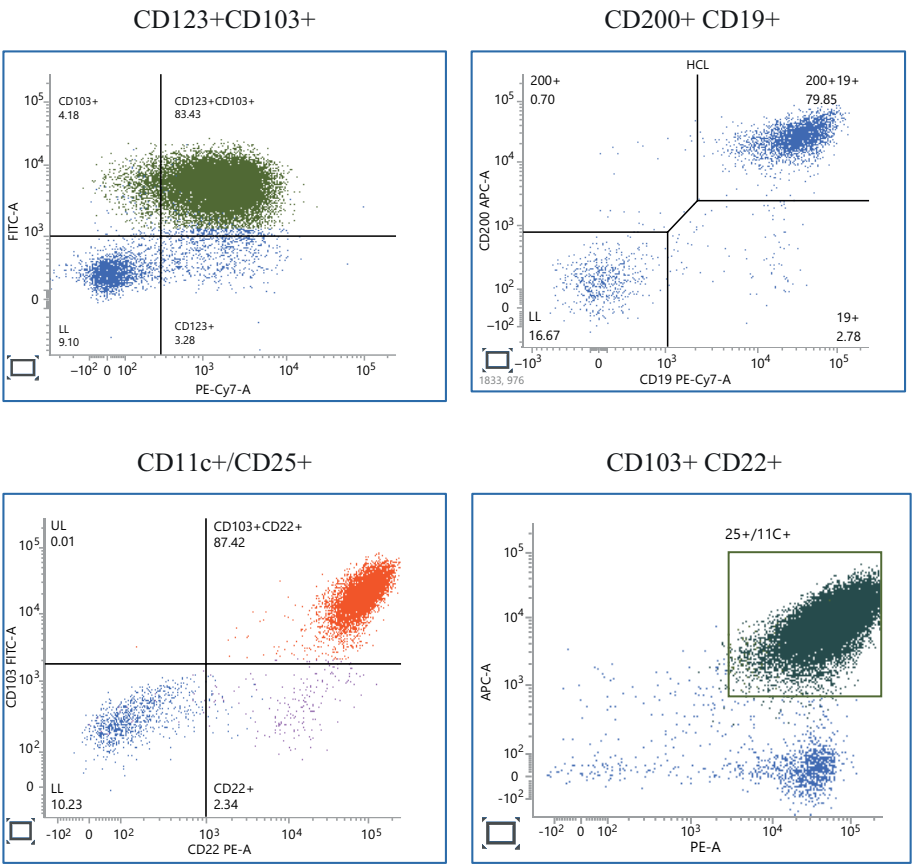

Figure 4. Classic HCL-phenotype on flow cytometry: CD11c+, CD103+, CD123+CD22+, CD25+, CD200+ cells.

TREATMENT OF CLASSIC HCL

A diagnosis of HCL does not necessarily entail treatment initiation in all patients. Indeed, asymptomatic patients (10% of all HCL patients) with moderate pancytopenia may remain stable and asymptomatic for years (1, 2). Such cases should be carefully followed in the outpatient setting until blood cell counts begin to decline. The frequency of visitation must be individualized to the clinical status of the patient. In addition, to ensure safe monitoring, the patient should also have a broad knowledge of the symptoms (30).

Symptomatic patients should however receive treatment for HCL. The indication for treatment initiation includes symptomatic splenomegaly, constitutive symptoms, recurrent infections and/or at least one significant cytopenia (absolute neutrophil count <1000/μL), hemoglobin concentration <11 g/dL, or platelet count <100,000/μL) (1, 2). Taking into account the myelosuppressive effect of

PNAs, it seems advisable to start the treatment before the counts decline to dangerous level.

FIRST-LINE TREATMENT

The purine nucleoside analogs cladribine (2-chlorodeoxyadenosine, 2-CdA) and pentostatin (deoxycoformycin, DCF), remain the standard first-line treatment for HCL and their design is still recognized as one of the greatest achievements in cancer therapy (Table 1) (31–43). Although 2-CdA and DCF have never been compared head-to-head, the two agents demonstrate similar long-term results regarding efficacy and toxicity. However, 2-CdA is more convenient for patients due to the shorter treatment duration, and hence is used more often than DCF (40, 41). In a study performed at Scripps Clinic in San Diego in 1990, all 12 patients responded to treatment, and 11 achieved complete remissions (CR) (43). Retrospective studies report overall response rates (ORR) of >95% and a complete remission (CR) rate >75%, with median relapse-free survival (RFS) up to 15 years. Many patients who were the first to be treated with PNAs are still alive 30 years later and remain in their first CR (42). A recently published European multicenter meta-analysis confirmed that 2-CdA has high efficacy as the first-line treatment. Data collected on 384 patients treated between 1969 and 2018 identified a 94% overall response rate (ORR), which was comparable with previous analyses, and a median OS of 25 years. Median PFS was 13 years, but even more importantly, 43% of patients were still free from progression after 22 years (37).

Cladribine follows different schedules and routes of administration. Originally, 2-CdA was administered at a dose of 0.1mg/kg/d for seven days in continuous intravenous infusion. However, the plasma concentration of 2-CdA does not differ between two-hour or continuous infusion, and so the drug is most commonly given as two-hour intravenous or subcutaneous infusions (44, 45). In addition, treatment with weekly 2-CdA infusions is equally effective as the standard 5-day 2-CdA administration (46, 47). Currently, 2-CdA is typically used at a dose of 0.12–0.14mg/kg/ day as a two-hour intravenous infusion for five to seven days, or 0.12–0.15 mg/kg/day in a two-hour intravenous infusion, once a week for six doses.

Treatment with 2-CdA might be complicated by fever present during or shortly following therapy, without other infectious symptoms or marked deterioration of general clinical status; this is usually attributed to cytokine release associated with rapid lysis of circulating hairy cells (48). In addition, neutropenia and infections can occur as symptoms of the natural history of the disease. On the other hand, these symptoms could arise as a consequence of PNA administration and their myelosuppressive and/or immunosuppressive activity (49). A complete diagnostic work-up and the prompt use of broad-spectrum antibiotics in combination with granulocyte colony stimulating factor (G-CSF) are commonly indicated. Cladribine is contraindicated in patients with active infection, and it is recommended to treat infections before beginning treatment with PNAs whenever possible. If immediate treatment initiation is needed, DCF at a reduced dose or interferon alpha (IFN-α) must be considered. Recently, BRAF inhibitors have been reported to be effective and safe in patients with newly diagnosed classic hairy cell leukemia and active infection (50).

TABLE 1 **Selected clinical trials of first line treatment with PNAs with longer follow-up in classic hairy cell leukemia**

Study	Treatment	No of pts/Follow-up	Response	Response duration and OS
Saven et al 1998 (31)	2-CdA 0.087 or 0.1 mg/kg/d ci × 7 days	349/median 52 m	ORR 98%, CR 91%, PR 7%	Median PFS for all responders 52 m, for CR 53m, for PR 37m
Goodman et al 2003 (32)	2-CdA 0.1 mg/kg/d ci × 7 days	207/NA	ORR 100%, CR 95%, PR 5% 95%/ Median 98m	Median PFS for all responders 98 m, OS 97% at 108 months
Cheson et al. 1998 (34)	2-CdA 0.1 mg/kg/d ci × 7 days	861/median 52 m	CR 50%, PR 37%	Median PFS > 44 m (range 1–63 + m)
Broccoli et al. 2022 (35)	2-CdA sc or iv	384/median 8.5 yrs	CR 72%, PR 22%	Median PFS 13 years, median OS not reached at 25 years
Inbar et al. 2018 (36)	2-CDA i.v. (62%) or sc (38%) × 5 days	203/median 5.2 yrs	NR.	OS at 10-year 94%, and at 20 yrs 75%
Broccoli et al. 2021 (37)	2-CdA 0.14 mg/kg sc or iv, for 5–7 days or once a week for 5 weeks.	122/followed between 1986 and 2018	ORR 86%, CR 54%	Median TTNT – 8.2 years, for PR 5.3 years ; for CR median NR at 25.8 years, median OS NR; probability of 65.7% alive at 25 years.
Grever et al 1995 (38)	DCF 2–4 mg/m^2 iv biweekly	154/median 57 m	OR 79%, PR 76%	Median RFS 82 m
Else et al (39)	DCF 4 mg/m^2 iv biweekly	188/median 14.0 yrs	OR96%, CR82%	Median PFS 10.5 yrs; Median RFS 16 yrs; OS at 15 yrs 78%

2-CdA: 2-chlorodeoxiadenosine, cladribine; ci: continuous infusion; CR: complete response; DCF: deoxycoformycin, pentostatin; DFS: disease free survival; iv: intravenously; NA: not available; NR: not reached; No: number of patients – month; ORR: overall response rate; OS: overal survival; PFS: progression free survival; PR: partial response; RFS: relapse free survival; sc: subcutaneoysly; TFI: treatment free interval; TTNT: time-to-next treatment; yrs: years

If PNAs are contraindicated, the BRAF inhibitors vemurafenib or dabrafenib can be used off-label in patients with neutropenia and infections. They can also be used before PNA therapy; in such cases, PNA therapy should be considered following amelioration of blood cell count and recovery from infection to obtain the best possible response in the treatment of HCL.

Another option for the treatment of HCL patients is the use of 2-CdA administered concurrently with eight weekly doses of rituximab, or with rituximab administered at least six months later, depending on minimal residual disease (MRD) status. In patients treated with 2-CdA plus concurrent rituximab, the CR rate was found to be 94% with minimal residual disease (MRD) being undetectable for a median of 6.5 years (51). However, this treatment is suggested rather for patients who are relapsed or refractory to PNA monotherapy (1, 2).

Pentostatin is typically administered intravenously at a dose of 4 mg/m^2 by bolus injection or diluted in a larger volume and given over 20 to 30 minutes once every other week. In the absence of major toxicity and with continuing improvement, the treatment should be continued until hematologic CR plus two additional doses following CR. In patients with active infection or any other increased risk of mortality or morbidity due to myelosupression, the pentostatin dose might be reduced to 2 mg/m^2 until clinical improvement is observed, with subsequent administration of standard doses (52).

Nowadays, there are limited indications for the use of IFN-α in the first-line treatment. The responses induced by IFN-α are partial and of short duration. Most patients eventually relapse, with a median response duration ranging from 6 to 25 months (53). The use of IFN-α may be used transiently to increase the neutrophil count and cure infection, prior to the initiation of treatment with PNA (54). Another potential indication for IFN-α treatment is the COVID-19 pandemic, to avoid the severe immunosuppression associated with PNA and rituximab use (55). Splenectomy is a life-saving procedure for splenic rupture due to splenomegaly. In patients refractory to IFN-α, it may be also be performed in pregnancy (56, 57).

TREATMENT OF RELAPSED/REFRACTORY HCL

Despite the remarkable success of PNAs, half of the patients experience at least one relapse of the disease later in life. As such, new therapeutic strategies are still needed to further improve PFS. At relapse, the same indications for treatment apply as for first-line therapy, including symptomatic disease or progressive cytopenias (1, 2). Patients with early relapse (< 24 months) are candidates for alternative therapies, preferentially with novel drugs or within clinical trials. In remission lasting 24–60 months, retreatment with PNA combined with rituximab should be considered. The addition of rituximab to 2-CdA at standard doses of 375mg/m^2 weekly for 6-8 doses, either concurrently or sequentially one month after 2-CdA, can improve the outcome (51, 58). Finally, patients who remain in CR for more than five years (>60 months) may be retreated with the initial therapy (1).

Bendamustin (70mg/m^2 or 90mg/m^2) in combination with rituximab (375 mg/m^2) given intravenously on days 1 and 15, for six cycles at four-week intervals demonstrated activity in 12 heavily pretreated HCL patients (59). The overall

response rate was 100%, with seven CRs. MRD was undetectable in six patients with CRs at 30–35 (median 31) months of follow-up.

MOXETUMOMAB PASUDOTOX

Moxetumomab pasudotox (Moxe), was originally discovered by Ira Pastan and Robert Kreitman in the National Cancer Institute, Center for Cancer Research (60, 61). Moxe is an anti-CD22 targeted recombinant immunotoxin produced by the fusion of a toxin fragment, Pseudomonas exotoxin A (PE38), with the Fv of the murine anti-CD22 antibody, which leads the toxin directly to the tumor cell. The drug was active and well tolerated in phase 1 and 3 studies performed in relapsed/refractory patients with HCL (60, 62–65). In the phase 1/2 study Moxe was used at a dose of 50-µg/kg every other day for three doses in four-week cycles (60, 62). Among 33 analyzed patients, the OR rate was 88%, with 64% CR. The CR duration was longer in MRD-negative patients. The median CR duration was 13.5 months in nine MRD-positive CRs, and 42.1 months in 11 MRD-negative CRs.

In a phase 3 trial, 80 patients were treated with Moxe at a dose of 40 µg/kg by 30 min intravenous infusion on days 1, 3, and 5 of a 28-day cycle (64). Treatment was continued for up to six cycles, or until CR with MRD negativity, disease progression or unacceptable toxicity. At a median follow-up of 24.6 months, 60 patients (75%) had responded to treatment, of which 27 had MRD-negative CR. The durable CR rate with hematologic response longer than 180 days was 36%, and CR longer than 360 days was 33%. Longer median hematologic remission was noted in MRD-negative patients (62.8 months) than in MRD-positive patients (12.0 months). Inferior results were observed in splenectomized patients. There is a single report on successful retreatment with Moxe in relapsed patients (65).

The most frequent adverse events (AEs) were easily manageable nausea, peripheral edema, headache, and pyrexia. Hemolytic uremic syndrome (HUS) and capillary leak syndrome (CLS) are black box warnings for Moxe, which was noted in eight patients (10%), before or on day 8 of treatment; these led to drug discontinuation (62–65). Thus, careful clinical and laboratory assessment are mandatory during treatment. In order to prevent life-threatening complications, adequate oral and intravenous hydration is recommended. All patients should receive intravenous fluids two to four hours before and after Moxe administration. On days 1-8 of each 28-day cycle, all patients should be hydrated with oral fluids, 250ml per hour, and not to go more than two to three hours at night without drinking. Thromboprophylaxis with low-dose aspirin is also considered (66).

Moxe was approved by the USA Food and Drug Administration (FDA – September 2018) and the European Medicines Agency (EMA – February 2021) for the treatment of adult patients with relapsed/refractory HCL who had received at least two prior systemic therapies, including treatment with purine analogues. However, in July 2021 the European Commission withdrew the approval for Moxe in the European Union. The withdrawal was performed at the request of the marketing authorization holder, AstraZeneca, for commercial reasons. Moxe is still commercially available in the USA.

BRAF INHIBITORS

The $BRAF^{V600E}$ mutation, the driving genetic event in HCL, provided the scientific rationale for the therapeutic use of BRAF-MEK pathway inhibitors in patients with classic HCL. The BRAF kinase inhibitors vemurafenib and dabrafenib are active drugs in patients with refractory and recurrent HCL; they can be used in monotherapy, or in combination with CD20 antibodies or MEK inhibitors (Table 2). In a phase-2 single-arm multicenter study performed in Italy and the USA, vemurafenib was given as a single drug at 960 mg twice daily for a median of 16–18 weeks (67). Most of the patients responded to treatment. Overall response rates were 96% (25/26) after a median of eight weeks in the Italian patients and 100% after a median of 12 weeks in the USA. Complete response rates were 34.6% and 41.7%, respectively. However, all the CRs were MRD positive, and the median relapse-free survival (RFS) was only nine months after treatment discontinuation (67).

Drug-related AEs were mostly of grade 1–2, and these included skin toxicity (rash, photosensitivity), arthralgia, fever, and elevated bilirubin level. All adverse events were reversible and easily manageable with dose-adjustments and symptomatic treatments including low-dose steroids and non-steroidal anti-inflammatory drugs. However, about 50% of patients required dose modifications due to AEs. Originally, the dosing and scheduling of BRAF inhibitors was extrapolated from standard treatments for melanoma and still remains a matter of debate. Based on the retrospective analysis of patients treated outside clinical trials with variables schedules of administration, we already know that BRAF inhibitors induce hematologic remission in all HCL patients, regardless of the dose used (68). However, higher doses of BRAF inhibitors (vemurafenib > 480mg BID, dabrafenib > 150mg BID) improve the quality of responses and prolong time to next treatment (TNT) when compared with low-dose BRAF inhibitors. Retreatment with BRAF inhibitors is equally effective only after the second course, but the responsiveness decreases with each successive cycle, with median TNT less than four months (68, 69). In those cases, when BRAF inhibitors are the only available treatment option, continuous treatment with low-dose BRAF inhibitors should be considered, as it may provide stabilization of the disease for more than one year (69).

Deeper and longer responses can be achieved when vemurafenib is combined with rituximab (70). In a phase 2 trial in patients with refractory or relapsed HCL, vemurafenib was administered at 960mg BID for eight weeks and rituximab at $375mg/m^2$ every two weeks for eight doses (70). CR was achieved in 26 of 30 patients (87%). Furthermore, undetectable MRD was observed in 17 (65%) of 26 patients in CR. MRD negativity correlated with longer survival without relapse. These excellent responses correlated with PFS, which amounted to 78% at a median follow-up of 37 months. In addition, this drug combination seems to be effective in patients previously treated with Moxe (71). Vemurafenib was also combined with obinutuzumab in previously untreated HCL patients in a phase 2 study (72). Vemurafenib was administered at a dose of 960 mg twice per day for four months and obinutuzumab at 1000 mg.iv. on days 1, 8 and 15 of month 2, and day 1 of months 3 and 4. MRD-negative CR was achieved in seven patients, and PR was noted in two patients at the end of treatment. All patients remained in remission with a median follow-up of 9.7 months.

TABLE 2 **Larger clinical trials with novel agents in hairy cell leukemia**

Study	Treatment regimen	No of pts/characteristics	Response	Response duration and OS
Kreitman et al. 2018, 2021 (63, 64)	Moxetumomab pasudotox 40 μg/kg i.v. on days 1, 3, and 5 every 28 days for ≤6 cycles	80/cHCL or HCL-v after ≥2 prior therapies	ORR – 80%, CR – 41%; CR MRD negative – 85%;	Median PFS 71.7 m for CR lasting ≥ 60 months, CR with HR ≥ 360 days –33%
Tiacci et al. 2015 (67)	Vemurafenib 960 mg p.o.twice daily for 16 or 18	50/cHCL, R/R after PNA	Italian study: ORR – 96%, CR – 35% U.S. study: OR –100%, CR –42%	Italian study: RFS 19m;U.S. study: 1-year PFS 73% and 1-year OS 91%
Dietrich et al 2016 (68)	Vemurafenib 240-1920 mg/d p.o. for median 90 days	21/cHCL, median TFD 8 yrs	ORR 95%, CR 40%	Median EFS 17m, OS at 12 m –88%.
Tiaccci et al. 2021 (70)	Vemurafenib 960 mg p.o. twice daily for 8 weeks + rituximab 375 mg/m^2 i.v. every two weeks	30/c HCL with a median of 3 previous therapies.	CR – 26/30 (87%), MRD negative CR 17 (65%)	PFS for median follow-up of 37 m 78%, RFS for median follow-up of 37 m 85%
Tiaccci et al. 2021 (73)	Dabrafenib 150 mg p.o. twice daily for 8–12 weeks	10/RR cHCL, median 3.5 prior ther therapies	ORR 80%, CR 30%, PR – 50%	OS 90% at median follow-up 64 m
Kreitman et al. 2018 (74)	Dabrafenib 150 mg p.o.twice daily) + trametinib 2 mg p.o. once daily	43/RR cHCL (49% received ≥ 4 prior treatments)	ORR -32/41 (78%); CR – 20 (49%), PR 12 (29%)	PFS and OS at 1 year – 97.6%
Rogers et al 2021 (76)	Ibrutinib: 420 mg – 840 mg p.o. daily-10	37/ cHCL –28 (76%) HCL-v – 9(24%)	ORR (CR and PR) at 32 weeks 24% and at 48 weeks 54%	Estimated 3yrs - PFS rate -73% and OS rate – 85%, median OS – 69 months

BM: bone marrow; CR: complete response; cHCL: classic HCL; EFS: event-free survival; HCL-v: HCL variant; HR: hematologic remission; MRD: minimal residual disease; ORR: overall response rate; OS: overall survival; PFS: progression free survival; p.o.: orally; PR: partial response; RR: refractory/relapsed; RFS: relapse-free survival; TFD: time from diagnosis; ther: therapies.

Another BRAF inhibitor, dabrafenib, was investigated in a phase 2 study in relapsed/refractory patients with classic HCL (73). Dabrafenib was given at a dose of 150 mg twice daily for eight weeks. If no CR was obtained after this time, the patients received an additional four-week treatment. ORR was 80% and CR 30%. The combination of BRAF inhibitors with MEK inhibitors is another strategy for enhancing treatment efficacy (74). In a phase 2 trial, dabrafenib was administered continuously at a dose of 150 mg BID with tramentinib 2 mg, once daily. Response was achieved in 78% of patients. PFS and OS rates were both 97.6% at 12 months, and PFS was 50% at 18 months. However, AEs led to dose reduction in 42% and treatment interruption in 56% of patients.

IBRUTINIB

The B-cell receptor (BCR) signaling pathway is a crucial pathway of B cells, both for their survival and for surface-mediated activation, proliferation, and differentiation. BCR signaling is also involved in HCL pathogenesis (75). Recently, ibrutinib was evaluated in a phase 2 study in 37 HCL patients including 28 with classic HCL and nine with HCL-v (76). Ibrutinib was given at a dose 420–840 mg daily until progression or unacceptable toxicity. Response was 24% at 32 weeks and 36% at 48 weeks. The OR rate at any time was 54% including seven CR, 13 PR and 10 patients had stable disease. Similar response rates were observed in patients with the classic HCL and HCL-v. The estimated 36-month PFS was 73% and OS 85%, with no differences between HCL and HCL-v. Even though the results are not as spectacular as with other novel drugs, ibrutinib remains an option for patients not suitable for other treatments (77).

HAIRY CELL LEUKEMIA VARIANT

Hairy cell leukemia variant (HCL-v) is a rare B-cell lymphoproliferative neoplasm, arising or locating primarily in the spleen; it is biologically distinct from classic HCL, being more aggressive and responding poorly to PNAs (78). In the fifth edition of the WHO Classification of Hematolymphoid Tumors (2022), HCL-v and prolymphocytic B-cell leukemia are reclassified as splenic B-cell lymphoma/leukemia with prominent nucleoli (5). The clinical course of HCL-v is more aggressive than classic HCL with a median OS of 7–9 years (79). Morphologically, HCL-v cells show hybrid features between classic HCL cells and prolymphocytes. The nuclei have a prominent nucleolus, similar to prolymphocytes, and the cytoplasm often has a variable number of cytoplasmic projections. Patients are usually symptomatic, with symptoms of anemia and/or bleeding depending on cytopenias, and with abdominal discomfort related to splenomegaly. White blood count is elevated with lymphocytosis in the PB smear, accompanied by anemia and thrombocytopenia. The absolute number of monocytes is normal, in contrast to classic HCL. The BM is hypercellular and may be easily aspirated. Peripheral lymphadenopathy is rare, but central lymphadenopathy with enlarged abdominal and retroperitoneal lymph nodes may be detected on CT scans. Hepatomegaly is reported in 20–30%.

A critical aspect of HCL-v diagnosis is immunophenotype. Leukemic cells strongly express the pan-B-cell markers CD19, CD20, CD22 and FMC7. Surface immunoglobulin expression is strong, with CD5 and CD23 usually negative. In contrast to classic HCL, CD25 and CD123 are negative but CD11c is always positive and CD103 is positive in 2/3 of HCL-v cases.

HCL-v lacks Annexin A1 expression and the *BRAF*^{V600E} point mutation, which is characteristic of classic HCL. A subset of patients also has activating mutations in *MAP2K1*, a gene that encodes MEK1, a downstream component of the BRAF-MEK-ERK signaling cascade. While there is no genetic mutation diagnostic of HCL-v, genetic profiling efforts have identified potential therapeutic targets (i.e., *MAP2K1, KDM6A, CREBBP, ARID1A, CCND3, U2AF1, KMT2C*).

PNAs are less active in HCL-v than in classic HCL. In addition, patient responses are poor and of short duration: only about a half the patients obtain PR, and the median time of response is 15 months (80). The current treatment of choice in previously untreated HCL-v patients is combination immunochemotherapy with 2-CdA and rituximab. In a phase 2 study 2-CdA was given intravenously, at a dose of 0.15mg/kg for five consecutive days with eight weekly doses of rituximab 375mg/m^2 in 20 patients, of whom eight were previously untreated and 12 with recurrent/refractory disease (81). The CR rate was 95%, and MRD negative CR in 80% of patients at six months after the end of treatment. The 5-year PFS was 63.3% and 10-year PFS was 44.3%. The duration of response was longer in the MRD-negative patients.

Ibrutinib is active in the treatment of HCL-v. Recently published data by Rogers et al. show the ORR increasing with time, from 24% at 32 weeks to 36% at 48 weeks, for both HCL-v and classic HCL (76). Splenectomy is still an option in the treatment of HCL-v as it corrects cytopenias due to hypersplenism and removes a significant bulk of disease. Historical results indicate 74% PRs lasting for 1–10 years (median: four years) (82, 83). Rituximab may be used as a consolidation therapy after splenectomy (84). In addition, splenic irradiation can be considered in elderly patients with high surgical risks (80).

CONCLUSION

Classic HCL is a rare type of B-cell chronic lymphoid leukemia characterized by marked splenomegaly, progressive pancytopenia, and reactive marrow fibrosis. A diagnosis of HCL is based on cytology and confirmed by flow cytometry studies using anti-B-cell antibodies against CD19, CD20 or CD22, as well as antibodies more specific to HCL, such as CD11c, CD25, CD103 and CD123. Most patients with classic HCL also demonstrate the *BRAF*^{V600E} mutation, which has been described as a disease-defining genetic event.

The purine nucleoside analogs 2-CdA and DCF are the drugs of choice in previously untreated patients with HCL. These agents induce durable and unmaintained, long-lasting CR in more than 70% of patients. Thanks to the use of these drugs, classic HCL has transformed from a disease with poor prognosis to a highly treatable disorder with near-normal survival. Rituximab is also active in HCL and can be given as a single agent or in combination with PNA. Immunotoxin, i.e., moxetumomab pasudotox, has been approved for the treatment of patients with

relapsed or refractory HCL who relapse after two or more prior systemic therapies. The BRAF inhibitors vemurafenib and dabrafenib exhibit remarkable activity in patients with classic HCL and are used in relapsed and refractory patients. However, these drugs have not yet been formally approved for the treatment of HCL. BRAF inhibitors are more active when combined with CD20 monoclonal antibodies. The BTK inhibitor ibrutinib is under investigation in patients with relapsed HCL and has demonstrated some activity.

HCL-v is characterized by lymphocytosis with lymphoid cells of relatively large size and prominent nucleoli, cytopenias without monocytopenia, atypical HCL immunophenotype without CD25 expression, and a lack of BRAF mutation. Biologically, this disease is more closely related to splenic lymphomas, and together with prolymphocytic B-cell leukemia, has been reclassified by the fifth edition of the WHO Classification of Haematolymphoid Tumors (2022) as splenic B-cell lymphoma/leukemia with prominent nucleoli.

Although HCL-v demonstrates poor response to single-agent purine analogs 2-CdA and DCF, which are very effective in classic HCL, better ORR and response duration were achieved by combining PNAs with rituximab. Indeed, the combination of 2-CdA with rituximab is now the recommended first-line treatment in this disease.

Acknowledgment: This research was supported by funding from the Medical University of Lodz, Poland (No. 503/1-093-01/503-11-004). We thank Edward Lowczowski from the Medical University of Lodz for editorial assistance.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

REFERENCES

1. Grever MR, Abdel-Wahab O, Andritsos LA, Banerji V, Barrientos J, Blachly JS, Call TG, Catovsky D, Dearden C, Demeter J, et al : Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. Blood. 2017;129(5):553–60. https://doi.org/10.1182/blood-2016-01-689422
2. Robak T, Matutes E, Catovsky D, Zinzani PL, Buske C; ESMO Guidelines Committee. Hairy cell leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26 (Suppl 5):v100-7. https://doi.org/10.1093/annonc/mdv200
3. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90. https://doi.org/10.1182/blood-2016-01-643569
4. Maitre E, Tomowiak C, Lebecque B, Bijou F, Benabed K, Naguib D, et al. Deciphering genetic alterations of hairy cell leukemia and hairy cell leukemia-like disorders in 98 patients. Cancers (Basel). 2022;14(8):1904. https://doi.org/10.3390/cancers14081904

5. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720–48. https://doi.org/10.1038/s41375-022-01620-2
6. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. CA Cancer J Clin. 2016;66(6):443–59. https://doi.org/10.3322/caac.21357
7. Dores GM, Matsuno RK, Weisenburger DD, Rosenberg PS, Anderson WF. Hairy cell leukaemia: a heterogeneous disease? Br J Haematol. 2008;142(1):45–51.https://doi.org/10.1111/j.1365-2141.2008.07156.x
8. Monnereau A, Slager SL, Hughes AM, Smith A, Glimelius B, Habermann TM, et al, Clavel J. Medical history, lifestyle, and occupational risk factors for hairy cell leukemia: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014;2014(48):115–24. https://doi.org/10.1093/jncimonographs/lgu004
9. Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, et al J.Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. Occup Environ Med. 2009;66(5):291–8. https://doi.org/10.1136/oem.2008.040972
10. Stewart DJ, Keating MJ. Radiation exposure as a possible etiologic factor in hairy cell leukemia (leukemic reticuloendotheliosis). Cancer. 1980;46(7):1577-80. https://doi.org/10.1002/1097-0142(19801001)46:7<1577::AID-CNCR2820460714>3.0.CO;2-L
11. Colovic MD, Jankovic GM, Wiernik PH. Hairy cell leukemia in first cousins and review of the literature. Eur J Haematol. 2001;67(3):185–8. https://doi.org/10.1034/j.1600-0609.2001.5790478.x
12. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364(24):2305–15. https://doi.org/10.1056/NEJMoa1014209
13. Xi L, Arons E, Navarro W, Calvo KR, Stetler-Stevenson M, Raffeld M, Kreitman RJ. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. Blood. 2012;119(14):3330–2. https://doi.org/10.1182/blood-2011-09-379339
14. Tiacci E, Schiavoni G, Forconi F, Santi A, Trentin L, Ambrosetti A, et al. Simple genetic diagnosis of hairy cell leukemia by sensitive detection of the BRAF-V600E mutation. Blood. 2012;119(1):192–5. https://doi.org/10.1182/blood-2011-08-371179
15. Oscier D, Stamatopoulos K, Mirandari A, Strefford J. The genomics of hairy cell leukaemia and splenic diffuse red pulp lymphoma. Cancers (Basel). 2022; 29:14(3):697. https://doi.org/10.3390/cancers14030697
16. Troussard X, Maître E, Cornet E. Hairy cell leukemia 2022: Update on diagnosis, risk-stratification, and treatment. Am J Hematol. 2022;97(2):226–236. https://doi.org/10.1002/ajh.26390
17. Tschernitz S, Flossbach L, Bonengel M, Roth S, Rosenwald A, Geissinger E. Alternative BRAF mutations in BRAF V600E-negative hairy cell leukaemias. Br J Haematol. 2014;165(4):529–33. https://doi.org/10.1111/bjh.12735
18. Forconi F, Sozzi E, Cencini E, Zaja F, Intermesoli T, Stelitano C, et al. Hairy cell leukemias with unmutated IGHV genes define the minor subset refractory to single-agent cladribine and with more aggressive behavior. Blood. 2009;114(21):4696–702. https://doi.org/10.1182/blood-2009-03-212449
19. Andrulis M, Penzel R, Weichert W, von Deimling A, Capper D. Application of a BRAF V600E mutation-specific antibody for the diagnosis of hairy cell leukemia.Am J Surg Pathol. 2012;36(12):1796–800. https://doi.org/10.1097/PAS.0b013e3182549b50
20. Maitre E, Cornet E, Salaün V, Kerneves P, Chèze S, Repesse Y, Damaj G, Troussard X. Immunophenotypic analysis of hairy cell leukemia (HCL) and hairy cell leukemia-like (HCL-like) disorders. Cancers (Basel). 2022;14(4):1050. https://doi.org/10.3390/cancers14041050
21. Puła A, Robak T. Hairy cell leukemia: a brief update on current knowledge and treatment prospects. Curr Opin Oncol. 2021;33(5):412–19. https://doi.org/10.1097/CCO.0000000000000771
22. Robak E, Jesionek-Kupnicka D, Robak T. Skin changes in hairy cell leukemia. Ann Hematol. 2021;100(3):615–25; https://doi.org/10.1007/s00277-020-04349-z
23. Robak P, Jesionek-Kupnicka D, Kupnicki P, Polliack A, Robak T. Bone lesions in hairy cell leukemia: Diagnosis and treatment. Eur J Haematol. 2020;105(6):682-91https://doi.org/10.1111/ejh.13505
24. Kreitman RJ. Hairy cell leukemia: present and future directions. Leuk Lymphoma. 2019;60(12):2869–2879. https://doi.org/10.1080/10428194.2019.1608536

25. Summers TA, Jaffe ES. Hairy cell leukemia diagnostic criteria and differential diagnosis. Leuk Lymphoma 2011; 2011;52(Suppl 2):6–10. https://doi.org/10.3109/10428194.2011.565435
26. Katayama I. Bone marrow in hairy cell leukemia. Hematol Oncol Clin North Am. 1988;2(4):585–602. https://doi.org/10.1016/S0889-8588(18)30586-0
27. Troussard X, Maître E, Cornet E. Hairy cell leukemia 2022: Update on diagnosis, risk-stratification, and treatment. Am J Hematol. 2022;97(2):226–36. https://doi.org/10.1002/ajh.26390
28. Wierda WG, Byrd JC, Abramson JS, et al. Hairy Cell Leukemia, Version 2.2018, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15(11):1414–1427. https://doi.org/10.6004/jnccn.2017.0165
29. Wotherspoon A, Attygalle A, Mendes LS. Bone marrow and splenic histology in hairy cell leukaemia. Best Pract Res Clin Haematol. 2015;28(4):200–7. https://doi.org/10.1016/j.beha.2015.10.019
30. Cross M, Dearden C. Hairy cell leukaemia. Curr Oncol Rep. 2020;22(5):42. https://doi.org/10.1007/s11912-020-00911-0
31. Saven A, Burian C, Koziol JA, Piro LD. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood. 1998;92(6):1918–26. https://doi.org/10.1182/blood.V92.6.1918
32. Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol. 2003;21(5):891–6. https://doi.org/10.1200/JCO.2003.05.093
33. Bohn JP, Neururer S, Pirklbauer M, Pircher A, Wolf D. Hairy cell leukemia patients have a normal life expectancy-a 35-year single-center experience and comparison with the general population. Cancers (Basel). 2022;14(5):1242. https://doi.org/10.3390/cancers14051242
34. Cheson BD, Sorensen JM, Vena DA, Montello MJ, Barrett JA, Damasio E, et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine via the Group C protocol mechanism of the National Cancer Institute: a report of 979 patients. J Clin Oncol. 1998;16(9):3007–15. https://doi.org/10.1200/JCO.1998.16.9.3007
35. Broccoli A, Argnani L, Cross M, Janus A, Maitre E, Troussard X, et al. A 3-decade multicenter European experience with cladribine as upfront treatment in 384 hairy cell leukemia patients. Blood Adv. 2022 May 18:bloodadvances.202200785. https://doi.org/10.1182/bloodadvances.2022007854
36. Inbar M, Herishanu Y, Goldschmidt N, Bairey O, Yuklea M, Shvidel L, et al. Hairy cell leukemia: retrospective analysis of demographic data and outcome of 203 patients from 12 medical centers in Israel. Anticancer Res. 2018;38(11):6423–9. https://doi.org/10.21873/anticanres.13003
37. Broccoli A, Argnani L, Nanni L, Terragna C, Sabattini E, Gabrielli G, et al. The treatment of hairy cell leukemia with a focus on long lasting responses to cladribine: A 30-year experience. Am J Hematol. 2021;96(10):1204–10. https://doi.org/10.1002/ajh.26287
38. Grever M, Kopecky K, Foucar MK, Head D, Bennett JM, Hutchison RE, et al. Randomized comparison of pentostatin versus interferon alfa-2a in previously untreated patients with hairy cell leukemia: an intergroup study. J Clin Oncol. 1995;13(4):974–82. https://doi.org/10.1200/JCO.1995.13.4.974
39. Else M, Dearden CE, Matutes E, Garcia-Talavera J, Rohatiner AZ, Johnson SA, et al.. Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis. Br J Haematol. 2009;145(6):733–40. https://doi.org/10.1111/j.1365-2141.2009.07668.x
40. Robak T, Błasińska-Morawiec M, Błoński J, Hellmann A, Hałaburda K, Konopka L, et al. 2-chlorodeoxyadenosine (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant: 7-year experience in Poland. Eur J Haematol. 1999 ;62(1):49–56. https://doi.org/10.1111/j.1600-0609.1999.tb01114.x
41. Paillassa J, Cornet E, Noel S, Tomowiak C, Lepretre S, Vaudaux S, et al. Analysis of a cohort of 279 patients with hairy-cell leukemia (HCL): 10 years of follow-up. Blood Cancer J. 2020;10(5):62. https://doi.org/10.1038/s41408-020-0328-z
42. Else M, Dearden CE, Catovsky D. Long-term follow-up after purine analogue therapy in hairy cell leukaemia. Best Pract Res Clin Haematol. 2015;28(4):217–229. https://doi.org/10.1016/j.beha.2015.09.004
43. Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. N Engl J Med. 1990;322(16):1117–21. https://doi.org/10.1056/NEJM199004193221605

44. Liliemark J, Juliusson G. Cellular pharmacokinetics of 2-chloro-2'-deoxyadenosine nucleotides: comparison of intermittent and continuous intravenous infusion and subcutaneous and oral administration in leukemia patients. Clin Cancer Res. 1995;1(4):385–90.
45. Juliusson G, Heldal D, Hippe E, Hedenus M, Malm C, Wallman K, et al. Subcutaneous injections of 2-chlorodeoxyadenosine for symptomatic hairy cell leukemia. J Clin Oncol. 1995;13(4):989–95. https://doi.org/10.1200/JCO.1995.13.4.989
46. Robak T, Jamroziak K, Gora-Tybor J, Blonski JZ, Kasznicki M, Dwilewicz-Trojaczek J, et al. Cladribine in a weekly versus daily schedule for untreated active hairy cell leukemia: final report from the Polish Adult Leukemia Group (PALG) of a prospective, randomized, multicenter trial. Blood. 2007;109(9):3672–5. https://doi.org/10.1182/blood-2006-08-042929
47. Zenhäusern R, Schmitz SF, Solenthaler M, Heim D, Meyer-Monard S, Hess U, et al. Randomized trial of daily versus weekly administration of 2-chlorodeoxyadenosine in patients with hairy cell leukemia: a multicenter phase III trial (SAKK 32/98). Leuk Lymphoma. 2009; 50(9):1501–11. https://doi.org/10.1080/10428190903131755
48. Juliusson G, Lenkei R, Tjønnfjord G, Heldal D, Liliemark J. Neutropenic fever following cladribine therapy for symptomatic hairy-cell leukemia: predictive factors and effects of granulocyte-macrophage colony-stimulating factor. Ann Oncol. 1995;6(4):371–5. https://doi.org/10.1093/oxfordjournals.annonc.a059186
49. Robak T, Robak P. Purine nucleoside analogs in the treatment of rarer chronic lymphoid leukemias. Curr Pharm Des. 2012;18(23):3373–88. https://doi.org/10.2174/138161212801227005
50. Shenoi DP, Andritsos LA, Blachly JS, Rogers KA, Moran ME, Anghelina M, Jones JA, Grever MR. Classic hairy cell leukemia complicated by pancytopenia and severe infection: a report of 3 cases treated with vemurafenib. Blood Adv. 2019;3(2):116–118. https://doi.org/10.1182/bloodadvances.2018027466
51. Chihara D, Arons E, Stetler-Stevenson M, Yuan CM, Wang HW, Zhou H, et al. Randomized phase II study of first-line cladribine with concurrent or delayed rituximab in patients with hairy cell leukemia. J Clin Oncol. 2020 ;38(14):1527–1538. https://doi.org/10.1200/JCO.19.02250
52. Andritsos LA, Dunavin N, Lozanski G, Jones JA, Blachly JS, Lucas DM, et al. Reduced dose pentostatin for initial management of hairy cell leukemia patients who have active infection or risk of hemorrhage is safe and effective. Haematologica. 2015;100(1):e18–20. https://doi.org/10.3324/haematol.2014.113290
53. Ahmed S, Rai KR. Interferon in the treatment of hairy-cell leukemia. Best Pract Res Clin Haematol. 2003;16(1):69–81. https://doi.org/10.1016/S1521-6926(02)00084-1
54. Maitre E, Cornet E, Troussard X. Hairy cell leukemia: 2020 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019;94(12):1413–22. https://doi.org/10.1002/ajh.25653
55. Grever M, Andritsos L, Banerji V, Barrientos JC, Bhat S, Blachly JS, et al. Hairy cell leukemia and COVID-19 adaptation of treatment guidelines. Leukemia. 2021;35(7):1864–72. https://doi.org/10.1038/s41375-021-01257-7
56. Habermann TM, Rai K. Historical treatments of in hairy cell leukemia, splenectomy and interferon: past and current uses. Leuk Lymphoma. 2011;52 (Suppl 2):18–20. https://doi.org/10.3109/10428194.2011.573033
57. Stiles GM, Stanco LM, Saven A, Hoffmann KD. Splenectomy for hairy cell leukemia in pregnancy. J Perinatol. 1998;18(3):200–1.
58. Chihara D, Kantarjian H, O'Brien S, Jorgensen J, Pierce S, Faderl S, et al. Long-term durable remission by cladribine followed by rituximab in patients with hairy cell leukaemia: update of a phase II trial. Br J Haematol. 2016;174(5):760–6. https://doi.org/10.1111/bjh.14129
59. Burotto M, Stetler-Stevenson M, Arons E, Zhou H, Wilson W, Kreitman RJ. Bendamustine and rituximab in relapsed and refractory hairy cell leukemia. Clin Cancer Res. 2013;19(22):6313–21. https://doi.org/10.1158/1078-0432.CCR-13-1848
60. Kreitman RJ, Tallman MS, Robak T, Coutre S, Wilson WH, Stetler-Stevenson M, et al. Phase I trial of anti-CD22 recombinant immunotoxin moxetumomab pasudotox (CAT-8015 or HA22) in patients with hairy cell leukemia. J Clin Oncol. 2012;30(15):1822–8. https://doi.org/10.1200/JCO.2011.38.1756
61. Janus A, Robak T. Moxetumomab pasudotox for the treatment of hairy cell leukemia. Expert Opin Biol Ther. 2019;19(6):501–508. https://doi.org/10.1080/14712598.2019.1614558

62. Kreitman RJ, Tallman MS, Robak T, Coutre S, Wilson WH, Stetler-Stevenson M, et al. Minimal residual hairy cell leukemia eradication with moxetumomab pasudotox: phase 1 results and long-term follow-up. Blood. 2018;131(21):2331–4. https://doi.org/10.1182/blood-2017-09-803072
63. Kreitman RJ, Dearden C, Zinzani PL, Delgado J, Karlin L, Robak T, et al. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. Leukemia. 2018;32(8):1768–77. https://doi.org/10.1038/s41375-018-0210-1
64. Kreitman RJ, Dearden C, Zinzani PL, Delgado J, Robak T, le Coutre PD, et al. Moxetumomab pasudotox in heavily pre-treated patients with relapsed/refractory hairy cell leukemia (HCL): long-term follow-up from the pivotal trial. J Hematol Oncol. 2021;14(1):35. https://doi.org/10.1186/s13045-020-01004-y
65. Yurkiewicz IR, Coutre S, Ghesquieres H, Pastan I, Kreitman RJ. Moxetumomab pasudotox as re-treatment for heavily-pretreated relapsed hairy cell leukemia. Leuk Lymphoma. 2021;62(11):2812–4. https://doi.org/10.1080/10428194.2021.1929959
66. Kreitman RJ, Pastan I. Contextualizing the Use of Moxetumomab Pasudotox in the Treatment of Relapsed or Refractory Hairy Cell Leukemia. Oncologist. 2020;25(1):e170–e177. https://doi.org/10.1634/theoncologist.2019-0370
67. Tiacci E, Park JH, De Carolis L, Chung SS, Broccoli A, Scott S, et al. Targeting mutant BRAF in relapsed or refractory hairy-cell leukemia. N Engl J Med. 2015;373(18):1733–47. https://doi.org/10.1056/NEJMoa1506583
68. Dietrich S, Pircher A, Endris V, Peyrade F, Wendtner CM, Follows GA, et al. BRAF inhibition in hairy cell leukemia with low-dose vemurafenib. Blood. 2016;127(23):2847–55. https://doi.org/10.1182/blood-2015-11-680074
69. Liebers N, Roider T, Bohn JP, Haberbosch I, Pircher A, Ferstl B, et al. BRAF inhibitor treatment in classic hairy cell leukemia: a long-term follow-up study of patients treated outside clinical trials. Leukemia. 2020;34(5):1454–57. https://doi.org/10.1038/s41375-019-0646-y
70. Tiacci E, De Carolis L, Simonetti E, Capponi M, Ambrosetti A, Lucia E, et al. Vemurafenib plus rituximab in refractory or relapsed hairy-cell leukemia. N Engl J Med. 2021;384(19):1810–23. https://doi.org/10.1056/NEJMoa2031298
71. Robak T, Janus A, Jamroziak K, Tiacci E, Kreitman RJ. Vemurafenib and rituximab in patients with hairy cell leukemia previously treated with moxetumomab pasudotox. J Clin Med. 2021;10(13):2800. https://doi.org/10.3390/jcm10132800
72. Park JH, Shukla M, Salcedo JM, Vemuri S, Kinoshita JC, Smith MD. et al. First line chemo-free therapy with the BRAF inhibitor vemurafenib combined with obinutuzumab is effective in patients with HCL. Blood 2019, 134 (Suppl. 1), 3998. https://doi.org/10.1182/blood-2019-124478
73. Tiacci E, De Carolis L, Simonetti E, Merluzzi M, Bennati A, Perriello VM, et al. Safety and efficacy of the BRAF inhibitor dabrafenib in relapsed or refractory hairy cell leukemia: a pilot phase-2 clinical trial. Leukemia. 2021;35(11):3314–18. https://doi.org/10.1038/s41375-021-01210-8
74. Kreitman RJ, Moreau P, Hutchings M, Gazzah A, Blay J-Y, Wainberg ZA, et al. Treatment with combination of dabrafenib and trametinib in patients with recurrent/refractory BRAF V600E-mutated hairy cell leukemia (HCL). Blood 2018; 132 (Supplement 1): 391. https://doi.org/10.1182/blood-2018-99-113135
75. Weston-Bell NJ, Hendriks D, Sugiyarto G, Bos NA, Kluin-Nelemans HC, Forconi F, Sahota SS. Hairy cell leukemia cell lines expressing annexin A1 and displaying B-cell receptor signals characteristic of primary tumor cells lack the signature BRAF mutation to reveal unrepresentative origins. Leukemia. 2013;27(1):241–5. https://doi.org/10.1038/leu.2012.163
76. Rogers KA, Andritsos LA, Wei L, McLaughlin EM, Ruppert AS, Anghelina M et al. Phase 2 study of ibrutinib in classic and variant hairy cell leukemia. Blood. 2021;137(25):3473–3483. https://doi.org/10.1182/blood.2020009688
77. Paillassa J, Safa F, Troussard X. Updates in hairy cell leukemia (HCL) and variant-type HCL (HCL-V): rationale for targeted treatments with a focus on ibrutinib. Ther Adv Hematol. 2022 Apr 13;13:20406207221090886. https://doi.org/10.1177/20406207221090886
78. Robak T. Hairy-cell leukemia variant: recent view on diagnosis, biology and treatment. Cancer Treat Rev. 2011;37(1):3–10. https://doi.org/10.1016/j.ctrv.2010.05.003

79. Matutes E. Diagnostic and therapeutic challenges in hairy ur leukemia-variant: where are we in 2021? Expert Rev Hematol. 2021;14(4):355–63. https://doi.org/10.1080/17474086.2021.1908121
80. Matutes E, Wotherspoon A, Catovsky D. The variant form of hairy-cell leukaemia. Best Pract Res Clin Haematol. 2003;16(1):41–56. https://doi.org/10.1016/S1521-6926(02)00086-5
81. Chihara D, Arons E, Stetler-Stevenson M, Yuan C, Wang HW, Zhou H, et al. Long term follow-up of a phase II study of cladribine with concurrent rituximab with hairy cell leukemia variant. Blood Adv. 2021;5(23):4807–16. https://doi.org/10.1182/bloodadvances.2021005039
82. Matutes E, Wotherspoon A, Brito-Babapulle V, Catovsky D. The natural history and clinico-pathological features of the variant form of hairy cell leukemia. Leukemia. 2001;15(1):184–186. https://doi.org/10.1038/sj.leu.2401999
83. Liu Q, Harris N, Epperla N, Andritsos LA. Current and Emerging Therapeutic Options for Hairy Cell Leukemia Variant. Onco Targets Ther. 2021;14:1797–1805. https://doi.org/10.2147/OTT.S242247
84. Yoshida T, Mihara K, Sugihara S, Arihiro K, Mino T, Sasaki N, Takihara Y. Splenectomy followed by administration of rituximab is useful to treat a patient with hairy cell leukemia-variant. Ann Hematol. 2013;92(5):711–3. https://doi.org/10.1007/s00277-012-1612-1

4

Flow Cytometry in the Diagnosis of Leukemias

Weijie Li

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

Author for correspondence: Weijie Li, Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, USA. Email: wli@cmh.edu

Cite this chapter as: Li W. Flow Cytometry in the Diagnosis of Leukemias. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 22 Aug 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-flow-cytometry

Abstract: Leukemia is a group of hematologic malignancies characterized by the proliferation of abnormal lymphoid or hematopoietic cells in the bone marrow and frequent involvement of peripheral blood and other organs. Leukemia can be classified as acute or chronic based on its rate of progression and specified as one of the many subtypes with other information incorporated according to the WHO classification. Common leukemias include acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia. With the tremendous improvement in instrumentation and reagents during the past several decades, flow cytometry has become a powerful immunophenotyping tool, and plays a critically important role in the diagnosis of various leukemias. Flow cytometry can quickly identify the abnormal cell population, characterize its phenotype, give lineage classification, make the diagnosis, or narrow down the differential list. It can also assess the clonality of a mature B-cell or T-cell population, and determine DNA ploidy, which is also very useful for making diagnosis or predicting prognosis. Correlation with morphology, clinical information, and sometimes cytogenetic/molecular findings is always necessary for accurate

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

interpretation of flow cytometry results. This chapter provides an overview of the principles and the significant roles of flow cytometry in the diagnosis of leukemias.

Keywords: flow cytometry; leukemia; acute lymphoblastic leukemia; acute myeloid leukemia; chronic leukemia

INTRODUCTION

Leukemia is a heterogeneous group of hematologic malignant neoplasms characterized by the proliferation of abnormal lymphoid or hematopoietic cells in the bone marrow (BM) and frequent involvement of peripheral blood (PB), spleen, lymph node, and other organs. Leukemia can be classified as acute or chronic based on its rate of progression. Acute leukemia progresses fast, and patients' condition deteriorates quickly without treatment. In contrast, chronic leukemia progresses slowly, and the patients may stay with the disease for a long period of time even without treatment. Acute leukemia is the malignancy of lymphoid or hematopoietic precursors (blasts). Based on cell origin, acute leukemia can be classified as acute lymphoblastic leukemia, acute myeloid leukemia, or acute leukemia of ambiguous lineage. These acute leukemias can be further classified into many subtypes with the incorporation of more clinical, phenotypic, and genetic information. Chronic leukemia is the malignancy of mature or maturing lymphoid or hematopoietic cells. Most of the maturing myeloid malignancies present as chronic leukemia. Although cases of many types of lymphomas exist with extensive BM and/or PB involvement, the terminology of "leukemia" is only used in the names of a few mature B-cell and T/NK cell neoplasms, which usually show predominant BM and/or PB involvement. Leukemia is diagnosed based on clinical information and laboratory testing results.

Flow Cytometry (FCM) is a technology capable of providing rapid multi-parameter analysis of single cells or particles. It measures simultaneously physical characters (size and complexity) and multiple surface/ intracellular markers of single cells, which allows for rapid and accurate phenotypic characterization of a cell population. Together with histomorphology and other information, multi-parameter FCM can quickly diagnose leukemia and accurately classify leukemia as a subtype as defined by World Health Organization (WHO) classification (1–3). FCM study can also assess the clonality of mature B-cells by evaluating immunoglobulin (Ig) light chain expression, and the clonality of mature αβ T-cells by evaluating T-cell receptor (TCR) Vβ repertoire or TCR β chain constant 1 (TRBC1) expression (4, 5). In addition, FCM can readily measure DNA content and determine DNA ploidy of a cell population, which is also helpful in making diagnosis and/or predicting prognosis. The fast turnaround time of FCM testing allows for the quick diagnosis or timely selection of the proper other ancillary tests.

Compared with immunohistochemistry (IHC), FCM is not only faster but also capable of easily and efficiently correlating multiple parameters on a single cell. Moreover, FCM can detect the antigen intensity and aberrant antigen expression pattern. The major disadvantage of FCM is the lack of correlation with histomorphology. In addition, FCM requires viable fresh samples, and decreased viability

of certain neoplasms often precludes accurate FCM analysis. This chapter provides an overview of the principles and the significant roles of FCM in the diagnosis of leukemias.

PRINCIPLES AND KEY ELEMENTS OF FLOW CYTOMETRY

With the tremendous improvement in instrumentation and reagents during the past several decades, FCM has become a very powerful tool applied in many research or clinical fields for a wide variety of purposes (6, 7).

Flow cytometers

Flow cytometers consist of three main systems: fluidics, optics, and electronics (Figure 1A). The fluidics system is responsible for transporting samples to the flow cell where the cells form a single file stream and cross the laser beam. A stream of solution (sheath fluid) surrounding the specimen is introduced into the instrument at higher pressure so that the cells in the specimen form a roughly single file stream due to the phenomenon called hydrodynamic focusing. The cells or particles in the single file stream travel through the interrogation point where they are illuminated by light from one or more lasers. Eventually, the fluid stream is drained into a waste container (Figure 1A). The optics system consists of light sources (lasers), lenses, filters, and detectors. Lasers illuminate the cells or particles, and dichroic mirrors (DMs) and optical filters (OFs) are used to direct the resulting light signals to the appropriate detectors. DMs allow the light of corresponding wavelengths to pass while reflecting the light of other wavelengths. OFs further narrow the wavelengths reaching a detector. The detectors are usually photomultiplier tubes (PMTs) or photodiode arrays (PDAs) that convert the signals to electrical impulses, which are measured and converted to digital information by the electronics system. The digital information is collected and interpreted by the analysis software (6). The connected computer system directly interfaces with the flow cytometer and controls its functions. Data analysis can be performed either on the computer connected to the flow cytometer or on other computers that have access to the data.

Specimens and reagents/ antibody panels

Specimens suitable for FCM analysis include PB, BM, cerebrospinal fluid, serous effusions, fine needle aspirations (FNAs), and fresh unfixed tissue. PB and BM aspirates must be anticoagulated. Sample preparation for FCM analysis varies according to the specimen types and the antigens to be analyzed. The cell suspension generated by mincing tissue should be filtered to remove large particles that may clog the cytometer tubing and/or bind antibodies non-specifically. In any specimen containing a large amount of PB, the red blood cells should be removed through a lysis process using either a commercially available reagent or a homemade ammonium chloride solution before running the sample in a flow cytometer.

The key reagents for FCM studies are fluorochrome-conjugated antibodies against cell surface, cytoplasmic or nuclear antigens. Table 1 lists the antigens

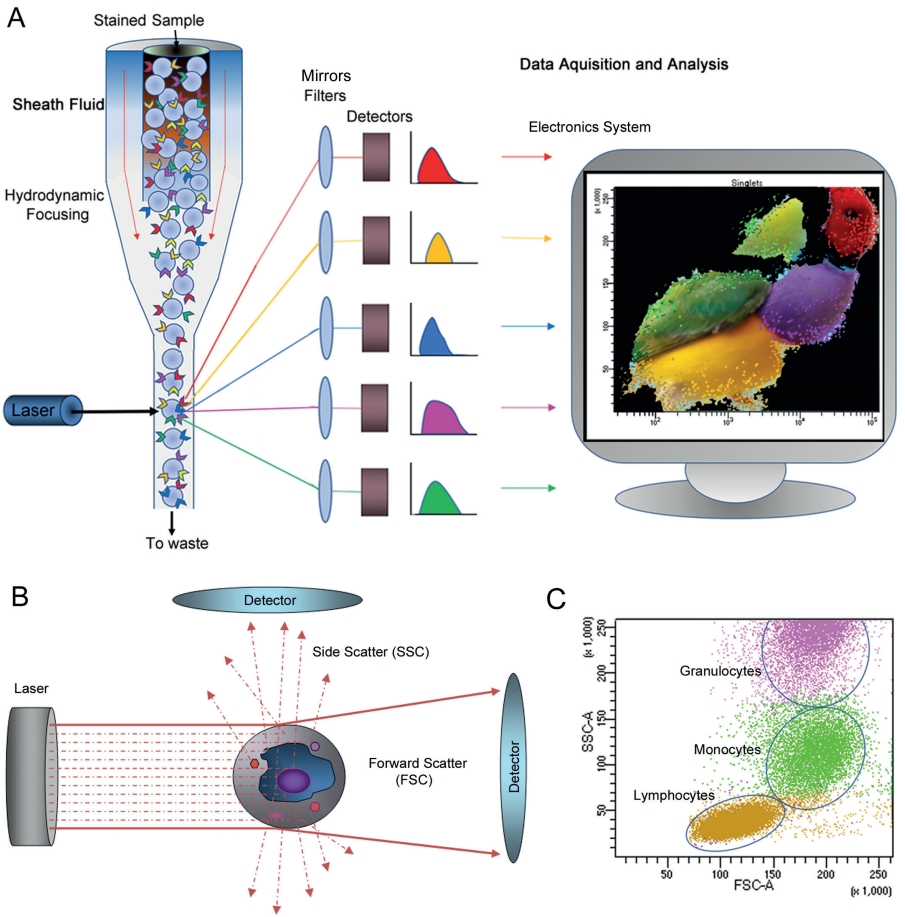

Figure 1. Schematic diagram of how flow cytometry works and light scatter of cells. A, After a liquid sample with stained cells is injected into a flow cytometer, the cells are forced to form a single file stream through hydrodynamic focusing. The cells in a single file stream are illuminated as they travel through a laser beam, and the optical and fluorescence signals of each cell are generated and detected by proper detectors that convert photons to electrical impulses. The electronics system measures the electrical impulses and converts these signals to digital information that is gathered and analyzed by specific software in a computer. **B**, Illustration of forward light scatter (FSC) and side light scatter (SSC) of a cell. **C,** Differentiation of peripheral blood leucocytes according to forward and side scatter properties.

commonly evaluated in the workup for hematologic malignancies. How many antibodies and how many tubes to run for a sample are dependent on the clinical indication and what type of flow cytometer used. A single-laser flow cytometer can evaluate three to five antigens simultaneously besides the light scatter properties. A flow cytometer with more lasers can evaluate more antigens at the same time. Eight to ten-color diagnostic antibody panels for hematologic neoplasms are commonly used currently in the United States. The combination of antibodies in

TABLE 1 **Surface or intracellular antigens commonly assessed by flow cytometry for lineage classification and immunophenotyping of hematolymphoid malignant neoplasms**

Lineage	Antigens
Stem cells	CD34, CD38, CD45
B cells	CD5, CD10, **CD19**, **CD20**, **CD22**, CD23, CD25, CD34, CD38, CD43, CD45, **CD79a**, CD103, CD200, FMC2, **cIgM**, **Kappa**, **Lambda**, LEF1, TdT
Plasma cells	CD19, CD20, CD38, CD45, CD56, CD117, CD138, **cKappa**, **cLambda**
T cells/NK cells	CD1a, **CD2**, **CD3**, CD4, **CD5**, CD7, **CD8**, CD10, CD16, CD25, CD26, CD30, CD34, CD45, CD56, **αβ-TCR**, **γδ-TCR**, TdT, **TRBC1**
Myelomonocytic cells	CD4, CD7, CD10, CD11b, CD11c, **CD13**, **CD14**, CD15, CD16, **CD33**, CD34, CD36, CD38, CD45, CD56, **CD64**, **CD65**, CD71, CD117, CD123, **cMPO**, **cLyso**, HLA-DR
Erythroblasts	CD34, CD36, CD38, CD45, CD71, CD117, **CD235a**
Megakaryoblasts	CD33, CD34, CD38, **CD41**, **CD42**, CD45, **CD61**, CD117, HLA-DR

The antigens in bold are lineage-specific markers. cIgM, cytoplasmic IgM; cKappa, cytoplasmic kappa; cLambda, cytoplasmic lambda; cLyso, cytoplasmic lysozyme; cMPO, cytoplasmic peroxidase; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase; TRBC1, TCR β chain constant 1.

one tube should be properly designed according to the antigens on the cells of interest, fluorochrome characteristics and clinical purpose. In general, the antibody against a weakly expressed antigen should be conjugated with a bright fluorochrome to increase the chance of detecting this antigen. Color compensation to minimize the spillover between different fluorochromes is very important in multicolor tubes. For diagnostic purposes, antibodies are typically combined as panels to answer specific questions about a cell population (8, 9). The screening panels should evaluate sufficient antigens to distinguish a neoplastic cell population from normal/reactive cells with a high degree of sensitivity.

Light scatter and fluorescence signals

Light scatter and fluorescence are the two types of signals generated and collected for a cell as it travels through the laser beam. Light scatter is used to measure the physical properties of a cell. Forward scatter (FSC) refers to the light scattered at narrow angles to the laser beam, and it is collected by a detector positioned in line with the laser beam on the opposite side of the sample stream (Figure 1B). The FCS is proportional to the surface area or size of the cell. Side scatter (SSC) refers to the light refracted or reflected by cytoplasmic organelles or nuclei of the cells, and it is collected by a detector positioned at approximately 90º to the laser beam. The SSC is proportional to the granularity and internal complexity of the

cell (Figure 1B). Together, FSC and SSC signals allow for the separation of cells in a specimen containing heterogeneous cell populations, e.g., three types of leukocytes (granulocytes, monocytes, and lymphocytes) in PB readily separated based on FSC and SSC characters (Figure 1C). When a cell crosses the laser beam, the fluorescent dye or fluorochrome attached to the cell is excited by light of a specific wavelength (excitation wavelength), then it absorbs the energy, gets excited, and emits light (fluorescence) at a longer wavelength (emission wavelength). In a flow cytometer, the fluorescent signals are detected by PMTs after being filtered by DMs and OFs. The intensity of the fluorescent signal is proportional to the expression level of the antigen, to which the corresponding fluorescently labeled antibody binds.

Data acquisition and analysis

Data acquisition refers to the data collection process by which the stained cells are running through a flow cytometer and illuminated by laser light, and the resulting signals are detected by the optics system. The number of cells, also known as events, required for evaluation is dependent on the purpose of the test and the nature of the specimen. For example, in the evaluation of a PB sample with predominantly leukemic cells, a relatively low number of viable cells (e.g., 20,000/tube) acquired is enough to characterize the phenotype of the leukemia. In contrast, minimal residual disease (MRD) detection usually requires a much larger number of viable cells (e.g., >500,000/tube) acquired to increase the sensitivity for detecting a very small population of residual malignant cells.

FCM data analysis requires specially designed software. Each flow cytometer usually has an associated software for data acquisition and analysis. Independent flow cytometry software, such as FCS Express, and FlowJo, with more functions is also commercially available. The flow cytometry data can be displayed as histograms or dot plots. The histograms is used for displaying a single parameter, while the dot plot is used to display two parameters and each dot represents one cell or particle. The stronger the signal is, the further along each scale the data are displayed. An antigen is called positive when its associated fluorescent signal is above the negative control (background signal). Since most of the specimens contain heterogeneous cell populations, an internal negative control is easily found and used for most antigens. However, due to the possible difference of nonspecific binding and autofluorescence levels, internal control may be misleading. Adding nonspecific Ig of the same isotype may work as better negative control and is used in some FCM tests.

Gating refers to the process of isolating cell populations within a heterogeneous cell sample according to their physical and phenotypical features, and searching for the abnormal cell population. Because normal leukocytes and hematopoietic precursors typically show consistent and reproducible patterns on SSC vs CD45 dot plot, SSC vs CD45 gating is the most commonly used initial step to separate various PB or BM cell populations. An abnormal cell population is identified based on the difference(s) of its phenotype from normal. These differences include: (i) abnormal increases or decreases of antigens normally expressed on the cells of interest; (ii) homogeneous expression patterns of antigens that normally show variable expression patterns; (iii) asynchronous antigen expression; and

(iv) aberrant expression of antigens of other cell types. To appreciate these abnormalities, the flow cytometrists must be familiar with the normal antigen expression pattern of all the cell populations in the specimen (10).

It is very important to have a strict quality assurance (QA) practice in an FCM laboratory to maintain consistency in FCM results. Quality control (QC) measures must be performed and documented regularly to ensure optimal instrument performance. Any change in the reagents, panels, instruments, or protocols must be carefully validated to make sure that the change does not affect the results. Clinical FCM laboratories should enroll in the available proficiency testing programs for the tests they are performing to make sure that their results are comparable to others.

FLOW CYTOMETRY IN THE DIAGNOSIS OF ACUTE LEUKEMIAS/ LEUKEMIAS OF BLASTS

Acute leukemia is a hematologic malignancy composed of abnormally proliferating lymphoid or hematopoietic precursors (blasts). The definition of acute leukemia is the presence of a significant number of abnormal blasts in BM according to the WHO classification (3). A value of ≥20% myeloblasts of total nucleated cells is required for making the diagnosis of acute myeloid leukemia (AML). Based on the incoming 5th edition of WHO classification (2), the 20% threshold is not needed for AML diagnosis if the blasts carry *PML::RARA, RUNX1-RUNX1T1, CBFB-MYH11, DEK::NUP214, RBM15::MRTFA, KMT2A* rearrangement, *MECOM* rearrangement, *NUP98* rearrangement, or *NPM1* mutation. In many treatment protocols, a value of ≥25% lymphoblasts is needed to make the diagnosis of acute lymphoblastic leukemia (ALL). If the abnormal precursors are predominantly localized in tissue, forming a solid mass lesion with no significant BM involvement, the diagnosis should be lymphoblastic lymphoma (LBL) or myeloid sarcoma depending on the blast type. LBL shares the same morphology, immunophenotype and biology with their ALL counterpart, so LBL and corresponding ALL are combined as one group (ALL/ LBL) by the current WHO classification (1, 3). FCM analysis can efficiently identify abnormal blast populations, give blasts lineage classification, aid in further WHO classification, and sometimes provide prognostic information (11, 12)

Identification of blast population

The identification of a large blast population, such as the ALL cases in Figure 2 and AML cases in Figure 3, is usually straightforward. Blasts usually show low SSC and dim to intermediate CD45 expression, and thus fall in the so-called blast region (low SSC and dim CD45) on CD45 vs SSC dot plot (13). However, not all the cells in the blast region are blasts. Basophils and plasmacytoid dendritic cells can also be found here, and normal B-cell precursors (hematogones) and early myeloid precursors are present here as well (10). The leukemic cells of certain acute leukemias may be seen outside of the blast region, e.g., acute promyelocytic leukemia (APL) typically showing intermediated to high SSC (Figure 3B). Therefore, careful evaluation of antigen expression profile is always necessary for the identification or

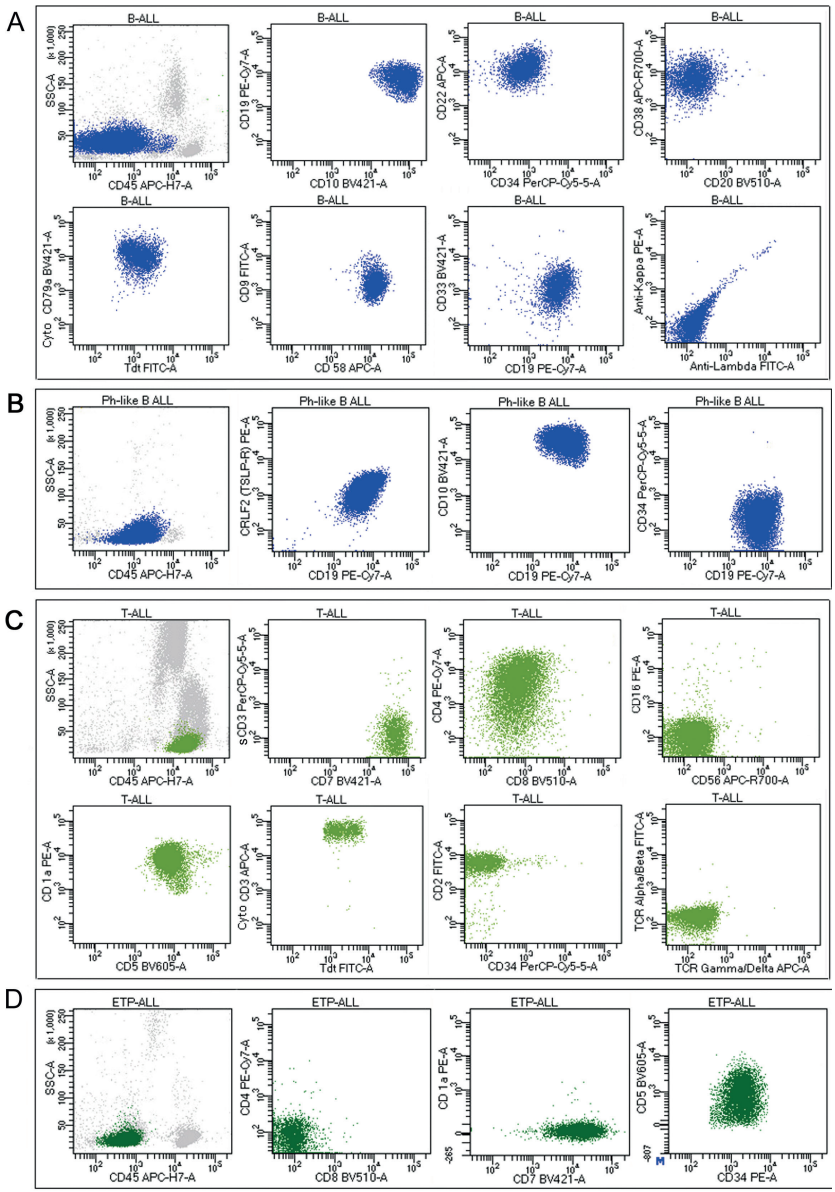

Figure 2. Acute lymphoblastic leukemia. Representative dot plots of acute lymphoblastic leukemia (ALL). **A**, Precursor B-cell ALL (B-ALL, blue) showing low side scatter, dim to negative CD45, expression of CD19, CD10, CD34, CD22, CD38, cytoplasmic (Cyto) CD79a, TdT, CD9, CD58, CD33, and no expression of CD20, kappa, and lambda. **B,** Philadelphia-like (Ph-like) B-ALL (blue) showing expression of CRLF2. **C,** T-cell ALL (T-ALL, green) showing low side scatter, intermediate to bright CD45, expression of CD7, CD4, CD8, CD1a, CD5, Cyto CD3, TdT, CD2, and no expression of surface CD3 (sCD3), CD16, CD56, CD34, TCR alpha beta, and TCR gamma delta. **D,** Early-T-precursor (ETP) ALL (dark green) being positive for CD7, CD34, CD5 (dim and partial), CD45 (dim to negative), and negative for CD4, CD8 and CD1a.

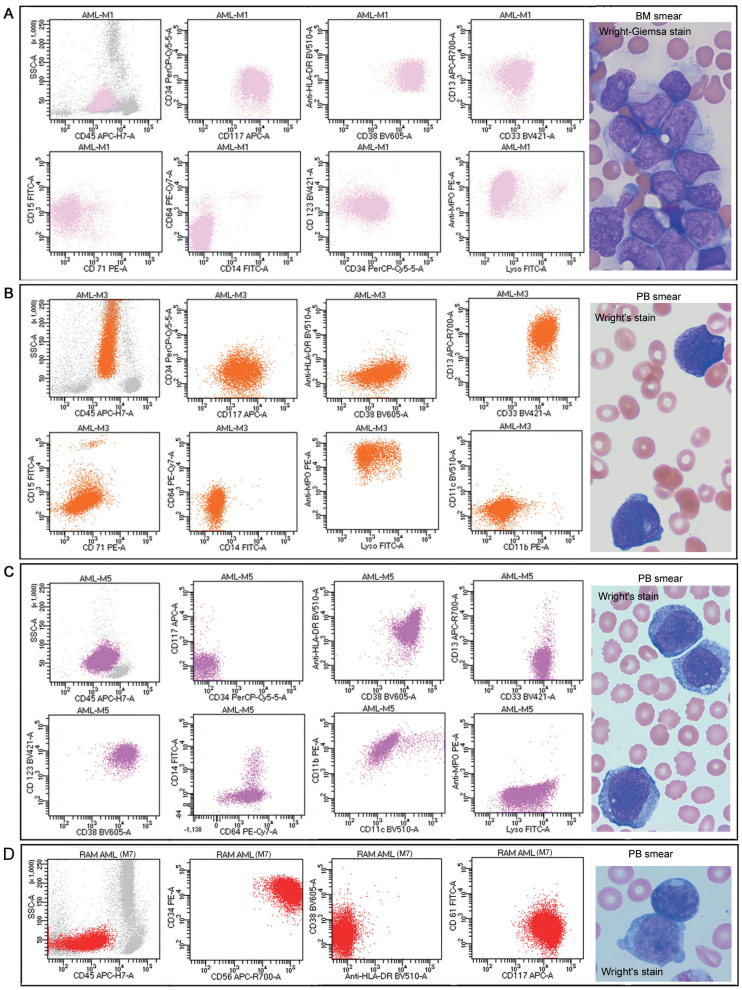

Figure 3. Acute myeloid leukemia. Representative flow cytometry dot plots and cytomorphology of acute myeloid leukemia (AML). **A,** AML-M1 (light pink) showing low side scatter, dim CD45, expression of CD34 (dim), CD117, HLA-DR, CD38, CD13, CD33, CD15, CD123 and myeloperoxidase (MPO), and no expression of CD71, CD64, CD14 and lysozyme. The blasts are large with irregular nuclei, fine chromatin, prominent nucleoli, abundant cytoplasm with thin single Auer rods and no or sparse granules. **B,** AML-M3 (acute promyelocytic leukemia) (orange) showing intermediate to high side scatter, intermediate CD45, expression of CD117, CD38, CD13, CD33, CD15 (dim), CD64 (dim and partial), CD71 (partial), MPO, lysozyme (partial), and no expression of CD34, HLA-DR, CD14, CD11b, and CD11c. The leukemic cells are large with round or irregular nuclei, abundant large azurophilic granules and multiple Auer rods (lower cell). **C,** AML-M5 (magenta) showing low to intermediate side scatter, intermediate to bright CD45, expression of HLA-DR, CD38, CD33, CD123, CD64, CD11b, CD11c, lysozyme, CD13 (small subset), CD14 (small subset), and no expression of CD117, CD34, and MPO. The blasts are large with round or oval nuclei, fine chromatin, inconspicuous nucleoli and abundant basophilic cytoplasm. **D,** AML-M7 with RAM phenotype (RAM-AML, red) being positive for CD34, CD56 (bright), CD117, CD61 (dim), CD38 (dim to negative), CD45 (dim to negative), and negative for HLA-DR. The blasts are medium to large in size with round or oval nuclei, fine chromatin, inconspicuous nucleoli and cytoplasmic blebs.

separation of the abnormal blast populations, especially in the specimens with a low number of blasts. Blasts often differ from mature cells by expressing immature markers and lacking antigens typically present on mature cells. For example, myeloblasts can be distinguished from mature or maturing myeloid cells by the expression of immature markers such as CD34 and CD117, and the absence of maturation markers such as CD11b, CD15, and CD16. Neoplastic blasts often have an abnormal phenotype that is different from that of normal hematopoietic precursors. Phenotypic abnormalities include altered expression level or pattern of antigens, aberrant expression of markers of another cell lineage, and asynchronous or dyssynchronous expression of antigens. For example, compared with hematogones (normal B-cell precursors in BM), leukemic cells of B-cell ALL (B-ALL) commonly show increased CD10, decreased CD38, aberrant expression of myeloid marker CD13 or CD33, and dyssynchronous co-expression of CD34 and CD20. The knowledge of normal precursor antigen expression patterns is especially important for evaluating post-treatment specimens, which commonly contain normal regenerative precursors and very low-level residual leukemia (14, 15).

Assignment of blast lineage

Accurately and quickly classifying an acute leukemia case as AML, B-ALL or T-ALL is very important for the timely selection of appropriate treatment. Leukemic blasts often show antigen expression profile resembling certain cell type and therefore can be assigned to that lineage by FCM analysis. As shown in Figure 2, lymphoblasts of ALL usually show low SSC and phenotype of precursor B cells or precursor T cells. Leukemic blasts from de novo B-ALL are almost always positive for CD19, cytoplasmic CD22 and cytoplasmic CD79a, and negative for surface Ig. Most of the B-ALL cases are positive for surface CD22, CD10, CD38, TdT and CD24. CD34 is commonly expressed, but its expression is quite variable, and often dim or partial. CD45 is usually dim and can be completely negative. CD20 is commonly negative or partially positive. B-ALL cases can be subclassified based on developmental stage as demonstrated by immunophenotyping, which is of little clinical significance nowadays and not required to be mentioned in pathology reports. For example, the majority of B-ALL cases express CD10 and are called common ALL (intermediate developmental stage); the B-ALL cases negative for CD10 are classified as pro-B ALL (early stage); the B-ALL cases expressing cytoplastic μ chain are classified as Pre-B ALL (late stage). Leukemic blasts from B-ALL can aberrantly express myeloid antigens (usually CD13 and CD33, occasionally CD15), and frequently show abnormal expression patterns of some non-specific markers such as CD58 and CD9 (Figure 2A), which are commonly included in the FCM panel for B-ALL MRD detection. Leukemic blasts of T-ALL are always positive for cytoplasmic CD3, usually positive for TdT, CD7, CD38, CD45 (dim), and variably positive for other pan T-cell antigens (surface CD3, CD2, CD4, CD5, CD8), T-cell receptor, CD10, and other immature markers (CD1a, CD34 and CD99) (Figure 2C). Some T-ALL cases can aberrantly express CD56, CD79a, CD117, or myeloid markers such as CD13 or CD33. In some cases, especially in the specimens collected after treatment, the immature markers can be very dim or completely absent, and the immature nature has to rely on detection of the absence of surface CD3, dual CD4/CD8 positivity or dual CD4/CD8 negativity (11). Early T-cell precursor T-ALL (ETP-ALL, Figure 2D) is defined

by a characteristic phenotype: CD8-, CD1a-, CD5- or dimly+ in <75% blasts, and positive for at least one of the myeloid or stem cell markers (CD11b, CD13, CD33, CD34, CD65, CD117, HLA-DR). ETP ALL is considered a high-risk T-ALL subtype, especially in adult patients (16), and it is listed as a separate entity in the WHO classification (1, 3).

The morphology and phenotype of AML cases vary greatly according to the differentiation direction and maturation level of the blasts. Leukemic blasts of AML with minimal differentiation (FAB-M0) or AML with no maturation (FAB-M1, Figure 3A) usually show low SSC, express early hematopoietic precursor antigens (CD34, CD38, and HLA-DR) and lack antigens associated with myeloid and monocytic maturation, such as CD11b, CD14, CD15, and CD65. Blasts express at least two myeloid-associated antigens, such as CD13, CD33 and CD117. CD38 and/or HLA-DR expression may be decreased. Rare cases may show asynchronous expression of CD11b or CD15. Myeloperoxidase (MPO) is usually positive in AML-M1, negative or minimally positive in AML-M0 by FCM. Blasts can aberrantly express CD7, or rarely other lymphoid markers. The leukemic blasts of AML with maturation (FAB M2) usually express multiple myeloid antigens associated with granulocytic differentiation, such as MPO, CD11b, CD13, CD15, CD33, and CD65. HLA-DR, CD34, CD38 and CD117 are usually positive but can be partially negative. Hypergranular variant of APL (FAB-M3) (Figure 3B) usually shows intermediate to high SSC and is characterized by absence or minimal expression of HLA-DR and CD34, and lack of leukocyte integrins CD11a, CD11b and CD18. CD13, CD33, CD64, CD117 and MPO are usually positive, while the granulocytic maturation markers CD15 and CD65 are usually negative or only weakly positive. In cases with microgranular morphology, there is a frequent expression of CD2 and CD34 by at least a portion of the leukemic cells. Leukemic blasts from AML with monocytic differentiation variably express the myeloid antigens CD13, CD15, CD33 (often bright), and CD65; they always express some monocytic markers such as CD14, CD64, CD4, CD163, CD11b, CD11c, CD36, and lysozyme. Most cases express HLA-DR, CD38, and CD123. CD34 and CD117 are positive in a subset of cases. MPO is often negative in acute monoblastic leukemia (FAB-M5a, Figure 3C), but may be partially positive in acute monocytic leukemia and acute myelomonocytic leukemia. Glycophorin A is the lineage-specific marker for acute erythroid leukemia (FAB M6), which is usually also positive for CD71, CD36, and CD117, and negative for CD34 and HLA-DR. CD41, CD61 and CD42b are specific markers for acute megakaryoblastic leukemia (FAB M7), which is often negative for CD34 and HLA-DR. The abnormal megakaryoblasts are usually positive for CD36, and often express CD13, CD33, and CD117, and may aberrantly express CD7, CD4 or CD56. Surface staining of CD41 and CD61 is less specific than their cytoplasmic staining due to possible adherence of platelets to blasts leading to false positive interpretation (17).

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) expresses CD4, CD56 (bright), CD36, CD38, CD43, CD45, CD71, HLA-DR, as well as plasmacytoid dendritic cell (PDC) associated markers (CD123, CD303, CD304, TCF4, TCL1) (2, 18). CD45 expression may range from dim to moderate. SSC of the tumor cells is usually low. CD7 and CD33 are relatively commonly expressed. TdT is positive in approximately one-third of the cases, and occasional cases may express CD117. Based on the WHO diagnostic criteria (2), BPDCN can be diagnosed in the presence of CD123 and one other PDC-associated marker in addition to CD4 and/or

CD56, or the presence of three PDC-associated markers and the absence of CD34 and other cell type-specific markers including CD3, CD14, CD19, lysozyme, and MPO. Given the presence of other hematologic malignancies with similar phenotypes, correlation with histomorphology, clinical information and IHC studies is always necessary to make a definitive diagnosis of BPDCN.

Lineage classification in some cases can be difficult. There are ~4% of acute leukemia cases with no overt evidence of differentiation along a single lineage. These cases can be separated into two groups: acute undifferentiated leukemias and mixed-phenotype acute leukemias (MPALs, Figure 4). MPAL may have one population of blasts expressing antigens belonging to two lineages (biphenotypic, Figure 4B), or have two distinct blast populations (bilineal, Figure 4C). There are defined criteria for assigning more than one lineage to a leukemic cell population for WHO classification (Figure 4A) (2). To qualify for B-cell lineage, the leukemic blasts with strong CD19 expression should have strong expression of at least one of the following: CD10, CD79a, and cytoplasmic CD22, or the leukemic blasts with weak CD19 expression should have at least two of these three other B-cell markers. For CD19 expression to be considered as strong, the intensity of CD19 in at least part of the leukemic blasts should reach 50% of hematogone level. CD3 (cytoplasmic or surface) is the only lineage-defining marker for T-cell, and its presence at a high intensity (at least partially >50% of the mature T-cell level) is enough to classify the blasts as T-cell lineage. Myeloid lineage is determined by the expression of cytoplasmic MPO, which can be detected by FCM, IHC stain or cytochemical stain. Monocytic lineage is determined by the diffuse expression of at least two of the following: nonspecific esterase, CD11c, CD14, CD64, and lysozyme. The most common combination is a myeloid lineage with a T-cell or B-cell lineage. Other combinations are also possible. No clear cut-off values on the percentage of positive blast cells have been given for these lineage-defining markers. MPO may be present at a low level on a small portion of leukemic lymphoblasts. Without other myeloid markers, this kind of MPO expression should not be interpreted as myeloid and lymphoid MPAL (3, 19). Acute undifferentiated leukemias lack antigens specific for any cell lineages as mentioned above, but often express HLA-DR, CD34, and CD38, and may express TdT.

Enumeration of blasts

FCM study can determine the precise percentage of blasts in the samples. However, the percentage of blasts by FCM often differs from that by the gold standard method — manual differential count of aspirate smears. There are several possible explanations for this discrepancy. First, the BM aspirate specimens submitted for FCM analysis are often not the first draw and contain fewer bone marrow spicules with greater dilution with PB. Second, there are likely unlysed erythrocytes in the sample, which increase the denominator for calculating blast percentage. Third, some blasts may be phenotypically difficult to capture and not included in the blast population. These are all possible reasons why blast percentage by FCM is lower than that by manual differential count. The blast percentage by FCM can also be higher due to decreased denominator resulting from the loss of nucleated erythroid precursors during the RBC lysis process. In addition, FCM analysis can identify neoplastic cells that might be missed by manual count because of atypical morphology or suboptimal smears. Moreover, the manual count may include

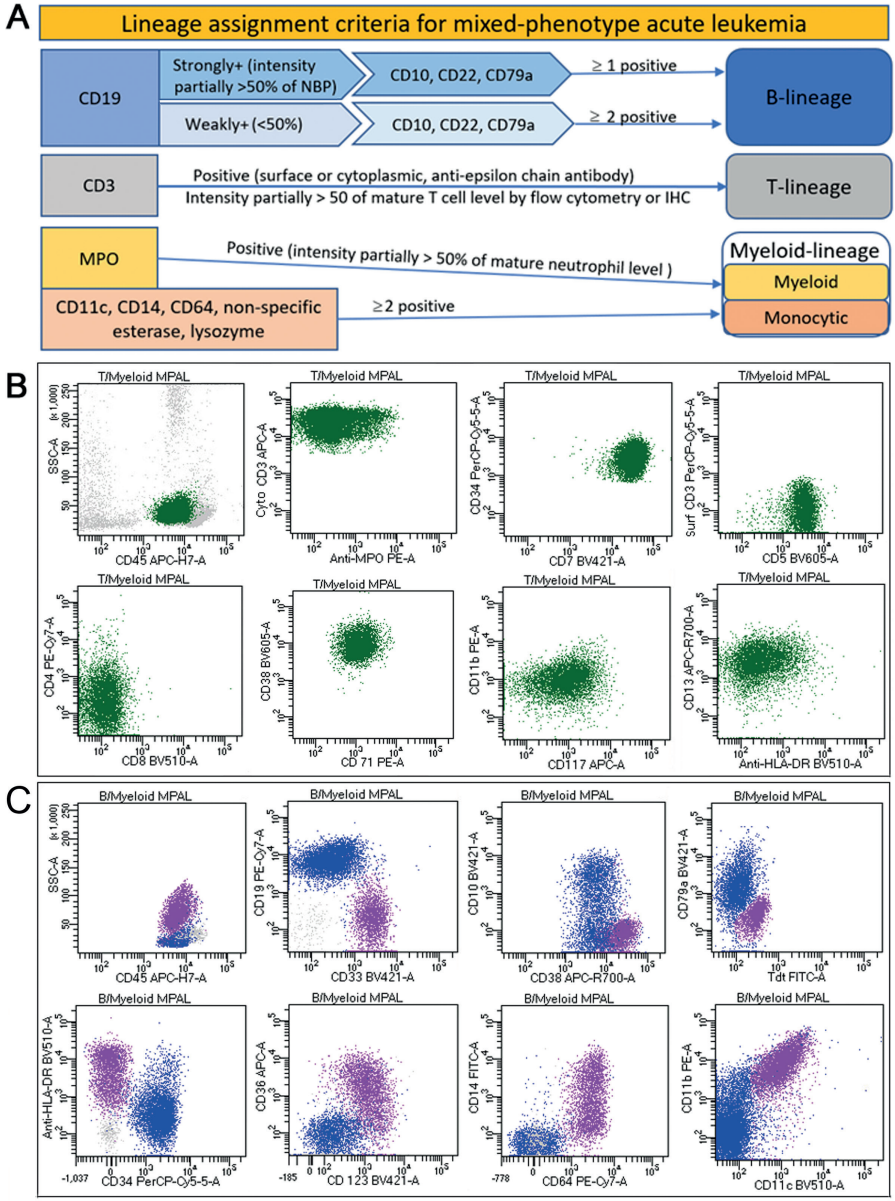

Figure 4. Mixed-phenotype acute leukemia (MPAL). A, WHO lineage assignment criteria for MPAL. NBP, normal B-cell precursor; MPO, myeloperoxidase. **B,** Biphenotypic T-cell/Myeloid MPAL showing one blast population (green) positive for cytoplasmic (Cyto) CD3, MPO (partial), CD7, CD34, CD5, CD4 (partial), CD38, CD71, CD11b, CD117, CD13, HLA-DR (partial), and negative for surface (Surf) CD3 and CD8. **C**, Bilineal B-cell/Monocytic MPAL showing two distinct blast populations: one (purple) with low to intermediate side scatter, positive for CD33, CD38, HLA-DR, CD36 (partial), CD123, CD64, CD14 (partial), CD11b and CD11c, and negative for others; the other (blue) with low side scatter, positive for CD19, CD38, CD10 (partial), CD79a, CD34, CD11b (partial), and negative for others.

some normal immature precursors, such as hematogones, as leukemic blasts. Therefore, it is necessary to perform both FCM and manual count for blast enumeration and investigate the cause of significant discrepancies.

Predicting cytogenetic abnormality and prognosis

Currently, cytogenetic/molecular testing is critical for further WHO classification and risk stratification of acute leukemias. The prognostic value provided by FCM immunophenotyping is most likely attributed to its prediction of certain cytogenetic/molecular subtypes. For example, CD10- B-ALL is commonly seen in B-ALL with *KMT2A* rearrangement, which is also commonly positive for CD15 and has a very poor prognosis. The detection of CRLF2 expression (Figure 2B) is associated with *CRLF2* gene alteration that is commonly seen in Philadelphia-like ALL and associated with poor prognosis (20). AML with t(8; 21)(q22;q22) commonly expresses B-cell markers such as CD19 and is associated with a favorable prognosis (21). Aberrant expression of CD2 in APL is associated with *FLT3–ITD* (22). However, FCM immunophenotyping should not be considered a surrogate tool for the detection of these subtypes due to the lack of specificity and sensitivity. As mentioned above, ETP-ALL (Figure 2D) is defined by the typical early-T-precursor immunophenotype and is associated with poor clinical outcomes in adult patients (16). AML with RAM phenotype (Figure 3D) is defined by the characteristic phenotype (bright CD56 expression, dim to absent CD38, dim to absent CD45, and absent HLA-DR), and is associated with a very poor prognosis (23).

FCM can measure DNA content and assess DNA ploidy for a cell population. This process involves staining cells with a DNA-binding fluorescent dye and then analyzing fluorescent signals of the cells of interest. A histogram is displayed with different ploidy patterns based on the DNA content of the cells in the sample (Figure 5). DNA ploidy is used for further classification of B-ALL and provides

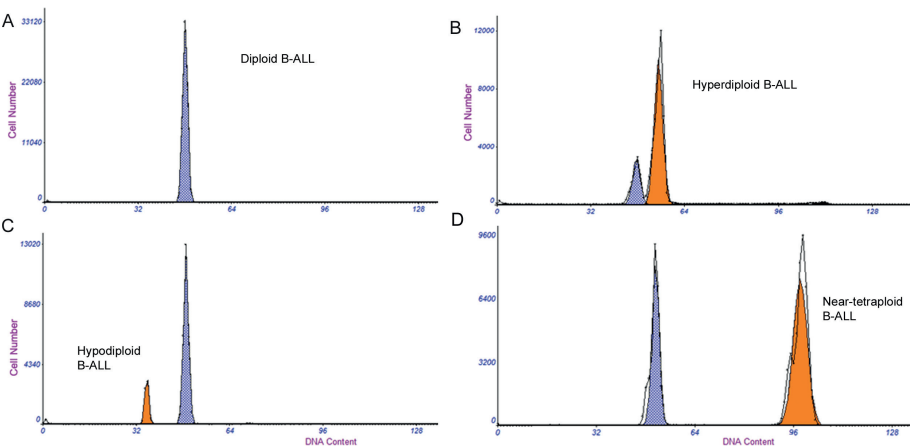

Figure 5. DNA ploidy histogram of precursor B-cell acute lymphoblastic leukemia (B-ALL). **A**, DNA diploid B-ALL (leukemic cells not separated from normal blood cells). **B,** DNA hyperdiploid B-ALL (orange color peak, DNA Index = 1.16). **C,** DNA hypodiploid B-ALL (orange color peak, DI = 0.73). D. Near-tetraploid B-ALL (orange color peak, DI = 1.90).

important prognostic information. High hyperdiploidy in B-ALL (usually DNA index ≥1.16, Figure 5B) is associated with a very favorable prognosis, while hypodiploidy (DNA index <1, Figure 5C) is associated with poor prognosis (1).

FLOW CYTOMETRY IN THE DIAGNOSIS OF MATURE B-CELL LEUKEMIAS

Mature B-Cell neoplasms consist of a heterogeneous group of lymphoid neoplastic diseases derived from B cells, which can be briefly divided into two groups: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma/leukemia /lymphoproliferative disorders (non-HL). Although many of the non-HLs can have a leukemic phase with significant BM and/or PB involvement, the "leukemia" term is only used in the names of a very limited number of non-HLs, i.e., chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL) and Splenic B-cell lymphoma/leukemia with prominent nucleoli (1). FCM is a very powerful tool for diagnosing non-HLs. FCM analysis can efficiently identify an atypical lymphoid population, differentiate neoplastic proliferation from reactive proliferation, characterize the phenotype of the neoplastic cell population, and assist in WHO classification. The neoplastic B cells can be distinguished from normal/reactive B cells by the identification of two main types of phenotypic abnormalities: immunoglobulin light chain restriction and aberrant antigen expression. These two types of abnormalities are present in most of the non-HL cases and can be efficiently identified by FCM analysis. Due to the large size, low proportion of tumor cells and T-cell resetting on tumor cells of HL, FCM is not useful in diagnosing HL.

Immunoglobulin light chain restriction/ B-cell clonality assessment

Neoplastic B cells express only one class of light chain (i.e., kappa or lambda) (Figure 6 A-D), whereas normal or reactive B cells always consist of a mixture of kappa-expressing B cells and lambda-expressing B cells (Figure 6E). The finding of only one class of light chain (light chain restriction) indicates a monoclonal B-cell proliferation. Light chain restriction may occasionally be seen in nonneoplastic reactive B-cell populations (24); therefore, it should not be considered equivalent to monoclonality, or by itself diagnostic of neoplasia. It should always be interpreted in combination with clinical information, morphology, and sometimes cytogenetic or molecular testing results. Identification of a large population of light chain-restricted B cells is usually straightforward, and it is reflected in an abnormal kappa-to-lambda ratio (Figure 6A-D). However, in the specimens with lots of admixed reactive B cells, evaluations of the kappa-to-lambda ratio may fail to identify a small clonal population. To increase the sensitivity, separate evaluations of B-cell subsets with distinct phenotypes and/or light scatter characters should be performed. When evaluating small populations of B cells for light chain restriction, caution should be exercised because reactive B cells may include small subsets of phenotypically identical cells. Some germinal center B (GCB) cells may show the absence of both classes of light chain, or may show one class of light chain excess, and they should not be interpreted as an abnormal CD10+ B-cell population (Figure 6F). It is important to include a prewash step to avoid false

negative results due to soluble antibodies in the samples interfering with the binding of fluorochrome-conjugated antibodies. It should be noted that B-lymphoblasts, plasma cells and thymic B cells are negative for surface Ig, and so are their neoplastic counterparts: B-ALL, plasma cell neoplasms, and primary mediastinal large B-cell lymphoma.

Aberrant B-cell antigen expression

FCM analysis can identify phenotypic deviations of mature B-cell neoplasms from normal antigen expression patterns of B-cells. The most easily recognized phenotypic aberrancy is the presence of antigens not normally expressed by B cells. For example, CD5, a T-cell antigen, can be aberrantly expressed in CLL and mantle cell lymphoma (MCL). It should be noted that a small population of mature B cells normally express CD5. These mature CD5+ B cells are often found in PB but may also be detected in lymph nodes or other tissue specimens, especially in pediatric patients or patients with autoimmune diseases (25). Therefore, interpretation of CD5 expression by B cells needs the correlation with other phenotypic features. Another type of phenotypic aberrancy is the abnormal expression of antigens not typically present in the B cells of a particular subset. For example, normal GCB cells are Bcl-2-, whereas Bcl-2 is positive in most other B-cell subsets. Abnormal Bcl-2 expression is found in most follicular lymphoma (FL) cases, which are derived from GCB cells and express GCB markers CD10 and BCL6. Other phenotypic aberrancies include alteration in the expression level of B-cell-associated antigens. For example, CLL often demonstrates decreased CD20, significantly decreased or absent FMC7, and detectable CD23 (17).

Classification of mature B-cell leukemias/ lymphomas

FCM immunophenotyping plays an important role in the classification of mature B-cell neoplasms. Each type of these diseases has some unique or relatively specific phenotypic features, and FCM findings in combination with histomorphologic features can make the final diagnosis in most cases. Most mature non-plasma cell B-cell neoplasms are positive for B-cell markers CD19, CD20, CD22, CD79a, and surface Ig (sIg), but the expression levels of these markers vary among the neoplasms. CLL is the most common leukemia in the Western world and is characterized by the clonal proliferation of abnormal mature CD5+ B cells in BM, PB, spleen and lymph nodes. CLL has a characteristic phenotype: CD20+ (weak), CD22+ (weak), CD23+ (often moderate to bright), FMC-7-, and sIg+ (weak with light chain restriction). This phenotype is enough to make the diagnosis in conjunction with the typical morphology in the majority of the cases. CD200 is consistently expressed in CLL, but not in MCL. The addition of CD200 in the panel has increased the sensitivity to identify CLL, even for the atypical cases (26). Lymphoid enhancer-binding factor 1 (LEF1) is highly expressed in CLL, but not detected in most other B-cell neoplasms, so it is considered a highly specific marker for CLL (27, 28). Including LEF1 in the FCM panel will increase the sensitivity and specificity for diagnosing CLL. MCL, another CD5+ small lymphocytic neoplasm, can also have significant PB involvement and should be differentiated from CLL. MCL usually shows a phenotype easily distinguished from CLL by FCM: CD20+ (moderate to bright), sIg+ (moderate to bright), CD23- or only

weakly+, FMC-7+, CD200-, LEF1-. The phenotype of MCL is more variable than that of CLL; hence, additional studies are usually needed to confirm the diagnosis. These studies include IHC stain for cyclin-D1 on paraffin section, cytogenetic study to identify the translocation t(11; 14)(q13;q32), or fluorescence in situ hybridization (FISH) analysis for *CCND1* gene rearrangement.

HCL is a rare indolent neoplasm of small mature B cells with characteristic hairy projections. It usually involves PB and diffusely infiltrates the BM and splenic red pulp. HCL has a very distinctive phenotype: CD20+ (bright), CD11c+ (bright), CD25+, CD103+, CD22+ (bright), sIg+ (intermediate to bright with light chain restriction), FMC-7+, CD23-, CD5-, and CD10-. This phenotype together with the characteristic morphology is enough for making the diagnosis of HCL. Occasionally classic HCL may show some deviations from this characteristic phenotype, e.g., CD10+, CD23+, CD103- or CD25-. The presence of phenotypic variations does not necessarily qualify the case as the HCL variant (vHCL) (29). The term vHCL, included in the new entity of splenic B-cell lymphoma/leukemia with prominent nucleoli in the 5th edition WHO classification, has been used to describe the cases with an unusual combination of morphologic, clinical, and phenotypic findings (1). vHCL usually presents with leukocytosis with no monocytopenia, and shows tumor cells with more variable morphology, often lacking the staining for TRAP, and negative for CD25, Annexin A1 and CD200. Marginal zone lymphoma (MZL) is usually distinguished from HCL by the lack of combined positivity for CD11c, CD25 and CD103, and the lack of bright staining for CD20 and CD22. Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of B-cell lymphomas variably positive for B-cell markers with light chain restriction. High FSC is usually present in DLBCL and it reflects the large size of the tumor cells and can be used as a feature to distinguish DLBCL from small mature B-cell neoplasms. It is worth remembering that occasionally any subtype of these B-cell neoplasms can have cases with an atypical phenotype. Correlation with morphologic features, clinical information, and sometimes cytogenetic/molecular findings is always required for accurate interpretation of FCM results.

Enumeration of clonal B cells

FCM numeration of clonal B cells, either through single-platform by using quantification beads or two-platform by using WBC count from hematology analyzer, is important to distinguish monoclonal B-cell lymphocytosis (MBL) from CLL. For the diagnosis of CLL, there must be a monoclonal B-cell count $\geq 5 \times 10^9$/L, with the characteristic morphology and phenotype of CLL in the PB. Individuals with a clonal CLL-like cell count $< 5 \times 10^9$/L and without lymphadenopathy, organomegaly, or other extramedullary disease are considered to have MBL. MBL is defined as the presence of a clonal B-cell population less than 5×10^9/L in the PB without other evidence of lymphoma or lymphoproliferative disorder. Most of the MBL cases present with the immunophenotype of CLL. Depending on the B-cell count, MBL can be classified as low count ($<0.5 \times 10^9$/L) or high count ($>0.5 \times 10^9$/L). Low-count MBL can be detected in up to 5% of adults over 40 years of age. Low-count MBL and high-count MBL have different prognoses, and some different biological and genetic characteristics (30). High-count MBL progresses to CLL at a rate of 1% to 2% per year, while low-count MBL rarely progresses to CLL (3, 30).

Diagnosing plasma cell leukemia

Plasma cell neoplasm (PCN) results from the expansion of a clone of abnormal terminally differentiated B-cells-- plasma cells. Plasma cell leukemia (PCL) is a rare form of PCN in which clonal plasma cells account for >20% of total leukocytes in PB. Identification of a large clonal plasma cell population with restricted cytoplasmic light chain expression is the basis for the diagnosis of PCL. CD38 and CD138 are two commonly used markers for the identification of plasma cells. CD38 is expressed by a wide variety of cell types at a lower intensity than plasma cells, while CD138 is more specific for plasma cells but is less sensitive. The neoplastic plasma cells often demonstrate an abnormal CD19- CD20- phenotype, which is different from most normal CD19+ CD20- plasma cells and CD19+ CD20+ mature B-cell neoplasms. Aberrant CD56 expression is identified in most cases of PCN (31). Decreased CD38 and/or CD138, abnormal gain of CD28, and loss of CD27 are common in PCN, and some PCN cases express CD117 (32). Although FCM is very useful in identifying abnormal plasma cell populations and making a distinction between plasma cell neoplasms and other mature B-cell malignancies, the diagnostic utility of FCM is limited by its difficulties in enumerating plasma cells. FCM usually identifies fewer plasma cells than manual count on the smears or IHC stain on biopsy sections. This discrepancy likely results from sampling difference, loss of plasma cells during sample processing, or a combination of both.

FLOW CYTOMETRY IN THE DIAGNOSIS OF MATURE T/NK CELL LEUKEMIAS

Mature T-cell or NK-cell neoplasms consist of a heterogeneous group of neoplastic diseases derived from T cells or NK cells. The mature T-cell or NK-cell neoplasms with “leukemia” in their names include T-prolymphocytic leukemia (T-PLL), T-large granular lymphocytic leukemia (T-LGL), NK-large granular lymphocytic leukemia (NK-LGL), adult T-cell leukemia/lymphoma (ATLL), aggressive NK-cell leukemia (A-NKL). Other T/NK cell neoplasms, such as Sezary syndrome (SS), also have leukemia presentation or leukemic phase even though they don’t have “leukemia” in their names. FCM is very useful in the diagnosis and classification of mature T/ NK-cell neoplasms. However, it is often more difficult to identify phenotypically abnormal mature T- or NK-cells than abnormal mature B cells, and FCM immunophenotypic features are usually not specific or diagnostic for any subtype of T/ NK-cell neoplasms.

Abnormal mature T-cell or NK-cell population can often be identified by FCM study through the detection of aberrant antigen expression. With the recent availability of specific antibodies against TRBC1, the clonality of an alpha beta T-cell population can also be assessed by a one-tube FCM assay (4). The application of clonality assessment by TRBC1 expression has increased the sensitivity and specificity of FCM analysis in the identification of an abnormal mature T-cell population.

Identification of restricted populations of T cells or NK cells/ clonality assessment

Alteration of CD4/CD8 ratio in T cells is not a useful indicator of clonality or neoplasia since it can be seen in many reactive or medical conditions. However, a significant deviation of the CD4/CD8 ratio from normal can raise concern for the presence of an abnormally restricted T-cell population. For example, an increased CD4/CD8 ratio in PB can lead to further evaluation of the expression of CD26 and CD7 on CD4+ T cells. Significant loss or absence of CD7 and/or CD26 in the CD4+ T-cell population points to the diagnosis of SS. The traditional FCM method for T-cell clonality assessment is to evaluate TCR Vβ repertoire. Normal T-cell populations are composed of a mixture of cells expressing variable Vβ family subtypes, while T-cell neoplasm is the expansion of a clone of T cells with restricted Vβ expression. Since there are more than 20 functional Vβ families and many more subfamilies, this FCM test for TCR Vβ repertoire is complex and labor intensive, and it consumes a large volume of samples and reagents with limited sensitivity. Therefore, it is not widely used in clinical FCM laboratories. FCM assessment of T-cell clonality is significantly improved with the recent advent of the antibody specific for TRBC1, one of two mutually exclusive TCR β-chain constant regions. Normal /reactive alpha beta T cells include a mixture of cells expressing TRBC1 or TRBC2 (TRBC1-) (Figure 6K), while the clonal T-cell population is either TRBC1 positive (Figure 6G-J) or negative. This FCM assay has been tested in many mature alpha beta T-cell neoplasms and proved very useful (4, 5). The panel should include antibodies for TCR αβ and/or TCR γδ besides TRBC1 because TRBC1 is normally negative in γδ T cells, which can serve as an internal negative control (Figure 6L). This assay is not useful for γδ or CD3-negative mature T-cell neoplasms due to the absence of the TCR β chain.

NK cells have no TCR, and hence cannot be assessed for clonality by using the TCR Vβ / TRBC1 FCM test or PCR for TCR gene rearrangement. FCM analysis of NK-receptor (NKR) expression has been developed to seek evidence of NK-cell clonality (33). Normal and reactive NK-cell populations express a variety of NKRs, whereas neoplastic NK-cell populations express a more restricted NKR repertoire.

Aberrant T-cell antigen expression

T-cell neoplasms often demonstrate altered expression of T-cell markers, which is often characterized by the complete loss of one or more pan-T-cell antigens. Rare extreme cases can lack multiple or all T-cell-associated antigens, showing a “null” phenotype, which can make cell lineage determination very difficult. CD5 and CD7 are the most frequently lost antigens in T-cell neoplasms. It should be noted that a small population of non-neoplastic CD7- T cells is well recognized in PB, BM and skin biopsy specimens, and CD7- T cells may expand in some reactive conditions, such as benign dermatoses. In addition, small subsets of T cells may lack CD5, e.g., CD3+CD5- γδ T cells. Some mature T-cell neoplasms can be dual CD4/8- or dual CD4/8+, which is more commonly seen in T ALL/LBL and can also be seen in some normal/reactive T-cell subsets (10, 34).

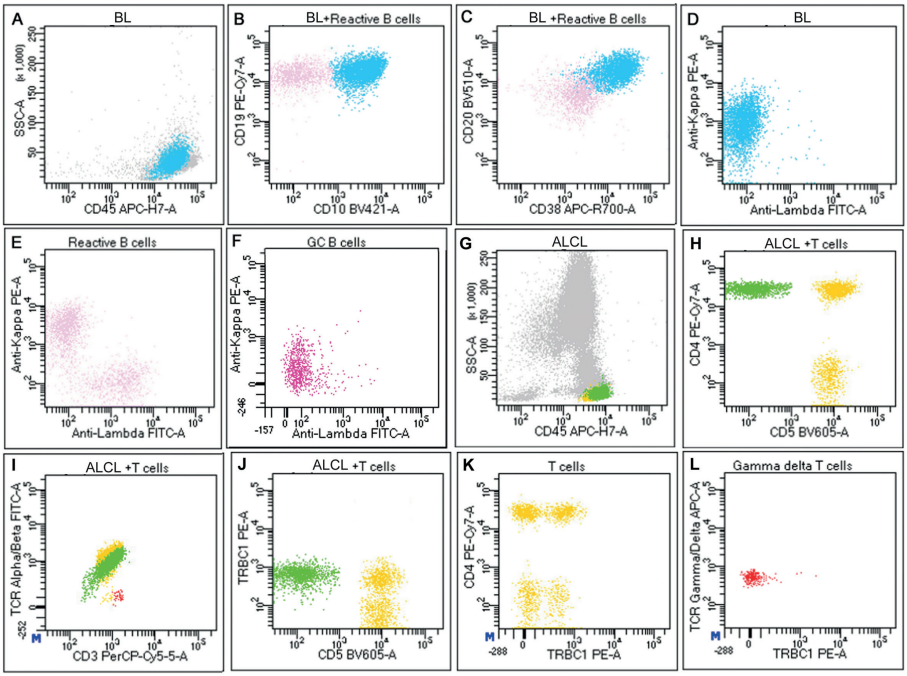

Figure 6. Assessment of clonality of B-cells and T-cells by flow cytometry. Burkitt leukemia (BL, blue) expressing CD45 (**A**), CD19 and CD10 (**B**), CD20 and CD38 (**C**), and showing kappa restriction (**D**). Reactive B cells (light pink) being polytypic (**E**). Germinal center B cells (magenta) showing kappa predominance and partial double-negativity for both kappa and lambda in a thyroid FNA sample from a patient with autoimmune thyroiditis. (**F**). Anaplastic large cell lymphoma (ALCL, green) being CD45+ (**G**), CD4+ and CD5-(**H**), TCR alpha beta+ and CD3+ (**I**), and expressing TRBC1 only (monotypic, **J**). Reactive CD4+ and CD4- T cells (golden) containing mixed TRBC1+ and TRBC1- populations (polytypic, **K**). Gamma delta T cells (red) always negative for TRBC1 (**L**).

The altered Altered staining intensity of T-cell antigens is more frequent than complete loss of them in T-cell and NK-cell neoplasms. However, these changes are more subtle and must be distinguished from the normal or reactive T/NK-cell subsets normally with these variations (10, 34). T/ NK-cell neoplasms can also aberrantly express antigens of other cell types, such as CD15, CD13, and CD33. Again, it should be noted that small subsets of normal/reactive T/NK cells can have markers more specific to other cell types, e.g., subsets of NK cells expressing CD19, CD33 or CD117 (10).

Classification of mature T-cell or NK-cell leukemias/ lymphomas

Although phenotypic heterogeneity exists in nearly all of the T-cell neoplasms, the common or typical features can be identified in most of the cases by FCM. SS and mycosis fungoides (SS/MF) commonly have a CD3+ CD4+ CD26- CD7- T-cell phenotype, which is not specific for these diseases and therefore should

be interpreted in conjunction with the clinical presentation and morphologic features. CD8+ MF exists and is more commonly seen in the pediatric population with a hypopigmented feature. Although SS and MF share lots of similarities in morphology and phenotype, they have different cell origins and should be considered two distinct diseases (35). The normal counterparts of SS tumor cells are circulating central memory T cells, while the counterparts of MF tumor cells are skin-resident memory T cells. ATLL can have a similar CD3+ CD4+ CD7- phenotype to SS/MF, but it usually shows strong CD25 expression and is positive for human T-cell leukemia virus-1 (HTLV-1), the etiologic agent of ATLL. Anaplastic large cell lymphoma (ALCL) primarily involves lymph nodes and skin but may involve BM, PB and any other sites with variable phenotype and morphology. ALCL is commonly CD4+, CD2+, CD25+, usually positive for cytotoxic granule-associated proteins and EMA, and often lacks multiple other T-cell antigens. IHC stains are usually required for the definite diagnosis of ALCL. CD30 is always positive, diffusely, and strongly with membrane and Golgi staining pattern. The diagnosis of ALK+ ALCL is established based on the expression of ALK-1 protein or the identification of *ALK* gene rearrangement. T-LGL usually demonstrates a CD8+ T-cell phenotype with a frequent decrease of CD5 and/or CD7 expression. Most T-LGL cases express CD57, CD16, cytotoxic granule-associated proteins granzyme-B, perforin and TIA-1, while the expression of CD56 is uncommon. A diagnosis of T-LGL is often established by combining this phenotype with morphologic and clinical features. Hepatosplenic T-cell lymphoma (HSTL) is frequently CD4-CD8- (occasionally CD4-CD8+) with the expression of CD56 and no expression of CD57 and CD5. Most HSTL cases express TCR γδ, demonstrate a nonactivated cytotoxic phenotype with expression of TIA-1 but no granzyme-B and perforin, and frequently demonstrate the cytogenetic abnormality of isochromosome 7q (36). T-PLL has a mature T-cell phenotype with expression of surface CD3 and most other T-cell antigens including CD2, CD5, and CD7. T-PLL is frequently CD4+, or dual CD4/8+, negative for CD25 and cytotoxic granule-associated proteins. A diagnosis of T-PLL can be established in most cases by the correlation of immunophenotype with morphology, clinical features and/or demonstration of *TCL1A/B* gene rearrangement. Enteropathy-associated T-cell lymphoma (EATL) is usually negative for CD4, CD5 and CD8, positive for CD3, CD7, CD103, and cytotoxic granule-associated proteins TIA-1, granzyme-B, and perforin. EATL is commonly associated with celiac disease. Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) shares some similarities in phenotype with EATL, but it is commonly CD8+, CD56+ and shows no clear association with celiac disease (3, 34, 37). As expected, the phenotype of PTCL, NOS is quite heterogeneous and often overlaps with other mature T-cell neoplasms. Other specified T-cell neoplasm entities must be excluded before a diagnosis of PTCL, NOS can be made.

NK-cell neoplasms, including NK-LGL, A-NKL, extranodal NK/T cell lymphoma (ENK/TCL), and EBV+ NK-cell lymphoproliferative diseases of childhood, have some overlapping phenotypic features, and it is important to distinguish the more aggressive neoplasms (A-NKL and ENK/TCL) from those with a more indolent course. A-NKL and ENK/TCL share many features, including an NK cell phenotype and the presence of EBV. They are usually positive for CD2, CD56, EBV, and cytotoxic granule-associated proteins TIA-1, granzyme-B, and perforin.

Surface CD3 is negative, but cytoplasmic CD3-epsilon is usually positive. CD4, CD5 and CD57 are usually negative (38). The distinction between them is usually made on clinical grounds. A-NKL presents with BM involvement, circulating neoplastic cells, cytopenias and constitutional symptoms, while ENK/TCL is a tissue-based mass lesion, commonly seen in the nasal cavity and surrounding area, and demonstrating angiocentric and angiodestructive growth pattern. NK-LGL, previously named chronic lymphoproliferative disorder of NK cells, is characterized by a persistent increase of NK cells in PB without an identified cause. NK-LGL is rare, not associated with EBV, and usually shows a chronic clinical course with many similarities with T-LGL. By FCM study, NK-LGL usually demonstrates a mature NK-cell phenotype with dim or absent CD2, CD7 and CD57, and restricted or abnormal NK-cell receptor expression (34).

FLOW CYTOMETRY IN THE DIAGNOSIS OF MATURE OR MATURING MYELOID LEUKEMIAS

Maturing myeloid neoplasms are a diverse group of neoplasms composed of neoplastic hematopoietic precursors at different maturation stages. Most of these diseases present as chronic leukemia. The diagnosis of these diseases is based on the combination of clinical information, blood cell counts, histomorphology of BM, and cytogenetic/molecular testing results. Although the aberrant phenotype of hematopoietic precursors is commonly detected in these neoplasms (39, 40), the diagnostic value of FCM immunophenotyping in these neoplasms is limited, and the significance of identifying phenotypic aberrancies in these diseases is not well-characterized. For the cases of most myeloproliferative neoplasms, FCM study is mainly used for the quantification of blasts to assist in detecting accelerated phase or transformation to AML. The advantages and limitations of FCM blast quantification are the same as those mentioned above in acute leukemia, and FCM cannot replace manual blast count.

CONCLUSION

FCM analysis plays a critically important role in the diagnosis of leukemias and other hematolymphoid malignancies. It can efficiently identify abnormal cell populations, differentiate neoplastic cell populations from normal/reactive cell populations, characterize the phenotype, give lineage assignments, and provide important information for WHO classification.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

REFERENCES

1. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720–1748. https://doi.org/10.1038/s41375-022-01620-2
2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703–1719. https://doi.org/10.1038/s41375-022-01613-1
3. Swerdlow SH, Campo E, Harris NL, et al (eds). World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. International Agency for Research on Cancer; Lyon, France: 2017.
4. Berg H, Otteson GE, Corley H, et al. Flow cytometric evaluation of TRBC1 expression in tissue specimens and body fluids is a novel and specific method for assessment of T-cell clonality and diagnosis of T-cell neoplasms. Cytometry B Clin Cytom. 2021;100(3):361–369. https://doi.org/10.1002/cyto.b.21881
5. Horna P, Shi M, Olteanu H, Johansson U. Emerging Role of T-cell Receptor Constant β Chain-1 (TRBC1) Expression in the Flow Cytometric Diagnosis of T-cell Malignancies. Int J Mol Sci. 2021 Feb 12;22(4):1817. https://doi.org/10.3390/ijms22041817
6. Adan A, Alizada G, Kiraz Y, et al. A. Flow cytometry: basic principles and applications. Crit Rev Biotechnol. 2017;37(2):163–176. https://doi.org/10.3109/07388551.2015.1128876
7. McKinnon KM. Flow Cytometry: An Overview. Curr Protoc Immunol. 2018;120:5.1.1–5.1.11. https://doi.org/10.1002/cpim.40
8. Wood B. 9-color and 10-color flow cytometry in the clinical laboratory. Arch Pathol Lab Med. 2006;130(5):680–90. https://doi.org/10.5858/2006-130-680-CACFCI
9. Wood BL, Arroz M, Barnett D, et al. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Cytometry B Clin Cytom. 2007;72 Suppl 1:S14–22. https://doi.org/10.1002/cyto.b.20363
10. Li W, Morgan R, Nieder R, et al. Normal or reactive minor cell populations in bone marrow and peripheral blood mimic minimal residual leukemia by flow cytometry. Cytometry B Clin Cytom. 2021;100(5):590–601. https://doi.org/10.1002/cyto.b.21968
11. DiGiuseppe JA, Wood BL. Applications of Flow Cytometric Immunophenotyping in the Diagnosis and Posttreatment Monitoring of B and T Lymphoblastic Leukemia/Lymphoma. Cytometry B Clin Cytom. 2019;96(4):256–265. https://doi.org/10.1002/cyto.b.21833
12. Weir EG, Borowitz MJ. Flow cytometry in the diagnosis of acute leukemia. Semin Hematol. 2001;38(2):124–38. https://doi.org/10.1016/S0037-1963(01)90046-0
13. Borowitz MJ, Guenther KL, Shults KE, et al. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. Am J Clin Pathol. 1993;100(5):534–40. https://doi.org/10.1093/ajcp/100.5.534
14. Loken MR, van de Loosdrecht A, Ogata K, et al. Flow cytometry in myelodysplastic syndromes: report from a working conference. Leuk Res. 2008;32(1):5–17. https://doi.org/10.1016/j.leukres.2007.04.020
15. McKenna RW, Asplund SL, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) and neoplastic lymphoblasts by 4-color flow cytometry. Leuk Lymphoma. 2004;45(2):277–85. https://doi.org/10.1080/1042819031000151950
16. Jain N, Lamb AV, O'Brien S, et al. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. Blood. 2016;127(15):1863–9. https://doi.org/10.1182/blood-2015-08-661702
17. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood. 2008;111(8):3941–67. https://doi.org/10.1182/blood-2007-11-120535
18. Julia F, Dalle S, Duru G, et al. Blastic plasmacytoid dendritic cell neoplasms: clinico-immunohistochemical correlations in a series of 91 patients. Am J Surg Pathol. 2014;38(5):673–80. https://doi.org/10.1097/PAS.0000000000000156

19. Guy J, Antony-Debré I, Benayoun E, et al. Flow cytometry thresholds of myeloperoxidase detection to discriminate between acute lymphoblastic or myeloblastic leukaemia. Br J Haematol. 2013;161(4):551–5. https://doi.org/10.1111/bjh.12277
20. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15. https://doi.org/10.1056/NEJMoa1403088
21. Chen X, Cherian S. Acute Myeloid Leukemia Immunophenotyping by Flow Cytometric Analysis. Clin Lab Med. 2017 Dec;37(4):753–769. https://doi.org/10.1016/j.cll.2017.07.003
22. Takenokuchi M, Kawano S, Nakamachi Y, et al. FLT3/ITD associated with an immature immunophenotype in PML-RARα leukemia. Hematol Rep. 2012;4(4):e22. https://doi.org/10.4081/hr.2012.e22
23. Eidenschink Brodersen L, Alonzo TA, Menssen AJ, et al. A recurrent immunophenotype at diagnosis independently identifies high-risk pediatric acute myeloid leukemia: a report from Children's Oncology Group. Leukemia. 2016;30(10):2077–2080. https://doi.org/10.1038/leu.2016.119
24. Kroft SH. Monoclones, monotypes, and neoplasia pitfalls in lymphoma diagnosis. Am J Clin Pathol. 2004 Apr;121(4):457–9. https://doi.org/10.1309/RR8AXUWM9FYX178A
25. Shirai T, Hirose S, Okada T, et al. CD5+ B cells in autoimmune disease and lymphoid malignancy. Clin Immunol Immunopathol. 1991;59(2):173–86. https://doi.org/10.1016/0090-1229(91)90016-4
26. Sandes AF, de Lourdes Chauffaille M, Oliveira CR, et al. CD200 has an important role in the differential diagnosis of mature B-cell neoplasms by multiparameter flow cytometry. Cytometry B Clin Cytom. 2014;86(2):98–105. https://doi.org/10.1002/cytob.21128
27. Amador-Ortiz C, Goolsby CL, Peterson LC, et al. Flow cytometric analysis of lymphoid enhancer-binding factor 1 in diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Clin Pathol. 2015;143(2):214–22. https://doi.org/10.1309/AJCPT32JDFIGHFHJ
28. Menter T, Dirnhofer S, Tzankov A. LEF1: a highly specific marker for the diagnosis of chronic lymphocytic B cell leukaemia/small lymphocytic B cell lymphoma. J Clin Pathol. 2015;68(6):473–8. https://doi.org/10.1136/jclinpath-2015-202862
29. Cessna MH, Hartung L, Tripp S, et al. Hairy cell leukemia variant: fact or fiction. Am J Clin Pathol. 2005;123(1):132–8. https://doi.org/10.1309/8QYTYQ1CLQMHQ9CL
30. Strati P, Shanafelt TD. Monoclonal B-cell lymphocytosis and early-stage chronic lymphocytic leukemia: diagnosis, natural history, and risk stratification. Blood. 2015;126(4):454–62. https://doi.org/10.1182/blood-2015-02-585059
31. Lin P, Owens R, Tricot G, Wilson CS. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. Am J Clin Pathol. 2004;121(4):482–8. https://doi.org/10.1309/74R4TB90BUWH27JX
32. Raja KR, Kovarova L, Hajek R. Review of phenotypic markers used in flow cytometric analysis of MGUS and MM, and applicability of flow cytometry in other plasma cell disorders. Br J Haematol. 2010;149(3):334–51. https://doi.org/10.1111/j.1365-2141.2010.08121.x
33. Morice WG, Kurtin PJ, Leibson PJ, Tefferi A, Hanson CA. Demonstration of aberrant T-cell and natural killer-cell antigen expression in all cases of granular lymphocytic leukaemia. Br J Haematol. 2003;120(6):1026–36. https://doi.org/10.1046/j.1365-2141.2003.04201.x
34. Craig JW, Dorfman DM. Flow Cytometry of T cells and T-cell Neoplasms. Clin Lab Med. 2017;37(4):725–751. https://doi.org/10.1016/j.cll.2017.07.002
35. Campbell JJ, Clark RA, Watanabe R, et al. (2010). Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. Blood. 116:767–71. https://doi.org/10.1182/blood-2009-11-251926
36. Yabe M, Miranda RN, Medeiros LJ. Hepatosplenic T-cell Lymphoma: a review of clinicopathologic features, pathogenesis, and prognostic factors. Hum Pathol. 2018;74:5–16. https://doi.org/10.1016/j.humpath.2018.01.005
37. Chen C, Gong Y, Yang Y, Xia Q, Rao Q, Shao Y, et al. Clinicopathological and molecular genomic features of monomorphic epitheliotropic intestinal T-cell lymphoma in the Chinese population: a study of 20 cases. Diagn Pathol. 2021;16(1):114. https://doi.org/10.1186/s13000-021-01173-5

38. El Hussein S, Patel KP, Fang H, Thakral B, Loghavi S, Kanagal-Shamanna R, et al. Genomic and Immunophenotypic Landscape of Aggressive NK-Cell Leukemia. Am J Surg Pathol. 2020;44(9):1235–1243. https://doi.org/10.1097/PAS.0000000000001518
39. Kern W, Bacher U, Schnittger S, et al. Multiparameter flow cytometry reveals myelodysplasia-related aberrant antigen expression in myelodysplastic/myeloproliferative neoplasms. Cytometry B Clin Cytom. 2013;84(3):194–7. https://doi.org/10.1002/cyto.b.21068
40. Ouyang J, Zheng W, Shen Q, et al. Flow cytometry immunophenotypic analysis of Philadelphia-negative myeloproliferative neoplasms: Correlation with histopathologic features. Cytometry B Clin Cytom. 2015;88(4):236–43. https://doi.org/10.1002/cytob.21215

5

Measurable Residual Disease Testing in Acute Leukemia: Technology and Clinical Significance

Weijie Li

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

Author for correspondence: Weijie Li, Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, USA. Email: wli@cmh.edu

Cite this chapter as: Li W. Measurable Residual Disease Testing in Acute Leukemia: Technology and Clinical Significance. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 21 Jul 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-measurable-residual-disease

Abstract: Measurable/minimal residual disease (MRD) is the strongest independent prognostic predictor in acute leukemia. Patients with undetectable MRD or good MRD response consistently demonstrate a lower risk of relapse and better survival outcomes compared with similarly treated patients with positive MRD or poor MRD response. MRD has already been used to guide risk-adapted therapies in routine care of patients with acute leukemia or in clinical trials in many countries. MRD can also be used as a surveillance biomarker with the potential to detect early relapse, and as a surrogate endpoint to speed up the testing and approval process for a new therapeutic agent. Multi-parametric flow cytometry and quantitative PCR are two methods commonly used for MRD detection. Recently, new techniques, such as digital PCR, next-generation sequencing, and next-generation flow cytometry, have also been applied in MRD detection and showed improved sensitivity and accuracy. These methods have

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

their own advantages and limitations. Despite tremendous advances in this field, there are still issues and questions regarding MRD testing methods and how to translate MRD information accurately into clinical and therapeutic applications. This chapter gives an overview of the methods and the clinical implications of MRD testing in acute lymphoblastic leukemia and acute myeloid leukemia.

Keywords: acute lymphoblastic leukemia; acute myeloid leukemia; measurable residual disease; minimal residual disease; multiparameter flow cytometry

INTRODUCTION

Detection of malignant cells that remain in the body (residual disease) during and after treatment for acute leukemia is the best way to monitor therapeutic response and predict relapse (Figure 1). In general, achieving a deeper response is associated with a better prognosis. Although most of the patients achieve complete remission (CR) according to clinical and morphological criteria, a large proportion of patients eventually relapse. It is clear that not all malignant cells are killed in the patients defined as being in CR, and the level of residual disease is strongly associated with relapse risk and survival outcomes (Figure 1).

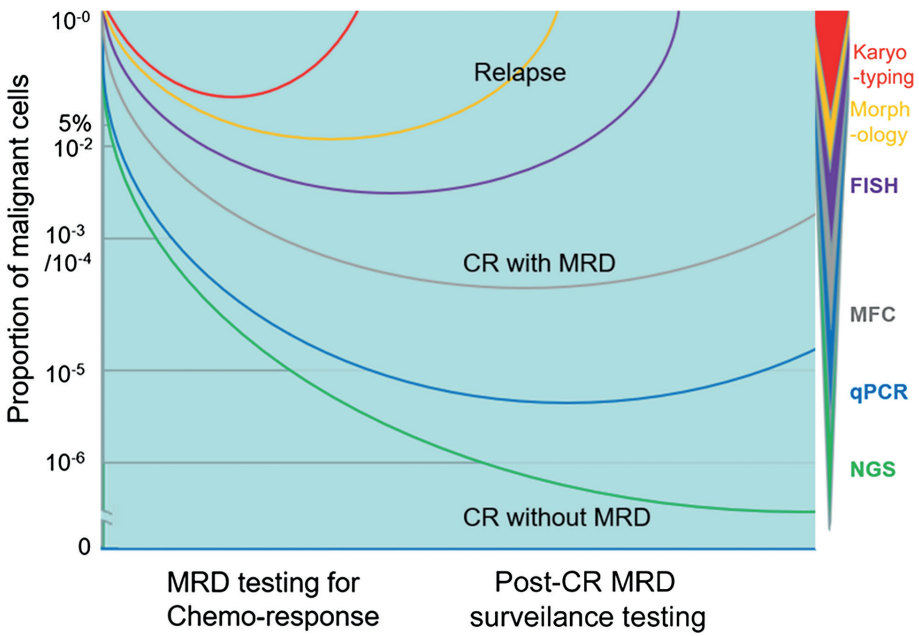

Figure 1. Diagram of the therapeutic response and relapse patterns of patients with hematologic malignancies based on MRD measured by different techniques with inconsistent sensitivities. CR, complete remission; FISH, fluorescence in situ hybridization; MFC, multiparametric flow cytometry; MRD, measurable residual disease; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction.

Morphology has a very limited capacity of assessing treatment response since only patients with high levels of residual disease (>1–5%) can be identified. The residual disease below the detection limit of morphology is referred to as measurable residual disease (MRD, also known as minimal residual disease). The preferred change of name from minimal RD to measurable RD is to emphasize the clinical importance of MRD and to reflect the result variation with respect to testing methods applied. Conventional chromosomal analysis (karyotyping) and fluorescence in situ hybridization (FISH) have no or very little role in MRD testing due to their low sensitivity. The useful methods suitable to detect MRD include multiparametric flow cytometry (MFC) and quantitative polymerase chain reaction (qPCR). Recently, more innovative techniques, such as digital PCR (dPCR), next-generation sequencing (NGS) and next-generation flow cytometry (NGF), are also applied in MRD detection (1–5). These methods have their own advantages and limitations (Table 1). During the last three decades, numerous

TABLE 1 Comparison of methods for measurable residual disease detection

Method	Sensitivity	Advantages	Disadvantages
Flow cytometry (LAIP+DFN)	10^{-3} to 10^{-5}	Fast (within few hours) High applicability Relatively inexpensive Information at cellular level Potential detecting phenotypic shift	Requires fresh sample and viable cells Requires high level of expertise Limited standardization
RT-qPCR for gene fusions	10^{-4} to 10^{-5}	Sensitive Relatively simple Standardized No need of patient-specific (PS) primers	Limited applicability Risk of cross contamination Can't detect small subclones or clonal evolution
RT-qPCR for IG/TCR gene rearrangements	10^{-4} to 10^{-5}	Sensitive Standardized with consensus guidelines	Requires diagnostic sample and PS primers Time consuming, labor-intensive, expensive Can't detect small subclones or clonal evolution
Digital PCR	10^{-3} to 10^{-5}	Sensitive Absolute quantification No need of standard curve Not affected by PCR inhibitors	Lack of standardization May require PS design Can't detect small subclones or clonal evolution
Next generation sequencing	10^{-6}	Highly sensitive No need of PS primers Wide applicability Potential to track small subclones and clonal evolution	Requires pretreatment specimen No standardization Requires high degree of informatics expertise Expensive

DFN, different from normal; LAIP, leukemia/lymphoma associated immunophenotype; PS, patient specific; RT-qPCR, real-time quantitative PCR

studies have been conducted, regarding the MRD detection methodologies and the clinical significance of MRD. These studies have demonstrated that MRD is an independent and the most powerful predictor of relapse and survival outcome (6–8). MRD testing has already become a part of routine care for some patients with acute leukemia, and the treatments for these patients are modified based on the MRD status. MRD can also be used as a surrogate endpoint to speed up the testing and approval process of a novel therapy or a new therapeutic product (9, 10). For patients in remission with maintenance therapy or off therapy, MRD testing can serve as a surveillance tool with potential to detect early relapse. This chapter gives an overview of the MRD testing methods and the clinical implications of MRD testing in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

METHODS FOR MRD TESTING

The technological advances have kept improving MRD detection methods, which have become more and more sensitive, more and more accurate, and subsequently capable of evaluating therapeutic responses at deeper and deeper levels. Classic microscopy and karyotyping have little or no value in MRD detection due to their low sensitivity. FISH is not considered as a sensitive method for MRD detection, although it can detect certain level of MRD depending on the target and how many nucleated cells screened. The useful methods suitable for MRD detection include multiparameter flow cytometry (MFC), real-time quantitative PCR (RT-qPCR), digital PCR (dPCR) and next-generation sequencing (NGS), which have been used in various clinical studies with different advantages and limitations (Table 1).

Multiparameter flow cytometry

MFC assesses the antigens present on the surface, cytoplasm, or nuclei of cells by using fluorochrome-conjugated specific monoclonal antibodies, as well as the physical characters of cells (size and complexity) by light scatter (Figure 2A). Antigens are expressed by malignant cells with variable degrees of difference from their normal counterpart cells. There are two strategies for MFC MRD detection: "Leukemia-Associated ImmunoPhenotype" (LAIP) and "Different from Normal" (DFN). The LAIP is identified at diagnosis by comparing the antigen expression profile of malignant cells to reference cell counterparts, through a panel of monoclonal antibodies. DFN defines malignant cells by recognizing immunophenotypic deviation from their normal counterpart population through the evaluation of antigen expression patterns (11). LAIP method is simple and easy to perform, and it can use a very limited number of antibodies. However, it needs diagnostic specimen and carries the risk of false positivity due to background noise and false negativity due to phenotypic changes. DFN does not need a pre-treatment immunophenotype, but its interpretation is more subjective and requires a higher level of expertise. It is recommended that both LAIP and DFN strategies should be used together to allow tracking of diagnostic and shifted leukemia phenotypes, whenever it is possible. Both strategies require expertise in the recognition of aberrant populations and exclusion of normal /reactive cell populations or potential backgrounds (11, 12).

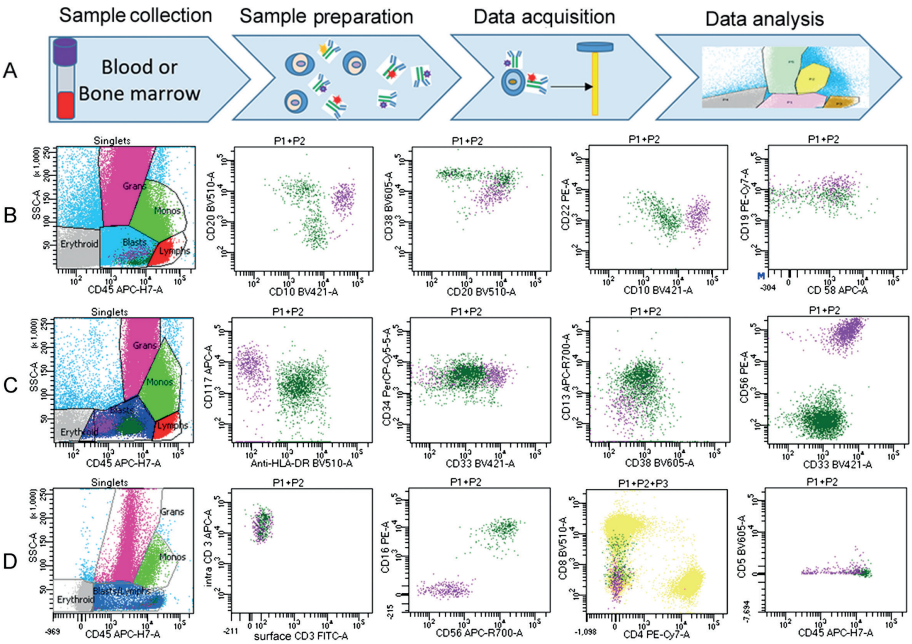

Figure 2. Measurable residual disease (MRD) detection by multi-parametric flow cytometry (MFC). A. The workflow of the MFC study is demonstrated. The nucleated cells of collected bone marrow or peripheral blood sample are incubated with fluorochrome-conjugated monoclonal antibodies, then run in a flow cytometer through the laser beam, and the signals of fluorescence and light scatters are collected and stored in the computer for analysis. **B.** Representative dot plots of MFC study on the bone marrow specimen (BM) of a precursor B-cell acute lymphoblastic leukemia (ALL) patient at the end of induction (EOI). The cell popution in dark green (P1, 0.45%) shows a normal antigen expression pattern, and is consistent with regenerating B-cell precursors (hematogones). The cell population in purple (P2, 0.07%) shows increased expression of CD10, CD19, CD20 and CD58, and decreased CD38, and is consistent with MRD. **C.** Representative dot plots of MFC study on the BM of an acute myeloid leukemia patient at the EOI. The cell popution in dark green (P1, 0.8%) shows a normal antigen expression pattern, and is consistent with regenerating myeloid precursors. The cell population in purple (P2, 0.2%) shows increased expression of CD33, CD117, decreased CD38, CD45, absent HLA-DR, and aberrant expression of CD56, and is consistent with MRD. **D.** Representative dot plots of MFC study on the BM of a T-cell ALL patient at the EOI. The cell popution in dark green (P1, 0.15%) is NK cells showing expression of intracellular CD3. The cell population in purple (P2, 0.1%) is positive for intracellular CD3, but is negative for surface CD3, CD4, CD5, CD8, CD16 and CD56, and is consistent with MRD. The cells in yellow (P3) are normal T cells.

MFC can be successfully applied to MRD testing for the majority of acute leukemia cases. MFC-MRD assay is relatively sensitive, and it can reach a sensitivity varying from 10^{-3} to 10^{-5} based on the disease and the panel used. The biggest advantage of MFC method is that it is fast and MRD results can be available within a few hours. The DFN strategy allows for the detection of phenotypic changes of MRD. Since the MFC assay measures proteins at the cellular level, the results can provide useful target information for monoclonal antibody treatment. However, this method has several limitations (Table 1). First, the test should be performed

shortly after sample collection, which could be an issue for the reference lab if the samples are received late due to delivery problems. Second, the assay requires a high level of technical expertise, and the interpretation can be very challenging, especially in cases with phenotypic changes (13, 14), although there are consensus guidelines published by the EuroFlow Consortium group (15, 16). Third, the assay is not fully standardized, and the interpretation and gating of relevant cell populations are quite subjective and time-consuming.

NGF takes advantage of innovative tools and procedures developed by the EuroFlow Consortium (17) and standardizes every step of the process from sample preparation to expert-guided automated reporting. NGF has overcome most of the obstacles mentioned above, and it has the advantages of objective expert-based panel design, fully standardized methodology, high reproducibility between laboratories, high sensitivity, and objective data analysis. EuroFlow-NGF MRD testing can reach a sensitivity of 10^{-6}, comparable to NGS, in multiple myeloma (MM), and it is applicable in 99% of MM cases and feasible in most laboratories (18). NGF and NGS showed good concordance in a recent comparison study (4). EuroFlow-NGF uses an automatic population separator, allowing to eliminate the inter-operator variability, and has a quality assessment of bone marrow (BM) cellularity by simultaneous detection of hematogones, erythroblasts, myeloid precursors, and/or mast cells (18). This information is important to ensure sample quality and identify significant hemodilution, which can lead to falsely low or negative results. The ability to analyze a complete immune profile, including T, B, and NK cells, as well as monocytes and other myeloid cell populations, at the time of MRD assessment, could also help to evaluate patients' immune system, which is likely another prognostic factor for survival.

The sample for MFC MRD detection can be peripheral blood (PB) or BM. In AML and B-cell ALL (B-ALL), MRD levels tend to be one or more logs higher in BM aspirate than in PB, whereas, in T-cell ALL (T-ALL), MRD levels are comparable in BM and PB (11, 19). In general, BM aspirate is typically the preferred specimen for MRD testing. However, since PB is an easily obtainable specimen without invasive procedures like BM aspiration, it can be used at an early time point of treatment to assess the kinetics of leukemia cell clearance, e.g., day 8 post-induction PB MRD assessment for B-ALL per the Children's Oncology Group (COG) protocol (20, 21). The BM aspirate sample for MRD assessment should be the first pull and less than 5ml to minimize hemodilution. BM samples should be anticoagulated with ethylenediaminetetraacetic acid (EDTA) or sodium heparin, transported at room temperature, and processed as soon as possible (ideally within 48 hours of collection). Sample preparation can be performed using two accepted techniques: (i) bulk lysis, followed by wash/stain/wash; or (ii) stain/lyse/wash or no-wash (22).

Acute lymphoblastic leukemia (ALL)

ALL is characterized by aggressive proliferation of lymphoblasts of either B-cell (B-ALL) or T-cell (T-ALL) in BM. MFC is a commonly used method for ALL MRD detection. In B-ALL, the specific challenge for MFC-MRD assay is to phenotypically distinguish leukemic cells from normal, regenerative B-cell precursors (also known as hematogones), which are commonly present in regenerative BM with consistent antigen expression patterns (Figure 2B, P1-green cell population).

The hematogones express CD34, CD10, and CD19 at early stage, gradually lose CD34, decrease CD10, gain CD20, and increase CD45 expression during maturation. CD38 is expressed at high level till the end stage. Leukemic B-lymphoblasts almost always show a certain deviation from normal antigen expression profile (Figure 2B, P2-purple cell population). The 6 or more-color panel including the backbone set (CD34, CD19, CD10, CD20, CD38 and CD45) can be used for most cases and reach a low limit of detection at 0.01% (23). Along with these six antigens, additional valuable markers for B-ALL MRD detection include CD13/CD33, CD9, CD58, CD22, and CD81, while other B-ALL diagnostic markers, such as CD79a, TdT, CD24, and surface immunoglobulins (IGs), are not very informative. The COG has a standardized 3-tube 6-color panel for B-ALL MRD detection, which is used for patients in COG studies. This panel includes CD34, CD19, CD10, CD20, CD38, CD45, CD9, CD58, CD13/33, CD71, CD3, and Syto16. Although it has limitations such as being not able to detect CD19-negative MRD due to its gating strategy and consuming more samples than a higher number panel, it is highly standardized with a good sensitivity (10^{-4}) for most of the cases. A highly sensitive standardized MFC B-ALL MRD assay has also been designed by the EuroFlow group (15), and it demonstrates that the application of a fully standardized bulk lysis protocol and two stepwise designed 8-color tubes (including the backbone panel plus CD81, and either CD66c/CD123 or CD73/CD304) allows highly sensitive MRD detection (up to 10^{-5}) in 99% of the B-ALL patients, when large numbers of events are acquired (>4 million cells).

The inclusion of other B-cell markers other than CD19 has been emphasized in recent years due to the increasing clinical use of immunotherapies targeting CD19 (i.e., blinatumomab and CAR-T 19). CD22 and CD24 are expressed in B lymphoblasts and can be essential for tracking B-ALL leukemic cells with down-regulation of CD19 as well as for the identification of CD22+ cases eligible for inotuzumab ozogamicin therapy (24). For the cases with immunotherapies, caution should always be exercised when interpreting the results, and appropriate modifications should be made for the panels.

Normal immature T-cells (thymocytes) are not present in BM and PB. Theoretically, it is easy to detect T-ALL MRD since any immature T cells detected in BM or PB will be considered MRD. T-ALL MRD backbone panels are set up to evaluate both the aberrant expression of mature T/NK-cell antigens (i.e., surface/cytoplasmic CD3, CD5, CD7, CD2, CD4, CD8, CD45, CD16, CD56) and immature markers (CD34, CD1a, TdT, CD99) (25). Based on our experience, the detection of T-ALL MRD is more challenging than B-ALL. T-cell immature markers (CD1a, CD34, CD99, TdT) are frequently absent or very dim, and CD45 is commonly quite bright in the residual leukemic cells after treatment. It is hard to tell the immature nature of residual T-ALL. We must rely on the DFN strategy to identify a T-cell population different from normal T cells. T cells are heterogenous and contain many subsets, which are phenotypically different to some extent. Knowing all these subsets is critical to accurately identify T-ALL MRD and avoid erroneous interpretation (12, 26). The strategies useful for most cases include looking for abnormal CD4/8 double-negative (DN), CD4/8 double-positive (DP), and/or sCD3- cCD3+ cells. Our T-ALL MRD panel studies all the cells in blast and lymphocyte regions (Figure 2D). For CD4/8 DN and sCD3- cases, NK cells could be an MRD mimicker, especially when they express cytoplasmic CD3 epsilon chain (Figure 2D, P1-green cell population). NK cells are present in almost all the

specimens and are usually easy to recognize with their expression of NK cell markers. For CD4/8 DN, sCD3+ cases, normal/reactive CD4/8 DN T cells (gamma delta T cells, NKT cells, or reactive T cells) could be a mimicker. The antigen expression level of other markers in these cells is normal, which helps to make the distinction. For CD4/8 DP cases, the reactive CD4/8 DP T cells could be a mimicker occasionally. Most of the reactive CD4/8 DP cells show the characteristic right angle and smear pattern on CD4 vs CD8 dot plot, and thus are easily recognizable.

Acute myeloid leukemia (AML)

AML is a heterogeneous group of malignant myeloid neoplasms characterized by the proliferation of abnormal myeloid progenitors, which differ in morphology and immunophenotype among the different subsets. It is not possible to have one MRD panel to fit all. According to ELN recommendations, the MFC-MRD assay should use a combined "LAIP-based DFN approach", by which the specific LAIP tracking is integrated into broad immunophenotypic profiling of BM cells. The panels should include core AML markers CD34, CD117, CD45, CD33, CD13, CD56, CD7, HLA-DR, and other selected or LAIP-related markers. Normal regenerative myeloid precursors have consistent and typical antigen expression patterns (Figure 2C, P1-green cell population), whereas leukemic myeloblasts almost always show some abnormal differentiation/maturation patterns (Figure 2C, P2-purple cell population). In cases with monocytic differentiation or a monocytic component, additional markers (e.g., CD64, CD14, CD11b, CD4) should be added (27, 28). Assessing MRD in monocytic AML is generally more challenging than in non-monocytic AML. This is mainly because neoplastic immature monocytes often do not express immature progenitor markers, such as CD34 and CD117, and lack expression of the monocytic marker CD14. They typically maintain the expression of CD15, CD33, CD36, and CD64 at levels close to normal mature monocytes with some degree of deviation. In a significant subset of monocytic AML cases, the MRD is present as a population of immature monocytes (usually CD14 low to negative) with immunophenotypic abnormalities in the expression of CD4, CD15, CD64, and/or HLA-DR, and aberrant CD56 expression at a moderate to high level (29). It should be noted that regenerating monocytes commonly express CD56, which usually shows a smear pattern and should not be considered residual leukemia (12). In cases with megakaryoblast differentiation, one or more megakaryoblastic markers (CD41, CD42, and CD61) should be added. It should be aware that adhesion of platelets to the cell surface can cause false positivity of these markers.

Overall, MFC MRD assays for AML are less sensitive than those for ALL, which usually shows more homogeneous and specific phenotypic aberrancies. In most AML cases, a low limit of detection of 0.1% can be achieved by the MFC method and this is the clinical decision-making point for most clinical studies. A lower level of MRD can be detected with an increased number of cells acquired and in cases with LAIP more significantly different from normal.

Leukemic stem cells (LSC) have been experimentally defined as leukemia-initiating cells, which are therapy-resistant and are thought to be the cellular reservoir of relapse in AML (30). Some studies have demonstrated the association of high LSC frequencies at the time of diagnosis with the presence of MRD and

subsequent poor prognosis (31–33). Therefore, LSC measurements are warranted to facilitate accurate risk stratification. LSCs can be immunophenotypically defined as CD34+/CD38- cells combined with an aberrant marker such as CD45RA, CLL-1, or CD123 (34). A significant advantage of LSC testing over MRD testing is that the former delivers a prognostic value both at diagnosis and after treatment, and in contrast to MRD, does not require comprehensive knowledge of normal hematopoietic cell differentiation patterns. A disadvantage is that the frequencies of CD34+CD38- LSCs are very low at follow-up, so LSC detection requires ideally 4 million events, likely best achieved with a one-tube assay (34, 35).

Polymerase chain reaction (PCR)

PCR is a technique that can quickly make copies of a piece of DNA and is the basis of many molecular tests. PCR-based MRD testing can target gene rearrangements, fusion genes resulting from chromosomal translocations, or gene mutations. RT-qPCR for antigen-receptor gene rearrangements or fusion genes has been well developed as an MRD detection method in acute leukemia. Recently, a more innovative PCR technique, droplet digital PCR (dPCR), has also been applied to MRD detection in hematologic malignancies.

Real-time quantitative PCR (RT-qPCR) for antigen-receptor gene rearrangements

IG and T-cell receptor (TCR) gene rearrangements are important physiological events for the development of normal B cells and T cells, respectively. The unique gene sequences of antigen receptors for each B or T cell result from a somatic rearrangement of separated gene segments (V, D and J) and random deletion or insertion of nucleotides at the junction between gene segments (36). Lymphoid malignancy is derived from the clonal proliferation of a single transformed lymphoid cell, and therefore all malignant cells will contain the same rearranged clonal IG and/or TCR genes. Although IG rearrangements are mostly found in B cells and TCR rearrangements in T cells, both B-lineage and T-lineage malignant cells can display cross-lineage rearrangements (37, 38). For example, up to 90% of B-ALL cases may exhibit TCR gene rearrangements (38), while 20% of T-ALL cases may have IG rearrangements (39). To identify these molecular markers at diagnosis, genomic DNA extracted from malignant lymphoid cells is amplified by PCR and subsequently sequenced. Allele-specific oligonucleotide (ASO)-primers are designed based on the sequence and used for RT-qPCR MRD testing (40, 41). Amplification conditions and sensitivity for each ASO-primer set are established on the diagnostic material serially diluted with normal mononuclear cells. This RT-qPCR protocol combined with fluorescence-labeled probes allows the detection of MRD at a sensitivity of up to 10^{-5} (42). This method of MRD detection is applicable for over 90% of ALL, CLL and other lymphoid malignancy cases (41, 43, 44).

As one of the most commonly used methods for MRD detection in lymphoid malignancies, RT-qPCR for IG/TCR gene rearrangements had been extensively standardized within the EuroMRD Consortium, and guidelines for the analysis and the interpretation of the results had been established (43). This method is

sensitive and widely applicable. It may fail in about 5–10% of the cases due to the absence of IG/TCR gene rearrangements or technical issues. The big drawback of this method is the requirement for establishing a patient-specific RT-qPCR assay, which is time-consuming and laborious. An adequate diagnostic sample is critical for the success of this assay. Occasionally the diagnostic DNA may be insufficient since diagnostic DNA is not only needed for the initial testing and establishment of the patient-specific assay, but also needed for generation of the standard curve of each MRD testing run. Another limitation of this method is the lack of ability to detect subclone or clonal evolution (45).

RT-qPCR for fusion genes

Gene fusion transcripts are other targets for MRD assessment by qPCR method. More than 40% of ALL cases and about a quarter of AML cases carry chromosomal translocations that generate chimeric transcripts. These abnormal gene rearrangements are the main driver events, likely expressed in all leukemic cells, and are stable during the disease. Therefore, they are potentially ideal targets for MRD testing (46–48). The most common fusion gene detected in adult B-ALL cases is *BCR-ABL1*, accounting for 25–30% of all cases. The most common fusion gene in pediatric B-ALL patients is *ETV6-RUNX1*, accounting for 25–30% of all cases. *KMT2A* gene rearrangement is the most common cytogenetic change in infant leukemia (occurring in about 80% of cases). *PML-RARA*, *RUNX1-RUNX1T1*, and *CBFB-MYH11* are the most common fusion genes detected in AML. These cytogenetic abnormalities have prognostic value, and their detection must be performed at diagnosis so that each patient can be monitored for MRD using a predefined marker throughout the disease. In the cases carrying the same translocation with different breakpoints, the RNA splicing process may produce the same fusion transcript or few splicing variants. Therefore, RNA is the optimal material to detect these lesions, since it allows the use of a small number of qPCR assays and offers the opportunity to apply the same primer set to all cases bearing the same translocation (46). Quantification of the gene fusion transcript using RNA samples is achieved by comparing the amplified product to a standard curve derived from the amplification of serial dilutions of a cell line or plasmid DNA.

A similar assay targeting mutated NPM1 has also been developed for MRD assessment in AML patients with *NPM1* mutations (49). This sensitive MRD assay can detect up to 1 malignant cell within 100,000 (10^{-5}) nucleated cells. The assays targeting the common fusion genes present in ALL and AML have been standardized by Europe Against Cancer (EAC) consortium and are widely used by clinical laboratories worldwide (46). This MRD detection assay is not patient-specific, which means that it does not need to design patient-specific primers, so it is relatively easy to perform and is not expensive. The limitation of this method is its limited applicability. In addition, the accuracy of MRD results by this assay may be affected by the variability in the number of RNA transcripts per malignant cell from patient to patient, and among different cells within the same patient.

Digital PCR (dPCR)

The dPCR technology is a newly developed technique based on sample splitting and Poisson statistics (50), and it has the potential to overcome the limitations of

conventional qPCR. The sample (RNA or DNA) is fractionated into thousands of droplets, where PCR amplification of the target gene occurs. The dPCR technique has been studied as an MRD detection method in multiple hematologic malignancies, and the data show that the dPCR assay has sensitivity and reproducibility at least comparable to the conventional qPCR method (1, 3, 51, 52). Recently, the clinical significance of dPCR MRD results has been reported in a study of pediatric ALL patients (53). The results showed that among "slow early responder" patients, most relapses occurred in cases with quantifiable dPCR MRD at day78, while patients with a negative or positive-not-quantified (PNQ) MRD by dPCR at day78 had a better outcome, indicating that MRD by dPCR can provide further risk stratification.

The dPCR method appears more accurate than RT-qPCR, and shows higher amplification efficiency, being less affected by the presence of inhibitors (54, 55). It is a high-throughput technology that produces an absolute quantification by amplifying target genes without a reference standard curve required. Hence, it has a lower chance of contamination. Compared with NGS, dPCR tends to show an inferior error rate, and it is faster and does not require a bioinformatics expert to analyze the results. The limitations of this method include the need for validation and the challenges of having to design an experiment for each assay. No guidelines for dPCR MRD assays have been established so far. A major standardization effort is underway within the EuroMRD Consortium.

Next-generation sequencing (NGS)

NGS is a high-throughput parallel sequencing technique that can produce millions of short-read sequences in a moment. The current available NGS platforms apply different approaches to achieve high-throughput sequencing. The general steps for a typical NGS run include DNA or RNA extraction from the samples, library preparation (DNA or cDNA fragmentation and adapter ligation, or PCR amplification), cluster generation, and finally sequencing (Figure 3A) (56). In a single experiment, NGS can provide accurate information on a DNA sequence and its alterations, such as mutations, insertions, deletions, or rearrangements. NGS is potentially applicable to all acute leukemia cases, but the interpretation of the data requires highly specialized bioinformatic approaches. There are three main types of NGS: whole genome sequencing (WGS), whole exome sequencing (WES), and targeted-gene sequencing. Targeted-gene sequencing method can provide profiling of several genes of interest simultaneously, and thus it is applicable to evaluate the mutations of several genes as potential targets for MRD assessment, and as measurable biomarkers for treatment (57).

Comparable to conventional qPCR methods, the first step of NGS MRD testing is also the identification of leukemia-specific clones using the diagnostic sample (Figure 3B, the first time point). However, in contrast to qPCR methods, the laborious design and testing of patient-specific assays are not needed since the same multiplex approach is applied to follow-up samples, with the index sequence(s) re-identified and quantified (Figure 3B, 2nd and 3rd time points). Moreover, the readout is more specific than qPCR testing, where false-positive results may occur due to nonspecific binding of the ASO primers. NGS MRD method targeting IG/TCR gene rearrangements can also detect clonal evolution and provide insight

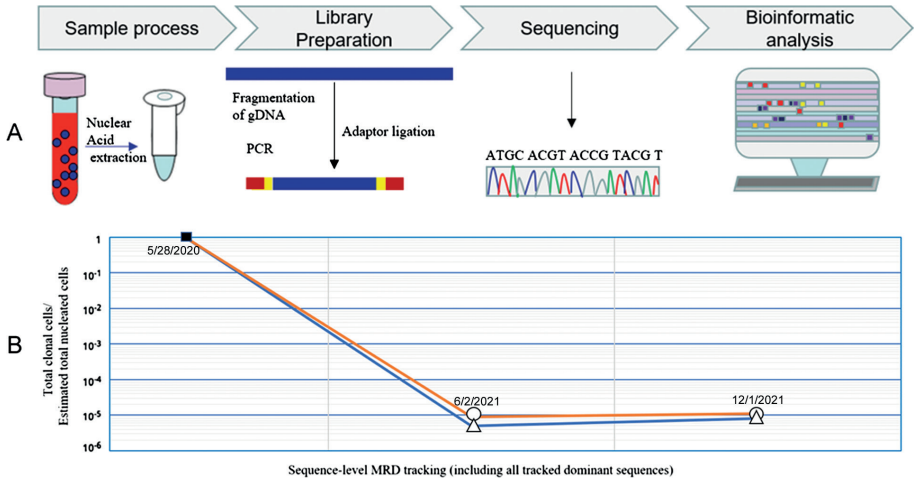

Figure 3. Next-generation sequencing (NGS) analysis for measurable residual disease (MRD). **A,** The workflow of NGS. **B,** An example of NGS MRD assay targeting T-cell receptor gamma (TCRG) gene rearrangements for a patient with T-cell lymphoblastic leukemia. NGS testing on the diagnostic bone marrow sample (5/28/2020) identified two major TCRG sequences. The follow-up bone marrow specimens showed 0.0005% MRD based on sequence A and 0.0009% MRD based on sequence B on 6/2/2021; 0.0008% MRD based on sequence A and 0.0011% MRD based on sequence B on 12/1/2021. ■, clonality test; Δ, tracking test: TCRG-sequence A; O, tracking test: TCRG-sequence B.

into the background repertoire of B and/or T cells. One disadvantage of NGS that had limited its use for MRD assessment was the sequencing error rate and its impact on the sensitivity of this technique compared to the methods discussed above (58). The introduction of error-corrected read technologies has overcome this limitation and greatly improved its sensitivity (59, 60). Another improvement is the use of Bayesian analytical techniques for mutation calling informed by site-specific error rates and prior clinical data regarding mutation frequencies (61). Overall, the current NGS MRD detection method reaches a higher sensitivity ($\leq 10^{-6}$) than other methods (2, 4, 62).

Besides IG/TCR gene rearrangements, gene mutations are also molecular markers commonly targeted by NGS MRD testing, especially in AML (62–64). Some of the mutations are not stable during the disease and can disappear or emerge at the time of relapse, as reported in the AML case with FLT3-ITD (65). Some of the persisting mutations such as *DNMT3A*, *ASXL1*, and *TET2* (64), collectively termed as DTA, known to be frequent in clonal hematopoiesis of indeterminate potential (CHIP) (66), do not have a prognostic value. Therefore, these mutations cannot be used as the target molecular markers for MRD detection in AML patients. Other limitations of this method include the requirement for a high-quality diagnostic DNA sample, and a high level of informatics expertise which is not widely available. In addition, currently, there are no uniform analytic and reporting standards for NGS MRD testing, which has complicated the comparison of different studies.

CLINICAL SIGNIFICANCE OF MRD DETECTION

There are many published studies investigating the clinical significance of MRD assessment in acute leukemia. Overall, MRD has consistently been demonstrated as one of the most powerful prognostic factors, and patients benefit from MRD-based risk-stratified management and therapy (6–8), although the clinical impact of MRD varies according to the diseases studied, patient age groups selected, MRD detection methods applied, chemotherapy regimen used, relative rates of allogeneic hematopoietic stem cell transplantation (allo-SCT), the timing of MRD assessment, and other factors. MRD testing can also serve as a surveillance tool with the potential to detect early relapse. In addition, MRD can be used as a surrogate endpoint to assess the therapeutic effect of a novel treatment or a new therapeutic product to speed up the testing and approval process (9, 10).

Prognostic prediction

The clinical significance of MRD has been extensively studied in ALL by either MFC or molecular methods for IG/TCR gene rearrangements (6). The early study of the COG measured the impact of different MFC MRD levels at the end of induction (EOI) on event-free survival (EFS) and showed that those with <0.01% MRD had an EFS of 88%, in contrast to an EFS below 60% for all other groups (20). Unfortunately, approximately two thirds of relapses occur in EOI MRD negative patients. To better explore the impact of MRD kinetics on the outcome, the COG measured MRD at an earlier time point (day 8 PB) by MFC. Patients with day 8 MRD > 1% had inferior outcomes compared to those with MRD ≤1% even if they cleared MRD by EOI (day 8 MRD-positive: 5-year EFS of 79% vs. 90%, if MRD-negative) (20). The AIEOP-BFM ALL 2000 trial evaluated the prognostic impact of molecular MRD at two different time points, day 33 and day 78 (equivalent to EOI and end of consolidation across different protocols worldwide), and the B-ALL patients (n = 3184) were classified by MRD status: MRD-standard risk if negative at a level of $<10^{-3}$ at day 33, MRD-intermediate risk if positive at day 33 but negative at day 78 and MRD-high risk if persistently positive at both time points. They found that MRD significantly correlated with outcome (5-year EFS of >90%, 78%, and 50% in the standard, intermediate and high-risk groups, respectively) (67). A later COG study on 7430 children with the National Cancer Institute (NCI) standard or high-risk B-ALL demonstrated a better 5-year disease-free survival (DFS) in a subset of patients with 0.01–0.1% MRD treated with augmented Berlin–Frankfurt–Munster therapy (ABFM) plus two interim maintenance and delayed intensification phases (68). This data suggest that intensification based on MRD response can rescue some unfavorable risk patients. Investigators at St. Jude Children's Research Hospital (SJCRH) investigated the impact of lower MRD thresholds based on the PCR MRD detection method in a cohort of 455 children with B-ALL and demonstrated that a persistent low-level disease of 0.001% to <0.01% was associated with a cumulate relapse risk of 12.7% compared to 5.0% for those with undetectable MRD (<0.001%) (69).

The prognostic values of MRD detected by IG/TCR gene qPCR in different genetic subtypes of B-ALL have also been studied (70). It was found that the risk

of relapse was strongly associated with MRD in all genetic subgroups. However, the relapse risk associated with a single MRD value varied significantly between genetic subgroups. These results suggest that a single threshold may not be appropriate for all subgroups, and individualized thresholds for different subtypes should be a better practice. For patients who receive allo-SCT, the detection of MRD (>0.01%) is associated with early post-transplant relapse and worse prognosis (71, 72).

The prognostic value of MRD in AML has also been well-demonstrated by many studies (7, 8, 27, 64, 73). Those with detectable MRD have higher cumulative incidence rates of relapse and shorter relapse-free survival (RFS) and/or OS than similarly treated patients without MRD. The strong association between detectable MRD and inferior clinical outcomes has been demonstrated at several timepoints throughout intensive AML therapy, which include early or mid-induction, after completion of one or two cycles of induction chemotherapy, after post-remission therapy, both before and after SCT, and after salvage chemotherapy for relapsed/refractory disease. Furthermore, the negative prognostic impact of a positive MRD test on outcomes has been found irrespective of MRD testing methods. Overall, the available data indicate that patients tested positive for MRD at any given timepoint, regardless of the detection methods used, have a higher risk of experiencing a relapse. On an individual patient level, results from MRD testing refine the prediction of RFS and OS to some degree, but the ability to accurately predict these outcomes remains limited (74). While different MRD detection methods can be used to provide prognostic information, it is important to note that the concordance between these assays is not 100%. Therefore, it may be most valuable to use different MRD assays in a complementary, rather than isolated manner. Retrospective studies have shown that when both MFC and NGS assays are used, patients without MRD by both methods have particularly good outcomes, patients with MRD by both methods have particularly poor outcomes, and patients with MRD by one method but not by the other have intermediate outcomes (64, 75).

Therapeutic implications of MRD

Given the strong prognostic value, MRD can be used to guide risk-adapted therapies. By tailoring therapies according to MRD response, patients with a high risk of relapse can selectively receive more aggressive therapy, such as allo-SCT in first CR, intensification of chemotherapy, or the introduction of novel therapeutic agents; while the patients with a low risk of relapse can receive reduced therapy to minimize therapy-related morbidity and mortality (76–78). Allo-SCT is associated with a reduced likelihood of relapse compared with nontransplant post-remission therapy but bears considerable risks of non-relapse-related morbidity and mortality. Allo-SCT has been shown to improve outcomes of patients with ALL and suboptimal MRD response to frontline chemotherapy (76). The study (79) also indicates that allo-SCT may be safely avoided in adolescent and adult ALL patients with good MRD response. A report from the Dutch COG has suggested that reduced chemotherapy in pediatric ALL patients who achieve MRD negativity is safe, and intensification of chemotherapy with or without allo-SCT can improve the outcomes of patients with suboptimal MRD response (77).

In addition to making decisions on whether to pursue allo-SCT in the first CR, MRD assessment can also identify patients who may benefit from novel therapies. This is especially important for those who may not be candidates for allo-SCT due to old age or significant comorbidities, as well as for those without an adequate donor. Inotuzumab and blinatumomab have shown significant promise in the management of relapsed/refractory ALL (80, 81). The apparently improved survival observed with these immunotherapies may be in part mediated through the higher MRD negativity rates achieved with these agents as compared to standard cytotoxic chemotherapy. The use of CD19-directed chimeric antigen receptor T cells (CAR T) in patients with relapsed/refractory ALL has also resulted in high rates of MRD negativity (82). Given the known significant impact of MRD response on long-term outcomes, these regimens leading to deeper remissions will ultimately translate into improved survival.

MRD also serves as a decision-making factor to identify AML patients for allo-SCT. The study on *NPM1*-mutated AML showed that DFS and OS were significantly improved by allo-SCT in those with suboptimal PB-MRD response, and allo-SCT provided no significant benefit to patients with *NPM1*-mutant AML who did not have detectable MRD or had good MRD response before allo-SCT (83). These data suggest that allo-SCT in the first CR might be a good option for patients with suboptimal MRD response, as is also supported by the study on t(8;21) AML (84). The GIMEMA AML1310 trial prospectively used MRD to guide SCT strategy in young adults with newly diagnosed AML (85). In this study, patients with intermediate-risk (IR) cytogenetic/molecular findings and detectable MRD after consolidation underwent allo-SCT and those without detectable MRD underwent autologous SCT (auto-SCT). Among these two groups of IR patients, there was no statistically significant difference in either 2-year OS (79% in MRD-negative vs. 70% in MRD-positive) or DFS (61% in MRD-negative vs. 67% in MRD-positive). These findings suggest that an MRD-directed selection of SCT consolidation may overcome the negative prognosis of MRD positivity in IR patients. It is also suggested that MRD status can reasonably be used to guide pre-SCT conditioning intensity (86). Recently, there have been quite a few studies evaluating MRD-directed approaches with the aim to eradicate MRD in patients with persistent or recurrent MRD after conventional therapy (8). These new approaches include the use of hypomethylating agents, FLT3 inhibitors, Venetoclax-based combinations, etc.

MRD monitoring and detection of early relapse

Since conversion from a negative to a positive MRD test result or an increase in MRD level over time is associated with overt disease recurrence, it is reasonable to consider MRD as a monitoring biomarker for routine surveillance and care of patients with acute leukemia following the completion of therapy. For example, the change from negative to detectable MRD by the RT-qPCR method in the cases of acute promyelocytic leukemia (APL) is almost always followed by hematologic relapse, although the interval between the MRD conversion and overt relapse can span more than one year (87). Change from negative to a

positive RT-qPCR test for *RUNX1/RUNXT1* transcripts in patients with t(8;21) AML is strongly indicative of disease recurrence, often with a very short latency from molecular to morphologic relapse (88). However, optimal timing for monitoring MRD and interval between tests are not well defined. More data are needed regarding the thresholds best suited to define relapse, the need and timing for confirmatory testing if a positive result is obtained, and how to approach patients with persistent molecular MRD at low levels. Early relapse detection may allow early therapeutic intervention to prevent overt relapse. However, the clinical benefit from early intervention based on MRD results needs to be further investigated.

MRD as a surrogate endpoint for new drug testing

The strong prognostic impact of MRD in hematologic malignancies has sparked the interest in using MRD as a surrogate efficacy biomarker to expedite the drug testing and approval process (9, 10, 89). The use of MRD as a surrogate endpoint could also decrease the clinical trial cost, as it would shorten the required time to conduct a large clinical trial. Important factors for establishing surrogacy include biological plausibility, results from studies demonstrating the prognostic value of the surrogate endpoint, and evidence from clinical trials showing that treatment effects on the surrogate endpoint correlate to treatment effects on the clinical outcome. Some data from clinical trials have demonstrated a therapeutic effect on both MRD responses and survival (90, 91), which supports the application of MRD as an adequate surrogate efficacy biomarker. The U.S. Food and Drug Administration (FDA) has issued a guidance document on the regulatory considerations for the use of MRD in clinical trials (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM623333.pdf). European medicines agency (EMA) *also publishes guideline on the use of MRD as a clinical endpoint in multiple myeloma* studies (https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-use-minimal-residual-disease-clinical-endpoint-multiple-myeloma-studies_en.pdf). Even with these guidelines, there remain challenges regarding how MRD can be utilized as a clinically meaningful endpoint. Technologies for detecting MRD are rapidly evolving, and the sensitivity of MRD testing keeps improving. With changes in assay techniques, the MRD thresholds and the goal of the treatment might become "moving targets". And the optimal timing for MRD assessment remains unclear and needs to be further investigated.

CONCLUSION

MRD testing is the best way to assess therapeutic response in-depth for patients with acute leukemias, and MRD status has been demonstrated as the strongest independent prognostic factor to predict relapse and survival outcomes. During the past three decades, technological advances have significantly improved MRD detection techniques, making MRD assays more and more sensitive and accurate. Currently, MRD assessment has already become a part of standard care in the management of acute leukemia patients, and the MRD results have been used to

guide risk-adapted therapies as routine care or in clinical trials. However, many questions remain regarding the best detection method, optimal timing and frequency of the tests, optimal assay-specific thresholds, and how to incorporate MRD information accurately into risk-adapted therapies. More studies are needed to fully answer these questions, and there should be ongoing efforts to standardize and harmonize the MRD testing methods and ensure that accurate results can be safely translated into clinical applications.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

Copyright and Permission Statement: The authors confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder(s), and all original sources have been appropriately acknowledged or referenced.

REFERENCES

1. Drandi D, Kubiczkova-Besse L, Ferrero S, et al. Minimal Residual Disease Detection by Droplet Digital PCR in Multiple Myeloma, Mantle Cell Lymphoma, and Follicular Lymphoma: A Comparison with Real-Time PCR. J Mol Diagn. 2015;17(6):652–660. https://doi.org/10.1016/j.jmoldx.2015.05.007
2. Wood B, Wu D, Crossley B, et al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. Blood. 2018;131(12):1350–1359. https://doi.org/10.1182/blood-2017-09-806521
3. Della Starza I, De Novi LA, Santoro A, et al. Digital droplet PCR and next-generation sequencing refine minimal residual disease monitoring in acute lymphoblastic leukemia. Leuk Lymphoma. 2019;60(11):2838–2840. https://doi.org/10.1080/10428194.2019.1607325
4. Medina A, Puig N, Flores-Montero J, et al. Comparison of Next-Generation Sequencing (NGS) and next-Generation Flow (NGF) for Minimal Residual Disease (MRD) Assessment in Multiple Myeloma. Blood Cancer J. 2020;10(10):108. https://doi.org/10.1038/s41408-020-00377-0
5. Paiva B, Puig N, Cedena MT, et al. Measurable Residual Disease by Next-Generation Flow Cytometry in Multiple Myeloma. J. Clin. Oncol. 2020;38:784–792.
6. Berry DA, Zhou S, Higley H, et al. Association of Minimal Residual Disease With Clinical Outcome in Pediatric and Adult Acute Lymphoblastic Leukemia: A Meta-analysis. JAMA Oncol. 2017;3(7):e170580. https://doi.org/10.1001/jamaoncol.2017.0580
7. Short NJ, Zhou S, Fu C, et al. Association of measurable residual disease with survival outcomes in patients with acute myeloid leukemia: a systematic review and meta-analysis. JAMA Oncol. 2020;6(12):1890–1899. https://doi.org/10.1001/jamaoncol.2020.4600
8. Aitken MJL, Ravandi F, Patel KP, Short NJ. Prognostic and therapeutic implications of measurable residual disease in acute myeloid leukemia. J Hematol Oncol. 2021;14(1):137. https://doi.org/10.1186/s13045-021-01148-5
9. Galimberti S, Devidas M, Lucenti A, et al. Validation of Minimal Residual Disease as Surrogate Endpoint for Event-Free Survival in Childhood Acute Lymphoblastic Leukemia. JNCI Cancer Spectr. 2018;2(4):pky069. https://doi.org/10.1093/jncics/pky069
10. Avet-Loiseau H, Ludwig H, Landgren O, et al. Minimal Residual Disease Status as a Surrogate Endpoint for Progression-free Survival in Newly Diagnosed Multiple Myeloma Studies: A Meta-analysis. Clin Lymphoma Myeloma Leuk. 2020;20(1):e30-e37. https://doi.org/10.1016/j.clml.2019.09.622

11. Chen X, Wood BL. Monitoring minimal residual disease in acute leukemia: technical challenges and interpretive complexities. Blood Rev. 2017; 31:63–75. https://doi.org/10.1016/j.blre.2016.09.006
12. Li W, Morgan R, Nieder R, et al. Normal or reactive minor cell populations in bone marrow and peripheral blood mimic minimal residual leukemia by flow cytometry. Cytometry B Clin Cytom. 2021;100(5):590–601. https://doi.org/10.1002/cyto.b.21968
13. Baer MR, Stewart CC, Dodge RK, et al. High frequency of immunophenotype changes in acute myeloid leukemia at relapse: implications for residual disease detection (Cancer and Leukemia Group B Study 8361). Blood. 2001;97(11):3574–3580. https://doi.org/10.1182/blood.V97.11.3574
14. Dworzak MN, Gaipa G, Schumich A, et al. Modulation of antigen expression in B-cell precursor acute lymphoblastic leukemia during induction therapy is partly transient: evidence for a drug-induced regulatory phenomenon. Results of the AIEOP-BFM-ALLFLOW-MRD-Study group. Cytometry B Clin Cytom. 2010; 78:147–153. https://doi.org/10.1002/cyto.b.20516
15. Theunissen P, Mejstrikova E, Sedek L, et al. EuroFlow Consortium. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129(3):347–357. https://doi.org/10.1182/blood-2016-07-726307
16. Kalina T, Brdickova N, Glier H, et al. Frequent issues and lessons learned from EuroFlow QA. J Immunol Methods. 2019;475:112520. https://doi.org/10.1016/j.jim.2018.09.008
17. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26:1908–1975. https://doi.org/10.1038/leu.2012.120
18. Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia. 2017;31(10):2094–2103. https://doi.org/10.1038/leu.2017.29
19. van der Velden VH, Jacobs DC, Wijkhuijs AJ, et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia. 2002;16(8):1432–1436. https://doi.org/10.1038/sj.leu.2402636
20. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood. 2008;111(12):5477–5485. https://doi.org/10.1182/blood-2008-01-132837
21. Setiadi A, Owen D, Tsang A, et al. The significance of peripheral blood minimal residual disease to predict early disease response in patients with B-cell acute lymphoblastic leukemia. Int J Lab Hematol. 2016;38(5):527–534. https://doi.org/10.1111/ijlh.12535
22. Muccio VE, Saraci E, Gilestro M, et al. Relevance of sample preparation for flow cytometry. Int J Lab Hematol. 2018;40(2):152–158. https://doi.org/10.1111/ijlh.12755
23. DiGiuseppe, J.A.; Wood, B.L. Applications of Flow Cytometric Immunophenotyping in the Diagnosis and Posttreatment Monitoring of B and T Lymphoblastic Leukemia/Lymphoma. Cytometry B Clin. Cytom. 2019; 96:256–265. https://doi.org/10.1002/cyto.b.21833
24. Liu Z, Li Y, Shi C. Monitoring Minimal/Measurable Residual Disease in B-Cell Acute Lymphoblastic Leukemia by Flow Cytometry during Targeted Therapy. Int. J. Hematol. 2021;113:337–343. https://doi.org/10.1007/s12185-021-03085-y
25. Fuda F, Chen W. Minimal/Measurable Residual Disease Detection in Acute Leukemias by Multiparameter Flow Cytometry. Curr Hematol Malig Rep. 2018;13(6):455–466. https://doi.org/10.1007/s11899-018-0479-1
26. Tembhare PR, Chatterjee G, Khanka T, et al. Eleven-marker 10-color flow cytometric assessment of measurable residual disease for T-cell acute lymphoblastic leukemia using an approach of exclusion. Cytometry B Clin Cytom. 2021;100(4):421–433. https://doi.org/10.1002/cyto.b.21939
27. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/Measurable Residual Disease in AML: A Consensus Document from the European LeukemiaNet MRD Working Party. Blood. 2018; 131:1275–1291. https://doi.org/10.1182/blood-2017-09-801498
28. Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2021;138(26):2753–2767. https://doi.org/10.1182/blood.2021013626

29. Wood BL. Acute Myeloid Leukemia Minimal Residual Disease Detection: The Difference from Normal Approach. Curr. Protoc. Cytom. 2020;93(1):e73. https://doi.org/10.1002/cpcy.73
30. Pollyea DA, Jordan CT. Therapeutic targeting of acute myeloid leukemia stem cells. Blood. 2017;129:1627–1635. https://doi.org/10.1182/blood-2016-10-696039
31. Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS ONE. 2014;9:e107587. https://doi.org/10.1371/journal.pone.0107587
32. Plesa A, Dumontet C, Mattei E, et al. High frequency of CD34+CD38-/low immature leukemia cells is correlated with unfavorable prognosis in acute myeloid leukemia. World J. Stem Cells. 2017;9(12):227–234. https://doi.org/10.4252/wjsc.v9.i12.227
33. Hanekamp D, Cloos J, Schuurhuis GJ. Leukemic stem cells: identification and clinical application. Int J Hematol. 2017;105:549–557. https://doi.org/10.1007/s12185-017-2221-5
34. Zeijlemaker W, Kelder A, Oussoren-Brockhoff YJ, et al. A simple one-tube assay for immunophenotypical quantification of leukemic stem cells in acute myeloid leukemia. Leukemia. 2016 Feb;30(2):439–46. https://doi.org/10.1038/leu.2015.252
35. Hanekamp D, Snel AN, Kelder A, et al. Applicability and reproducibility of acute myeloid leukaemia stem cell assessment in a multi-centre setting. Br. J. Haematol. 2020;190(6):891–900. https://doi.org/10.1111/bjh.16594
36. Cazzaniga G, Biondi A. Molecular monitoring of childhood acute lymphoblastic leukemia using antigen receptor gene rearrangements and quantitative polymerase chain reaction technology. Haematologica. 2005;90:382–390.
37. van Dongen JJ, Langerak AW, Brüggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17:2257–2317. https://doi.org/10.1038/sj.leu.2403202
38. Szczepanski T, Beishuizen A, Pongers-Willemse MJ, et al. Cross lineage T cell receptor gene rearrangements occur in more than ninety percent of childhood precursor-B acute lymphoblastic leukemias: alternative PCR targets for detection of minimal residual disease. Leukemia. 1999;13:196–205. https://doi.org/10.1038/sj.leu.2401277
39. Szczepanski T, Pongers-Willemse MJ, Langerak AW, et al. Ig heavy chain gene rearrangements in T-cell acute lymphoblastic leukemia exhibit predominant DH6-19 and DH7-27 gene usage, can result in complete V-D-J rearrangements, and are rare in T-cell receptor alpha beta lineage. Blood. 1999;93:4079–4085. https://doi.org/10.1182/blood.V93.12.4079
40. Germano G, del Giudice L, Palatron S, et al. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. Leukemia. 2003;17:1573–1582. https://doi.org/10.1038/sj.leu.2403008
41. Della Starza I, Cavalli M, Del Giudice I, et al. Comparison of two real-time quantitative polymerase chain reaction strategies for minimal residual disease evaluation in lymphoproliferative disorders: correlation between immunoglobulin gene mutation load and real-time quantitative polymerase chain reaction performance. Hematol Oncol. 2014;32:133–138. https://doi.org/10.1002/hon.2095
42. Verhagen OJ, Willemse MJ, Breunis WB, Wijkhuijs AJ, Jacobs DC, Joosten SA, et al. Application of germline IGH probes in real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia. Leukemia. 2000;14:1426–1435. https://doi.org/10.1038/sj.leu.2401801
43. van der Velden VH, Cazzaniga G, Schrauder A, et al. European Study Group on MRD detection in ALL (ESG-MRD-ALL). Analysis of minimal residual disease by Ig/TCR gene rearrangements: Guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21:604–611. https://doi.org/10.1038/sj.leu.2404586
44. Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18–20 September 2008. Leukemia. 2010;24:521–535. https://doi.org/10.1038/leu.2009.268
45. Szczepanski T, van der Velden VH, Raff T, et al. Comparative analysis of T-cell receptor gene rearrangements at diagnosis and relapse of T-cell acute lymphoblastic leukemia (T-ALL) shows high stability of clonal markers for monitoring of minimal residual disease and reveals the occurrence of second T-ALL. Leukemia. 2003;17:2149–2156. https://doi.org/10.1038/sj.leu.2403081

46. Gabert J, Beillard E, van der Velden VHJ, et al. Standardization and quality control studies of "real-time" quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia-a Europe Against Cancer program. Leukemia. 2003;17:2318–2357. https://doi.org/10.1038/sj.leu.2403135
47. Jourdan E, Boissel N, Chevret S, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood. 2013;121:2213–2223. https://doi.org/10.1182/blood-2012-10-462879
48. Corbacioglu A, Scholl C, Schlenk RF, et al. Prognostic impact of minimal residual disease in CBFB-MYH11- positive acute myeloid leukemia. J Clin Oncol. 2010;28:3724–3729. https://doi.org/10.1200/JCO.2010.28.6468
49. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N Engl J Med. 2016;374:422–433. https://doi.org/10.1056/NEJMoa1507471
50. Huggett JF, Whale A. Digital PCR as a novel technology and its potential implications for molecular diagnostics. Clin Chem. 2013;59:1691–1693. https://doi.org/10.1373/clinchem.2013.214742
51. Della Starza I, Del Giudice I, Menale L, et al. Minimal residual disease (MRD) detection by digital-droplet-PCR (ddPCR) in lymphoid malignancies. Haematologica. 2018;103:S1–129.
52. Coccaro N, Anelli L, Zagaria A, et al. Droplet digital PCR is a robust tool for monitoring minimal residual disease in adult philadelphia-positive acute lymphoblastic leukemia. J Mol Diagn. 2018;20:474–482. https://doi.org/10.1016/j.jmoldx.2018.03.002
53. Della Starza I, Nunes V, Lovisa F, et al. Droplet Digital PCR Improves IG-/TR-based MRD Risk Definition in Childhood B-cell Precursor Acute Lymphoblastic Leukemia. Hemasphere. 2021;5(3):e543. https://doi.org/10.1097/HS9.0000000000000543
54. Hindson CM, Chevillet JR, Briggs HA, et al. Absolute quantification by droplet digital PCR versus analog real time PCR. Nat Methods. 2013;10:1003–1005. https://doi.org/10.1038/nmeth.2633
55. Yang R, Paparini A, Monis P, et al. Comparison of next-generation droplet digital PCR (ddPCR) with quantitative PCR (qPCR) for enumeration of Cryptosporidium oocysts in faecal samples. Int J Parasitol. 2014;44:1105–1113. https://doi.org/10.1016/j.ijpara.2014.08.004
56. Buermans HP, den Dunnen JT. Next generation sequencing technology: Advances and applications. Biochim Biophys Acta. 2014;1842(10):1932–1941. https://doi.org/10.1016/j.bbadis.2014.06.015
57. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–2221. https://doi.org/10.1056/NEJMoa1516192
58. Ommen HB. Monitoring minimal residual disease in acute myeloid leukaemia: a review of the current evolving strategies. Ther Adv Hematol. 2016;7(1):3–16. https://doi.org/10.1177/2040620715614529
59. Ahn J, Hwang B, Young Kim H, et al. Asymmetrical barcode adapter-assisted recovery of duplicate reads and error correction strategy to detect rare mutations in circulating tumor DNA. Sci Rep. 2017;7:46678. https://doi.org/10.1038/srep46678
60. Crowgey EL, Mahajan N, Wong WH, et al. Error-corrected sequencing strategies enable comprehensive detection of leukemic mutations relevant for diagnosis and minimal residual disease monitoring. BMC Med Genomics. 2020;13(1):32. https://doi.org/10.1186/s12920-020-0671-8
61. Gerstung M, Papaemmanuil E, Campbell PJ. Subclonal variant calling with multiple samples and prior knowledge. Bioinformatics. 2014;30(9):1198–1204. https://doi.org/10.1093/bioinformatics/btt750
62. Sánchez R, Ayala R, Martínez-López J. Minimal Residual Disease Monitoring with Next-Generation Sequencing Methodologies in Hematological Malignancies. Int J Mol Sci. 2019;20(11):2832. https://doi.org/10.3390/ijms20112832
63. Klco JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. JAMA. 2015;314:811–822. https://doi.org/10.1001/jama.2015.9643
64. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med. 2018;378:1189–1199. https://doi.org/10.1056/NEJMoa1716863
65. Ottone T, Zaza S, Divona M, et al. Identification of emerging FLT3 ITD-positive clones during clinical remission and kinetics of disease relapse in acute myeloid leukaemia with mutated nucleophosmin. Br J Haematol. 2013;161:533–540. https://doi.org/10.1111/bjh.12288

66. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood. 2017;130:742–752. https://doi.org/10.1182/blood-2017-02-769869
67. Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: Results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010;115:3201–3214. https://doi.org/10.1182/blood-2009-10-248146
68. Borowitz MJ, Wood BL, Devidas M, et al. Assessment of end induction minimal residual disease (MRD) in childhood B precursor acute lymphoblastic leukemia (ALL) to eliminate the need for day 14 marrow examination: A Children's Oncology Group study. J. Clin. Oncol. 2013;31(Suppl. 15):10001. https://doi.org/10.1200/jco.2013.31.15_suppl.10001
69. Stow P, Key L, Chen X, et al. Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. Blood. 2010;115(23):4657–4663. https://doi.org/10.1182/blood-2009-11-253435
70. O'Connor D, Enshaei A, Bartram J, et al. Genotype-Specific Minimal Residual Disease Interpretation Improves Stratification in Pediatric Acute Lymphoblastic Leukemia. J. Clin. Oncol. 2018;36:34–43. https://doi.org/10.1200/JCO.2017.74.0449
71. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: The ALL-REZ BFM Study Group. J. Clin. Oncol. 2009;27:377–384. https://doi.org/10.1200/JCO.2008.17.6065
72. Umeda K, Hiramatsu H, Kawaguchi K, et al. Impact of pretransplant minimal residual disease on the post-transplant outcome of pediatric acute lymphoblastic leukemia. Pediatr. Transplant. 2016;20:692–696. https://doi.org/10.1111/petr.12732
73. Freeman SD, Hills RK, Virgo P, et al. Measurable residual disease at induction redefines partial response in acute myeloid leukemia and stratifies outcomes in patients at standard risk without NPM1 mutations. J Clin Oncol. 2018;36(15):1486–1497. https://doi.org/10.1200/JCO.2017.76.3425
74. Othus M, Wood BL, Stirewalt DL, et al. Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. Leukemia. 2016;30:2080–2083. https://doi.org/10.1038/leu.2016.120
75. Getta BM, Devlin SM, Levine RL, et al. Multicolor flow cytometry and multigene next-generation sequencing are complementary and highly predictive for relapse in acute myeloid leukemia after allogeneic transplantation. Biol Blood Marrow Transplant. 2017;23:1064–1071. https://doi.org/10.1016/j.bbmt.2017.03.017
76. Gokbuget N, Kneba M, Raff T, et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood. 2012;120:1868–1876. https://doi.org/10.1182/blood-2011-09-377713
77. Pieters R, de Groot-Kruseman H, Van der Velden V, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016;34(22):2591–2601. https://doi.org/10.1200/JCO.2015.64.6364
78. Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013;14(3):199–209. https://doi.org/10.1016/S1470-2045(12)70600-9
79. Ribera JM, Oriol A, Morgades M, et al. Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. J Clin Oncol. 2014;32(15):1595–1604. https://doi.org/10.1200/JCO.2013.52.2425
80. Topp MS, Gokbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B- precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. The Lancet Oncology. 2015;16:57. https://doi.org/10.1016/S1470-2045(14)71170-2

81. Dahl J, Marx K, Jabbour E. Inotuzumab ozogamicin in the treatment of acute lymphoblastic leukemia. Expert Rev Hematol. 2016;9(4):329–334. https://doi.org/10.1586/17474086.2016.1143771
82. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507–1517. https://doi.org/10.1056/NEJMoa1407222
83. Balsat M, Renneville A, Thomas X, et al. Postinduction minimal residual disease predicts outcome and benefit from allogeneic stem cell transplantation in acute myeloid leukemia with NPM1 mutation: a study by the Acute Leukemia French Association Group. J Clin Oncol. 2017;35(2):185–193. https://doi.org/10.1200/JCO.2016.67.1875
84. Zhu HH, Zhang XH, Qin YZ, et al. MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial. Blood. 2013;121(20):4056–4062. https://doi.org/10.1182/blood-2012-11-468348
85. Venditti A, Piciocchi A, Candoni A, et al. GIMEMA AML1310 trial of risk-adapted, MRD-directed therapy for young adults with newly diagnosed acute myeloid leukemia. Blood. 2019;134(12):935–945. https://doi.org/10.1182/blood.2018886960
86. Hourigan CS, Dillon LW, Gui G, et al. Impact of conditioning intensity of allogeneic transplantation for acute myeloid leukemia with genomic evidence of residual disease. J Clin Oncol. 2020;38(12):1273–1283. https://doi.org/10.1200/JCO.19.03011
87. Diverio D, Rossi V, Avvisati G, et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/ RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. Blood. 1998;92:784–789. https://doi.org/10.1182/blood.V92.3.784
88. Rücker FG, Agrawal M, Corbacioglu A, et al. Measurable residual disease monitoring in acute myeloid leukemia with t(8;21)(q22;q22.1): results from the AML Study Group. Blood. 2019;134:1608–1618. https://doi.org/10.1182/blood.2019001425
89. Walter RB, Ofran Y, Wierzbowska A, et al. Measurable residual disease as a biomarker in acute myeloid leukemia: theoretical and practical considerations. Leukemia. 2021;35(6):1529–1538. https://doi.org/10.1038/s41375-021-01230-4
90. Hanekamp D, Ngai LL, Janssen JJ, et al. Early assessment of clofarabine effectiveness based on measurable residual disease, including AML stem cells. Blood. 2021;137(12):1694–1697. https://doi.org/10.1182/blood.2020007150
91. Platzbecker U, Middeke JM, Sockel K, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. Lancet Oncol. 2018;19(12):1668–1679. https://doi.org/10.1016/S1470-2045(18)30580-1

6

Acute Myeloid Leukemia with Myelodysplasia Related Changes

Motoharu Shibusawa[1] • Tetsuya Tanimoto[2]

[1]IMS group Shinmatsudo Central General Hospital, Department of Hematology, Chiba, Japan; [2]Navitas Clinic Kawasaki, Kanagawa, Japan

Author for Correspondence: Motoharu Shibusawa, Shinmatsudo Central General Hospital, Department of Hematology, Chiba, Japan. Email: m_sibusawa@hotmail.com

Cite this chapter as: Shibusawa M, Tanimoto T. Acute Myeloid Leukemia With Myelodysplasia Related Changes. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 05 Aug 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-aml-mrca

Abstract: Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) is one of the common subtypes of AML. It accounts for approximately 20% of newly diagnosed AML. The World Health Organization classification 2017 defines AML-MRC as acute leukemia with ≥20% blasts in the peripheral blood or bone marrow with morphological features of myelodysplasia, or occurring in patients with a prior history of myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), with MDS related cytogenetic abnormalities, and the absence of specific genetic abnormalities of AML with recurrent genetic abnormalities. In diagnosing AML-MRC, there are three main criteria; the presence of (i) dysplasia; (ii) chromosomal abnormalities associated with AML-MRC; and (iii) a prior history of MDS or MDS/MPN. Therefore, AML-MRC is a heterogeneous disease, and the prognosis of each AML-MRC varies widely. AML-MRC is usually treated with chemotherapy, and

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

hematopoietic stem cell transplantation is one of the treatment options. Prognostic factors should be considered in planning a treatment strategy for each case.

Keywords: acute leukemia; acute myeloid leukemia with myelodysplasia related changes; acute myeloid leukemia; myelodysplasia; myelodysplasia related changes

INTRODUCTION

Acute myeloid leukemia (AML) is a hematopoietic cell neoplasm which is characterized by expansion of leukemic cells in the bone marrow. The World Health Organization (WHO) classification 2017 classifies AML and related precursor neoplasms into subtypes as follows: AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes (AML-MRC), therapy-related myeloid neoplasms (t-AML), AML not otherwise specified (AML-NOS), myeloid sarcoma, and myeloid proliferations associated with Down syndrome (1).

The term of AML-MRC was first introduced by the WHO classification 2008. It included AML cases with a history of myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), or with myelodysplasia-related cytogenetic abnormalities, or morphologic evidence of dysplasia in 50% or more of the cells in two or more myeloid lineages (2). In the revised WHO classification 2017, it defines AML-MRC as acute leukemia with ≥20% blasts in the peripheral blood or bone marrow with morphological features of myelodysplasia, or occurring in patients with a prior history of MDS or MDS/MPN, with MDS related cytogenetic abnormalities and the absence of specific genetic abnormalities characteristic of AML with recurrent genetic abnormalities (3). Therefore, there are three main criteria when patients are classified in this category: (i) those with the presence of dysplasia; (ii) those with MDS related cytogenetic abnormalities; and (iii) those with a prior history of MDS or MDS/MPN. According to a previous report (4), the reasons for AML-MRC diagnosis is as follows:(i) the majority of patients were diagnosed based on only one reason—20% for the presence of dysplasia, 56.8% for MDS related cytogenetics abnormalities, and 5.8% for a prior history of MDS or MDS/MPN; (ii) 16.8% of patients had two reasons—7.4% for MDS-related cytogenetics abnormalities and prior history of MDS or MDS/MPN, 7.4% for the presence of dysplasia and MDS related cytogenetics, and 2.1% for the presence of dysplasia and a history of MDS or MDS/MPN, and (iii) only 0.5% of patients had all three reasons.

In the upcoming 5th edition of the WHO classification for myeloid and histiocytic/dendritic tumors (5), the name of AML-MRC has been changed to AML, myelodysplasia-related (AML-MR). Morphology alone as a diagnostic premise to make a diagnosis of AML-MR is removed. Since more detailed updates of this disease as seen in the printed 5th edition book won't be available until the end of 2022, the description in this chapter is mostly based on the revised 4th edition. This chapter describes the epidemiology, morphological and cytogenetic features, diagnosis, clinical features, treatment, prognosis, and predictive factors of AML-MRC.

EPIDEMIOLOGY

According to a report from the United States, the age-adjusted incidence of AML is 4.3 per 100,000 persons annually. Incidence increases with age, with a median age at diagnosis of 68 years. Males develop AML 1.2–1.6 times more likely than females (6). As for the AML-MRC, according to a previous report from China (4), it accounted for 22.2% of all newly diagnosed AML. The median age of AML-MRC patients was 61 years (range 16–87). In addition, comparing AML-MRC patients with AML-NOS patients, AML-MRC patients had significantly older ages ($p < 0.001$). AML-MRC was also male-predominant (male to female ratio, 1.79:1) (4).

MORPHOLOGICAL MANIFESTATIONS OF DYSPLASIA

The findings of dysplasia observed in MDS (7) are shown in Table 1. Diagnosing AML-MRC solely based on the presence of dysplasia requires the presence of dysplasia in ≥50% of the cells in at least two hematopoietic cell lineages (hematopoietic cell lineages are neutrophils and their precursors, erythroid cells, and megakaryocytes) (3). It should be noted that several factors, including deficiency of vitamin B12, folic acid, and copper, can cause morphological dysplasia and cytopenia (7). Even so, the findings of nuclear hyposegmentation (pseudo-Pelger anomaly) (Figure 1A), ring sideroblasts (Figure 1B), micromegakaryocytes (Figure 1C) correlate most strongly correlate with MDS (8).

TABLE 1 **Morphological manifestations of dysplasia observed in myelodysplastic syndrome**

Dysgranulopoiesis	Dyserythropoiesis	Dysmegakaryopoiesis
Small or unusually large size	Nuclear	Micromegakaryocytes
Nuclear hyposegmentation (pseudo Pelger Huët)	Nuclear budding	Nuclear hypolobation
Nuclear hypersegmentation	Internuclear bridging	Multinucleation
Decreased granules; agranularity	Karyorrhexis	
Pseudo Chédiak Higashi granules	Multinuclearity	
Döhle bodies	Megaloblastoid changes	
Auer rods	Cytoplasmic	
	Ring sideroblasts	
	Vacuolization	
	Periodic acid Schiff (PAS) positivity	

From Myelodysplastic syndromes in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th Edition; 2017. p.102, cited in.

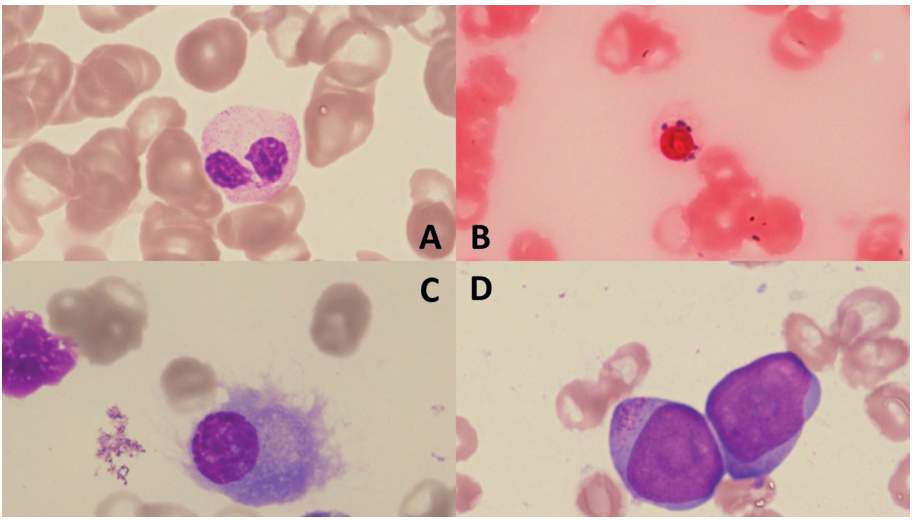

Figure 1. Microscopic picture of bone marrow smear. A: Nuclear hyposegmentation (pseudo-Pelger anomaly, Wright–Giemsa staining, ×1,000). The two lobes are joined by thin filaments and have coarse nuclear chromatin. **B:** Ring sideroblast (Iron stain, ×1,000). Iron granules are present in more than five and cover at least a third of the nuclear circumference. **C:** Micro megakaryocyte (Wright–Giemsa staining, ×1,000). Megakaryocyte has mononuclear and is less than promyelocytes in size. **D:** Blasts observed in acute myeloid leukemia with myelodysplasia-related changes (Wright–Giemsa staining, ×1,000). Blasts presented with loose reticular chromatin and a high nucleus-to-cytoplasm ratio. Some blasts showed cytoplasmic nucleoli and granules.

According to a previous report, among AML-MRC patients according to the WHO classification 2008, 31.9% of patients had the presence of dysplasia in one hematopoietic lineage. In 27.0% of patients, dysplasia was observed in two cell lineages, whereas trilineage dysplasia was found in only 8.8% of patients. In addition, multilineage dysplasia was observed in a higher frequency in cases with a history of prior MDS or MDS/MPN compared to those without (66.7% vs. 32.5%; P = 0.001) and also, cases with MDS related cytogenetic abnormalities compared those without (49.1% vs. 4.0%; P = 0.035), respectively (9).

ASSESSMENT OF THE PERCENTAGE OF BLASTS

Blasts in an AML-MRC case are shown in Figure D. Morphologically, the blasts found in AML-MRC vary from case to case, but it is important to know that pure erythroid leukemia (M6), according to the classical French-American-British classification, is associated with a prior history of MDS (7).There are several things to note in assessing the percentage of blasts. Firstly, the percentage of blasts in the peripheral blood may be higher than the percentage of blasts in the bone marrow. (10) Secondly, a bone marrow biopsy may be useful in assessing the percentage of blasts in the bone marrow. When a patient has myelofibrosis in the bone marrow, aspiration can be “dry tap”. In such a case, immunostaining of bone marrow biopsy is the only way to assess the percentage of blasts (11).

CHROMOSOME ABNORMALITIES

Cytogenetic abnormalities sufficient for the diagnosis of AML-MRC (3) are shown in Table 2. There are a few things to keep in mind in AML-MRC diagnosis. Firstly, trisomy 8, del(20q), and loss of Y chromosome are observed to be common in MDS patients, but these abnormalities are not considered to be specific to AML-MRC. Therefore, the presence of these chromosomal abnormalities is not sufficient for AML-MRC diagnosis. Secondly, AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) and AML with t(6;9)(p23;q34.1) may present with multilineage dysplasia. However, these cases should be classified as AML with recurrent genetic abnormalities (3). The percentages of cytogenetic abnormalities observed in AML-MRC patients as follows: complex karyotype in 77% of patients, monosomy 5 or del(5q) in 6%, monosomy 7 or del(7q) in 16%, concurrent chromosome 5 or 7 abnormalities in 1%, del(13q) in 1%, and i(17) in 0.5% (12).

TABLE 2 **Cytogenetic abnormalities sufficient for the diagnosis of acute myeloid leukemia with myelodysplasia-related changes**

Complex karyotype	**≥3 abnormalities**
Unbalanced abnormalities	Loss of chromosome 7 or del(7q)
	del(5q) or t(5q)
	Isochromosome 17q or t(17p)
	Loss of chromosome 13 or del(13q)
	del(11q)
	del(12p) or t(12p)
	idic(X)(q13)
Balanced abnormalities	t(11;16)(q23.3;p13.3)
	t(3;21)(q26.2;q22.1)
	t(1;3)(p36.3;q21.2)
	t(2;11)(p21;q23.3)
	t(5;12)(q32;p13.2)
	t(5;7)(q32;q11.2)
	t(5;17)(q32;p13.2)
	t(5;10)(q32;21)
	t(3;5)(q25.3;q35.1)

From Acute myeloid leukaemia with myelodysplasia-related changes. in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th; 2017. p.151, cited in.

GENETIC MUTATIONS

The frequency of genetic mutations (present in >10% of patients) detected in AML-MRC patients according to the WHO classification 2017 is as follows: the most frequent mutation was TP53 (39%), followed by ASXL1 (20%), NRAS (17%), DNMT3A (16%), SRSF2 (14%), TET2 (14%) and U2AF1 (14%) (12). In the diagnosis of AML-MRC, the following should be noted that AML cases with NPM1 and/or FLT3 mutations or mutations of CEBPA may present with multilineage dysplasia. However, these AML cases should be classified as AML with recurrent genetic abnormalities (3).

DIAGNOSIS

The diagnostic criteria for AML-MRC are shown in Table 3. Diagnosing AML-MRC requires taking a history of the presence or absence of a prior history of MDS or MPN. In addition, it also requires taking a history of the presence or absence of prior use of anticancer drugs and having radiation therapy to rule out t-AML. Furthermore, evaluation of microscopic findings and chromosomal analysis are necessary. Finally, the detected chromosomal abnormalities must be confirmed that they are not chromosomal abnormalities, which should be classified as AML with recurrent genetic abnormalities.

CLINICAL FEATURES

According to a retrospective analysis of Chinese patients (4), laboratory data of AML-MRC patients were as follows: white blood cell count (WBC), 7.5 × 10^9/L (range 0.3–375.9); hemoglobin level (Hb), 71 g/L (range 30–146); platelet

TABLE 3 Diagnostic criteria of acute myeloid leukemia with myelodysplasia-related changes

1	≥20% blood or marrow blasts	
2	Any of the following	History of myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm
		Myelodysplastic syndrome related cytogenetic abnormality
		Mutilineage dysplasia
3	Absence of both of the following	Prior cytotoxic or radition therapy for an unrelated disease
		Recurrent cytogenetic abnormality as discribed in acute myeloid leukemia with recurrent genetic abnormalities

The diagnosis of acute myeloid leukemia with myelodysplasia-related changes requires the three of the above criteria to be met. From Acute myeloid leukaemia with myelodysplasia-related changes. in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th; 2017. p.150, cited in.

count, 44 × 10^9/L (range 1–458); and lactate dehydrogenase (LDH) level, 387 IU (range 86–5986) (4). According to the 2017 European leukemia Net (ELN) criteria, 80.4% of cases were assigned to the intermediate-risk group. The remaining 19.6% of cases were classified into the unfavorable risk group. It is noteworthy that there were no patients assigned to the favorable risk group (4). Furthermore, in the study comparing 190 AML-MRC patients and 667 AML-NOS patients, AML-MRC patients had significantly older age ($p < 0.001$), lower Hb level ($p < 0.001$), and lower WBC count ($p < 0.001$), and higher male-to-female ratio ($p = 0.006$). As for clinical outcomes, AML-MRC patients had significantly lower complete remission (CR) rates (65.3% vs. 76.2%; $p = 0.005$) (4).

TREATMENT

There is no established standard of care for AML-MRC. AML-MRC is usually treated with chemotherapy, and hematopoietic stem cell transplantation is one of the treatment options.

Chemotherapy

Outcomes of chemotherapy treatment for AML-MRC have been reported. AML-MRC patients aged 60–75 years were treated in three ways (4): (i) an idarubicin and Ara-C (IA/DA) group; (ii) decitabine, aclarubicin, Ara-C, and granulocyte colony-stimulating factor (DAC+CAG) group; and (iii) supportive care. Among patients classified as the intermediate risk group based on the 2017 ELN criteria, CR rate of IA/DA group and DAC+CAG group were 60% and 63.6%, respectively, and overall survival (OS) of both groups were 6 and 6.5 months, respectively. In the patients classified as the unfavorable risk group, the CR rate of the IA/DA group and the DAC+CAG group were 52.2% and 59.3%, respectively. OS of the IA/DA group and the DAC+CAG group were 4.5 and 6.2 months, respectively (4). Outcomes of Liposomal Daunorubicin-Cytarabine (CPX-351) treatment for AML-MRC have also been reported. 188 AML patients including 131 patients with AML-MRC, 53 patients with t-AML, and 2 patients with other subtype of AML were treated with CPX-351. CR rate was 47%. After a median follow-up of 9.3 months, the median OS was 21 months, and 1 year OS rate was 64%. In multivariate analysis, complex karyotype predicted lower response ($p = 0.0001$), while pretreatment with hypomethylating agents ($p = 0.02$) and adverse risk (based on ELN 2017) ($p < 0.0001$) were associated with lower OS (13).

Allogenic hematopoietic stem cell transplant

A previous study reported the treatment outcome of 60 AML-MRC patients (according to the WHO classification 2008) who had undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT). In the patients' characteristics, there were no significant differences between the AML-MRC group and AML-NOS group in age, gender, performance status, hematopoietic cell transplantation comorbidity index, conditioning regimen, and

graft-versus-host disease prophylaxis, but the AML-MRC group had a higher frequency of non-CR disease status at allo-HSCT (48% vs. 27%, p = 0.01), unfavorable cytogenetic risk (40% vs. 9%, p < 0.0001), and underwent bone marrow transplantation (92% vs. 78%, p = 0.04) from HLA matched unrelated donor (75% vs. 41%, p = 0.0002). Despite the above differences in the patients' characteristics, there were no significant differences in the 2-year OS, cumulative incidence of relapse (CIR), and non-relapse mortality (NRM) between the two groups (2-year OS, 48% vs. 59%; 2-year CIR, 37% vs. 35%; 2-year NRM, 19% vs. 13%) (14). Thus, allo-HSCT is considered a treatment option for AML-MRC.

Prognosis

Among AML-MRC cases, according to the WHO classification 2017, the median OS was 7.6 months (95% confidence interval, 5–10.6 months) (15). As prognostic factors, age, LDH (4), the presence of MDS related cytogenetic abnormalities, monosomal or complex karyotype, and history of MDS or MDS/MPN (15) have been reported. However, it should be noted that the presence of dysplasia is not a prognostic factor. Actually, there were no significant differences in OS and event-free survival among AML-MRC patients having dysplasia in zero vs. one vs. two vs. three hematopoietic cell lineages. (16)

CONCLUSION

AML-MRC is one of the common subtypes of AML and accounts for approximately 20% of newly diagnosed AML. Diagnosing AML-MRC requires taking a history regarding the presence or absence of prior MDS or MDS/MPN and prior anticancer drug/radiotherapy treatment. There are three main criteria for AML-MRC diagnosis:the presence of dysplasia, chromosomal abnormalities associated with AML-MRC, and a prior history of MDS or MDS/MPN. AML-MRC is a heterogeneous disease, and the prognosis varies widely among patients. AML-MRC is usually treated with chemotherapy, and HSCT is one of the treatment options. In a planning treatment strategy, prognostic factors should be considered individually for each case.

Conflict of Interest: TT reports personal fees from Medical Network Systems Inc., and Bionics co., ltd, outside the submitted work. Dr. Shibusawa declares no conflicts of interest.

REFERENCES

1. Arber DA, Brunning RD, Beau MML, Falini B, Vardiman JW, Porwit A, et al. Acute myeloid leukaemia and related precursor neoplasms. In: Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, S Jaffe Elaine, A Pileri Stefano, Harald Stein, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th Edition ed. Lyon: International Agency for Research on Cancer; 2017. p. 129–71.
2. Yin CC, Medeiros LJ, Bueso-Ramos CE. REVIEW: Recent advances in the diagnosis and classification of myeloid neoplasms - comments on the 2008 WHO classification. International Journal of Laboratory Hematology. 2010;32(5):461–76. https://doi.org/10.1111/j.1751-553X.2010.01246.x
3. Arber DA, Brunning RD, Orazi A, Bain BJ, Porwit A, Beau MML, et al. Acute myeloid leukaemia with myelodysplasia-related changes. In: H Swerdlow Steven, Elias Campo, Nancy Lee Harris, S Jaffe Elaine, A Pileri Stefano, Harald Stein, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th Edition ed. Lyon: International Agency for Research on Cancer; 2017. p. 150–2.
4. Wang L, Chu X, Wang J, An L, Liu Y, Li L, et al. Clinical Characteristics and Optimal Therapy of Acute Myeloid Leukemia with Myelodysplasia-related-changes: A Retrospective Analysis in a Cohort of Chinese Patients. Turkish Journal of Hematology. 2021. https://doi.org/10.4274/tjh.galenos.2021.2021.0009
5. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703–19. https://doi.org/10.1038/s41375-022-01613-1
6. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Rev. 2019;36:70–87. https://doi.org/10.1016/j.blre.2019.04.005
7. Hasserjian RP, Orazi A, Brunning RD, Germing U, Beau MML, Porwit A, et al. Myelodysplastic syndromes. In: Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, S Jaffe Elaine, A Pileri Stefano, Harald Stein, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th Edition ed. Lyon: International Agency for Research on Cancer; 2017. p. 97–120.
8. Bowen D, Culligan D, Jowitt S, Kelsey S, Mufti G, Oscier D, et al. Guidelines for the diagnosis and therapy of adult myelodysplastic syndromes. British Journal of Haematology. 2003;120(2):187–200. https://doi.org/10.1046/j.1365-2141.2003.03907.x
9. Miesner M, Haferlach C, Bacher U, Weiss T, Macijewski K, Kohlmann A, et al. Multilineage dysplasia (MLD) in acute myeloid leukemia (AML) correlates with MDS-related cytogenetic abnormalities and a prior history of MDS or MDS/MPN but has no independent prognostic relevance: a comparison of 408 cases classified as "AML not otherwis. Blood. 2010;116(15):2742–51. https://doi.org/10.1182/blood-2010-04-279794
10. Amin HM, Yang Y, Shen Y, Estey EH, Giles FJ, Pierce SA, et al. Having a higher blast percentage in circulation than bone marrow: clinical implications in myelodysplastic syndrome and acute lymphoid and myeloid leukemias. Leukemia. 2005;19(9):1567–72. https://doi.org/10.1038/sj.leu.2403876
11. Orazi A. Histopathology in the Diagnosis and Classification of Acute Myeloid Leukemia, Myelodysplastic Syndromes, and Myelodysplastic/Myeloproliferative Diseases. Pathobiology. 2007;74(2):97–114. https://doi.org/10.1159/000101709
12. Montalban-Bravo G, Kanagal-Shamanna R, Class CA, Sasaki K, Ravandi F, Cortes JE, et al. Outcomes of acute myeloid leukemia with myelodysplasia related changes depend on diagnostic criteria and therapy. Am J Hematol. 2020;95(6):612–22. https://doi.org/10.1002/ajh.25769
13. Rautenberg C, Stölzel F, Röllig C, Stelljes M, Gaidzik V, Lauseker M, et al. Real-world experience of CPX-351 as first-line treatment for patients with acute myeloid leukemia. Blood Cancer J. 2021;11(10):164. https://doi.org/10.1038/s41408-021-00558-5

14. Ikegawa S, Doki N, Kurosawa S, Yamaguchi T, Sakaguchi M, Harada K, et al. Allogeneic hematopoietic stem cell transplant overcomes poor prognosis of acute myeloid leukemia with myelodysplasia-related changes. Leuk Lymphoma. 2016;57(1):76–80. https://doi.org/10.3109/10428194.2015.1063148
15. Fang H, He R, Chiu A, Viswanatha DS, Ketterling RP, Patnaik MS, et al. Genetic Factors in Acute Myeloid Leukemia With Myelodysplasia-Related Changes. Am J Clin Pathol. 2020;153(5):656–63. https://doi.org/10.1093/ajcp/aqz206
16. Haferlach T, Schoch C, Löffler H, Gassmann W, Kern W, Schnittger S, et al. Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies. J Clin Oncol. 2003;21(2):256–65. https://doi.org/10.1200/JCO.2003.08.005

7

Secondary Acute Myeloid Leukemia: Pathogenesis and Treatment

Debora Capelli • Diego Menotti • Alessandro Fiorentini • Francesco Saraceni • Attilio Olivieri

Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy

Author for correspondence: Debora Capelli, Hematology department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy. Email: debora.capelli@ospedaliriuniti.marche.it

Cite this chapter as: Capelli D, Menotti D, Fiorentini A, Saraceni F, Olivieri A. Secondary Acute Myeloid Leukemia: Pathogenesis and Treatment. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 22 Aug 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-secondary-acute-myeloid-leukemia

Abstract: Secondary acute myeloid leukemia includes acute myeloid leukemia that arises either from a previous myeloid hematologic disease such as myelodysplastic syndrome, chronic myeloproliferative syndrome, or myelodysplastic/myeloproliferative overlap syndromes or from a previous chemotherapy or radiotherapy performed for another disease. Secondary acute myeloid leukemia is characterized by a worse prognosis than its de novo counterparts, with a 5-year overall survival of <30% despite an advanced insight into pathogenesis and new available treatments. The best therapeutic strategy is to achieve complete remission with a negative minimal residual disease followed by hematopoietic stem cell transplantation; however, advanced age of patients at diagnosis, multiple comorbidities, and lower rate of complete remission makes these approaches available only for a small fraction of secondary acute myeloid leukemia patients. In this chapter, we discuss the epidemiology, pathogenesis, and prognostic

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

factors of secondary acute myeloid leukemia. Also, we discuss the main treatments currently available for eligible patients (fit patients) and non-eligible patients (unfit patients) for intensive chemotherapy and future treatment perspectives.

Keywords: acute myeloid leukemia; secondary acute myeloid leukemia; acute myeloid leukemia with myelodysplastic-related changes; therapy-related acute myeloid leukemia.

INTRODUCTION

Acute myeloid leukemia (AML) with myelodysplastic related changes (MRC) are diagnosed based on clinical, cytogenetic, and morphologic criteria, reviewed in the 2008 WHO classification as those with a previous history of myelodysplastic syndrome (MDS), or myeloproliferative neoplasia (MPN) with specific cytogenetic alterations, or myelodysplastic changes in more than 50% of two or more cell lineages (1). The updated 2016 WHO classification excludes AML with dysplastic changes with NPM1 and CEBPA biallelic mutations or del(9q) from the MRC subgroup (2, 3). Next generation sequencing helped the comprehension of the pathogenesis of these entities, identifying mutations of RUNX1, TP53, SETBP1, epigenetic regulators, and spliceosome genes as those characterizing MRC-AML (4). Methylation of transcription factors, bone marrow microenvironment alterations such as neo angiogenesis, pro-inflammatory changes, and fibrosis acquisition, characterize the evolution of MDS into AML (5, 6). The most accepted model of leukemic evolution of MDS is the "two-hit" model with sequential blockade of genes regulating cell differentiation, such as TET2 or RUNX1, followed by alteration of genes regulating cell proliferation and survival (FLT3, NPM1, IDH1) (7, 8). Increased expression of the altered anti-apoptotic protein bcl-2 results in further stimulation of expansion of the dysplastic clone, which acquires a survival advantage over normal hematopoietic cells (8).

Secondary AMLs include both MRC and therapy-related AML (t-AML). This latter entity is defined as AML arising after exposure to chemotherapy or radiotherapy for previous cancer or autoimmune diseases and was first recognized by the 2008 WHO classification (1). Latency between primary disease and t-AML depends on age at diagnosis of the primary malignancy, type of cytotoxic treatment, cumulative dose, and dose intensity and its development might be influenced by genetic predisposition. Current ELN 2022 guidelines for diagnosis and cure of the disease removed t-AML and AML evolving from previous MDS or MDS/MPN, considering them only as diagnostic qualifiers (9). AML with myelodysplasia-related gene mutations (ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2) and those with myelodysplasia-related cytogenetic abnormalities took the place of t-AML and MRC-AML, as the new entities of secondary AML, underlying the prognostic significance of molecular and cytogenetic features. Secondary AMLs are indeed characterized by an extremely poor prognosis and still represent a challenge for cure.

PATHOGENESIS

Chen et al. have shown that MDS is a disease characterized by several subclones with mutations in TET2, U2AF1 and TP53 genes which might acquire additional mutations, such as NOTCH2 and KMT2C, or later mutations associated with leukemic transformation such as RUNX1, NRAS and NTRK3. These complex subclonal mutations occur in stem cells determining MDS expansion or evolution into AML via variable mechanisms. Sequential single cell analysis technique may help the comprehension of these complex transformation mechanisms better than new generation sequencing (10). Lindsely et al. (11) analyzed mutations in 194 patients with secondary AML or t-AML (therapy-related acute myeloid leukemia) and in 105 de novo AML patients, identifying four different groups of mutations:

(i) *Secondary type mutations specific to secondary AML*: SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR and STAG2 capable of sustaining ineffective hematopoiesis, already at the diagnosis of MDS, without leading to the development of leukemia, which persist even after treatment, at clonal remission.
(ii) *De novo mutations:* NPM1, present in 5.4% of secondary AML, MLL/11q23 rearrangements, and CBF.
(iii) *Mutations in the TP53 gene (15.1% of secondary AML):* Acquired early in small subclones, less frequently expressing other mutations, but often associated with complex karyotype (12), expressed in 21.4% of de novo forms, characterized by poor prognosis.
(iv) *Pan-AML:* Mutations such as those of myeloid transcription factors (RUNX1, CEBPA, GATA2) and signal transduction proteins (FLT3 or RAS pathway), present in 78% of secondary AML, expressed equally in secondary and de-novo AML, absent in MDS, are responsible for leukemic evolution and disappear at remission.

Finally, the authors suggested three distinct ontogenetic patterns of AML MRC: the first is characterized by the presence of secondary type mutations, causing ineffective hematopoiesis, associated with a greater number of driver mutations, driving the evolution into AML; the second having de novo or pan-AML mutations, driving the evolution into AML themselves; and the third having TP53 mutations, acquired early, and associated with a lower number of driver mutations in comparison with the first group.

Lindsley et al. observed that similar genetic data were found in de-novo and t-AML patients, suggesting that these mechanisms are also applicable in t-AML pathogenesis and that the analysis of mutations might help to recognize MRC-AML in elderly de-novo AML patients, when secondary and TP53 mutations were observed (11). Another scenario characterizes MRC-AML secondary to MPN. Twenty-five per cent of MPN evolves in MRC-AML (13). Epigenetic regulators and mRNA splicing factors mutations, and primary "triple negative" myelofibrosis are more likely to undergo leukemic transformation. Mutations in splicing factor genes are associated with progression to secondary myelofibrosis and essential thrombocythemia (ET); TP53 mutations predict the risk of leukemic

transformation, and IDH1/2 mutations increase from 1–4% of chronic phase to 22% in blast-phase MPN (14).

Therapy-related AML

The WHO 2016 defines t-AML as those forms of AML that arise following exposure to chemo and/or radiotherapy for a previous neoplastic or not neoplastic disease. Breast cancer, non-Hodgkin lymphomas, and Hodgkin lymphomas are the three primary malignancies most frequently associated with the development of t-AML (15). Data from the Surveillance Epidemiology and End Result (SEER) register show that the incidence of t-AML increased from 0.04/100,000 people in the period 2001–2007 to 0.2/100,000 people in the period 2008–2014 (16). t-AML accounts for about 8% of all AML diagnoses with median age of diagnosis depending on the primary tumor, ranging between 40–66 years, which is reported to be 57.8 years in a retrospective analysis of 6 prospective multicenter trials of the German-Austrian AML Study Group (AMLSG) (15). It is associated with a poor prognosis, with a lower overall survival compared to de novo AML (15, 17, 18). The pathogenesis of t-AML is complex and still not fully known. To date, the most accredited theories include: (i) direct damage to the hematopoietic stem cells and the bone-marrow microenvironment mediated by chemo and radiotherapy; and (ii) pre-existing clonal hematopoiesis (19, 20).

The two classes of drugs that are associated with the development of t-AML are alkylating agents and topoisomerase II inhibitors (TOPII inhibitors). They generate double-stranded DNA breaks (DSBs) leading to growth arrest and cell apoptosis. If DSBs are not repaired, they can generate chromosomal alterations and genomic instability characteristic of these drugs (20, 21) (21–23). Radiotherapy determines either direct damage to the DNA, which can cause single or DSB, or an indirect damage to DNA through the generation of reactive oxygen species that can interact with DNA or nuclear proteins leading to modifications of DNA bases and/or DSBs (24, 25). Alkylating agents and radiotherapy often result in chromosome 5 and chromosome 7 abnormalities, complex karyotype, and TP53 mutation. AML occurs with a latency of 5–7 years and is often preceded by MDS. TopII inhibitors cause chromosomal translocations that most often involve the KMT2A genes on chromosome 11q23, RUNX1 on 21q22 and PML/RARA. The development of leukemia has a shorter period ranging from 1 to 3 years and is almost never preceded by a MDS (20, 26–28).

Bone marrow microenvironment, consisting of pluripotent mesenchymal cells and their descendants, endothelium of bone marrow sinusoids, fibroblasts, reticular cells, adipocytes, and catecholaminergic fibers, among others, regulate almost all functions of hematopoietic stem cells (HSC); it is therefore not surprising that the altered functions of these cells, caused by therapy, can contribute to the pathogenesis of t-AML (29–31). Cytotoxic agents cause the release of numerous pro-inflammatory cytokines (TNF alpha, IL-6, TGF beta) and the generation of free radicals that damage both the mesenchymal cells and the autonomous nerve fibers of the niche. Their altered functions, in several mouse models, have been shown to be sufficient for the onset of AML (32, 33).

Another pathogenetic mechanism hypothesizes that the presence of a clonal hematopoiesis of uncertain significance (CHIP) which, under the selective

pressure of chemo and radiotherapy, gain a proliferative advantage over the normal counterpart (34). This is supported by a case-control study where up to 71% of patients with a primary neoplasia had a contemporary CHIP; in these patients, TP53 mutation was present in 16% of cases. The cumulative incidence of t-AML was 30% in patients with a concomitant CHIP vs only 7% in those who did not have CHIP (35, 36). Furthermore, patients with t-AML present mutations on TP53 gene more frequently than patients with de-novo AML (33% vs 5–10% respectively) and these mutations are found, albeit with very low frequencies, in the bone marrow cells of those same individuals even before chemotherapy (37–41).

The development of t-AML is also a multistep process in which numerous somatic mutations accumulate, resulting in a progressive proliferative advantage and the arrest of differentiation in the pre-leukemic clone. The most frequently mutated genes in t-AML grouped into several functional groups are: (i) epigenetic regulators (TET2, DNMT3A, IDH1/IDH2, EZH2, ASXL1); (ii) regulators of the RNA spliceosome machinery (SRSF2, SF3B1, U2AF1); (iii) regulators of transcription (TP53, RUNX1); (iv) regulators of signaling pathways (FLT3) (37–39).

The t-AMLs are associated with poor prognosis with an estimated overall survival of 7–10 months (40), complete response (CR) rates of about 28–30% (41), and a shorter duration of response than de novo forms even after consolidation (42). The poor prognosis of these neoplasms depends on both patient-related factors and AML-related factors. Patient-related factors include older age at diagnosis, reduced bone marrow reserve from previous therapy as well as damage to the microenvironment, altered function of other organs as a complication of previous therapy, the need for prolonged immunosuppressive therapy due to a previous solid organ transplant, and the presence of the previous neoplasm still active. AML-related factors include TP53 mutation, and complex or monosomic karyotype, which are much more frequent in t-AML than de novo AML (43). However, the diagnosis of t-AML per se, does not contraindicate eligibility for intensive chemotherapy as some disease subgroups, such as those associated with the t(8;21), inv(16)(p13q22)/t(16;16)(p13;q22) or the t(15;17), have high CR rates (above 70%) and a 2-year overall survival of about 50% with this therapeutic approach, albeit still lower than the de novo counterpart (44, 45).

DEFINITION OF FITNESS TO INTENSIVE AND NON-INTENSIVE CHEMOTHERAPY

Scoring systems capable of predicting early mortality after intensive chemotherapy have not shown sufficient accuracy and reproducibility to ensure objective selection of patients fit for intensive chemotherapy. Rather, a recent work (46) has validated the 'Ferrara' criteria, selected by a group of experts from the Italian Society of Hematology (SIE), the Italian Society of Experimental Hematology (SIES), and the Italian Group for Bone Marrow Transplantation (GITMO), as a model capable of distinguishing patients fit for intensive chemotherapy, fit for non-intensive chemotherapy, or unfit for non-intensive chemotherapy (47). A list of 24 conceptual criteria was selected using a analytic hierarchy process-based consensus process, and on the basis of the pairwise comparisons of these

criteria, the members of the panel proposed the definition of unfitness to intensive chemotherapy as the presence of at least one of the following nine criteria: (i) age older than 75 years; (ii) congestive heart failure or documented cardiomyopathy with an EF ≤50%; (iii) documented pulmonary disease with DLCO ≤65% or FEV1 ≤65%, or dyspnea at rest or requiring oxygen, or any pleural neoplasm or uncontrolled lung neoplasm; (iv) dialysis and age older than 60 years or uncontrolled renal carcinoma; (v) liver cirrhosis Child B or C, or documented liver disease with marked elevation of transaminases (>3 times normal values) and an age older than 60 years, or any biliary tree carcinoma or uncontrolled liver carcinoma or acute viral hepatitis; (vi) active infection resistant to anti-infective therapy; (vii) current mental illness requiring psychiatric hospitalization, institutionalization or intensive outpatient management, current cognitive status that produces dependence (as confirmed by the specialist) not controlled by the caregiver; (viii) ECOG performance status ≥3 not related to leukemia; and (ix) any other comorbidity that the physician judges to be incompatible with conventional intensive chemotherapy). Patients fit for intensive chemotherapy are therefore those lacking all these clinical conditions.

The unfitness to non-intensive chemotherapy was defined as the fulfillment of at least one of the following six criteria: (i) refractory congestive heart failure; (ii) documented pulmonary disease with DLCO ≤65% or FEV1 ≤65%, or dyspnea at rest or requiring oxygen, or any pleural neoplasm or uncontrolled lung neoplasm; (iii) liver cirrhosis Child B or C or acute viral hepatitis; (iv) active infection resistant to anti-infective therapy; (v) current mental illness requiring psychiatric hospitalization, institutionalization or intensive outpatient management, or current cognitive status that produces dependence (as confirmed by the specialist) not controlled by the caregiver; and (vi) uncontrolled neoplasia. Patients fit for non-intensive chemotherapy do not fulfill any of these clinical conditions.

Obviously, these criteria did not take into account mortality rates related to newer low-intensity therapies, such as Venetoclax, IDH, and FLT3 inhibitors, associated or not with low-intensity chemotherapy or hypomethylating agents, where their accuracy has yet to be validated and explored, but they remain, at present, the best available clinical instrument for defining fitness to non-intensive chemotherapy.

TREATMENT OF PATIENTS FIT FOR INTENSIVE CHEMOTHERAPY

The current standard therapy for patients fit for chemotherapy is CPX-351, a liposomal formulation of cytarabine and daunorubicin in a fixed 5:1 molar ratio, approved by FDA in 2017. A 5-year follow up of a Phase III trial in secondary AML confirmed an overall survival benefit of CPX over the standard 7+3, with a 10% increase in survival due to reduced early mortality and higher transplantation rate compared with 7+3 (gain of survival due to reduced early mortality and higher transplant rate in comparison to 7+3 (48). The transplant landmark analysis showed a higher number of transplanted patients, slightly older, in the CPX arm than in the 7+3 arm, having a 3-year overall survival rate of 56% versus 23% respectively (49). Improved transplant outcomes were not due to a decrease in

relapse, but to an improvement in non-relapse mortality with CPX, due to better tolerance of this induction approach compared to 7+3. This pivotal Phase III trial did not have the MRD data, but three real life data, the Italian, the French, and the German experience, showed respectively 57%, 57%, and 64% MRD negativity in responders (50–52). There is lack of data on the effect of CPX combination in FLT3 and IDH mutations. FLT3-mutated MRC-AML, fit for intensive therapy, should receive 3+7 with midostaurin; IDH-mutated patients do not yet have defined regimen for target therapy, but when ivosidenib and enasidenib achieve registration, they will represent an attractive therapeutic option.

CPX-351 combinations

V-FAST phase Ib trial accrued patients based on molecular profile: those with FLT3 and IDH2 wild type received CPX-351 plus venetoclax, those with either FLT3-ITDs or TKDs received CPX plus midostaurin, and those with IDH2 positive received CPX plus enasidenib (53). Venetoclax was administered as a short schedule, days 1–14, instead of days 1–28 of VIALE-A, to reduce hematological toxicity (Arm A). Midostaurin was administered along with the RATIFY schedule from days 8 through 21 (Arm B). Enasidenib was administered on days 8 to 28 (Arm C). Arm A enrolled 20 patients obtaining 50% CR/CRi (CR with incomplete hematological recovery rate), with similar median time to absolute neutrophil count (ANC) and platelet recovery in comparison to CPX351 phase III trial (48), in a very poor prognosis setting represented by 86% of the patients with intermediate and high-risk profiling, and nearly 30% of the patients with TP53 mutations. Thirty-day and 60-day mortality were low. The other two arms need to enroll additional patients before giving reliable results, but preliminary safety data show acceptable hematological recovery and a 0% early mortality rate, with responses achieved in all treated patients. The study is active, but not recruiting. MRC-AML might express FLT3 mutations. In the phase III study, 13% of patients had a FLT3 mutation on the CPX-351 arm versus 20% on the 3+7 arm. Trials combining FLT3 inhibitors with CPX351, quizartinib, and gilteritinib are ongoing and other regimens like the CLIA scheme (cladribine, idarubicin, cytarabine with gilteritinib) are under evaluation (NCT02115295). Trials on combination treatment with azacytidine, venetoclax, gilteritinib, and quizartinib are also under evaluation (NCT03661307, NCT04140487).

CPX-351 in patients with MDS and prior hypomethylating agent exposure

Phase III data of CPX-351 did not show an advantage in comparison to 7+3 in patients with secondary AML and prior MDS, who received prior therapy with hypomethylating agents, such as azacitidine and decitabine. A retrospective analysis compared outcomes of 242 patients affected by AML secondary to MDS who were pre-treated with hypomethylating agents, after three induction strategies: CPX-351 versus 7+3 versus CLAG-M (cladribine, cytarabine, G-CSF, and mitoxantrone) (54). Patients receiving the CLAG-M regimen, achieved a 53% CR/CRi rate, higher than that observed with CPX-351 (41%) and 7+3 (32%), with similar median overall survival accounting for 7.27, 7.07 and 7.63 months respectively. The sample size and multicenter enrollment make this real-life experience

indicative of real-world outcomes, even with the limitation of being a retrospective study. Patients receiving less than four cycles of hypomethylating agents had a better response rate of 64%, and 6-months analysis showed that those receiving CPX-351, followed by allogeneic transplant, had a better overall survival in comparison to all other patients. Allogeneic transplant conferred a survival advantage in all treatment arms.

Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HCST) represents the only curative option for the treatment of MRC and t-AML. A retrospective Italian study showed a median overall survival of 58.8 months in patients after HCST (55). Litzow et al. analyzed 545 t-AML undergoing HCST and showed an overall survival of 22% at 5 years. The rapid identification of a suitable donor and the choice of a bridge to transplant with better extra hematological tolerance are the foundations of a successful cure. In a phase III trial, 31% of patients transplanted after CPX-351 treatment were over 70 years vs 15% transplanted after 3+7. Elderly t-AML patients who achieved a response after CPX-351 had better post HCST overall survival and a higher rate of transplantability, compared with those responding after 3+7 (56).

A retrospective analysis of the European Group for Bone marrow Transplant (EBMT) analyzed 802 secondary AML patients, median age of 59.6 years, undergoing HSCT after a myeloablative conditioning (MAC) in 40% of cases and a reduced-intensity conditioning (RIC) in 60%. They showed a 2-year cumulative recurrence incidence (RI) of 37%, leukemia-free survival (LFS) of 40%, overall survival (OS) of 46%, non-relapse mortality (NRM) of 23%, and chronic graft-versus-host disease (cGVHD) of 39%, with similar results between conditioning regimens. Patients in the MAC group had better RI (hazard ratio [HR], 1.79; P <. 05), LFS (HR, 1.43; P = .02) and OS (HR, 1.53; P = .005) in comparison with those receiving a RIC regimen. There was no difference in the cumulative incidence of NRM (HR, 1.38; P = .15) (57).

Allogeneic HCST is the best long-term treatment strategy for high-risk patients compared with chemotherapy alone (58). Myeloablative regimens had a lower risk of recurrence and higher LFS and overall survival than RICs, with no statistically significant difference in NRM. The decrease in the incidence of recurrence is concordant with a recent phase III study by Scott et al. in which patients with AML/MDS who received myeloablative conditioning had a statistically significant higher recurrence-free survival rate and a nonsignificant trend toward improved overall survival (59). This study showed no difference in NRM between the two conditioning groups, suggesting improvement in supportive care and management of post-transplant complications (60).

TREATMENTS OF PATIENTS UNFIT FOR INTENSIVE CHEMOTHERAPY

The criteria of patients who are unfit for intensive chemotherapy is defined above under "*Definition of fitness to intensive and non-intensive chemotherapy*".

Venetoclax and hypomethylating agents

The high expression of bcl2 protein in AML blasts and the preliminary efficacy and safety of venetoclax in monotherapy (61) are the reasons for the VIALE-A phase III trial. It randomized 145 patients on azacitidine alone and 286 patients on azacitidine plus venetoclax. The CR/CRi was 67% with a hazard ratio of 0.56 for the combination group whereas it was 23% CR/CRi for azacitidine alone (62). Even though the data on the combination of hypomethylating agent and venetoclax are encouraging, they are modest in TP53-mutated patients, and the addition of venetoclax to the 10-day decitabine scheme did not result in any particular benefit with regards to overall survival and relapse-free survival compared to historical results with 10-day decitabine alone (63). VIALE-C showed that the combination of low dose cytarabine and venetoclax did not result in survival advantage, in comparison with low dose cytarabine alone in secondary AML (64). Outcomes of t-AML patients, representing 8–9% of all enrolled subjects, were not reported in these trials.

A retrospective observational study analyzed 217 patients treated with CPX-351 and 439 patients treated with venetoclax/azacitidine; the patients had a balanced distribution of European LeukemiaNet risk, high risk mutations (TP53, ASXL1, RUNX1), FLT3, IDH, and hematopoietic cell transplantation-specific comorbidity index (HCT-CI) (65). Overall survival, tolerance, and early mortality were similar in both two groups, but infections and febrile neutropenia were more frequent in CPX-351 patients vs venetoclax/azacitidine, with a median overall survival of 13 months in the CPX-351 group and 11 months in the combination group. Multivariate analysis did not identify any predictive factors of response to therapy, but HSCT was associated with significantly improved survival. These outcomes were confirmed when analysis was restricted to patients who met the eligibility criteria of the phase III CPX-351 trial. The main pitfall of the study is the lack of MRD data.

The phase III AZA-AML-001 study comparing azacitidine vs conventional care regimens including 7+3 intensive chemotherapy, low doses of ara-c (LDAC), and supportive care showed a survival advantage in the azacitidine arm vs conventional care regimens arm with one-year survival of 46% vs 32%, respectively (66). The study enrolled 488 elderly patients with newly diagnosed AML, and of these, 262 had AML with myelodysplasia-related changes (AML-MRC). Even in the AML-MRC subgroup, the survival advantage in the azacitidine arm over the conventional care regimens arm was maintained (one-year survival 44.3% vs 27.2% respectively). Within the patient's group with AML-MRC, overall survival was higher in those with multilinear dysplasia on morphologic examination than in patients with cytogenetic alterations defining AML-MRC (67). The median overall survival in patients with morphologic dysplasia in the azacitidine arm was 16.3 months vs 5.3 months in patients with cytogenetic alterations diagnostic for AML-MRC (68).

Decitabine administered for five consecutive days was compared to treatment choice (TC) (LDAC or supportive care) in 485 older patients with newly diagnosed AML in a phase III randomized multicenter trial (69). In this trial, decitabine did not demonstrate a significant improvement in median overall survival compared to TC (7.7 months vs. 5 months) and this result was also true for 171 patients with secondary AML (69, 70). In a phase II study, in 19 patients with AML-MRC

aged ≥60 years, decitabine administered for 10 consecutive days (71) showed an overall response rate (CR + CRi) of 74% (95% CI: 49–91%). Although hypomethylating agents are commonly used in the treatment of AML-MRC patients, to date, no head-to-head comparison between azacitidine and decitabine has been performed. However, from the combined analysis of the five published phase III randomized control trials on hypomethylating agents seem to suggest an overall survival advantage of azacitidine over decitabine (HR for azacitidine 0.67, 95% CI: 0.56–0.79, P < 0.00001; HR for decitabine 0.86, 95% CI: 0.73–1.02, P = 0.08) (72).

JAK inhibitors

Ruxolitinib as monotherapy resulted in modest responses in phase II studies in transformed JAK2 mutated MPNs (73, 74). Decitabine alone extends survival to 9–10 months in advanced MPN with a better safety profile than intensive chemotherapy regimens (75, 76). Azacitidine and ruxolitinib combination was explored in chronic phase primary myelofibrosis (PMF) in a phase II study at the MD Anderson Cancer Center resulting in high rates of reduction of splenomegaly and fibrosis (77). A combination of ruxolitinib 50 mg, twice a day, and decitabine was explored in a phase I/II study in blastic phase (BP) patients with an overall response rate of 61% and a median overall survival of 8.4 months (78). Mascarenhas et al. combined ruxolitinib at a reduced dosage of 25 mg twice daily for the induction cycle and 10 mg twice daily for subsequent cycles in combination with decitabine 20 mg/sm for 5 days, in a phase II study enrolling 25 PMF patients in AP/BP (advanced phase/blastic phase), achieving an overall response rate of 44% and a median overall survival of 9.5 months. The survival data equaled that of intensive chemotherapy followed by HCST, (23) but it appears that the addition of ruxolitinib, results in better responses in terms of rate and duration, compared to decitabine alone, due to the reduction in splenomegaly (median reduction, –54.8%) with a positive impact on quality of life, unaffected by TP53 expression (79).

Future Perspectives

The peculiar biological characteristics of MRC and t-AML, induce chemoresistance and poor tolerance to the classic 7+3. CPX-351 and hypomethylating agents/venetoclax represent the current standard of care, but the mutational landscape deserves new possible target therapy in the repertoire of future clinical trials. IDH inhibitors (IDHi), antibody targeting CD47, and anti TP53 drug eprenetapopt (APR246) might represent possible target agents deserving further evaluation in combination with hypomethylating agents in MRC-AML. New MDM2 (murine double minute 2) inhibitors, and BET (bromodomain and extraterminal) inhibitors also show activity in advanced, accelerated-phase PMF, and could be extended to AML evolving from an underlying MPN. Several phase I and II clinical trials have shown promising results in this unfavorable setting and support the rational for the design of future trial of combinations of new drugs. For instance, anti PD-1 nivolumab in association with azacitidine provided a CR/CRi rate of 39% in 31 relapsed refractory secondary AML (80). The small molecule TP53 inhibitor eprenetapopt was able to restore mutated TP53 and showed a 56%

CR rate in a phase II trial in combination with azacytidine (81). The anti-CD47 antibody magrolimab (Hu5F9-G4) demonstrated a 67% CR/CRi rate in combination with azacytidine in TP53-mutated AML with an overall survival of 12.9 months (82). The bispecific DART molecule targeting CD3-CD123 achieved a 39% CR in a phase I/II study in 38 relapse/refractory AML (83). Menin inhibitor SNDX-5613 showed a 24% CR/Cri and a 50% overall response rate in a phase I study, with NPM1, MLL or KMT2A AML mutations, expressed by 40% of AML, predicting response (84). CPX-351, hypomethylating agents+venetoclax, 7+3+midostaurin are the current golden standard for the treatment of unfavorable disease respectively in fit, unfit, and FLT3 mutant MRC and t-AML. Poor cytogenetic and molecular risk, in addition to the older age of these patients, are the main limitations for cure, and allogeneic transplant remains the only curative option. Double or triple combinations of some of the above drugs with hypomethylating agents and/or venetoclax or CPX-351 might deserve further exploration in future phase III trials, tailoring therapies, based on molecular repertoire and patient fitness, aiming to increase both cure and quality of life.

CONCLUSION

Secondary AML are extremely heterogeneous diseases, characterized by poor prognosis. The study of the pathogenesis of this entity has led to the identification of a number of mutations specific to secondary AML (11). The latest revision of the ELN guidelines for the diagnosis and treatment of AML not only lowered the cut off of blasts to 10%, by identifying the new entity of AML/MDS in the presence of these specific mutations, but also eliminated the entity of MRC AML, replacing it with the 'AML with myelodisplastic related gene mutations', having at least one of the pathognomonic mutations (ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2) and lacking a specific cytogenetic alteration, diagnostic of AML with myelodysplasia-related cytogenetic abnormalities. This means that the prognostic significance of AML molecular features overcomes that of a previous history of hematological disorder or exposition to chemotherapy and radiotherapy, underlying the correlation between such mutations and resistance and refractoriness to standard therapies. CPX-351 remains the gold standard in patients eligible for intensive chemotherapy, with the chance of a potential improvement after combination with venetoclax and FLT3 and IDH inhibitors, currently under investigation (53). A better understanding of the pathogenesis of the disease may guide preclinical research for future targets, worthy of tailored therapies, especially in patients pretreated with hypomethylating agents, where current real-world experience shows improved OS in transplanted patients after CPX-351 (54). Current supportive therapies, which can certainly be implemented, have already determined an improved outcome of HSCT after MAC conditioning, as shown in a recent EBMT survey, with an NRM rate of 23%, similar to that observed after RIC conditioning (57), which was still burdened by worse LFS, without a significant reduction in OS. Therefore, the 2-year recurrence incidence of 37% remains the main obstacle to overcome, to improve the cure of the disease. An area of future research will certainly be the modulation of minimal residual disease after transplantation, through maintenance therapy with FLT3 inhibitors

and the use of pre-emptive therapy with target and immunologic drugs, such as anti-CD47 or anti-CD123 monoclonal antibodies, in addition to currently available hypomethylating agents.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

Copyright and Permission Statement: The authors confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder(s), and all original sources have been appropriately acknowledged or referenced.

REFERENCES

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A. et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood. 2009;114(5):937–951. https://doi.org/10.1182/blood-2009-03-209262
2. Falini B, Macijewski K, Weiss T, Bacher U, Schnittger S, Kern W. et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (NPM1). Blood. 2010;115(18):3776–3786. https://doi.org/10.1182/blood-2009-08-240457
3. Bacher U, Schnittger S, Macijewski K, Grossmann V, Kohlmann A, Alpermann T. et al. Multilineage dysplasia does not influence prognosis in CEBPA-mutated AML, supporting the WHO proposal to classify these patients as a unique entity. Blood. 2012;119(20):4719–4722. https://doi.org/10.1182/blood-2011-12-395574
4. Baer C, Walter W, Stengel A, Hutter S, Meggendorfer M, Kern W. et al. Molecular Classification of AML-MRC Reveals a Distinct Profile and Identifies MRC-like Patients with Poor Overall Survival. Blood. 2019;134:2735. https://doi.org/10.1182/blood-2019-128234
5. Porwit A, Saft L. The AML-MDS interface-Leukemic transformation in myelodysplastic syndromes. J. Hematopathol. 2011;4:69–79. https://doi.org/10.1007/s12308-011-0088-6
6. Jiang Y, Dunbar A, Gondek LP, Mohan S, Rataul M, O'Keefe C. et al. Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. Blood. 2009;113(6):1315–1325. https://doi.org/10.1182/blood-2008-06-163246
7. Nolte F, Hofmann WK. Molecular mechanisms involved in the progression of myelodysplastic syndrome. Future Oncol. 2010;6(3):445–455. https://doi.org/10.2217/fon.09.175
8. Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood. 2000;96(12):3932–3938. https://doi.org/10.1182/blood.V96.12.3932
9. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H. et al. Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. Blood. 2022;blood.2022016867. https://doi.org/10.1182/blood.2022016867
10. Chen J, Kao YR, Sun D, Todorova TI, Reynolds D, Narayanagari SR. et al. Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level. Nat Med. 2019;25(1):103–110. https://doi.org/10.1038/s41591-018-0267-4
11. Lindsley R, Mar B, Mazzola E, Grauman P, Shareef S, Allen S. et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125(9):1367–1376. https://doi.org/10.1182/blood-2014-11-610543
12. Rücker FG, Schlenk RF, Bullinger L, Kayser S, Teleanu V, Kett H. et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. 2012;119(9):2114–2121. https://doi.org/10.1182/blood-2011-08-375758

13. Pasca S, Chifotides HT, Verstovsek S, Bose P. Mutational landscape of blast phase myeloproliferative neoplasms (MPN-BP) and antecedent MPN. Int Rev Cell Mol Biol. 2022;366:83–124. https://doi.org/10.1016/bs.ircmb.2021.02.008
14. Chifotides HT, Masarova L, Alfayez M, Daver N, Alvarado Y, Jabbour E. et al. Outcome of patients with IDH1/2-mutated post-myeloproliferative neoplasm AML in the era of IDH inhibitors. Blood Adv. 2020;4(21):5336–5342. https://doi.org/10.1182/bloodadvances.2020001528
15. Kayser S, Döhner K, Krauter J, Köhne CH, Horst HA, Held G. et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117(7):2137–2145. https://doi.org/10.1182/blood-2010-08-301713
16. Guru Murthy GS, Hamadani M, Dhakal B, Hari P, Atallah E. Incidence and survival of therapy related myeloid neoplasm in United States. Leuk Res. 2018;71:95–99. https://doi.org/10.1016/j.leukres.2018.07.013
17. Granfeldt Østgård LS, Medeiros BC, Sengeløv H, Nørgaard M, Andersen MK, Dufva IH. et al. Epidemiology and clinical significance of secondary and therapy-related acute myeloid leukemia: a national population-based cohort study. J Clin Oncol. 2015;33(31):3641–3649. https://doi.org/10.1200/JCO.2014.60.0890
18. Hulegårdh E, Nilsson C, Lazarevic V, Garelius H, Antunovic P, Rangert Derolf Å. et al. Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: a report from the Swedish Acute Leukemia Registry. Am J Hematol. 2015;90(3):208–214. https://doi.org/10.1002/ajh.23908
19. Heuser M. Therapy-related myeloid neoplasms: does knowing the origin help to guide treatment? Hematology Am Soc Hematol Educ Program. 2016;2016(1):24–32. https://doi.org/10.1182/asheducation.V2016.1.24.088333
20. McNerney ME, Godley LA, Le Beau MM. Therapy-related myeloid neoplasms: when genetics and environment collide. Nat Rev Cancer. 2017;17(9):513–527. https://doi.org/10.1038/nrc.2017.60
21. Sill H, Olipitz W, Zebisch A, Schulz E, Wölfler A. Therapy-related myeloid neoplasms: pathobiology and clinical characteristics. Br J Pharmacol. 2011;162(4):792–805. https://doi.org/10.1111/j.1476-5381.2010.01100.x
22. Cowell IG, Austin CA. Mechanism of generation of therapy related leukemia in response to antitopoisomerase II agents. Int J Environ Res Public Health. 2012;9(6):2075–2091. https://doi.org/10.3390/ijerph9062075
23. Leone G, Fianchi L, Pagano L, Voso MT. Incidence and susceptibility to therapy-related myeloid neoplasms. Chem Biol Interact. 2010;184(1–2):39–45. https://doi.org/10.1016/j.cbi.2009.12.013
24. Rassool FV, Gaymes TJ, Omidvar N, Brady N, Beurlet S, Pla M. et al. Reactive oxygen species, DNA damage, and error-prone repair: a model for genomic instability with progression in myeloid leukemia? Cancer Res. 2007;67(18):8762–8771. https://doi.org/10.1158/0008-5472.CAN-06-4807
25. Klymenko SV, Bink K, Trott KR, Bebeshko VG, Bazyka DA, Dmytrenko IV. et al. MLL gene alterations in radiation-associated acute myeloid leukemia. Exp Oncol. 2005;27(1):71–75.
26. Qian Z, Joslin JM, Tennant TR, Reshmi SC, Young DJ, Stoddart A. et al. Cytogenetic and genetic pathways in therapy-related acute myeloid leukemia. Chem Biol Interact. 2010;184(1–2):50–57. https://doi.org/10.1016/j.cbi.2009.11.025
27. Hashimoto A, Takada K, Horiguchi H, Sato T, Iyama S, Murase K. et al. Combination chemotherapy of azacitidine and cetuximab for therapy-related acute myeloid leukemia following oxaliplatin for metastatic colorectal cancer. Case Rep Oncol. 2014;7(2):316–322. Published 2014 May 17. https://doi.org/10.1159/000363100
28. Larson RA. Therapy-related myeloid neoplasms. Haematologica. 2009;94(4):454–459. https://doi.org/10.3324/haematol.2008.005157
29. Gao X, Xu C, Asada N, Frenette PS. The hematopoietic stem cell niche: from embryo to adult. Development. 2018;145(2):dev139691. Published 2018 Jan 22. https://doi.org/10.1242/dev.139691
30. Boulais PE, Frenette PS. Making sense of hematopoietic stem cell niches. Blood. 2015;125(17):2621–2629. https://doi.org/10.1182/blood-2014-09-570192
31. Szade K, Gulati GS, Chan CKF, Kao KS, Miyanishi M, Marjon KD. et al. Where hematopoietic stem cells live: the bone marrow niche. Antioxid Redox Signal. 2018;29(2):191–204. https://doi.org/10.1089/ars.2017.7419

32. Tang C, Li MH, Chen YL, Sun HY, Liu SL, Zheng WW. et al. Chemotherapy-induced niche perturbs hematopoietic reconstitution in B-cell acute lymphoblastic leukemia. J Exp Clin Cancer Res. 2018;37(1):204. Published 2018 Aug 29. https://doi.org/10.1186/s13046-018-0859-3
33. Lucas D, Scheiermann C, Chow A, Kunisaki Y, Bruns I, Barrick C. et al. Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. Nat Med. 2013;19(6):695–703. https://doi.org/10.1038/nm.3155
34. Ok CY, Patel KP, Garcia-Manero G, Routbort MJ, Peng J, Tang G. et al. TP53 mutation characteristics in therapy-related myelodysplastic syndromes and acute myeloid leukemia is similar to de novo diseases. J Hematol Oncol. 2015;8:45. Published 2015 May 8. https://doi.org/10.1186/s13045-015-0139-z
35. Hsu JI, Dayaram T, Tovy A, De Braekeleer E, Jeong M, Wang F. et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell. 2018;23(5):700–713.e6. https://doi.org/10.1016/j.stem.2018.10.004
36. Czader M, Orazi A. Therapy-related myeloid neoplasms. Am J Clin Pathol. 2009;132(3):410–425. https://doi.org/10.1309/AJCPD85MCOHHCOMQ
37. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A. et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–2074. https://doi.org/10.1056/NEJMoa1301689
38. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS. et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012;481(7382):506–510. Published 2012 Jan 11.
39. Gill H, Leung AY and Kwong YL. Molecular and cellular mechanisms of myelodysplastic syndrome: implications on targeted therapy. Int J Mol Sci. 2016;17(4):440. Published 2016 Mar 24. https://doi.org/10.3390/ijms17040440
40. Morton LM, Dores GM, Schonfeld SJ, Linet MS, Sigel BS, Lam CJK. et al. Association of chemotherapy for solid tumors with development of therapy-related myelodysplastic syndrome or acute myeloid leukemia in the modern era. JAMA Oncol. 2019;5(3):318–325. https://doi.org/10.1001/jamaoncol.2018.5625
41. Kantarjian HM, Estey EH, Keating MJ. Treatment of therapy-related leukemia and myelodysplastic syndrome. Hematol Oncol Clin North Am. 1993;7(1):81–107. https://doi.org/10.1016/S0889-8588(18)30259-4
42. Larson RA, Wernli M, Le Beau MM, Daly KM, Pape LH, Rowley JD. et al. Short remission durations in therapy-related leukemia despite cytogenetic complete responses to high-dose cytarabine. Blood. 1988;72(4):1333–1339. https://doi.org/10.1182/blood.V72.4.1333.1333
43. Chua CC, Fleming S, Wei AH. Clinicopathological aspects of therapy-related acute myeloid leukemia and myelodysplastic syndrome. Best Pract Res Clin Haematol. 2019 Mar. 32(1):3–12. https://doi.org/10.1016/j.beha.2019.02.007
44. Aldoss I, Pullarkat V. Therapy-related acute myeloid leukemia with favorable cytogenetics:still favorable? Leuk Res. 2012;36(12):1547–1551. https://doi.org/10.1016/j.leukres.2012.09.008
45. Strickland SA, Vey N. Diagnosis and treatment of therapy-related acute myeloid leukemia. Crit Rev Oncol Hematol. 2022;171:103607. https://doi.org/10.1016/j.critrevonc.2022.103607
46. Palmieri R, Othus M, Halpern AB, Percival MM, Godwin CD, Becker PS. et al. Accuracy of SIE/SIES/GITMO Consensus Criteria for Unfitness to Predict Early Mortality After Intensive Chemotherapy in Adults With AML or Other High-Grade Myeloid Neoplasm. J Clin Oncol. 2020;38:4163–4174. https://doi.org/10.1200/JCO.20.01392
47. Ferrara F, Barosi G, Venditti A, Angelucci E, Gobbi M, F Pane F. et al. Consensus-based definition of unfitness to intensive and non-intensive chemotherapy in acute myeloid leukemia: a project of SIE, SIES and GITMO group on a new tool for therapy decision making. Leukemia. 2013;27:997–9. https://doi.org/10.1038/leu.2012.303
48. Lancet JE, Uy GL, Newell LF, Lin TL, Ritchie EK, Stuart RK. et al. CPX-351 versus 7+3 cytarabine and daunorubicin chemotherapy in older adults with newly diagnosed high-risk or secondary acute myeloid leukaemia: 5-year results of a randomised, open-label, multicentre, phase 3 trial. Lancet Haematol. 2021;8(7):e481–e491. https://doi.org/10.1016/S2352-3026(21)00134-4
49. Lin TL, Rizzieri DA, Ryan DH, Schiller GJ, Kolitz JE, Uy GL. et al. Older adults with newly diagnosed high-risk/secondary AML who achieved remission with CPX-351: phase 3 post hoc analyses. Blood Adv. 2021;5(6):1719–1728. https://doi.org/10.1182/bloodadvances.2020003510

50. Guolo F, Minetto P, Clavio M, Miglino M, Marcolin R, Passanante M. et al. Prognostic Impact of Minimal Residual Disease Assessment in Elderly Patients with Secondary Acute Myeloid Leukemia. a Comparison between CPX-351 and Intensified Fludarabine-Based Regimens. Blood 2021;138 (Supplement 1): 3442. https://doi.org/10.1182/blood-2021-153060
51. Chiche E, Rahmé R, Bertoli S, Dumas PY, Micol JB, Hicheri Y. et al. Real-life experience with CPX-351 and impact on the outcome of high-risk AML patients: a multicentric French cohort. Blood Adv. 2021;5(1):176–184. https://doi.org/10.1182/bloodadvances.2020003159
52. Rautenberg C, Stölzel F, Röllig C, Stelljes M, Gaidzik V, Lauseker M. et al. Real-world experience of CPX-351 as first-line treatment for patients with acute myeloid leukemia. Blood Cancer J. 2021;11(10):164. Published 2021 Oct 4. https://doi.org/10.1038/s41408-021-00558-5
53. Pullarkat VA, Levis M, Mannis GN, Strickland SA, Lin TL, Faderl S, Chakravarthy D, Chandrasekaran V, Cheung RS, Erba HP.Preliminary Results By Age Group of Treatment with CPX-351 Plus Venetoclax in Adults with Newly Diagnosed AML: Subgroup Analysis of the V-FAST Phase 1b Master Trial. Blood (2021) 138 (Supplement 1): 1268. https://doi.org/10.1182/blood-2021-148054
54. Talati C, Goldberg AD, Przespolewski A, Chan O, Ali NA, Kim J. et al. Comparison of induction strategies and responses for acute myeloid leukemia patients after resistance to hypomethylating agents for antecedent myeloid malignancy. Leuk Res. 2020;93:106367. https://doi.org/10.1016/j.leukres.2020.106367
55. Fianchi L, Pagano L, Piciocchi A, Candoni A, Gaidano G, Breccia M. et al. Characteristics and outcome of therapy-related myeloid neoplasms: Report from the Italian network on secondary leukemias. Am J Hematol. 2015;90(5):E80–E85. https://doi.org/10.1002/ajh.23966
56. Litzow MR, Tarima S, Pérez WS, Bolwell BJ, Cairo MS, Camitta BM. et al. Allogeneic transplantation for therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood. 2010;115(9):1850–1857. https://doi.org/10.1182/blood-2009-10-249128
57. Sengsayadeth S, Gatwood KS, Boumendil A, Labopin M, Finke J, Ganser A. et al. Conditioning intensity in secondary AML with prior myelodysplastic syndrome/myeloproliferative disorders: an EBMT ALWP study. Blood Adv. 2018;2(16):2127–2135. https://doi.org/10.1182/bloodadvances.2018019976
58. Kayser S, Dohner K, Krauter J, Kohne CH, Horst HA, Held G. et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117(7):2137–2145. https://doi.org/10.1182/blood-2010-08-301713
59. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL. et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol. 2017;35(11):1154–1161. https://doi.org/10.1200/JCO.2016.70.7091
60. Devine SM, Owzar K, Blum W, Mulkey F, Stone RM, Hsu JW. et al. Phase II study of allogeneic transplantation for older patients with acute myeloid leukemia in first complete remission using a reduced-intensity conditioning regimen: results from Cancer and Leukemia Group B 100103 (Alliance for Clinical Trials in Oncology)/Blood and Marrow Transplant Clinical Trial Network 0502. J Clin Oncol. 2015;33(35):4167–4175. https://doi.org/10.1200/JCO.2015.62.7273
61. Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T. et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. Cancer Discov. 2016;6(10):1106–1117. https://doi.org/10.1158/2159-8290.CD-16-0313
62. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH. et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. N Engl J Med. 2020;383(7):617–629. https://doi.org/10.1056/NEJMoa2012971
63. Kim K, Maiti A, Loghavi S, Pourebrahim R, Kadia TM, Rausch CR. et al. Outcomes of TP53-mutant acute myeloid leukemia with decitabine and venetoclax. Cancer. 2021;127(20):3772–3781. https://doi.org/10.1002/cncr.33689
64. Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Laribi K. et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. Blood. 2020;135(24):2137–2145. https://doi.org/10.1182/blood.2020004856
65. Matthews AH, Perl AE, Luger SM, Loren AW, Gill SI, Porter DL. et al. Real-world effectiveness of CPX-351 vs venetoclax and azacitidine in acute myeloid leukemia. Blood Adv. 2022;bloodadvances.2022007265. https://doi.org/10.1182/bloodadvances.2022007265

66. Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH. et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood. 2015;126(3):291–299. https://doi.org/10.1182/blood-2015-01-621664
67. Seymour JF, Döhner H, Butrym A, Wierzbowska A, Selleslag D, Jang JH. et al. Azacitidine improves clinical outcomes in older patients with acute myeloid leukaemia with myelodysplasia-related changes compared with conventional care regimens. BMC Cancer. 2017;17(1):852. Published 2017 Dec 14. https://doi.org/10.1186/s12885-017-3803-6
68. Koenig KL, Sahasrabudhe KD, Sigmund AM, Bhatnagar B. AML with Myelodysplasia-Related Changes: Development, Challenges, and Treatment Advances. Advances. Genes (Basel). 2020;11(8):845. Published 2020 Jul 24. https://doi.org/10.3390/genes11080845
69. Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J. et al. Multicenter, Randomized, Open-Label, Phase III Trial of Decitabine Versus Patient Choice, With Physician Advice, of Either Supportive Care or Low-Dose Cytarabine for the Treatment of Older Patients with Newly Diagnosed Acute Myeloid Leukemia. J Clin Oncol. 2012;30(21):2670–2677. https://doi.org/10.1200/JCO.2011.38.9429
70. Mayer J, Arthur C, Delaunay J, Mazur G, Thomas XG, Wierzbowska A. et al. Multivariate and subgroup analyses of a randomized, multinational, phase 3 trial of decitabine vs treatment choice of supportive care or cytarabine in older patients with newly diagnosed acute myeloid leukemia and poor- or intermediate-risk cytogenetics. BMC Cancer. 2014;14:69. Published 2014 Feb 6. https://doi.org/10.1186/1471-2407-14-69
71. Blum W, Garzon R, Klisovic RB, Schwind S, Walker A, Geyer S. et al. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. Proc Natl Acad Sci U S A. 2010;107(16):7473–7478. https://doi.org/10.1073/pnas.1002650107
72. Yun S, Vincelette ND, Abraham I, Robertson KD, Fernandez-Zapico ME, Patnaik MM. Targeting epigenetic pathways in acute myeloid leukemia and myelodysplastic syndrome: A systematic review of hypomethylating agents trials. Clin Epigenetics. 2016;8:68. Published 2016 Jun 14. https://doi.org/10.1186/s13148-016-0233-2
73. Eghtedar A, Verstovsek S, Estrov Z, Burger J, Cortes J, Bivins C. et al. Phase 2 study of the JAK kinase inhibitor ruxolitinib in patients with refractory leukemias, including postmyeloproliferative neoplasm acute myeloid leukemia. Blood. 2012;119(20):4614–4618. https://doi.org/10.1182/blood-2011-12-400051
74. Pemmaraju N, Kantarjian H, Kadia T, Cortes J, Borthakur G, Newberry K. et al. A phase I/II study of the Janus kinase (JAK)1 and 2 inhibitor ruxolitinib in patients with relapsed or refractory acute myeloid leukemia. Clin Lymphoma Myeloma Leuk. 2015;15(3):171–176. https://doi.org/10.1016/j.clml.2014.08.003
75. Mascarenhas J, Navada S, Malone A, Rodriguez A, Najfeld V, Hoffman R. Therapeutic options for patients with myelofibrosis in blast phase. Leuk Res. 2010;34(9):1246–1249. https://doi.org/10.1016/j.leukres.2010.05.008
76. Kennedy JA, Atenafu EG, Messner HA, Craddock KJ, Brandwein JM, Lipton JH. et al. Treatment outcomes following leukemic transformation in Philadelphia-negative myeloproliferative neoplasms. Blood. 2013;121(14):2725–2733. https://doi.org/10.1182/blood-2012-10-464248
77. Masarova L, Bose P, Pemmaraju N, Daver NG, Zhou L, Pierce S. et al. Prognostic value of blasts in peripheral blood in myelofibrosis in the ruxolitinib era. Cancer. 2020;126(19):4322–4331. https://doi.org/10.1002/cncr.33094
78. Bose P, Verstovsek S, Cortes JE, Tse S, Gasior Y, Jain N. et al. A phase 1/2 study of ruxolitinib and decitabine in patients with post-myeloproliferative neoplasm acute myeloid leukemia. Leukemia. 2020;34(9):2489–2492. https://doi.org/10.1038/s41375-020-0778-0
79. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF. et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9):799–807. https://doi.org/10.1056/NEJMoa1110557
80. Daver N, Garcia-Manero G, Basu S, Boddu PC, Alfayez M, Cortes JE. et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II study. Cancer Discov. 2019;9(3):370–383. https://doi.org/10.1158/2159-8290.CD-18-0774

81. Cluzeau T, Sebert M, Rahmé R, Cuzzubbo S, Walter-petrich A, Lehmann che J. et al. APR-246 combined with azacitidine (AZA) in TP53 mutated myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). A phase 2 study by the Groupe Francophone Des Myélodysplasies (GFM). Blood 2019;134 (Supplement_1): 677. https://doi.org/10.1182/blood-2019-125579
82. Sallman D, Asch A, Kambhampati S, Al Malki M, Zeidner J, Donnellan W. et al. The first-in-class anti-CD47 antibody magrolimab combined with azacitidine is well-tolerated and effective in AML patients: phase 1b results. Presented at: American Society of Hematology (ASH) Annual Meeting and Exposition, Virtual meeting December 5–8, 2020. https://doi.org/10.1016/S2152-2650(21)01337-9
83. Uy GL, Aldoss I, Foster MC, Sayre PH, Wieduwilt MJ, Advani AS. et al. Flotetuzumab as salvage immunotherapy for refractory acute myeloid leukemia. Blood. 2021;137(6):751–762. https://doi.org/10.1182/blood.2020007732
84. Bai H, Zhang SQ, Lei H, Wang F, Ma M, Xin M. Menin-MLL protein-protein interaction inhibitors: a patent review (2014–2021). Expert Opin Ther Pat. 2022;32(5):507–522. https://doi.org/10.1080/13543776.2022.2045947

8

Myeloid/Lymphoid Neoplasms with Eosinophilia and Platelet Derived Growth Factor Receptor Alpha (PDGFRA) Rearrangement

Prabhjot Kaur • Wahab A. Khan

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH, USA

Author for correspondence: Prabhjot Kaur, Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756. Email Prabhjot.Kaur@hitchcock.org

Cite this chapter as: Kaur K, Khan WA. Myeloid/Lymphoid Neoplasms with Eosinophilia and Platelet Derived Growth Factor Receptor Alpha (PDGFRA) Rearrangement. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 14 Aug 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-pdgfra-rearrangement

Abstract: Myeloid and lymphoid neoplasms with eosinophilia and *PDGFRA* rearrangements are recognized as a distinct entity within the section of "Myeloid/Lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK)". Based on the WHO classification of tumors of Hematopoietic and Lymphoid tissues (5th Ed, 2022), these neoplasms also comprise of *PDGFRB* rearrangement, *FGFR1* gene rearrangements, *JAK2* rearrangement, *FLT3* rearrangement, *ETV6*::*ABL1* fusion as well as some others. Of note, these are recently defined entities and are still evolving. In this chapter, practical issues regarding diagnosis, cytogenetic testing, molecular studies, therapeutics, and clinical

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

implications are discussed. Differential diagnosis in consideration for eosinophilia and hypereosinophilic syndrome are further highlighted.

Keywords: *CHIC2* deletion; *FIP1L1-PDGFRA* neoplasm; hypereosinophilic syndrome; myeloid/lymphoid neoplasms; *PDGFRA* rearrangement

INTRODUCTION

Myeloid/Lymphoid neoplasms with eosinophilia (M/LN-Eo) harboring gene rearrangements of *PDGFRA*, *PDGFRB, FGFR1*, *JAK2* were first recognized as a separate category of hematolymphoid neoplasms, by the World Health Organization (WHO) in 2008 (1). This entity was further revised in WHO-2016 (2) and WHO-5th Edition (3), as neoplasms within this category shared some features. These include: (i) eosinophilia, (but, not always); (ii) presence of a fusion gene product or a mutation that results in expression of an aberrant tyrosine kinase with therapeutic implications; and (iii) cell origin postulated to be a mutated pluripotent (lymphoid-myeloid) stem cell. Currently, genetic abnormalities defining myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK) include *PDGFRA* rearrangement, *PDGFRB* rearrangement, *FGFR1* rearrangement, *JAK2* rearrangement, *FLT3* rearrangement, *ETV6::ABL1* fusion and additional tyrosine kinase fusions such as *ETV6::FGFR2*, ETV6::NTRK3; *RANBP2::ALK; BCR::RET; FGFR1OP::RET* (1).

The MLN-TK category of neoplasms usually present as chronic myeloid entities with eosinophilia (usually >1.5 × 10^9/L) such as chronic eosinophilic leukemia (CEL), atypical chronic myeloid leukemia (aCML) or chronic myelomonocytic leukemia (CMML), among others. These neoplasms can rarely present as acute myeloid leukemia (AML) or B/T lymphoblastic leukemia/lymphoma (B/T LBLL). They are negative for the Philadelphia (Ph) chromosome or the presence of a *BCR-ABL1* fusion (2, 4–7). Most of these entities are recognized when they present with eosinophilia, which can prompt molecular cytogenetic interrogation for *PDGFRA*, *PDGFRB*, *FGFR1*or *PCM1-JAK2* abnormalities. Neoplasms that lack eosinophilia have mostly been detected as a serendipitous finding in sequencing studies.

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND *PDGFRA* REARRANGEMENTS

Myeloid/Lymphoid neoplasms with eosinophilia and *PDGFRA* rearrangements is a rare syndrome with striking male predominance (M: F approximately 16:1 overall). The reported median age is 40 (range 7–77 years) (7–10). The clinical findings include fatigue, pruritus, respiratory, cardiac, or pulmonary symptoms. Pulmonary symptoms include dyspnea and cough with components of both restrictive and obstructive pulmonary disease. Cardiac symptoms may include endomyocardial fibrosis, Loeffler endocarditis and valvular regurgitation. Asymptomatic cases have also been reported (5). Absolute eosinophil counts range from 5.4 to 71.7 × 10^3/µl (median 12.5 × 10^3/µl). The serum tryptase is

elevated (>12 ng/mL) with a range from 6.5–45 ng/mL (median 24 ng/mL). Serum Vit B12 is also increased (8, 11, 12). These neoplasms usually present as CEL with significant involvement of the mast cell lineage and sometimes the neutrophil lineage. Rarely, they present as AML, T-LBLL or with transformation as B-LBLL with accompanying eosinophilia (5). Organ damage occurs as a result of leukemic infiltration or release of cytokines, enzymes or other proteins by eosinophils.

Microscopy

Peripheral blood findings are significant for eosinophilia which are usually mature eosinophils (Figure 1). Rare numbers of eosinophil precursors can be seen. Abnormal morphology with sparse granulation, cytoplasmic vacuolation, abnormal hyper- or hyposegmentation can be seen. These changes are also observed in reactive etiologies and therefore not significantly helpful. There can be neutrophilia, while monocytosis and basophilia are not common. Anemia and thrombocytopenia can be seen. Bone marrow aspirates demonstrate numerous eosinophils and eosinophilic precursors (4, 13–15) (Figure 1). The bone marrow core biopsies are hypercellular with increased eosinophils and precursors. The megakaryocytes demonstrate normal morphology or are myeloproliferative neoplasm-like (MPN-like), myelodysplastic syndrome-like (MDS-like), or mixed MDS/MPN-like. Mast cells are usually increased and present as scattered or loose aggregates.

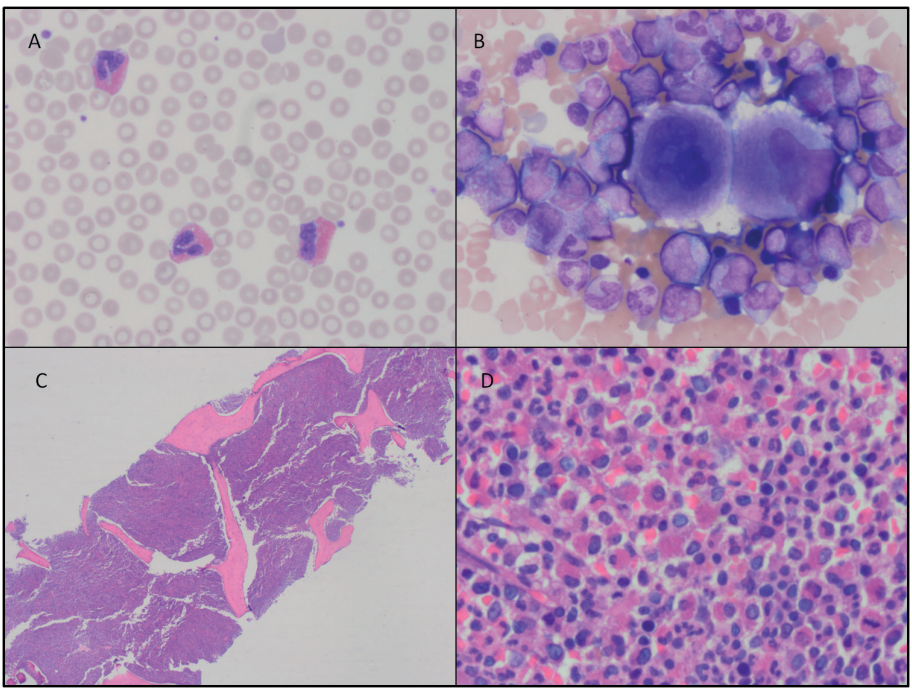

Figure 1. Microscopy. **A.** Peripheral smear with absolute eosinophilia. **B.** Aspirate with increased eosinophils **C, D.** Hypercellular marrow with increased eosinophils.

The dense compact aggregates of spindled mast cells typically associated with systemic mastocytosis appear to be rare in *FIP1L1-PDGFRA* fusion positive cases (9).

They can be seen as atypical spindle shaped mast cells which show aberrant expression of CD2 and/or CD25 and/or CD30 (Figure 2). Mast cell aggregates are usually not typically obvious in routine hematoxylin and eosin-stained sections and are highlighted by immunohistochemical stains for mast cell tryptase or CD117 (C-KIT). Using immunohistochemistry or flow cytometry, the mast cells are shown to be positive for CD117, CD25 +/- and CD2 +/- expression. Bone marrow (BM) fibrosis is also observed in some of these cases. Myeloid sarcoma/extramedullary involvement can be seen in a significant subset (7). The most common extramedullary sites are lymph node, epidural or spinal masses, and rarely cutaneous involvement or oral mass. These usually demonstrate infiltration with maturing myeloid elements and associated eosinophilia containing various degrees of fibrosis. Necrosis can be seen in these extramedullary sites. Immature cells/blasts are seen in myeloid sarcoma, monocytic sarcoma or B/T lymphoblastic lymphoma/leukemia. In cases presenting with T-LBL, B-LBL, or T-cell lymphoma, the diagnosis of the M/LN-Eo with *PDGFRA* rearrangement is suspected either based on the BM findings or have been incidentally discovered by RNA sequencing analysis (7). This illustrates the importance of BM examination and maintaining a low threshold for fluorescence *in situ* hybridization (FISH) or RNA-sequencing testing in the assessment of the extramedullary tumors.

Cell of origin

Pluripotent stem cell can give rise to eosinophils, neutrophils, monocytes, mast cells, T cells or B cells (16). This was demonstrated by performing nested reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR and FISH studies on purified cell populations from patients presenting with these neoplasms and the fusion gene was detected in eosinophils, neutrophils, mast cells, T cells, B cells and monocytes, suggesting that the mutation arises in a pluripotential hematopoietic progenitor cell capable of giving rise to multiple lineages. The basis for the preferential expansion of eosinophils and mast cells remains unclear.

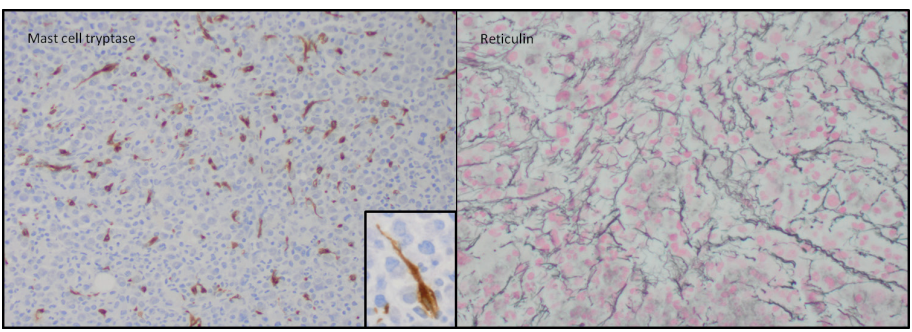

Figure 2. Mast cell. Immunostain highlights increased singly distributed abnormal spindle shaped mast cells. Reticulin stain highlight increased reticulin fiber deposition.

Also, the fusion gene maybe detected in a lineage separate from the presenting neoplasm. For example, lymphocytosis may not be seen even in cases presenting with B or T cell lineage neoplasms (16).

Genetics

The most common genetic variant in the group of M/LN-Eo and *PDGFRA* rearrangement family is the *FIP1L1-PDGFRA* fusion, resulting from a submicroscopic 800-kb interstitial deletion on chromosome 4; del(4)(q12q12). This fusion creates a tyrosine kinase leading to a gain of function event and is responsive to imatinib (4). This fusion is cryptic by karyotype analysis but can be detected by FISH (Figures 3, 4). The FISH test for *FIP1L1-PDGFRA* can be performed by using a three-color probe strategy (*SCFD2*, *LNX*, *PDGFRA*) wherein a deletion of *LNX* (*CHIC2*) locus with retention of the flanking *SCFD2* and *PDGFRA* loci is indicative of the *FIP1L1-PDGFRA* fusion (4, 14). Additional fusion partners of *PDGFRA* that have been described, including *BCR*, *ETV6*, *KIF5B*, *CDK5RAP2*, *STRN*, *TNKS2*, and *FOXP1*. Most *PDGFRA* partner genes can be detected by FISH. Other modalities for detection include next generation sequencing (NGS), RNA fusion assays or if an unbalanced rearrangement suspected; single-nucleotide polymorphism chromosomal microarray approach (SNP-CMA) to detect gains or losses (17).

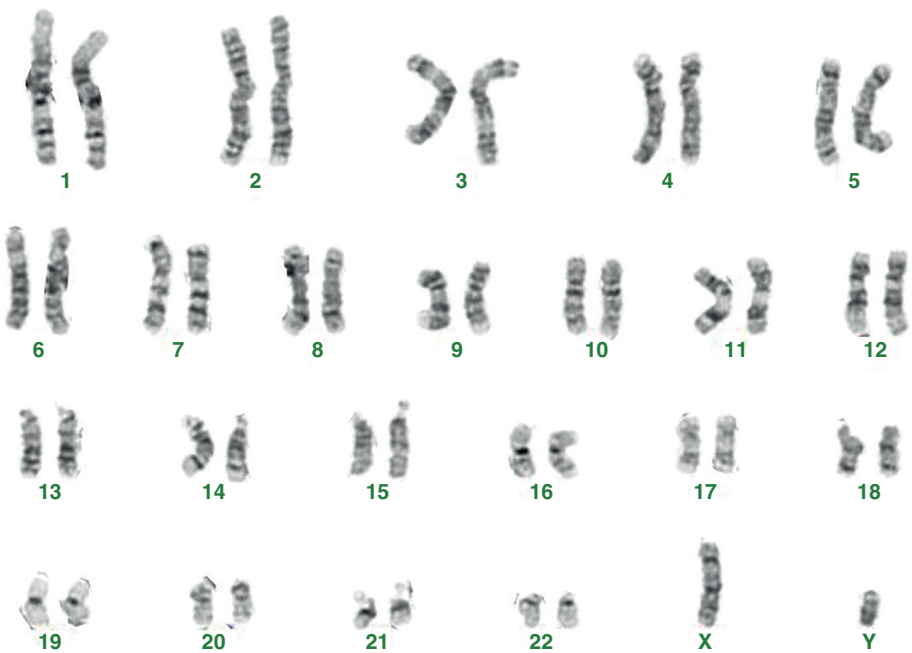

Figure 3. Cytogenetics. The karyotype in M/Ln with Eo and PDGFRA rearrangement is usually normal.

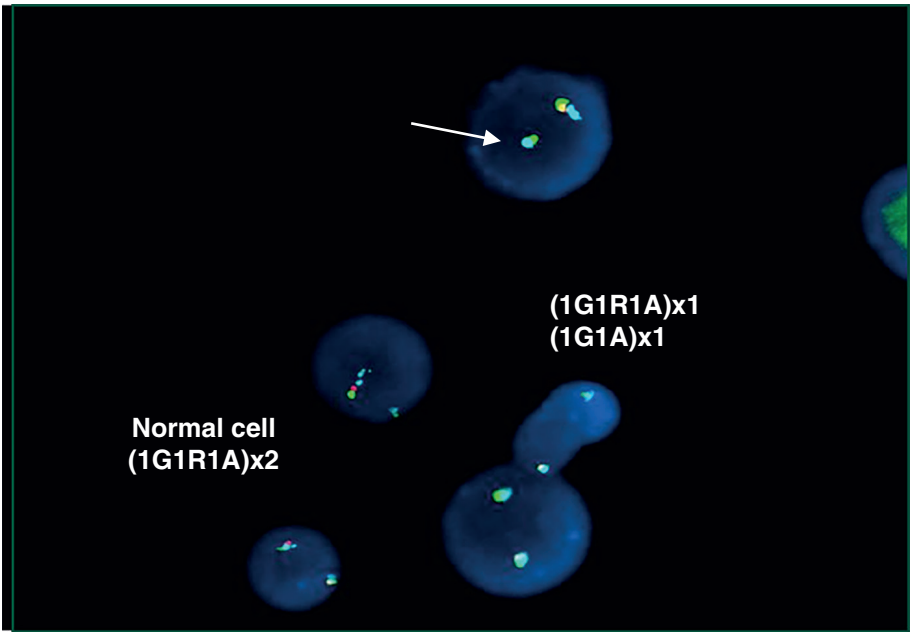

Figure 4. FISH demonstrating a *CHIC2* deletion. FISH performed on fixed nuclei using a tri-color DNA FISH probe strategy (Abbott Molecular) shows a fusion probe signal pattern as a result of a submicroscopic deletion within chromosome 4q12, involving CHIC2. Loss of the spectrum red signal (CHIC2 gene) is noted for one of the two probe signals (arrow). G (green), A (aqua), and R (red) notations refer to the FISH probe spectrum color.

Pathobiology, treatment, and prognosis

M/LN-Eo with abnormalities of *PDGFRA* result from the formation of a fusion gene, or rarely from a mutation resulting in the expression of an aberrant tyrosine kinase. Tyrosine kinase enzymes transfer phosphate from ATP to specific amino acids on substrate proteins (18–23). Phosphorylation of the substrate proteins leads to the activation of signal-transduction pathways that influence cell growth, differentiation, and death. The most studied neoplasm with aberrant tyrosine kinase pathway is chronic myeloid leukemia (CML). This is discussed briefly as its pathobiology is relevant to neoplasms placed in the category of M/LN-Eo with tyrosine kinase fusions, namely *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2*, among others defined within this entity.

CML, as we know, is associated with a recurring chromosomal abnormality due to translocation of genetic material with the formation of the Philadelphia chromosome (Ph Chr) (24). The Ph chr is a derivative chromosome 22 resulting from a reciprocal exchange between the long arms of chromosomes 9 and 22 at cytobands 9q34 and 22q11.2. The translocation, t(9;22), results in the juxtaposition of 3' DNA sequences derived from the *ABL* proto-oncogene on chromosome 9 with 5' sequences of the breakpoint cluster region (*BCR*) gene on chromosome 22, forming a fusion well-known as, *BCR-ABL1*. *BCR-ABL1* produces a chimeric

messenger RNA from which a fusion BCR-ABL1 oncoprotein is translated. The length of the BCR-ABL1 chimeric protein varies and is determined by the breakpoint within the *BCR* gene. *ABL1* encodes a tyrosine kinase that is tightly regulated, whereas the activity of BCR-ABL1 fusion protein is autonomous and markedly increased relative to that of normal ABL1 protein. Chronic-phase CML is driven by the constitutively active BCR-ABL1 tyrosine kinase protein, which activates multiple pathways, leading to the malignant expansion of myeloid cells through the stimulation of mitosis, the disruption of cyto-adherence, regulatory control by stromal cells, and the inhibition of apoptosis. Differentiation and maturation of the leukemic clone is relatively intact in chronic-phase CML, however *BCR-ABL1* fusion is also thought to promote genomic instability. This can ultimately lead to secondary mutations and to the blast phase. The BCR-ABL1 oncoprotein activates its substrate by the phosphorylation of one of its tyrosine residues. This subsequently activates other downstream effector molecules. Imatinib mesylate inhibits the tyrosine kinase activity of the BCR-ABL1 oncoprotein, by occupying the ATP binding site whereby the action of BCR-ABL1 is inhibited, preventing phosphorylation of its substrate (18–21, 24, 25).

Given the above historical context of the well-known tyrosine kinase mentioned, the platelet derived growth factor receptor (PDGFR A/B) belongs to a similar family of receptor tyrosine kinase (RTK). PDGFR family of receptors (Figure 5), consist of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular split kinase domain connected by a kinase insert. Ligand binding causes activation, dimerization, and phosphorylation of tyrosine sites. These phosphotyrosines then act as docking sites for Src homology 2 (SH2)

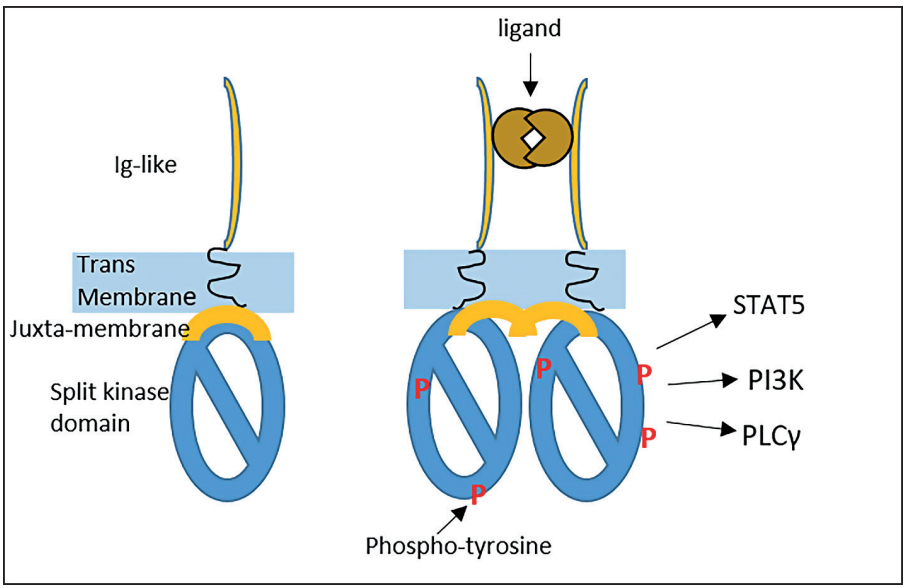

Figure 5. PDGFR A receptor with tyrosine kinase activity. (Adapted from Federica Toffalini, Jean-Baptiste Demoulin, Blood, 2010).

domains of a variety of signal transduction proteins, including phosphatidylinositol 3-kinase (PI3K), phospholipase Cγ (PLCγ), and SRC family kinases, which are shared by type III RTK. A substantial number of adaptor molecules containing SH2 domains, such as Grb2, Grb7, Grb10, Shc, Crk, APS, and Nck provide a diverse set of connections between receptors and signaling pathways, such as mitogen-activated protein. *PDGFR* gene mutations or chimeric proteins resulting from translocations lead to ligand independent constitutive activation of PDGFR. Imatinib mesylate inhibits the tyrosine kinase activity of the mutant PDGFR as well as chimeric PDGFR and other oncoproteins like PDGFRB, cKIT (23, 26–28). In 2003, Cools et al. (4) described an 800kb deletion on chromosome 4q12 which leads to the creation of a *FIP1L1-PDGFRA* fusion gene containing the 5' portion of *FIP1L1* and the 3' portion of *PDGFRA*. This fusion tyrosine kinase receptor is constitutively active and has been shown to induce self-phosphorylation and phosphorylation of the STAT5 pathway (8). The 800kb deletion on chromosome 4q12 display a normal karyotype as mentioned above. However, the *FIP1L1-PDGFRA* fusion may be detected by either RT-PCR or FISH studies (12, 29). As the fusion breakpoints in the *FIP1L1* gene are variable, the size of the product amplified by RT-PCR may vary between patients, and primers must be constructed to account for all known translocation breakpoints. These can be circumvented by the standard quantitative polymerase chain reaction. The screening test is based on detection of overexpression of a 3' region of PDGFRA or a 3' region of PDGFRB which is a possible indicator of an underlying fusion gene. The normal *FIP1L1* and *PDGFRA* genes are closely located on chromosome 4q12 because of which the common "fusion probe" strategy, employed to detect balanced translocations, cannot be used for detection of this abnormality by FISH.

As mentioned earlier, a tri-color FISH strategy is now in use, with one probe located in the 800kb region deleted by the *FIP1L1-PDGFRA* fusion (including the cysteine-rich hydrophobic domain 2 (*CHIC2*) gene and two probes flanking the fusion breakpoints. Deletion of the intervening sequence with retention of the probes telomeric and centromeric to the breakpoint serves as a surrogate marker for the *FIP1L1-PDGFRA* fusion gene. The tri-color FISH method identifies the *FIP1L1/PDGFRA* fusion caused by interstitial deletion of *CHIC2* and other structural rearrangements of *PDGFRA*, albeit the translocation partner may not be readily known, at single cell resolution. Most of these clinical assays are fairly new, especially the PCR assay designed by Erben et al. (30) and well-defined criteria do not exist on the preferred modality for diagnosis and follow-up. The FISH assay is, most times, used for initial diagnosis and RT-PCR assays for follow-up studies or detection of minimal residual disease. There are rare cases where translocations involving the *PDGFRA* gene result in novel chimeric genes (27, 31–34). Other fusion partners of *PDGFRA* include *BCR*, *ETV6*, *KIF5B*, *CDK5RAP2*, *STRN*, *TNKS2*, and *FOXP1*. These translocations can be suggestive by karyotype analysis but require targeted FISH, RT-PCR, or other genomic-scale mapping to identify the partner genes involved. Point mutations in *PDGFRA* have also been identified and are detected by NGS (7, 29, 33, 35). Recognition of this entity is of crucial clinical importance as the *FIP1L1-PDGFRA* fusion protein and the additional translocations involving the *PDGFRA* gene are potently inhibited by imatinib. Low dose (100 mg/day) imatinib therapy in *FIP1L1-PDGFRA* rearrangement positive patients leads to rapid normalization of eosinophil counts, offering the potential to limit the end organ damage (such as endocardial fibrosis) observed in patients

with chronic hypereosinophilia. The neoplasms with *FIP1L1-PDGFRA* are likely responsive also to dasatinib, nilotinib, sorafenib and midostaurin (PKC412) (36). Clinical remission is usually achieved in a month with molecular remission in 3 months. The treatment is not usually discontinued, however there are considerations on potentially limiting or gradually stopping the drug. Of note, resistance to imatinib has also been reported (14, 18, 22, 36, 37).

DIFFERENTIAL DIAGNOSIS OF ENTITIES PRESENTING WITH EOSINOPHILIA

Eosinophils are derived from myeloid progenitors in bone marrow through the action of hematopoietic cytokines such as interleukin (IL)-3, IL-5, and granulocyte-macrophage colony–stimulating factor (GM-CSF). Eosinophils are activated by various stimuli such as tissue injury, viral and bacterial infections, allergens, benign and malignant neoplasms. On activation, eosinophils produce cytotoxic cytostimulatory proteins such as eosinophil peroxidase, major basic protein, eosinophil cationic proteins, cytokines such as Interlukin-2 (IL-2), IL3, IL-4, IL-5, IL-8, Il-10, IL-12, IL-13, and TGF alpha/beta and GM-CSF, chemokines (CCL3/MPI-1alpha, CCL5/RANtes, CCL7/MCP-3, CCL8/MCP-2), lipid mediators (leukotrienes, prostaglandins), and growth factors (heparin-binding epidermal growth factor-like binding protein). These have anti-parasitic and bactericidal activities and modulate immediate allergic reactions and inflammatory responses. However, persistent eosinophilia can result in irreversible organ damage, affecting the heart, lung, skin, gastrointestinal tract, and the central nervous system. Significant cardiovascular complications include endomyocardial fibrosis and intravascular thrombosis (38–40).

Eosinophilia is defined as greater than 0.5 eosinophils × 10^9/L blood. Hypereosinophilia (HE) is defined by >1.5 eosinophils × 10^9/L blood on 2 examinations (interval ≥1 month). Tissue HE is defined by the following: (i) percentage of eosinophils in BM section exceeds 20% of all nucleated cells; and/or (ii) Pathologist is of the opinion that tissue infiltration by eosinophils is extensive; and/or (iii) marked deposition of eosinophil granule proteins (in the absence or presence of eosinophils). Eosinophilia/HE can be seen in reactive conditions such as infections with parasites such as helminth infections, scabies, allergic bronchopulmonary aspergillosis, drug reactions, chronic graft-versus-host disease, chronic inflammatory disorders (e.g., inflammatory bowel disease), autoimmune diseases, pulmonary diseases (e.g., hypersensitivity pneumonitis, Loffler's), and collagen vascular diseases. Secondary HE is usually cytokine driven and is considered a paraneoplastic process due to dysregulated production of IL3, IL5 by the neoplastic cells. It can be seen in Hodgkin's Lymphoma, B- or T-cell lymphoma/leukemia, Langerhans cell histiocytosis or solid tumors/malignancy. Primary or clonal HE is seen in neoplastic hematologic disorders such as CML with eosinophilia, AML with inv(16) and eosinophilia (AML-M4-eo), *JAK2* V617F positive myeloproliferative neoplasm (MPN) with eosinophilia. It is also observed in aggressive systemic mastocytosis, myelodysplastic syndrome or MDS/MPN overlap syndromes with eosinophilia, CEL, hematopoietic neoplasms with eosinophilia and abnormalities in *PDGFRA/B*, *FGFR1* mutations, and lymphocytic variant of

hypereosinophilic syndrome (HES). HES is defined as HE with organ damage such as fibrosis (lung, heart, digestive tract, skin, and others), thrombosis with or without thromboembolism, cutaneous or mucosal erythema, edema/angioedema, ulceration, pruritus, and eczema, as well as peripheral or central neuropathy with chronic or recurrent neurologic deficit. Viewed in terms of the HES, primary HES is analogous to clonal eosinophilia; secondary HES is cytokine driven eosinophilia and idiopathic HES is end-organ damage directly attributable to HE and no discernible underlying cause of the HE (14, 38, 41–43). The algorithm that may be followed for diagnostic evaluation for eosinophilia is shown in Figure 6.

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND *PDGFRB* REARRANGEMENT

This is a distinct type of myeloid neoplasm that is seen in association with rearrangement of *PDGFRB* at cytoband 5q32, the most common being t(5;12)(q32;p13.2) with formation of the *ETV-PDGFRB* fusion gene. Clinically, it is seen more in males (male-to-female ratio is 2:1) with a wide age range (8–72 years)

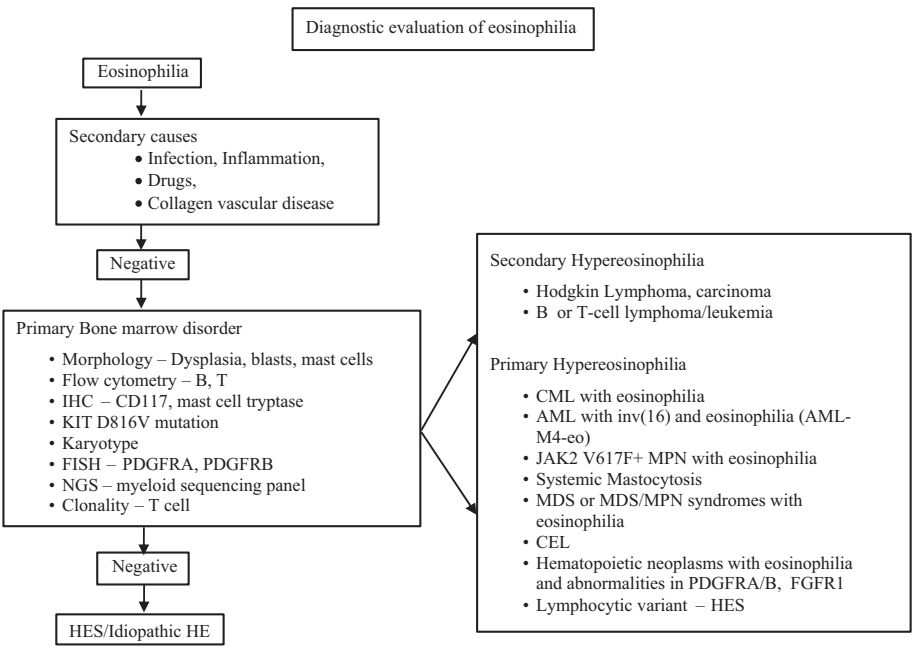

Figure 6. Diagnostic evaluation of eosinophilia. AEC, absolute eosinophil count; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; RT-PCR, reverse transcription polymerase chain reaction. Adapted from Pozdnyakova O, Orazi A, Kelemen K,et al. AmJ Clin Pathol. 2021;155(2):160–78.

and median age of onset in the late 40s. Most present with hepatosplenomegaly, skin infiltration or cardiac disease that may lead to cardiac failure. Serum tryptase is moderately elevated. The hematologic features are most often of CEL or CMML with eosinophilia. Other presentations include aCML with eosinophilia, MPNs with eosinophilia and rarely as AML or juvenile myelomonocytic leukemia (JMML). This entity usually presents with leukocytosis with increased neutrophils, eosinophils, monocytes, and precursors with a median white blood count of 34.4×10^9/L ($6.8–116 \times 10^9$/L) and a median absolute eosinophil count (AEC) of 4.44×10^9/L ($0.07–73.5 \times 10^9$/L). Rarely basophils can be increased. The bone marrow is hypercellular with granulocytic proliferation and eosinophilia. MDS-like megakaryocytes (small hypolobated and forms with abnormal nuclear lobation) are seen whereas MPN-like megakaryocytes are not seen. There can be myelofibrosis and mast cells with spindle-shaped morphology can be seen. These cells can be seen scattered or in loose aggregates, with aberrant CD25 co-expression. Blast are <20% in the peripheral blood or BM. Besides the *ETV6-PDGFRB* gene fusion, at-least 30 additional partner genes have been described with *PDGFRB*, many of which are also imatinib-responsive (7, 14, 28, 44). Cryptic rearrangements are described, in which case FISH/RT-PCR/RNA sequencing can be recommended. Of note, *PDGFRB* fusion genes associated with Ph-like B-ALL are excluded. These Ph-like B-ALL present with *IKZF1* deletion, 7p-, IKZF1/CEP7, rearrangements in *ABL1*, *ABL2, PDGFRB*, JAK2, CRLF2, and *P2RY8*. The screening technique used by Erben et al. (30), is also useful in the detection of these rearrangements (7, 14)). It is important to distinguish Ph-like B-lymphoblastic leukemia/lymphoma from myeloid/lymphoid neoplasms with *PDGFRA*, *PDGFRB* neoplasm. Myeloid/lymphoid neoplasms with Eos cases that present with B-ALL differ from Philadelphia-like B-ALL by the presence of an underlying MPN (7, 14).

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA ASSOCIATED WITH *FGFR1* REARRANGEMENT

The aforementioned category has traditionally been considered as stem cell leukemia/lymphoma with rearrangements involving chromosome 8p11 (FGFR1). These are aggressive, rare, pluripotent stem cell disorder with poor prognosis. The WHO 2016 monogram defines these entities as MPN or MDS/MPN with prominent eosinophilia. In some cases, these present with neutrophilia or monocytosis, AML, T- or B-ALL, and/or mixed-phenotype acute leukemia with eosinophilia. The *FGFR1* disease entities can also show the presence of t(8;13)(p11.2;q12) or a variant translocation leading to *FGFR1* rearrangement. These genetic aberrancies can be demonstrated in myeloid cells, lymphoblasts, or both. They usually have multiple partner genes –most often Zn finger gene on chromosome 13. The PB and BM show features of CML, CEL, MPN-unclassifiable, aCML, or MDS/MPN. Somatic mutations are common in *FGFR1*-rearranged cases with *RUNX1* mutations. These do not respond to TKI (imatinib) therapy. Aggressive chemotherapy with stem cell transplant is the best curative option. Newer modalities include Pemigatinib, an FGFR inhibitor are being explored (45, 46).

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND JAK2 REARRANGEMENTS

These are rare with no more than 30 cases described. These usually present in males with median age of 47 years (1). The most often cited example is t(8;9)(p22;p24.1)/*PCM1-JAK2* that fuses the Janus activated kinase 2 gene (*JAK2*) with the human autoantigen pericentriolar material gene 1 (*PCM1*), resulting in a constitutively activated tyrosine kinase. Structural variants involve *ETV6-JAK2* and *BCR-JAK2* (47). Clinical features are variable with presentation as chronic myeloid neoplasm (MPN/MDS) or acute leukemia (AML, ALL). The morphology shows a triad of features that include hypercellular with eosinophils, aggregates of erythroblasts, and marrow fibrosis. As both myeloid and lymphoid presentation is seen, it is consistent with the origin from pluripotent hematopoietic cells. They can be given targeted therapy with the *JAK2* inhibitor ruxolitinib or stem cell transplant. The *PCM1-JAK2* fusions do not respond to imatinib (45, 46, 48, 49). Nilotinib or dasatinib may be more effective than imatinib to induce durable complete remissions in ETV6-ABL1 positive patients.

The salient features of myeloid/lymphoid neoplasms associated with eosinophilia and tyrosine kinase gene fusions are:

- These arise from pluripotent stem cells as both myeloid & lymphoid proliferations.
- They usually present with eosinophilia.
- These are aberrant tyrosine kinase driven neoplasms and can be treated with tyrosine kinase inhibitors.
- The disease presentations include: MPNs, MDSs, MDS/MPN, AML, B/T-LBL/ALL, or mixed-lineage neoplasms.
- Cryptic rearrangements are recognized in both genes; *PDGFRA, PDGFRB.*
- Cases in these categories of neoplasm that present as B-lymphoblastic lymphoma/leukemia differ from de novo Philadelphia-like B-ALL by the presence of an underlying MPN.

CHRONIC EOSINOPHILIC LEUKEMIA (CEL)

This entity is considered under myeloproliferative neoplasms and not under the umbrella of myeloid/lymphoid neoplasms associated with eosinophilia and rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1,* or *PCM1-JAK2* fusion. CEL, a disease of elderly males, who may present asymptomatic or with constitutional symptoms such as weight loss, night sweats, fever, angio-edema, and other features seen in patients with eosinophilia. As defined by the WHO 2016, the diagnostic criteria include:

(i) Eosinophilia ($>1.5 \times 10^3/\mu L$) for at least 4 weeks
(ii) Exclude BCR-ABL1+ CML, PV, ET, PMF, CNL, CMML, aCML,
(iii) Exclude PDGFRA, PDGFRB, FGFR1, PCM-JAK2, ETV-JAK2, BCR-JAK2 fusion.

(iv) Blasts are <20% of all cells (No diagnostic features of AML with inv(16) (p13.1q22), t(16;16) (p13.1q22), t(8;21)(q22;q22.1), and other diagnostic features of AML are present)
(v) Demonstrate morphology and cytogenetic and/or molecular genetic abnormality or blasts >2% in PB, or >5% and <19% in BM. As per the latest WHO 5th edition the criteria of increased blasts (≥2% in peripheral blood or 5–19% in bone marrow) as an alternative to clonality is eliminated (1).

The most striking feature is peripheral blood eosinophilia on microscopy are mostly mature forms and rarely immature forms of eosinophilic myelocytes and promyelocytes. Eosinophils may show sparse granulation, cytoplasmic vacuolation, hyper or hypo segmentation, but are not specific to the disease and can be seen in reactive conditions as well. The bone marrow is hypercellular with dysplasia that can be seen in megakaryocytes, erythroid precursors, or granulocytes. A recent paper by Wang et al. provide more specific criteria that can help in separating this entity from reactive etiologies, such as dysplasia in megakaryocytes, and erythroids and granulocyte in greater than 20% of the cells (15). In addition, any of the following two features can help in separating the entities: hypercellularity (>20–30% age appropriate), abnormal eosinophils, myelofibrosis, or G: E ratio>10 (15). Evidence of cytogenetic, FISH, and molecular clonality may include trisomy 8 or isochromosome 17 [i(17q)], loss of chromosome 7. Somatic mutation in *ASXL1*, *TET2*, *E2Z2*, *KIT*, *M541L*, X-linked polymorphism of HUMARA and *PGK* genes or variants that have been seen in other myeloid neoplasms such as *TP53*, *EZH2*, *SETBP1*, *NRAS*, *CSF3R*, *JAK2* are also observed (50). CEL is diagnosed when all reactive etiology for eosinophilia has been excluded and there is no evidence of a lymphoproliferative or myeloproliferative disorder. The positive criteria for diagnosis of CEL is demonstration of morphologic abnormality and a clonal chromosomal abnormality. Given that the identification of a *FIP1L1-PDGFRA* fusion gene provides evidence for a clonal population of eosinophils, neoplasms with *PDGFRA-FIP1L1* fusions were initially also classified as CEL, before the separate categorization for MLN TK was introduced in WHO 2008. It is worth noting that a subset of patients with HES/CEL, without well-defined genetic abnormality, also respond to imatinib therapy. They presumably possess other, not-yet characterized, tyrosine kinase abnormalities that drive the HE (4, 29, 51, 52). Patients with CEL are usually treated with hydroxyurea, interferon-α or allo hematopoietic stem cell transplant (HSCT) (6, 15).

LYMPHOCYTIC VARIANT OF HES

Persistent HE has been seen in a subset of patients that show abnormal T-lymphocytes identified either phenotypically by flow cytometry or clonal T-cell populations as delineated by PCR and considered as a 'lymphoproliferative variant' of HE. Briefly, the normal CD4+ T helper cells are divided into Th1, Th2 cells. Th1 secrete interleukin-2 (IL2), interferon gamma, and tumor necrosis factor and are involved in cell-mediated immunity. Th2 secrete IL4 (i.e., it stimulates the production of IgE antibodies) and IL5 (i.e., promotes the differentiation and activation of eosinophils). In some patients, there is expansion of an abnormal proliferation of Th2 type cells,

through production of IL-5 and other cytokines is thought to contribute to persistent eosinophilia. The T cells are phenotypically abnormal and reported aberrancies are (CD2+/3+/4+/5+/7↓↓); (CD2+/3+/7+/8+/5↓↓), (CD2+/4+/3–/7–), $CD3^{+}4^{-}8^{-}\alpha\beta^{+}$). They are clonal as demonstrated by karyotype analysis and instability of 6q, partial 6q or 10p deletions have been reported. T cell receptor gene rearrangements are also seen. It is currently unclear whether these T cell proliferations represent unusual reactive processes or are possibly early peripheral blood involvement by a subtle T-cell lymphoma or leukemia. Clinically these patients present with cutaneous lesions, pruritus, erythroderma, urticarial, angioedema, serum hyper IgE, and polyclonal hypergammaglobulinemia. There is no standard therapy for lymphoproliferative variant of eosinophilia. However, use of corticosteroid therapy along with low-dose oral cyclophosphamide or methotrexate as steroid-sparing agents has been reported (52–54).

SYSTEMIC MASTOCYTOSIS WITH EOSINOPHILIA

Mastocytosis is a rare neoplasm and includes three disease types: systemic mastocytosis (SM), cutaneous mastocytosis and mast cell sarcoma. SM is a clonal disorder of mast cells characterized by involvement of at least one extracutaneous organ, with or without evidence of skin lesions. The constellation of clinical features includes gastrointestinal symptoms, cutaneous symptoms, splenomegaly, anemia, and a subset of cases of SM may present with eosinophilia. The diagnosis of SM is based on the presence of one major and one minor, or 3 minor criteria. The major criterion is defined by the presence of two or more dense mast cell aggregates (>15 mast cells/aggregate) in bone marrow or extracutaneous organ. Minor criteria include the presence of >25% spindled, immature or atypical mast cells; *KIT* point mutation at codon 816; or aberrant expression of CD2, +/- CD25, +/-CD30 on mast cells or serum tryptase greater than >20 ng/mL.

Some of the P*DGFRA/FIP1L1* positive MPNs present with spindled, CD25+ mast cells and elevated tryptase levels, and therefore may meet the three minor criteria used for the diagnosis of systemic mastocytosis. These have, in the past been delineated as systemic mastocytosis with *FIP1L1-PDGFRA* fusion (9, 13). One large study, however, highlighted distinct clinical and biological differences between neoplasms with *FIP1L1-PDGFRA* rearrangements and cases diagnostic for systemic mastocytosis (11, 13). In particular, *FIP1L1-PDGFRA*+ cases display a marked male predominance, with cardiac and pulmonary symptoms being more common and gastrointestinal symptoms being less common than in systemic mastocytosis, irrespective of eosinophilia. While tryptase levels are often elevated in *FIP1L1-PDGFRA*+ patients, the elevation is moderate (usually <50 ng/mL) in contrast to systemic mastocytosis which frequently displays tryptase levels >100 ng/mL. Finally, the *KIT* codon 816 mutations typical of systemic mastocytosis are not identified in *FIP1L1-PDGFRA* positive cases. The demonstration of distinct clinical, morphologic, and genetic features in *FIP1L1-PDGFRA*+ cases warrant their distinction from systemic mastocytosis. The current WHO classification has therefore recognized *FIP1L1-PDGFRA*+ myeloproliferative neoplasm as a distinct clinicopathologic entity based on molecular pathogenesis and molecularly targeted therapy (1).

CONCLUSION

This chapter defines criteria for *PDGFRA*-associated myeloid/lymphoid neoplasms. Entities included within Myeloid/Lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions are discussed with reference to the latest edition of WHO classification of tumors of hematopoietic and lymphoid tissues (5th edition 2022). The current technologies including FISH use for the diagnosis are discussed. Finally, the differential diagnosis of entities that present with eosinophilia are discussed.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

Copyright and Permission Statement: The authors confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder(s), and all original sources have been appropriately acknowledged or referenced

REFERENCES

1. Swerdlow SH, International Agency for Research on Cancer., World Health Organization. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: International Agency for Research on Cancer; 2008. 439 p. p.
2. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90. https://doi.org/10.1182/blood-2016-01-643569
3. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703–19. https://doi.org/10.1038/s41375-022-01613-1
4. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. The New England journal of medicine. 2003;348(13):1201–14. https://doi.org/10.1056/NEJMoa025217
5. Metzgeroth G, Walz C, Score J, Siebert R, Schnittger S, Haferlach C, et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia. 2007;21(6):1183–8. https://doi.org/10.1038/sj.leu.2404662
6. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405. https://doi.org/10.1182/blood-2016-03-643544
7. Pozdnyakova O, Orazi A, Kelemen K, King R, Reichard KK, Craig FE, et al. Myeloid/Lymphoid Neoplasms Associated With Eosinophilia and Rearrangements of PDGFRA, PDGFRB, or FGFR1 or With PCM1-JAK2. American journal of clinical pathology. 2021;155(2):160–78. https://doi.org/10.1093/ajcp/aqaa208
8. Gotlib J, Cools J, Malone JM, 3rd, Schrier SL, Gilliland DG, Coutré SE. The FIP1L1-PDGFRalpha fusion tyrosine kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. Blood. 2004;103(8):2879–91. https://doi.org/10.1182/blood-2003-06-1824
9. Pardanani A, Brockman SR, Paternoster SF, Flynn HC, Ketterling RP, Lasho TL, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood. 2004;104(10):3038–45. https://doi.org/10.1182/blood-2004-03-0787

10. Bain BJ, Fletcher SH. Chronic eosinophilic leukemias and the myeloproliferative variant of the hypereosinophilic syndrome. Immunol Allergy Clin North Am. 2007;27(3):377–88. https://doi.org/10.1016/j.iac.2007.06.001
11. Maric I, Robyn J, Metcalfe DD, Fay MP, Carter M, Wilson T, et al. KIT D816V-associated systemic mastocytosis with eosinophilia and FIP1L1/PDGFRA-associated chronic eosinophilic leukemia are distinct entities. The Journal of allergy and clinical immunology. 2007;120(3):680–7. https://doi.org/10.1016/j.jaci.2007.05.024
12. Vandenberghe P, Wlodarska I, Michaux L, Zachée P, Boogaerts M, Vanstraelen D, et al. Clinical and molecular features of FIP1L1-PDFGRA (+) chronic eosinophilic leukemias. Leukemia. 2004;18(4):734–42. https://doi.org/10.1038/sj.leu.2403313
13. Pardanani A, Ketterling RP, Brockman SR, Flynn HC, Paternoster SF, Shearer BM, et al. CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib mesylate therapy. Blood. 2003;102(9):3093–6. https://doi.org/10.1182/blood-2003-05-1627
14. Reiter A, Gotlib J. Myeloid neoplasms with eosinophilia. Blood. 2017;129(6):704–14. https://doi.org/10.1182/blood-2016-10-695973
15. Wang SA, Hasserjian RP, Tam W, Tsai AG, Geyer JT, George TI, et al. Bone marrow morphology is a strong discriminator between chronic eosinophilic leukemia, not otherwise specified and reactive idiopathic hypereosinophilic syndrome. Haematologica. 2017;102(8):1352–60. https://doi.org/10.3324/haematol.2017.165340
16. Robyn J, Lemery S, McCoy JP, Kubofcik J, Kim YJ, Pack S, et al. Multilineage involvement of the fusion gene in patients with FIP1L1/PDGFRA-positive hypereosinophilic syndrome. Br J Haematol. 2006;132(3):286–92. https://doi.org/10.1111/j.1365-2141.2005.05863.x
17. Kumar S, Razzaq SK, Vo AD, Gautam M, Li H. Identifying fusion transcripts using next generation sequencing. Wiley Interdiscip Rev RNA. 2016;7(6):811–23. https://doi.org/10.1002/wrna.1382
18. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. The Journal of pharmacology and experimental therapeutics. 2000;295(1):139–45.
19. Buchdunger E, Zimmermann J, Mett H, Meyer T, Müller M, Druker BJ, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res. 1996;56(1):100–4.
20. Carroll M, Ohno-Jones S, Tamura S, Buchdunger E, Zimmermann J, Lydon NB, et al. CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins. Blood. 1997;90(12):4947–52. https://doi.org/10.1182/blood.V90.12.4947
21. Kennedy JA, Hobbs G. Tyrosine Kinase Inhibitors in the Treatment of Chronic-Phase CML: Strategies for Frontline Decision-making. Current hematologic malignancy reports. 2018;13(3):202–11. https://doi.org/10.1007/s11899-018-0449-7
22. Tefferi A. Molecular drug targets in myeloproliferative neoplasms: mutant ABL1, JAK2, MPL, KIT, PDGFRA, PDGFRB and FGFR1. J Cell Mol Med. 2009;13(2):215–37. https://doi.org/10.1111/j.1582-4934.2008.00559.x
23. Toffalini F, Demoulin JB. New insights into the mechanisms of hematopoietic cell transformation by activated receptor tyrosine kinases. Blood. 2010;116(14):2429–37. https://doi.org/10.1182/blood-2010-04-279752
24. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300(5894):765–7. https://doi.org/10.1038/300765a0
25. Savage DG, Antman KH. Imatinib mesylate--a new oral targeted therapy. The New England journal of medicine. 2002;346(9):683–93. https://doi.org/10.1056/NEJMra013339
26. Cools J, Stover EH, Wlodarska I, Marynen P, Gilliland DG. The FIP1L1-PDGFRalpha kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia. Current opinion in hematology. 2004;11(1):51–7. https://doi.org/10.1097/00062752-200401000-00008
27. Curtis CE, Grand FH, Musto P, Clark A, Murphy J, Perla G, et al. Two novel imatinib-responsive PDGFRA fusion genes in chronic eosinophilic leukaemia. Br J Haematol. 2007;138(1):77–81. https://doi.org/10.1111/j.1365-2141.2007.06628.x

28. David M, Cross NC, Burgstaller S, Chase A, Curtis C, Dang R, et al. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. Blood. 2007;109(1):61–4. https://doi.org/10.1182/blood-2006-05-024828
29. Roche-Lestienne C, Lepers S, Soenen-Cornu V, Kahn JE, Laï JL, Hachulla E, et al. Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics. Leukemia. 2005;19(5):792–8. https://doi.org/10.1038/sj.leu.2403722
30. Erben P, Gosenca D, Müller MC, Reinhard J, Score J, Del Valle F, et al. Screening for diverse PDGFRA or PDGFRB fusion genes is facilitated by generic quantitative reverse transcriptase polymerase chain reaction analysis. Haematologica. 2010;95(5):738–44. https://doi.org/10.3324/haematol.2009.016345
31. Baxter EJ, Hochhaus A, Bolufer P, Reiter A, Fernandez JM, Senent L, et al. The t(4;22)(q12;q11) in atypical chronic myeloid leukaemia fuses BCR to PDGFRA. Hum Mol Genet. 2002;11(12):1391–7. https://doi.org/10.1093/hmg/11.12.1391
32. Trempat P, Villalva C, Laurent G, Armstrong F, Delsol G, Dastugue N, et al. Chronic myeloproliferative disorders with rearrangement of the platelet-derived growth factor alpha receptor: a new clinical target for STI571/Glivec. Oncogene. 2003;22(36):5702–6. https://doi.org/10.1038/sj.onc.1206543
33. Score J, Curtis C, Waghorn K, Stalder M, Jotterand M, Grand FH, et al. Identification of a novel imatinib responsive KIF5B-PDGFRA fusion gene following screening for PDGFRA overexpression in patients with hypereosinophilia. Leukemia. 2006;20(5):827–32. https://doi.org/10.1038/sj.leu.2404154
34. Tashiro H, Shirasaki R, Noguchi M, Gotoh M, Kawasugi K, Shirafuji N. Molecular analysis of chronic eosinophilic leukemia with t(4;10) showing good response to imatinib mesylate. International journal of hematology. 2006;83(5):433–8. https://doi.org/10.1532/IJH97.05180
35. Pardanani A, Lasho T, Barraco D, Patnaik M, Elala Y, Tefferi A. Next generation sequencing of myeloid neoplasms with eosinophilia harboring the FIP1L1-PDGFRA mutation. American journal of hematology. 2016;91(3):E10-1. https://doi.org/10.1002/ajh.24273
36. Lierman E, Michaux L, Beullens E, Pierre P, Marynen P, Cools J, et al. FIP1L1-PDGFRalpha D842V, a novel panresistant mutant, emerging after treatment of FIP1L1-PDGFRalpha T674I eosinophilic leukemia with single agent sorafenib. Leukemia. 2009;23(5):845–51. https://doi.org/10.1038/leu.2009.2
37. Jain N, Khoury JD, Pemmaraju N, Kollipara P, Kantarjian H, Verstovsek S. Imatinib therapy in a patient with suspected chronic neutrophilic leukemia and FIP1L1-PDGFRA rearrangement. Blood. 2013;122(19):3387–8. https://doi.org/10.1182/blood-2013-07-516500
38. Klion AD. Eosinophilia: a pragmatic approach to diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2015;2015:92–7. https://doi.org/10.1182/asheducation-2015.1.92
39. O'Sullivan JA, Bochner BS. Eosinophils and eosinophil-associated diseases: An update. The Journal of allergy and clinical immunology. 2018;141(2):505–17. https://doi.org/10.1016/j.jaci.2017.09.022
40. Wechsler ME, Munitz A, Ackerman SJ, Drake MG, Jackson DJ, Wardlaw AJ, et al. Eosinophils in Health and Disease: A State-of-the-Art Review. Mayo Clin Proc. 2021;96(10):2694–707. https://doi.org/10.1016/j.mayocp.2021.04.025
41. Kelemen K, Saft L, Craig FE, Orazi A, Nakashima M, Wertheim GB, et al. Eosinophilia/Hypereosinophilia in the Setting of Reactive and Idiopathic Causes, Well-Defined Myeloid or Lymphoid Leukemias, or Germline Disorders. American journal of clinical pathology. 2021;155(2):179–210. https://doi.org/10.1093/ajcp/aqaa244
42. Roufosse F, Cogan E, Goldman M. The hypereosinophilic syndrome revisited. Annu Rev Med. 2003;54:169–84. https://doi.org/10.1146/annurev.med.54.101601.152431
43. Roufosse FE, Goldman M, Cogan E. Hypereosinophilic syndromes. Orphanet J Rare Dis. 2007;2:37. https://doi.org/10.1186/1750-1172-2-37
44. Schwaller J, Anastasiadou E, Cain D, Kutok J, Wojiski S, Williams IR, et al. H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived growth factor receptor beta gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). Blood. 2001;97(12):3910–8. https://doi.org/10.1182/blood.V97.12.3910
45. Macdonald D, Reiter A, Cross NC. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. Acta Haematol. 2002;107(2):101–7. https://doi.org/10.1159/000046639

46. Reiter A, Walz C, Watmore A, Schoch C, Blau I, Schlegelberger B, et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. Cancer Res. 2005;65(7):2662–7. https://doi.org/10.1158/0008-5472.CAN-04-4263
47. Schwaab J, Naumann N, Luebke J, Jawhar M, Somervaille TCP, Williams MS, et al. Response to tyrosine kinase inhibitors in myeloid neoplasms associated with PCM1-JAK2, BCR-JAK2 and ETV6-ABL1 fusion genes. American journal of hematology. 2020;95(7):824–33. https://doi.org/10.1002/ajh.25825
48. Bain BJ, Ahmad S. Should myeloid and lymphoid neoplasms with PCM1-JAK2 and other rearrangements of JAK2 be recognized as specific entities? Br J Haematol. 2014;166(6):809–17. https://doi.org/10.1111/bjh.12963
49. Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, et al. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood. 1997;90(7):2535–40. https://doi.org/10.1182/blood.V90.7.2535
50. Wang SA, Tam W, Tsai AG, Arber DA, Hasserjian RP, Geyer JT, et al. Targeted next-generation sequencing identifies a subset of idiopathic hypereosinophilic syndrome with features similar to chronic eosinophilic leukemia, not otherwise specified. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2016;29(8):854–64. https://doi.org/10.1038/modpathol.2016.75
51. Baccarani M, Cilloni D, Rondoni M, Ottaviani E, Messa F, Merante S, et al. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRalpha-positive hypereosinophilic syndrome. Results of a multicenter prospective study. Haematologica. 2007;92(9):1173–9. https://doi.org/10.3324/haematol.11420
52. Parreira L, Tavares de Castro J, Hibbin JA, Marsh JC, Marcus RE, Babapulle VB, et al. Chromosome and cell culture studies in eosinophilic leukaemia. Br J Haematol. 1986;62(4):659–69. https://doi.org/10.1111/j.1365-2141.1986.tb04089.x
53. Cogan E, Schandené L, Crusiaux A, Cochaux P, Velu T, Goldman M. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. The New England journal of medicine. 1994;330(8):535–8. https://doi.org/10.1056/NEJM199402243300804
54. Vaklavas C, Tefferi A, Butterfield J, Ketterling R, Verstovsek S, Kantarjian H, et al. 'Idiopathic' eosinophilia with an Occult T-cell clone: prevalence and clinical course. Leukemia research. 2007;31(5):691–4. https://doi.org/10.1016/j.leukres.2006.10.005

9

Novel Aspects of Leukemia Pharmacogenomics

Deyanira Escalante-Bautista[1,2] • Rosas-Vargas Haydeé[2] • Doris Cerecedo[1]

[1]Laboratorio de Hematobiología, ENMyH, Instituto Politécnico Nacional, Mexico City, Mexico; [2]Unidad de Investigación Médica en Genética Humana, UMAE Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social IMSS, Av. Cuauhtémoc 330, Col Doctores, Delegación Cuauhtémoc, 06720, Mexico City, Mexico.

Author for Correspondence: Doris Cerecedo, Laboratorio de Hematobiología, Escuela Nacional de Medicina y Homeopatía, I.P.N., Guillermo Massieu Helguera No. 239, Col. La Escalera Ticomán, 07320, Ciudad de México (CDMX), Mexico. E-mail: dcereced@ipn.mx

Cite this chapter as: Escalante-Bautista D, Haydeé RV, Cerecedo D. Novel Aspects of Leukemia Pharmacogenomics. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 21 Aug 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-pharmacogenomics

Abstract: Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children between the ages of 2 and 6. It is more frequent in boys than in girls. Currently, the overall cure rate of childhood ALL is approximately 75–80%. Integrated genomic analyses of patients with ALL have advanced the knowledge of the biological basis of ALL and have contributed to identifying subtypes, dysregulated pathways, and therapeutic targets that have resulted in the assignment of stratification categories and improvement of treatment strategies. Genomic studies in pediatric ALL patients have demonstrated chromosomal alterations during the evolution of the disease that directly influence the response to treatment and prognosis. Hence, the proper stratification of patients for identifying risks to prescribe the best treatment is crucial in the management of patients with ALL. Current risk stratification and treatment algorithms include cytogenetic alterations, clinical parameters, and levels of minimal residual disease. All these

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

features are integrated to establish the clinical management of patients with ALL for surveillance of treatment success or for the identification of alternative therapeutic approaches. This chapter focuses on the genetic variations that affect the response to most of the chemotherapy drugs used for ALL.

Keywords: genetic variants in acute lymphoblastic leukemia; leukemia pharmacogenomics; pharmacogenetic testing in childhood acute lymphoblastic leukemia; protective pharmacogenetic variants in acute lymphoblastic leukemia; stratification and treatment of patients with acute lymphoblastic leukemia

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a group of neoplasms derived from B- and T-lineage lymphoid precursors and are classified based on their biological and molecular characteristics. The typical B-lineage ALL is observed in most cases (85%), whereas T-lineage ALL is associated with a lymphomatous mass in the mediastinum or other sites. In the last 27 years, there has been an increase in the incidence, prevalence, and mortality of leukemia worldwide; in 2017, there were 0.52 million incident cases, 2.43 million prevalent cases, and 0.35 million deaths, which are often observed in older patients and unhealthy young people (1, 2). A higher incidence among Latino patients has been observed in Mexico, with 57.6 cases observed per 100,000 individuals in a population (3), whereas the 5-year overall survival (OS) is only 50–65% in contrast to the OS of patients who developed the disease in other regions, with an OS corresponding to > 90% and a cure rate of 85%.

Environmental risk factors for childhood leukemia include ionizing and nonionizing radiation (4), chemicals (such as hydrocarbons and pesticides), and parental tobacco use; cigarettes have also been established as being risk factors for leukemia. Ethnicity is also an epidemiological condition for ALL, as it is a poor prognostic factor in Latino populations (5, 6); in addition, the incidence of this disease has increased over the last decade (7). Although the feasible cause remains unknown, socioeconomic status, environmental risks, genetic mutations, or a combination of these factors may contribute to ALL development.

The treatment efficacy has been successful for most patients, and risk factors such as sex, ethnicity, and number of leucocytes have become diminished (8); therefore, its clinical outcome has exceptionally improved. In developed regions, the OS is 5 years, and it has increased over the same period from 60% to approximately 90% for children younger than 15 years and from 28% to more than 75% for adolescents aged 15 to 19 years. Childhood and adolescent cancer survivors require close monitoring because cancer therapy side effects may persist or develop months or years after treatment. Specific information about the incidence, type, and monitoring of late effects in childhood and adolescent cancer survivors is available elsewhere (9).

According to the World Health Organization protocols established in 2008, the diagnoses of ALL include the study of cell morphology, immunophenotype, and genetics/cytogenetics (10, 11). Identification of the morphological bone

marrow cells to differentiate from acute myeloid leukemia (AML) is the first strategy to diagnose ALL. When considering the cellular heterogeneity of ALL subtypes, flow cytometry immunophenotyping is the optimal method for confirming ALL diagnoses and for monitoring minimal residual disease (MRD).

B-cell ALL and T-cell ALL are characterized by recurrent cytogenetic changes (12); therefore, cytogenetics is of great value for the diagnosis, risk stratification, disease monitoring, and treatment selection of ALL. Recent advancements in conventional cytogenetics techniques, such as fluorescence *in situ* hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), array comparative genomic hybridization (aCGH), and next-generation sequencing (NGS), have improved classical cytogenetics technologies (13–15).

Pharmacogenomics is the combination of pharmacology and genomics that studies the influence of a person's genetic makeup on response to pharmacotherapy. This chapter focuses on the molecular and genetic basis of known polymorphisms that affect response to drugs most used to treat ALL.

STRATIFICATION AND TREATMENT OF PATIENTS WITH ALL

In general, ALL treatment is designed based on the risk of failure rate, thus allowing for the identification of some clinical characteristics to stratify patients and potentially influence the prognosis. The clinical features include ages less than 1 year and older than 10 years, a white blood cell count (WBC) greater than 50.000–100,000/ml, and the involvement of sanctuary organs (16). Due to recent advances in treatment regimens, the outcomes of T-ALL have improved. The most useful prognostic factor is the response to early treatment, which is estimated by the clearance of leukemic cells from the blood or bone marrow that depends on drug sensitivity or resistance of leukemic cells; additionally, early response is dependent on the pharmacodynamics of the drugs and the pharmacogenetics of the host. Minimal residual disease (MDR) is defined by the presence of 0.01% or more ALL cells and has become a crucial factor for risk stratification in childhood ALL. In addition, it represents a risk of relapse, particularly when measured during or at the end of remission-induction therapy (17).

Current treatment for ALL includes four phases that occur over 2–3 years: induction, consolidation, intensification, and long-term maintenance (18). Pediatric patients with persistent minimal residual ALL are directed to receive an allogeneic hemopoietic cell transplantation that generates a 5-year OS of 79% for low-risk patients and 8% for high-risk patients (19), whereas the OS is 45% in adults. Therefore, the outcome is discouraging compared to the results observed in children (20). It must be assumed that the population of cells from which the tumor arises (cancer stem cells) expresses quiescence and drug resistance, thus hindering the efforts to eradicate them from a patient (21). The treatment phases are as follows:

- *Induction chemotherapy*: This treatment seeks to eliminate malignant burden cells and to restore bone marrow function (22–24).
- *Consolidation therapy*: The goal of this treatment is to prevent the onset of therapy-resistant clones (23).

- *Intensification therapy.* The aim of this treatment is to improve the outcome of patients with a slow early response to therapy (25, 26).
- *Maintenance therapy*: This treatment represents the longer phase and lasts from 2 to 3 years (27).

During this time, clinical features (such as myelosuppression) must be avoided, as it is a predictor of risk relapse (28).

A central nervous system (CNS) prophylaxis should simultaneously be considered with systemic chemotherapy; however, it has been associated with late neurocognitive deficits, endocrinopathy, secondary cancers, and excess late mortality. Therefore, cranial irradiation should be directed to patients with CNS involvement at the time of diagnosis; new therapeutic approaches can include serial intensive intrathecal chemotherapy with methotrexate (MTX) alone or MTX, cytarabine, and hydrocortisone in conjunction with high-dose intravenous MTX and cytarabine (29).

Although hematopoietic stem cell transplantation (HSCT) remains a viable option for those patients with high risk or relapsed ALL, the data on HSCT in patients with disease survival (DS) are limited, and the role in relapsed patients with DS remains unclear (30, 31).

Toxicity

The success of modern treatment approaches for childhood ALL that yield 5-year OS rates above 90% is the result of intense chemotherapy and the respective early response to directed chemotherapy according to treatment stratification by somatic mutations and the optimized use of traditional antileukemic agents, as well as the inclusion of broad-spectrum antibiotics to eliminate opportunistic infections (32). However, a high mortality index of leukemia patients has been observed due to the toxicity of the therapy (rather than by the leukemia itself). To standardize the wide-ranging diversities in toxicity manifestations, the US National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v6.0 is available for review at the following site: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50.

CTCAE has defined and graded the toxicities observed during childhood ALL therapy; according to 15 international childhood ALL study groups (Ponte di Legno Toxicity Working Group, or PTWG), the CTCAE has developed consensus definitions for acute toxicities (33). These definitions include mucositis (34), central neurotoxicity (35), peripheral neuropathy (36), bone toxicities (osteonecrosis) (37), thromboembolism (38), sinusoidal obstruction syndrome (veno-occlusive disease) (39), endocrinopathies (especially corticosteroid-induced adrenal insufficiency and hyperglycemia) (40), high-dose MTX-related nephrotoxicity (41) (42), asparaginase-associated hypersensitivity (43), asparaginase-associated pancreatitis (44), and hyperlipidemia (45). Fortunately, most chemotherapeutic drugs have been subjected to pharmacogenetic studies that are useful for adjusting the doses from the beginning of the treatment in individual patients to improve the outcome and to minimize the risks of acute or late side effects.

PHARMACOGENETICS

The accelerated and simultaneous development of molecular pharmacology, biotechnology, and genomics have contributed to revolutionizing the basic principles of therapy and drug development. Pharmacogenetics (PGx) is the branch of pharmacology that focuses on the study of genetic factors that influence the variability of drug responses among patients. As a discipline, it integrates knowledge of the pharmacokinetics (metabolism or disposition of drugs) and pharmacodynamics (efficacy or toxicity of drugs) of each drug (46). Specifically, it focuses on the study of genetic variations in sequences encoding enzymes, drug metabolizers, drug transporters, and drug targets, as well as the effect of the presence of genetic variants on the difference between drug efficacy and toxicity (47).

Over the past decade, PGx has been widely incorporated into pharmacological research and drug development initiatives. The implementation of personalized medicine has future goals of developing polygenic models that accurately predict pharmacological responses and toxicity in individual patients, as well as the use of these models to prospectively personalize treatment regimens to improve efficacy and safety through a better understanding of the patient's pharmacogenetic characteristics (48).

Currently available tools that support personalized medicine and provide up-to-date information for individualizing therapy include the PharmGKB platform (available at http://www.pharmgkb.org), which is a massive resource that provides specialists with relevant information regarding genetic variations and different drug responses. The second PGx resource is the U.S. Food and Drug Administration (FDA) drug labeling database (https://www.fda.gov/drugs). Another knowledge resource is the Clinical Practice Guidelines (CPG) (https://www.nccih.nih.gov/health/providers/clinicalpractice), which lists a set of guidelines for specific diagnostic cases, along with recommended therapeutic action plans (49). Researchers in this area continue to emphasize that their studies should update the general treatment protocols to strengthen them and to improve their effectiveness in patients who are diagnosed with ALL. The treatments that are used are chemotherapy and bone marrow transplantation, and it has been proposed to adopt a personalized treatment (not a generalized treatment) for improving the appropriate doses, which can be designed according to the particular genetic background of each individual (50).

Genetic variants associated with the risk of developing ALL chemotherapy toxicity

Currently, there are several alternative treatment protocols for ALL and all these protocols share many common points. Their implementation has demonstrated very good results or efficacy but also toxicity, thus making this disease an important target for PGx. The typical course of treatment is composed of three main phases and lasts between 2 and 3 years, according to the risk stratification of the disease. Recently, reviews have been published focusing on the evidence of the different responses to drugs in patients diagnosed with leukemia (51).

However, a major disadvantage of PGx research in leukemia is that in each phase of the treatment protocol, patients receive a combination of different drugs and the corresponding toxicities, which are often overlapping, and include hepatotoxicity and myelosuppression. In addition, drug-gene interactions are sometimes influenced by drug–drug interactions, as in the case of 6-mercaptopurine (6-MP) and MTX. Consequently, this circumstance makes it difficult to determine exactly which drug is causing toxicity or to determine the efficacy of PGx for adjusting the dose and for improving efficacy (52).

L-asparaginase

L-asparaginase is one of the drugs that is widely used in the initial chemotherapeutic treatment of ALL (53). Asparaginase is an enzyme originating from several bacterial sources; however, only asparaginases from *Escherichia coli* and *Erwinia chrysanthemi* are used in medicine (54). The function of this enzyme is to catalyze the hydrolysis of the amino acid asparagine (Asn) into aspartic acid (Asp) and ammonia. Leukemic cells do not synthesize Asp (unlike healthy cells); therefore, they depend on its exogenous supply with this mechanism, thus causing the death of the leukemic cell. Among the most representative genetic variants associated with hypersensitivity or toxicity induced by the treatment of this enzyme are *GRIA1* rs4958351, the *ATF5* T1562C variant, *HLA-DRB1* *07:01, *HLA-DQB1* *02:02, rs9272131 close to the *HLA-DQA1* gene, *NFATC2* rs6021191, and *CNOT3* rs73062673. Furthermore, the *PRSS1* rs4726576 variant has been reported to be associated with the risk of developing pancreatitis. Although the presence of the variants or their role in the development of asparaginase hypersensitivity is not yet clear, this effect has been observed in different populations; therefore, it is important to continue with this type of study to provide a more in-depth understanding (55).

Glucocorticoids

Glucocorticoids are part of the induction phase of the chemotherapeutic treatment of ALL. They exert their activity by reducing cell proliferation and by promoting apoptosis or cell cycle arrest by binding to intracytoplasmic glucocorticoid receptors. However, this drug is associated with toxicity in the presence of several variants, such as the haplotype *ABCB1*rs1128503 rs2032582 rs1045642, IL-10rs1800896, or haplotype *NR3C1* rs6189, rs6190, which affects glucocorticoid sensitivity (56). The rs10989692 variants that are close to the *GRIN3A* gene, as well as the *GSTP1* rs1695 and rs1138272 variants, have shown an association with the presence of side effects. Although there have been several studies with this association, there have been considerable discrepancies between the mentioned findings; thus, there is not enough evidence of the effects, and more data are required to consider these variants within routine pharmacogenomic tests (57–60).

Vincristine

Vincristine has the affinity to bind to tubulin dimers, thereby preventing the formation of microtubules and causing the arrest of mitosis and the death of

leukemic cells in metaphase. The presence of toxicity-associated variants has been described in children with ALL who have developed neurotoxicity. Examples of these variants include the *CYP3A5*3* (rs776746) and *CYP3A5*6* (rs10264272 or rs924607), located within the promoter region of the 72 kDa centrosomal protein CEP72. Heterozygous or homozygous genotypes of *CEP72* have been related to neuropathy in different populations; the latter variant is already described in the PharmGKB platform and is associated with neurotoxicity induced by vincristine (11, 23–26).

Methotrexate

MTX is a folate pathway inhibitor and is currently an important component of ALL treatment. MTX suppresses DNA synthesis by competitively inhibiting the enzyme dihydrofolate reductase (DHFR). Genetic variants located in genes encoding enzymes involved in metabolism or transport can significantly affect the absorption, metabolism, excretion, and activity of the drug (27). This drug has a prolonged use during chemotherapeutic treatment; it is also one of the most studied drugs due to the adverse effects that are presented during its administration. Among the genetic variants associated with toxicity due to the administration of this drug, there are some variants located in genes related to cellular processes or leukemogenesis, such as *CCND1* or *ARID5B*. Another important group of genes with pharmacogenetic significant variants are those encoding enzymes involved in the folate pathway, including *DHFR, ITPA, MTR, TYMS*, and *MTHFR*. This group of proteins is key to the *de novo* synthesis of purines and pyrimidines. One of the most studied genes is *MTHFR*. Two variants (rs1801133 and rs1801131) are able to modify the protein sequence, thus consequently causing a reduction in MTHFR activity and an increase in intracellular MTX concentration. The genes encoding input and output drug transport proteins also play an important pharmacogenetic role, particularly the members of the SLC family (*SLC19A1*, *SLC22A1*, *SLC28A8, SLCA6*, and *SLC29A1*). The most relevant variants of this group are *SLCO1B1* rs11045879, rs4149081, and rs4149056, with the presence of these genes having been reported to affect the elimination of MTX, thus causing gastrointestinal and hematological toxicities. Another family of transporters is the ABC family of ATP-binding cassettes (ABCC1, ABCB1, ABCC2, ABCG2, and ABCC4), which are the main MTX output transporters, and the presence of variants in different genes that encode these transporters has been associated with a lower concentration of the different proteins of this family or a decrease in their enzymatic function; as a result, an increase in intracellular MTX levels is observed (1, 28–32).

Thiopurines

6-Mercaptopurine and 6-thioguanine are purine analogs that are metabolically transformed into thioguanine nucleotides (TGNs) that are capable of incorporating into DNA, thus leading to cell death. The presence of variants in different genes has been associated with adverse effects; in addition, thiopurine S-methyltransferase (*TPMT*) is the most studied gene in terms of its pharmacogenomics. This knowledge is useful for the benefit of patients through the

individualization of therapy. Three common variants of the *TPMT* gene (rs1800462, rs1800460, and rs1142345) account for most cases of inherited *TPMT* deficiency. *TPMT* and thiopurines represent one of the first and best documented gene-drug pairs in pharmacogenomics. In addition, the variants *NUDT15**2* (rs746071566) and *NUDT15**3* (rs116855232) in the pharmacogenetic NUDT15 are associated with 6-MP intolerance and are involved in the elimination of 6-MP. An important fact is that these effects been corroborated in multiple studies; thus, the presence of these variants in its sequence is key for the treatment of ALL. Protein kinase C and casein kinase substrate in protein 2 of neurons (*PACSIN2*) have become the focus of attention in the pharmacogenomics of thiopurine drugs, as the presence of the rs2413739 variant demonstrated the strongest association with TPMT activity, although there have been few studies on the association results that have shown congruence in the results (33–40). However, more research is needed to replicate some of these findings, and more concerted efforts are needed to apply this evidence to clinical settings to reduce toxicity from ALL treatment in the pediatric population.

GST genes

The clearance of drugs, such as glucocorticoids, vincristine, anthracyclines, and cyclophosphamide, occurs through the action of a family of enzymes generically named glutathione S-transferase (GST), which are responsible for the inactivation of xenobiotics. The most common polymorphisms described in ALL that influence the risk of treatment success are deletions of the GSTM1 and GSTT1 genes and the A313G substitution in the GSTP1 gene (*GSTP1*B*) (rs1695) (61).

PROTECTIVE PHARMACOGENETIC VARIANTS

As described in the previous section, research studies have focused on the general detection of new genetic variants associated with the metabolism and effect of drugs. These studies have typically focused on the search for, and characterization of risk variants associated with one or more of the adverse effects of either a particular drug or the set of drugs that are used in some phase of treatment. The main reason for this focus is that when a new drug undergoes preclinical and clinical trials, its safety and efficacy are assessed in terms of benefits over risks; thus, when the drug reaches the market, it is assumed to meet the established safety requirements. However, the existence of both risk and protective variants may have a population distribution that was not representative in the testing phases. For comparisons, control groups are integrated with patients whose adverse effects under the same treatment have been null or of a lower risk level, which is ideally matched by age and sex, as well as by follow-up time. Under these circumstances, some genetic variants have been described that are designated as being protective because they are more frequently observed in control groups than in risk groups and are significantly associated with lower toxicities and plasma drug levels. Some examples of such genetic variants are included below, with reference only to the toxic effects of MTX because of space limitations, while also noting that there are variants associated with protection from other drugs and other pathways.

Methotrexate toxicity protective variants

Among the variants that are protective against the toxicity of MTX, the drug that is commonly used in the treatment of ALL is methylenetetrahydrofolate reductase (MTHFR). Among the most studied and relevant drugs are the MTHFR variants rs1801133 and rs1801131. Although studies are numerous, significant evidence of a protective effect is scarce. Hasse et al. (62) described the association between MTHFR rs1801133 and lower blood methotrexate levels, lower risks of anemia and leukopenia, and a lower rate of cycles with infection from a study in Caucasian children. Additionally, Giletti et al. (63) observed that the MTHFR rs1801133 variant has strong protective effects against hematological toxicity caused by MTX. Furthermore, in a Japanese population of pediatric ALL patients, Fukushima et al. (64) described the protection given by the presence of the C allele of the *MTHFR A1298C* (rs1801131) variant that is expressed in lower liver toxicity in carriers. Furthermore, we must not forget that the phenotype of each individual, in addition to the environmental variables that are not discussed in this chapter, are the result of the combination of genetic variants and their interactions. With this observation in mind, there are numerous examples of studies in which haplotypes, and not individual variants, exert a protective effect. For example, patients carrying the *MTHFR 677C-1298C* haplotype have significantly lower plasma concentrations of MTX, as well as less frequent MTX-related toxicities during therapy (65).

Another important molecule involved in folate metabolism and corresponding MTX metabolism is dihydrofolate reductase, which is encoded by the *DHFR* gene, with the *DHFR-* rs1650694 variant of this gene having a clear protective association against hematological toxicity in adult Uruguayan patients with ALL (63).

Regarding the genes encoding the ABC transporter family, two Mexican groups have described protective associations. Zaruma-Torres et al. (66) found that the *ABCB1* rs1128503 and *ABCC5* rs3792585 variants are associated with a protective effect against methotrexate-mediated myelosuppression in children with ALL. Likewise, Ramírez-Pacheco et al. (67) demonstrated that the *ABCB1* rs1045642 variant is protective against leukopenia in homozygotes for the C allele in Mexican children with ALL. Furthermore, Lopez-López E et al. (68) reported that the presence of the G allele of the *ABCC4* rs9516519 variant is associated with lower plasma MTX concentrations and lower toxicity in Spanish children with ALL.

ACTIONABLE PHARMACOGENETIC VARIANTS IN THE TREATMENT OF LEUKEMIA

From the perspective of pharmacogenomics, actionable variants include all the genetic variants that affect drug responses. Under this broad definition, it is correct to include the aforementioned risks and protective variants under this denomination because they are considered in therapeutic decision-making, mainly in the adjustments of doses at which a drug is prescribed or for the use of alternative drugs. These adjustments follow the indications of clinical guidelines that have been developed by organizations such as the Clinical Pharmacogenetic Implementation Consortium (CPIC) or the FDA, when considering the individual's genetic information.

A relevant example of such guidelines is the guideline containing dosing recommendations for thiopurines that are used in the treatment of leukemias, including mercaptopurine for lymphoid neoplasms and thioguanine for myeloid leukemias (69). Dosing guidelines are based on thiopurine methyltransferase (*TPMT*) and nudix hydrolase 15 (*NUDT15*) gene genotypes. Depending on the diplotypes in their different combinations, individuals are classified into normal, intermediate, intermediate potential, poor, and indeterminate metabolizers, for each of which there are specific recommendations for dose adjustments. As the frequencies of each genetic variant may differ between the populations, the relevance of these factors also differs between the populations; therefore, it is important to consider the ancestry of each patient (70, 71). In addition to the reduced risk of unwanted effects, another benefit of lowering the dose of mercaptopurine in the maintenance phase in patients carrying low-activity TPMT alleles is the reduced risk of secondary malignancies (72).

In the case of genes related to methotrexate sensitivity or toxicity, genome-wide association studies (GWAS) have shown that some *SLCO1B1* variants are useful as a reference for dosage adjustments, with a significant decrease in gastrointestinal toxicity associated with faster methotrexate clearance (73, 74), which is key in patients who are treated with high doses of the drug (75).

The list of pharmacogenomic variants related to the drugs that are used in the therapy of leukemia is limited, as only those variants for which there is strong evidence of interactions with one or more drugs are included. However, information continues to be gathered from studies in different populations, and there are variants that stand out as candidates to be considered for validation as actionable variants that are recognized by the specialized organizations mentioned above. Examples of such variants include the human leukocyte antigen haplotypes *HLA-DRB1* *07:01, *HLA-DRB1**16:02, *HLA-DQA1**02:01, and *HLA-DQB1**02:02, which have been linked to asparaginase hypersensitivity (76, 77).

Another drug that is commonly used in the treatment of leukemia is vincristine, which is associated with a risk of neuropathy. Some variants in the cytochromes p450 *CYP3A4* and *CYP3A5*, as well as the variant *CEP72* rs924607 encoding centrosomal protein 72 (78, 79), have been described that produce changes in its expression and that serve as a reference for modifying drug doses. However, the results from different studies have been contradictory, which mainly concerns the *CYP* isoforms (80–82), and this effect is most likely related to the ancestry and genetic background of the studied populations (83). The relevant genetic variants associated with toxicity of chemotherapy in children with ALL described above are summarized in Table 1.

Although interventions that have been implemented as part of the algorithm defining the treatment strategy for leukemia patients are still rare, their use is an extremely valuable tool in reducing deaths and severe adverse events related to chemotherapy. It is essential to expand studies that are focused on both the discovery of new variants that are likely to be actionable and their validation in populations with different ancestry so that the benefits of pharmacogenomics can be extended on a global scale.

TABLE 1 **Relevant genetic variants associated with toxicity of chemotherapy in children with ALL**

Gene	Variant	Level&	Drug	Gene	Variant	Level	Drug
ABCB1	rs1128503 #	3	Methotrexate	ITPA	rs1127354	4	Mercaptopurine
ABCB1	rs1045642 #	3	Methotrexate	MTHFD1	rs2236225	3	Methotrexate
ABCC2	rs717620	3	Methotrexate	MTHFR	rs1801133 #	4	Mercaptopurine
ABCC4	rs7317112	3	Methotrexate	MTHFR	rs1801131 #	4	Methotrexate
ABCC4	rs9516519#	3	Methotrexate	MTHFR	rs1801131	4	Methotrexate
ABCC5	rs3792585	NA	Methotrexate	MTHFR	rs1801133	2A	Methotrexate
ABCG2	rs2231142	4	Methotrexate	MTR	rs1805087	4	Methotrexate
ARID5B	rs4948496	3	Methotrexate	MTRR	rs1801394	3	Methotrexate
BCL2L11	rs2241843	3	Corticosteroids	NFATC2	rs6021191	3	Asparaginase
BCL2L11	rs724710	3	Corticosteroids	NUDT15	rs746071566	3	Mercaptopurine
BMP7	rs79085477	3	*	NUDT15	rs766023281	3	Mercaptopurine
CAT	rs10836235	3	Anthracyclines and related substances	NUDT15	NUDT15*1; NUDT15*2; NUDT15*3	1A	Mercaptopurine
CCND1	rs9344	3	Methotrexate	PACSIN2	rs2413739	3	Mercaptopurine
CEP72	rs924607 @	3	Vincristine	PACSIN2	rs2413739	3	Mercaptopurine; Methotrexate
CPA2	rs199695765	3	Asparaginase	PNPLA3	rs738409	3	***
DHFR	rs1650694#	NA	Methotrexate	SERPINE1	rs6092	3	Dexamethasone

(Continued)

TABLE 1 **Relevant genetic variants associated with toxicity of chemotherapy in children with ALL (*Continued*)**

Gene	Variant	Level&	Drug	Gene	Variant	Level	Drug
DHFR	rs442767	3	Methotrexate	SHMT1	rs1979277	3	Methotrexate
DHFR	rs70991108	3	Methotrexate	SLCO1B1	rs4149081	3	Methotrexate
DHFR	rs408626	3	Methotrexate	SLCO1B1	rs4149056	3	Mercaptopurine; methotrexate
DOK5	rs117532069	3	*	SLCO1B1	rs11045879	3	Mercaptopurine
DROSHA	rs639174	3	**	SLCO1B1	rs11045879	4	Methotrexate
FOLH1	rs61886492	3	Mercaptopurine; Methotrexate	SOD2	rs4880	3	Asparaginase
GOGH	rs11545078	3	Methotrexate	TPMT	TPMT*1; TPMT*21; TPMT*33; TPMT*34	3	Thioguanine
GNMT	rs10948059	3	Mercaptopurine	TPMT	rs1142345	3	Mercaptopurine
GRIA1	rs4958381	3	Asparaginase	TPMT	TPMT*1; TPMT*21; TPMT*33; TPMT*34	3	Mercaptopurine
GSTP1	rs1695	3	Mercaptopurine; methotrexate	TPMT	TPMT*1; TPMT*21; TPMT*33; TPMT*34	3	Thioguanine
HLA-DRB1	rs17885382[@]	3	Asparaginase	TPMT	TPMT*1; TPMT*21; TPMT*33; TPMT*34	3	Mercaptopurine
ITPA	rs7270101	3	Mercaptopurine; methotrexate	TYMS	rs11280056	3	Methotrexate
ITPA	rs1127354	3	Methotrexate	TYMS	rs45445694	3	Methotrexate

[#]Also described as protective variant or [@]actionable variant. [&]Level of evidence to PharmaGKB: Level 1, variant with proven association; level 2, moderate levelof support; 3, low level of evidence supporting the association; and 4, very little information and discrepancies between results. NA not annotated. *Cyclophosphamide; cytarabine; daunorubicin; dexamethasone; doxorubicin; methotrexate; pegaspargase; prednisone; thioguanine; vincristine. **Cyclophosphamide; cytarabine; daunorubicin; mercaptopurine.

PHARMACOGENETIC TESTING IN CHILDHOOD ALL

There is currently sufficient evidence demonstrating the need for the implementation of personalized medicine. Regarding childhood ALL, there are two established pharmacogenetic tests that detect variants in the TPMT and NUD15 genes (due to the fact that the presence of variants in these genes interferes with the metabolism of drugs such as thioguanine and 6 mercaptopurine, which impacts their enzymatic function), thus requiring dose adjustments in patients who present these genotypes. The most commonly available clinical PGx tests that are used worldwide are those that detect variants of these genes, but many institutions still do not offer these tests, especially in developing countries. The tests are accredited by the Clinical Pharmacogenetics Implementation Consortium (CPIC), the FDA, and the European Medicines Agency (EMA) (41, 42).

There are significant challenges associated with drug implementation, such as laboratory or hospital infrastructure factors, costs, profit, and lack of knowledge or skepticism of physicians. However, we can conclude that the efficacy of different anti-leukemia drugs is affected by genetic variants; therefore, it may be much more cost-effective and practical to perform a preventive PGx test to avoid adverse effects, thus improving the patient's quality of life and allowing for the implementation of an individualized therapy that improves survival prognoses (43).

CONCLUSION

ALL is the most common pediatric cancer and is characterized by the expression of lymphoid cell surface markers. The treatment effectiveness has improved for most patients, as risk factors such as sex, ethnicity, and the number of leucocytes has become diminished. The specific molecular alterations and modifications in critical pathways of leukemogenesis have been achieved with the use of modern tools that have increased our knowledge related to lymphoblastic leukemias, which has allowed for the improvement of the survival of patients suffering from this disease. In addition, current functional studies of basic genetic alterations identified in ALL patients have contributed to a better understanding of ALL pathogenesis and the management of this disease. The application of individualized strategies, especially in children, based on the integration of knowledge related to the biology of tumor cells, the pharmacodynamics of the drugs, and particularly the pharmacogenetics that values the impact of multiple mutations in the genome of the host to determine the patient's response to drug therapy, could guarantee a better outcome for each patient.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

REFERENCES

1. Lin X, Wang J, Huang X, Wang H, Li F, Ye W, et al. Global, regional, and national burdens of leukemia from 1990 to 2017: a systematic analysis of the global burden of disease 2017 study. Aging (Albany NY). 2021;13(7):10468–89. https://doi.org/10.18632/aging.202809
2. Zhu C, Yang G, Li H, Du D, Lin Y. Electrochemical sensors and biosensors based on nanomaterials and nanostructures. Analytical chemistry. 2015;87(1):230–49. https://doi.org/10.1021/ac5039863
3. Quiroz E, Aldoss I, Pullarkat V, Rego E, Marcucci G, Douer D. The emerging story of acute lymphoblastic leukemia among the Latin American population - biological and clinical implications. Blood Rev. 2019;33:98–105. https://doi.org/10.1016/j.blre.2018.08.002
4. Jabo B, Morgan JW, Martinez ME, Ghamsary M, Wieduwilt MJ. Sociodemographic disparities in chemotherapy and hematopoietic cell transplantation utilization among adult acute lymphoblastic and acute myeloid leukemia patients. PLoS One. 2017;12(4):e0174760. https://doi.org/10.1371/journal.pone.0174760
5. Perez-Saldivar ML, Fajardo-Gutierrez A, Bernaldez-Rios R, Martinez-Avalos A, Medina-Sanson A, Espinosa-Hernandez L, et al. Childhood acute leukemias are frequent in Mexico City: descriptive epidemiology. BMC Cancer. 2011;11:355. https://doi.org/10.1186/1471-2407-11-355
6. Barrington-Trimis JL, Cockburn M, Metayer C, Gauderman WJ, Wiemels J, McKean-Cowdin R. Rising rates of acute lymphoblastic leukemia in Hispanic children: trends in incidence from 1992 to 2011. Blood. 2015;125(19):3033–4. https://doi.org/10.1182/blood-2015-03-634006
7. Howlader N, Mariotto AB, Woloshin S, Schwartz LM. Providing clinicians and patients with actual prognosis: cancer in the context of competing causes of death. J Natl Cancer Inst Monogr. 2014;2014(49):255–64. https://doi.org/10.1093/jncimonographs/lgu022
8. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360(26):2730–41. https://doi.org/10.1056/NEJMoa0900386
9. Late Effects of Treatment for Childhood Cancer (PDQ(R)): Health Professional Version. PDQ Cancer Information Summaries. Bethesda (MD)2002.
10. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–51. https://doi.org/10.1182/blood-2009-03-209262
11. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985–2000). Leukemia. 2010;24(2):320–34. https://doi.org/10.1038/leu.2009.253
12. Harrison CJ. Cytogenetics of paediatric and adolescent acute lymphoblastic leukaemia. Br J Haematol. 2009;144(2):147–56. https://doi.org/10.1111/j.1365-2141.2008.07417.x
13. Gijsbers AC, Ruivenkamp CA. Molecular karyotyping: from microscope to SNP arrays. Horm Res Paediatr. 2011;76(3):208–13. https://doi.org/10.1159/000330406
14. Riegel M. Human molecular cytogenetics: From cells to nucleotides. Genet Mol Biol. 2014;37(1 Suppl):194–209. https://doi.org/10.1590/S1415-47572014000200006
15. Coccaro N, Anelli L, Zagaria A, Specchia G, Albano F. Next-Generation Sequencing in Acute Lymphoblastic Leukemia. Int J Mol Sci. 2019;20(12). https://doi.org/10.3390/ijms20122929
16. Onciu M. Acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23(4):655–74. https://doi.org/10.1016/j.hoc.2009.04.009
17. Campana D. Role of minimal residual disease monitoring in adult and pediatric acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23(5):1083–98, vii. https://doi.org/10.1016/j.hoc.2009.07.010
18. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381(9881):1943–55. https://doi.org/10.1016/S0140-6736(12)62187-4
19. Bacigalupo A, Soraru M, Dominietto A, Pozzi S, Geroldi S, Van Lint MT, et al. Allogeneic hemopoietic SCT for patients with primary myelofibrosis: a predictive transplant score based on transfusion

requirement, spleen size and donor type. Bone Marrow Transplant. 2010;45(3):458–63. https://doi.org/10.1038/bmt.2009.188
20. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol. 2011;29(5):532–43. https://doi.org/10.1200/JCO.2010.30.1382
21. Nassar D, Blanpain C. Cancer Stem Cells: Basic Concepts and Therapeutic Implications. Annu Rev Pathol. 2016;11:47–76. https://doi.org/10.1146/annurev-pathol-012615-044438
22. Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, Sather HN, et al. Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia. 2010;24(2):285–97. https://doi.org/10.1038/leu.2009.262
23. Kato M, Manabe A. Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int. 2018;60(1):4–12. https://doi.org/10.1111/ped.13457
24. Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. Lancet Oncol. 2010;11(11):1096–106. https://doi.org/10.1016/S1470-2045(10)70114-5
25. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166–78. https://doi.org/10.1056/NEJMra052603
26. Vrooman LM, Silverman LB. Childhood acute lymphoblastic leukemia: update on prognostic factors. Curr Opin Pediatr. 2009;21(1):1–8. https://doi.org/10.1097/MOP.0b013e32831f1f24
27. Schmiegelow K, Nielsen SN, Frandsen TL, Nersting J. Mercaptopurine/Methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. J Pediatr Hematol Oncol. 2014;36(7):503–17. https://doi.org/10.1097/MPH.0000000000000206
28. Schmiegelow K, Heyman M, Gustafsson G, Lausen B, Wesenberg F, Kristinsson J, et al. The degree of myelosuppression during maintenance therapy of adolescents with B-lineage intermediate risk acute lymphoblastic leukemia predicts risk of relapse. Leukemia. 2010;24(4):715–20. https://doi.org/10.1038/leu.2009.303
29. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617):1030–43. https://doi.org/10.1016/S0140-6736(08)60457-2
30. Hitzler JK, He W, Doyle J, Cairo M, Camitta BM, Chan KW, et al. Outcome of transplantation for acute lymphoblastic leukemia in children with Down syndrome. Pediatr Blood Cancer. 2014;61(6):1126–8. https://doi.org/10.1002/pbc.24918
31. Goto H, Kaneko T, Shioda Y, Kajiwara M, Sakashita K, Kitoh T, et al. Hematopoietic stem cell transplantation for patients with acute lymphoblastic leukemia and Down syndrome. Pediatr Blood Cancer. 2015;62(1):148–52. https://doi.org/10.1002/pbc.25245
32. Pui CH, Yang JJ, Hunger SP, Pieters R, Schrappe M, Biondi A, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol. 2015;33(27):2938–48. https://doi.org/10.1200/JCO.2014.59.1636
33. Schmiegelow K, Attarbaschi A, Barzilai S, Escherich G, Frandsen TL, Halsey C, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17(6):e231-e9. https://doi.org/10.1016/S1470-2045(16)30035-3
34. Rask C, Albertioni F, Schroder H, Peterson C. Oral mucositis in children with acute lymphoblastic leukemia after high-dose methotrexate treatment without delayed elimination of methotrexate: relation to pharmacokinetic parameters of methotrexate. Pediatr Hematol Oncol. 1996;13(4):359–67. https://doi.org/10.3109/08880019609030842
35. Mrakotsky CM, Silverman LB, Dahlberg SE, Alyman MC, Sands SA, Queally JT, et al. Neurobehavioral side effects of corticosteroids during active treatment for acute lymphoblastic leukemia in children are age-dependent: report from Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. Pediatr Blood Cancer. 2011;57(3):492–8. https://doi.org/10.1002/pbc.23060
36. Diouf B, Crews KR, Lew G, Pei D, Cheng C, Bao J, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. JAMA. 2015;313(8):815–23. https://doi.org/10.1001/jama.2015.0894
37. den Hoed MA, Pluijm SM, te Winkel ML, de Groot-Kruseman HA, Fiocco M, Hoogerbrugge P, et al. Aggravated bone density decline following symptomatic osteonecrosis in children with

acute lymphoblastic leukemia. Haematologica. 2015;100(12):1564–70. https://doi.org/10.3324/haematol.2015.125583
38. Farinasso L, Bertorello N, Garbarini L, Gajno TM, Barisone E, Artesani L, et al. Risk factors of central venous lines-related thrombosis in children with acute lymphoblastic leukemia during induction therapy: a prospective study. Leukemia. 2007;21(3):552–6. https://doi.org/10.1038/sj.leu.2404560
39. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). Semin Liver Dis. 2002;22(1):27–42. https://doi.org/10.1055/s-2002-23204
40. Teuffel O, Kuster SP, Hunger SP, Conter V, Hitzler J, Ethier MC, et al. Dexamethasone versus prednisone for induction therapy in childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Leukemia. 2011;25(8):1232–8. https://doi.org/10.1038/leu.2011.84
41. Forster VJ, van Delft FW, Baird SF, Mair S, Skinner R, Halsey C. Drug interactions may be important risk factors for methotrexate neurotoxicity, particularly in pediatric leukemia patients. Cancer Chemother Pharmacol. 2016;78(5):1093–6. https://doi.org/10.1007/s00280-016-3153-0
42. de Miguel D, Garcia-Suarez J, Martin Y, Gil-Fernandez JJ, Burgaleta C. Severe acute renal failure following high-dose methotrexate therapy in adults with haematological malignancies: a significant number result from unrecognized co-administration of several drugs. Nephrol Dial Transplant. 2008;23(12):3762–6. https://doi.org/10.1093/ndt/gfn503
43. Liu C, Kawedia JD, Cheng C, Pei D, Fernandez CA, Cai X, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. Leukemia. 2012;26(11):2303–9. https://doi.org/10.1038/leu.2012.102
44. Knoderer HM, Robarge J, Flockhart DA. Predicting asparaginase-associated pancreatitis. Pediatr Blood Cancer. 2007;49(5):634–9. https://doi.org/10.1002/pbc.21037
45. Bhojwani D, Darbandi R, Pei D, Ramsey LB, Chemaitilly W, Sandlund JT, et al. Severe hypertriglyceridaemia during therapy for childhood acute lymphoblastic leukaemia. Eur J Cancer. 2014;50(15):2685–94. https://doi.org/10.1016/j.ejca.2014.06.023
46. Cheok MH, Pottier N, Kager L, Evans WE. Pharmacogenetics in acute lymphoblastic leukemia. Semin Hematol. 2009;46(1):39–51. https://doi.org/10.1053/j.seminhematol.2008.09.002
47. Roden DM, Altman RB, Benowitz NL, Flockhart DA, Giacomini KM, Johnson JA, et al. Pharmacogenomics: challenges and opportunities. Ann Intern Med. 2006;145(10):749–57. https://doi.org/10.7326/0003-4819-145-10-200611210-00007
48. Crews KR, Cross SJ, McCormick JN, Baker DK, Molinelli AR, Mullins R, et al. Development and implementation of a pharmacist-managed clinical pharmacogenetics service. Am J Health Syst Pharm. 2011;68(2):143–50. https://doi.org/10.2146/ajhp100113
49. Kim DC, Wang X, Yang CR, Gao JX. A framework for personalized medicine: prediction of drug sensitivity in cancer by proteomic profiling. Proteome Sci. 2012;10 Suppl 1:S13. https://doi.org/10.1186/1477-5956-10-S1-S13
50. Giacomini KM, Brett CM, Altman RB, Benowitz NL, Dolan ME, Flockhart DA, et al. The pharmacogenetics research network: from SNP discovery to clinical drug response. Clin Pharmacol Ther. 2007;81(3):328–45. https://doi.org/10.1038/sj.clpt.6100087
51. Inaba H, Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica. 2020;105(11):2524–39. https://doi.org/10.3324/haematol.2020.247031
52. Maamari D, El-Khoury H, Saifi O, Muwakkit SA, Zgheib NK. Implementation of Pharmacogenetics to Individualize Treatment Regimens for Children with Acute Lymphoblastic Leukemia. Pharmgenomics Pers Med. 2020;13:295–317. https://doi.org/10.2147/PGPM.S239602
53. Li L, Sajdyk T, Smith EML, Chang CW, Li C, Ho RH, et al. Genetic Variants Associated With Vincristine-Induced Peripheral Neuropathy in Two Populations of Children With Acute Lymphoblastic Leukemia. Clin Pharmacol Ther. 2019;105(6):1421–8. https://doi.org/10.1002/cpt.1324
54. Franca R, Rebora P, Bertorello N, Fagioli F, Conter V, Biondi A, et al. Pharmacogenetics and induction/consolidation therapy toxicities in acute lymphoblastic leukemia patients treated with AIEOP-BFM ALL 2000 protocol. Pharmacogenomics J. 2017;17(1):4–10. https://doi.org/10.1038/tpj.2015.83

55. Hegyi M, Arany A, Semsei AF, Csordas K, Eipel O, Gezsi A, et al. Pharmacogenetic analysis of high-dose methotrexate treatment in children with osteosarcoma. Oncotarget. 2017;8(6):9388–98. https://doi.org/10.18632/oncotarget.11543
56. Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Mol Cell Endocrinol. 2009;300(1–2):7–16. https://doi.org/10.1016/j.mce.2008.10.001
57. Lin KT, Wang LH. New dimension of glucocorticoids in cancer treatment. Steroids. 2016;111:84–8. https://doi.org/10.1016/j.steroids.2016.02.019
58. Zhou ZW, Chen XW, Sneed KB, Yang YX, Zhang X, He ZX, et al. Clinical association between pharmacogenomics and adverse drug reactions. Drugs. 2015;75(6):589–631. https://doi.org/10.1007/s40265-015-0375-0
59. Eipel OT, Nemeth K, Torok D, Csordas K, Hegyi M, Ponyi A, et al. The glucocorticoid receptor gene polymorphism N363S predisposes to more severe toxic side effects during pediatric acute lymphoblastic leukemia (ALL) therapy. Int J Hematol. 2013;97(2):216–22. https://doi.org/10.1007/s12185-012-1236-1
60. Gasic V, Zukic B, Stankovic B, Janic D, Dokmanovic L, Lazic J, et al. Pharmacogenomic markers of glucocorticoid response in the initial phase of remission induction therapy in childhood acute lymphoblastic leukemia. Radiol Oncol. 2018;52(3):296–306. https://doi.org/10.2478/raon-2018-0034
61. Borst L, Buchard A, Rosthoj S, Wesolowska A, Wehner PS, Wesenberg F, et al. Gene dose effects of GSTM1, GSTT1 and GSTP1 polymorphisms on outcome in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2012;34(1):38–42. https://doi.org/10.1097/MPH.0b013e3182346cdd
62. Haase R, Elsner K, Merkel N, Stiefel M, Mauz-Korholz C, Kramm CM, et al. High dose methotrexate treatment in childhood ALL: pilot study on the impact of the MTHFR 677C>T and 1298A>C polymorphisms on MTX-related toxicity. Klin Padiatr. 2012;224(3):156–9. https://doi.org/10.1055/s-0032-1304623
63. Giletti A, Vital M, Lorenzo M, Cardozo P, Borelli G, Gabus R, et al. Methotrexate pharmacogenetics in Uruguayan adults with hematological malignant diseases. Eur J Pharm Sci. 2017;109:480–5. https://doi.org/10.1016/j.ejps.2017.09.006
64. Fukushima H, Fukushima T, Sakai A, Suzuki R, Nakajima-Yamaguchi R, Kobayashi C, et al. Polymorphisms of MTHFR Associated with Higher Relapse/Death Ratio and Delayed Weekly MTX Administration in Pediatric Lymphoid Malignancies. Leuk Res Treatment. 2013;2013:238528. https://doi.org/10.1155/2013/238528
65. Kaluzna E, Strauss E, Zajac-Spychala O, Gowin E, Swiatek-Koscielna B, Nowak J, et al. Functional variants of gene encoding folate metabolizing enzyme and methotrexate-related toxicity in children with acute lymphoblastic leukemia. Eur J Pharmacol. 2015;769:93–9. https://doi.org/10.1016/j.ejphar.2015.10.058
66. Zaruma-Torres F, Lares-Asseff I, Reyes-Espinoza A, Loera-Castaneda V, Chairez-Hernandez I, Sosa-Macias M, et al. Association of ABCB1, ABCC5 and xanthine oxidase genetic polymorphisms with methotrexate adverse reactions in Mexican pediatric patients with ALL. Drug Metab Pers Ther. 2015;30(3):195–201. https://doi.org/10.1515/dmpt-2015-0011
67. Ramirez-Pacheco A, Moreno-Guerrero S, Alamillo I, Medina-Sanson A, Lopez B, Moreno-Galvan M. Mexican Childhood Acute Lymphoblastic Leukemia: A Pilot Study of the MDR1 and MTHFR Gene Polymorphisms and Their Associations with Clinical Outcomes. Genet Test Mol Biomarkers. 2016;20(10):597–602. https://doi.org/10.1089/gtmb.2015.0287
68. Lopez-Lopez E, Ballesteros J, Pinan MA, Sanchez de Toledo J, Garcia de Andoin N, Garcia-Miguel P, et al. Polymorphisms in the methotrexate transport pathway: a new tool for MTX plasma level prediction in pediatric acute lymphoblastic leukemia. Pharmacogenet Genomics. 2013;23(2):53–61. https://doi.org/10.1097/FPC.0b013e32835c3b24
69. Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. Clin Pharmacol Ther. 2019;105(5):1095–105. https://doi.org/10.1002/cpt.1304

70. Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 2015;33(11):1235–42. https://doi.org/10.1200/JCO.2014.59.4671
71. Snowdon JL, Weeraratne D, Huang H, Brotman D, Xue S, Willis VC, et al. Clinical insights into hematologic malignancies and comparative analysis of molecular signatures of acute myeloid leukemia in different ethnicities using an artificial intelligence offering. Medicine (Baltimore). 2021;100(51):e27969. https://doi.org/10.1097/MD.0000000000027969
72. Levinsen M, Rotevatn EO, Rosthoj S, Nersting J, Abrahamsson J, Appell ML, et al. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: influence on cure rates and risk of second cancer. Pediatr Blood Cancer. 2014;61(5):797–802. https://doi.org/10.1002/pbc.24921
73. Trevino LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. J Clin Oncol. 2009;27(35):5972–8. https://doi.org/10.1200/JCO.2008.20.4156
74. Ramsey LB, Panetta JC, Smith C, Yang W, Fan Y, Winick NJ, et al. Genome-wide study of methotrexate clearance replicates SLCO1B1. Blood. 2013;121(6):898–904. https://doi.org/10.1182/blood-2012-08-452839
75. Schulte RR, Choi L, Utreja N, Van Driest SL, Stein CM, Ho RH. Effect of SLCO1B1 Polymorphisms on High-Dose Methotrexate Clearance in Children and Young Adults With Leukemia and Lymphoblastic Lymphoma. Clin Transl Sci. 2021;14(1):343–53. https://doi.org/10.1111/cts.12879
76. Kutszegi N, Yang X, Gezsi A, Schermann G, Erdelyi DJ, Semsei AF, et al. HLA-DRB1*07:01-HLA-DQA1*02:01-HLA-DQB1*02:02 haplotype is associated with a high risk of asparaginase hypersensitivity in acute lymphoblastic leukemia. Haematologica. 2017;102(9):1578–86. https://doi.org/10.3324/haematol.2017.168211
77. Liu S, Gao C, Wu Y, Lin W, Li J, Xue T, et al. HLA-DRB1*16:02 is associated with PEG-asparaginase hypersensitivity. Pharmacogenomics. 2021;22(17):1135–42. https://doi.org/10.2217/pgs-2021-0107
78. Wright GEB, Amstutz U, Drogemoller BI, Shih J, Rassekh SR, Hayden MR, et al. Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy Implicates Pharmacokinetic and Inherited Neuropathy Genes. Clin Pharmacol Ther. 2019;105(2):402–10. https://doi.org/10.1002/cpt.1179
79. Klumpers MJ, Brand A, Hakobjan M, Gattuso G, Schiavello E, Terenziani M, et al. Contribution of common and rare genetic variants in CEP72 on vincristine-induced peripheral neuropathy in brain tumour patients. Br J Clin Pharmacol. 2022;88(7):3463–73. https://doi.org/10.1111/bcp.15267
80. Egbelakin A, Ferguson MJ, MacGill EA, Lehmann AS, Topletz AR, Quinney SK, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2011;56(3):361–7. https://doi.org/10.1002/pbc.22845
81. Zgheib NK, Ghanem KM, Tamim H, Aridi C, Shahine R, Tarek N, et al. Genetic polymorphisms in candidate genes are not associated with increased vincristine-related peripheral neuropathy in Arab children treated for acute childhood leukemia: a single institution study. Pharmacogenet Genomics. 2018;28(8):189–95. https://doi.org/10.1097/FPC.0000000000000345
82. Sawaki A, Miyazaki K, Yamaguchi M, Takeuchi T, Kobayashi K, Imai H, et al. Genetic polymorphisms and vincristine-induced peripheral neuropathy in patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone therapy. Int J Hematol. 2020;111(5):686–91. https://doi.org/10.1007/s12185-020-02832-x
83. Sims RP. The effect of race on the CYP3A-mediated metabolism of vincristine in pediatric patients with acute lymphoblastic leukemia. J Oncol Pharm Pract. 2016;22(1):76–81. https://doi.org/10.1177/1078155214553143

10

Prognostic and Predictive Biomarkers in Precursor B-cell Acute Lymphoblastic Leukemia

Lei Zhang • Sahibu Sultan M. Habeebu • Weijie Li

Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA

Author for correspondence: Lei Zhang, Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, USA. Email: lzhang@cmh.edu

Cite this chapter as: Zhang L, Habeebu SSM, Li W. Prognostic and Predictive Biomarkers in Precursor B-cell Acute Lymphoblastic Leukemia. In: Li W. editor. *Leukemia*. Brisbane (AU): Exon Publications. Online first 30 Sep 2022.

Doi: https://doi.org/10.36255/exon-publications-leukemia-biomarkers-lymphoblastic-leukemia

Abstract: Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a hematologic malignancy characterized by clonal proliferation of abnormal B-cell precursors in the bone marrow. Most of the B-ALL cases are diagnosed in children, although it can present at any age. Thanks to the tremendous advances in our understanding of its biology, identification of more and more prognostic and predictive biomarkers, and application of individualized risk-adjusted treatment, B-ALL has become the most curable malignancy in children, with a long-term survival rate close to 90% in newly diagnosed patients. However, the prognosis of B-ALL remains dismal in adults and children with relapse. Relapsed B-ALL continues to be the leading cause of cancer-related death in children and young adults. Risk stratification is currently based on age, white blood cell count, early therapeutic response, and chromosomal abnormalities such as ploidy and translocations. Recent advances in molecular diagnostic technologies have led to a rapid expansion of the list of

In: Li W (Editor). *Leukemia*. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA. ISBN: 978-0-6453320-7-0. Doi: https://doi.org/10.36255/exon-publications-leukemia

molecular biomarkers associated with B-ALL, which show promise to improve the accuracy of risk prediction, and eventually achieve better risk-adapted treatment and clinical outcome. In this chapter, we provide an overview of the prognostic and predictive biomarkers in B-ALL, including some recently identified genomic alterations with significant prognostic impact.

Keywords: genetic biomarkers for acute lymphoblastic leukemia; immunophenotypic biomarkers for acute lymphoblastic leukemia; molecular biomarkers for acute lymphoblastic leukemia; prognostic biomarkers in B-ALL; predictive biomarkers in B-ALL

INTRODUCTION

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a hematologic malignancy resulting from clonal proliferation of abnormal B-cell precursors (B-lymphoblasts) in bone marrow (BM). Most B-ALL cases are diagnosed in young children, although it can occur at any age. ALL, composed of approximately 85% B-ALL and 15% T-cell ALL (T-ALL), is the most common malignancy in children, accounting for approximately 30% of all pediatric cancer cases. The estimated number of new B-ALL cases in the USA is close to 5000 each year (1, 2). Thanks to the tremendous advances in our understanding of the biology of this disease, identification of more and more prognostic and predictive biomarkers, risk stratification and risk-adjusted treatment, B-ALL has become the most curable malignancy in children, with a long-term survival rate close to 90%. However, the prognosis of B-ALL remains dismal in adults and pediatric patients with relapse (1, 2). Relapse and chemotherapy related morbidity and mortality remain the big challenges to oncologists, and B-ALL continues to be the leading cause of cancer-related death in children and young adults. Therefore, new prognostic / predictive biomarkers and more accurate risk stratification are needed to further improve individualized treatment and achieve better clinical outcomes.

A biomarker is defined as a characteristic that is measured as an indicator of normal biological processes, pathological processes, or responses to an exposure or therapeutic intervention. Biomarkers could be molecular, morphologic, radiographic, physiological, or phenotypic. Molecular biomarkers are molecules that provide specific information about a given disease, or predisposition to a given disease. Molecular biomarkers are of many types, including characteristic chromosomal numerical or structural alterations, DNA sequence variants including indels, altered RNA, and altered/aberrant or new proteins. Molecular biomarkers play an increasingly important role in risk stratification and risk-adapted therapy of B-ALL.

Risk stratification of B-ALL is currently based on age, white blood cell count (WBC), early therapeutic response, chromosomal abnormalities such as ploidy, and translocations. Recent advances in molecular diagnostic technologies have led to a rapid expansion of the list of genetic biomarkers associated with B-ALL, which help identify new subtypes and show promise to improve the accuracy of risk stratification, and eventually achieve better personalized treatment and clinical outcomes. In this chapter, we provide an overview of the prognostic and

predictive biomarkers in B-ALL, including some recently identified genomic alterations with impact on clinical outcomes. The genetic biomarkers predicting toxicity and resistance to chemotherapy drugs are not included in this chapter; they are described in the chapter of *Novel Aspects of Leukemia Pharmacogenomics* (3).

CLINICAL CLASSIFICATION OF MOLECULAR BIOMARKERS

Based on their role in patient management, molecular biomarkers are classified into three groups: diagnostic biomarkers, prognostic biomarkers, and predictive biomarkers. Diagnostic biomarkers are molecules or molecular alterations that are pathognomonic for their associated diseases or are highly unique to the diseases. The presence of a diagnostic biomarker is essential for the diagnosis of the disease associated with it. Moreover, a diagnostic biomarker can be the initiator of the disease process, e.g., *BCR::ABL1* fusion in *BCR::ABL1* positive B-ALL. Some diagnostic biomarkers are indicators of an increased predisposition to their associated diseases, e.g., germline mutations in *ETV6*, *RUNX1*, *PAX5,* and *IKZF1* in B-ALL.

Prognostic biomarkers provide information about the biology or natural history of their associated diseases. They are essential for the clinical risk stratification of patients for different cancer treatment protocols. There may be many prognostic biomarkers for one malignancy, and one biomarker may be prognostic for more than one disease entity. In addition, a biomarker can be both diagnostic and prognostic. Like diagnostic biomarkers, a prognostic biomarker may also be the initiator of the disease or an indicator of increased predisposition to the disease.

Predictive biomarkers indicate the likelihood that a given cancer will or will not respond to a specific treatment. Positive predictive biomarkers are associated with positive or enhanced response to therapy, while negative predictive biomarkers predict resistance to therapy. Positive predictive biomarkers are often targets for targeted therapies. Some biomarkers are both prognostic and predictive. The common approaches for investigating molecular biomarkers are:

- *Proteins:* Flow cytometry (FCM), immunohistochemistry, and cytochemistry.
- *Chromosome abnormalities:* Karyotyping, and Fluorescence In-Situ Hybridization (FISH).
- *DNA:* PCR, Sanger sequencing, microarray, Multiplex Ligation-dependent Probe Amplification (MLPA), and NGS (single gene, targeted or panel, whole exome, and whole genome).
- *RNA:* RNASeq, Gene expression profiling, Reverse Transcription-PCR.

GENETIC BIOMARKERS

Genetic abnormalities are used as important diagnostic, prognostic, and predictive biomarkers to help early disease detection, risk stratification, and guide treatment. Cytogenetic abnormalities served as the basis for the listed entities in the 2022 5th edition of WHO B-ALL classification (4). However, technological

advances in molecular technology have greatly enhanced our ability to detect driver mutations in B-ALL. Applying technologies such as next generation sequencing (NGS) in the form of whole exome sequencing, whole genome sequencing, transcriptome sequencing (RNA-seq), and deletion-duplication analysis, has led to the description of 23 distinct genetic subtypes of B-ALL (5). Some of these subtypes are very rare. Significant abnormalities are described below and summarized in Figure 1 and Table 1.

Hyperdiploidy

More than 46 chromosomes represent the largest cytogenetic subgroup in childhood B-ALL. It can be subdivided into two groups, high hyperdiploidy (51–65 chromosomes or DNA index (DI) ≥ 1.16) and low hyperdiploidy (47–50 chromosomes or DI 1.0–1.16) with a different prognosis for each group.

High hyperdiploidy (HHyper) is a favorable prognostic factor, presenting in up to 30% of children and 10% of adults with B-ALL (6). Typically, children with HHyper B-ALL achieve negative minimal residual disease (MRD) after induction treatment and have excellent cure rates, with 5-year event-free survival (EFS) and overall survival (OS) rates of ~75%, and ~ 90%, respectively. The most commonly gained chromosomes are 4, 6, 8, 10, 14, 17, 18, 21 and X with the presence of double trisomy (+4, +10) or triple trisomy (+4, +10, +17) being a prognostic factor of a very low risk of relapse (7). Trisomy 18 also has been associated with a favorable prognosis, while gain of extra copies of chromosomes 5 and 20 has been associated with relatively poor prognosis compared with those lacking these trisomies (8).

Although HHyper is generally associated with a favorable prognosis, there are still approximately 20% of HHyper childhood B-ALL cases that relapse. Several co-existent or secondary genetic abnormalities are thought to have an impact on prognosis. The structural abnormality i(17q) and gain of 1q are correlated with poor outcome in some studies (9). A recent study shows evidence that duplication 1q is an independent adverse factor on the disease-free survival (DFS) of HHyper patients (10). The recurrent translocations, t(9;22)(q34;q11), t(12;21)(p13;q22), t(1;19)(q23;p13), and t(4;11)(q21;q23) have also been reported in 1–4% of HHyper B-ALL. In these cases, the prognostic impact of the translocation is believed to override the beneficial effect of the high hyperdiploidy (11). Previous data showed that patients with both t(9;22) and hyperdiploidy have better outcome compared to t(9;22) in a diploid background (10). Also, mutations targeting genes encoding histone modifiers (e.g., *CREBBP*) and the RTK-RAS pathway (e.g., *FLT3*) are common in patients with HHyper. Mutations in these genes as well as *IKZF1* deletion have been detected at a higher incidence in relapsed specimens in comparison with diagnostic B-ALL samples (12, 13).

Low hyperdiploidy (LHyper) is an unfavorable prognostic factor in B-ALL, presenting in 10–11% of pediatric and 10–15% of adult cases with increasing incidence with age (6). The gained chromosomes generally include chromosomes X, 21 and 14. Studies have shown that patients with LHyper have worse OS and relapse-free survival (RFS), a significantly shorter median time to first relapse and less frequently achieve second complete remission than the patients with normal karyotype and miscellaneous abnormalities (14).

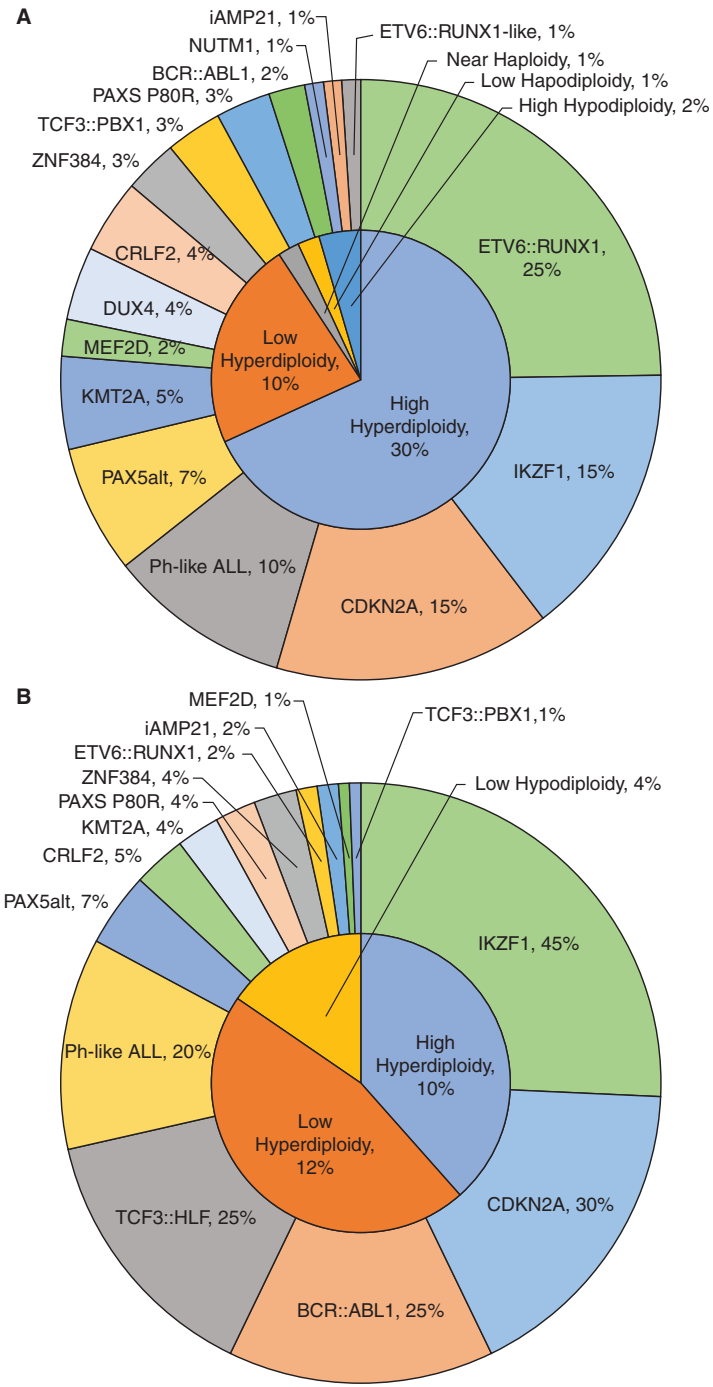

Figure 1. Pie chart of frequencies of the major subgroups of B-ALL. A, Pediatric B-ALL. **B,** Adult B-ALL.

TABLE 1 **Major genetic biomarkers in B-ALL**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
High Hyperdiploidy	N/A	N/A	51–65 chromosomes	Excellent prognosis	N/A	Associated with mutations in genes encoding histone modifiers (e.g., *CREBBP*) and RTK-RAS pathway (e.g., *FLT3*)
Low Hyperdiploidy	N/A	N/A	47–50 chromosomes	Poor prognosis	N/A	-
Near Haploidy	N/A	N/A	24–29 chromosomes	Very Poor prognosis	Bcl2 inhibitors	May be associated with alterations in *NF1*, *NRAS*, *KRAS*, *FLT3*, etc.
Low Hypodiploidy	N/A	N/A	30–39 chromosomes	Very poor prognosis	Bcl2 inhibitors	Associated with *TP53* mutations (>90%)
High hypodiploidy	N/A	N/A	40–44 chromosomes	Poor prognosis	N/A	-
BCR::ABL1	*BCR*: 22q11; *ABL1*: 9q34.12	*ABL1*: Many diverse functions, including antigen receptor signaling in lymphocytes	Translocation	Poor prognosis; improved by TKIs	Positive; responds to ABL1 TKIs	High risk ALL.80% with *IKZF1* deletion
BCR::ABL1-like	N/A	N/A	Gene fusions (Table 2)	Poor prognosis	N/A	High risk ALL
CDKN2A	9p21.3	Cell cycle regulation, tumor suppression	Deletion/ hypermethylation	Poor prognosis	Negative	High risk ALL

(Continued)

TABLE 1 **Major genetic biomarkers in B-ALL (*Continued*)**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
CRLF2	Xp22.33 and Yp11.2	B cell development through activation of the JAK-STAT pathway	Gene fusions/point mutation	Poor prognosis	Negative; trial of JAK TKIs	Standard and high risk ALL
DUX4	4q35.2	*DUX4*: Transcriptional activator	Gene fusions	Good prognosis	N/A	Most frequent partner is *IGH*. CD2 and CD371 positive.
ERG	21q22.2	*ERG*: Transcriptional regulator of hematopoiesis	Deletion	Good prognosis	N/A	Occurs exclusively in the DUX4r subgroup.
ETV6::RUNX1	*ETV6*: 12p13; *RUNX1*: 21q22	Transcriptional regulation	Translocation	Excellent prognosis	N/A	-
ETV6::RUNX1-like	N/A	N/A	Translocation	Poor prognosis	N/A	>80% occur in children. *ETV6* and *IKZF1* alterations
iAMP21	21q	N/A	Amplification	Poor prognosis	N/A	High risk ALL. Associated with RAS signaling pathway gene mutations,
IKZF1	7p12.2	B-cell development regulation	Deletion/point mutation/gene fusion	Poor prognosis	Resistance to ABL1 TKIs	High risk ALL. Older age at diagnosis. Emerging subtype IKZF1plus with very poor prognosis.

(Continued)

TABLE 1 Major genetic biomarkers in B-ALL (*Continued*)

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
KMT2A	11q23	Transcription factor; regulates gene expression during hematopoiesis	Translocation/ inversion	Very poor prognosis	Resistance to glucocorticoids	High risk ALL. CD10 negative, CD15, CD33 and CD68 positive.
MEF2D	1q22	Control of cell growth, survival and apoptosis	Gene fusions	Poor prognosis	Responds to HDAC9 inhibitors	CD10 negative, CD38 bright. *IKZF1* and *CDKN2A/B* deletion
NUTM1	15q14	Role in regulating cell proliferation.	Gene fusions	Excellent prognosis	HDAC inhibitors	Rare; occurs mainly in infants
PAX5 P80R	9p13	*PAX5*: Transcription factor; regulates B-lineage specific genes	Hotspot mutation (PAX5 p.Pro80Arg mutation)	Intermediate prognosis; poor prognosis in children.	N/A	Accompanied by inactivation of the second *PAX5* allele in all cases.
PAX5alt	9p13	Transcription factor; regulates early stages of B cell development	Gene fusions/ deletion/ amplification	Intermediate prognosis; poor prognosis in adults.	N/A	High risk ALL. More than 20 fusion partners including *ETV6* etc.

(Continued)

TABLE 1 **Major genetic biomarkers in B-ALL (*Continued*)**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
TCF3::HLF	*TCF3*: 19p13.3; *HLF*: 17q22	*TCF3*: Lymphopoiesis; required for B and T cell development; *HLF*: Transcriptional activator	Translocation	Very poor prognosis	N/A	High risk ALL. *PAX5* deletion and RAS pathway gene mutations.
TCF3::PBX1	*TCF3*: 19p13.3; *PBX1*: 1q23.3	*TCF3*: See above. *PBX1* regulates many embryonic processes including hematopoiesis.	Translocation	Intermediate to excellent prognosis	N/A	Intermediate risk ALL due to modern therapy. High risk of CNS leukemia and relapse. Expresses cytoplasmic immunoglobulin μ heavy chain
ZNF384	12p12	Transcription factor	Gene fusions	Intermediate prognosis; but varies with different partners	FLT3 inhibitors	CD13 and CD33 positive, negative or weak CD10.
IGH::IL3	*IGH*: 13q32; *IL3*: 5q31.1	*IL3*: cytokine controlling the production and differentiation of hematopoietic progenitor cells	Translocation	Poor prognosis	N/A	Present with reactive hypereosinophilia and lack of peripheral blasts.

Hypodiploidy

Hypodiploidy may be defined as the loss of one or more chromosomes and constitutes ~5% of B-ALL cases across all age groups (6). Hypodiploidy B-ALL is defined by most studies as ≤44 chromosomes and may be further divided into three groups: (i) high-hypodiploid (40–44 chromosomes), (ii) low-hypodiploid (30–39 chromosomes), and (iii) near-haploid (24–29 chromosomes) B-ALL. Near-haploid and low-hypodiploid B-ALL cases display significantly poorer clinical outcomes in comparison with high-hypodiploid pediatric B-ALL (6, 15, 16).

Near-haploid B-ALL presents in approximately 0.5% of pediatric B-ALL and has not been reported in adult ALL. It usually retains disomies for chromosomes 8, 10, 14, 18, 21, X and Y (17). In this subtype, up to 70.6% of patients may harbor mutations in receptor tyrosine kinase (RTK) and RAS pathway genes including *NF1*, *NRAS*, *KRAS*, *FLT3* and *PTPN1*. In addition, deletions of *IKZF3* and histone cluster at chromosome 6p22 are frequently observed. Other frequently detected abnormalities in this subtype include alterations in *CREBBP* and *PAG1*, deletions involving *CDKN2A/B, RB1*, and *PAX5*, and point mutations in *EP300* and *EZH2* (18).

Low hypodiploid B-ALL (LHypo) presents in 0.5% of pediatric patients and approximately 4% of adult patients and its frequency increases with age (19). Retained chromosomes generally include 1, 5, 6, 8, 10, 11, 14, 18, 19, 21, 22, X and Y. The characteristic molecular abnormality in LHypo B-ALL is *TP53* mutation, which is detected in >90% of both pediatric and adult patients in this subtype. Moreover, due to the frequent loss of chromosome 17, *TP53* mutations are found to be homozygous in virtually all LHypo B-ALL. In approximately 50% of pediatric LHypo B-ALL, *TP53* mutations are also found in non-tumor cells indicating germline *TP53* mutation associated with Li-Fraumeni syndrome. In contrast to pediatric cases, *TP53* mutations in LHypo adult B-ALL are somatic and are not found in non-tumor and remission samples (18, 20). In addition to *TP53* mutations, other cryptic cytogenetic or molecular genetic abnormalities frequently found in LHypo B-ALL include *IKZF2/Helios* loss, *RB1* alterations, *CDKN2A/B* alterations, and *CREBBP* mutations (18, 20).

Both near-haploid and low hypodiploid genomes can undergo endoreduplication resulting in doubling of the hypodiploid chromosome complement (Figure 2), which occurs in 60–65% of hypodiploid B-ALL. Often both hypodiploid and hyperdiploid (doubled) clones are present at the same time (16). A doubled clone has a modal chromosome number of 50–78, so-called “masked hypodiploidy”, which may be the only clone observed at diagnosis and may not be differentiated from a high-hyperdiploid or triploid clone cytogenetically (16). As hyperdiploidy with more than 50 chromosomes and hypodiploidy are associated with different prognoses, it is crucial to distinguish between true hyperdiploidy and masked hypodiploid B-ALL. A single nucleotide polymorphism (SNP) array should be performed to detect loss of heterozygosity (LOH) (Figure 2B), which is a very characteristic feature for masked hypodiploidy (19).

ETV6::RUNX1 Fusion – t(12;21)

The *ETV6::RUNX1* fusion (formerly called *TEL::AML1* fusion) is one of the most frequent genetic alterations that initiate B-cell lymphoblastic leukemogenesis. It results from the cytogenetically cryptic translocation t(12;21)(p13.2;q22.1), and

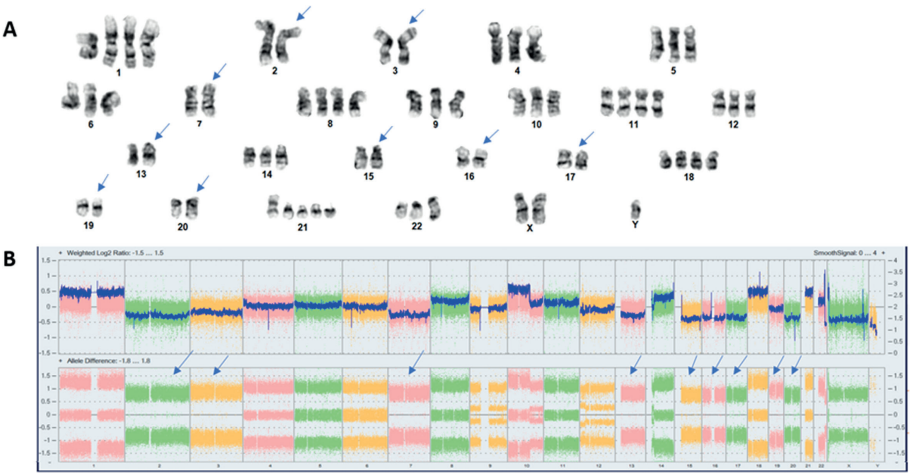

Figure 2. Doubled low hypodiploid B-ALL confirmed by microarray. A, A karyotype with 68 chromosomes which could be high hyperdiploid (good prognosis) or doubled low hypodiploid (poor prognosis). **B,** Microarray shows copy neutral loss of heterozygosity for the chromosomes with two copies (2, 3, 7, 13, 15, 16, 17, 19 and 20) (arrowed) confirming doubled low hypodiploid.

has been reported in 25 – 30% of pediatric B-ALL and 1 – 4% of adult B-ALL (2). *ETV6* is a transcriptional repressor which acts as a tumor suppressor. It is frequently involved in translocations, with at least 41 translocation partners discovered so far (21), with *RUNX1* as the most frequent partner in B-ALL. Despite the high prevalence of *ETV6-RUNX1* fusion in childhood B-ALL, the consensus now is that this fusion alone is unlikely to be responsible for causing overt leukemia, and a postnatal second-hit is required for completion of B-ALL leukemic transformation (22). Numerous secondary abnormalities have been reported in the *ETV6::RUNX1* subgroup with deletion of the non-translocated *ETV6* allele as the most common, followed by deletion of 6q and 9p, amongst others (23). *ETV6::RUNX1* fusion in B-ALL is associated with an excellent prognosis, with OS estimated at 94% at 5 years and 88% at 10 years (6). Recent larger studies have shown that the good prognosis associated with *ETV6::RUNX1* fusion remains even in the presence of additional genetic abnormalities (24).

ETV6::RUNX1 Fusion-Like

ETV6::RUNX1 fusion-like B-ALL shares similar gene expression and immunophenotype profiles (CD27 positive, CD44 low to negative) with *ETV6::RUNX1* fusion B-ALL but lacks the fusion gene (25). More than 80% of *ETV6::RUNX1*-like cases occur in children, accounting for 2–3% of pediatric B-ALL (6). Enriched genetic abnormalities in this subtype include *ETV6* deletion or rearrangement with *IKZF1* or deletion of *ELMO1*, *IKZF1* and *ARPP21*, deletions of histone gene cluster on 6p22.2, *BTG1* aberrations, as well as other chromosome rearrangements such as *TCF3::FLI1* and *FUS::ERG* fusion (5, 25). The prognosis of this subtype is yet to be determined. The average 5-year EFS is 66.7%, thus higher-intensity therapy may be considered for this subtype (26).

BCR::ABL1 Fusion – t(9;22)

BCR::ABL1 fusion presents in 1–3% of pediatric B-ALL and approximately 25% of adult B-ALL with increased frequency with age and is a poor prognostic biomarker (27). The majority of *BCR::ABL1* fusion results from t(9;22)(q34.1;q11.2) [Philadelphia chromosome positive (Ph+)] and gives rise to a constitutively active tyrosine kinase that can activate many pathways including RAS, RAC, PI3K/AKT/mTOR, NF-κB and JAK/STAT. Other genetic abnormalities frequently associated with Ph+ B-ALL include deletions of *IKZF1* (80%), *PAX5* (50%), *CDKN2A/B* (50%) and *EBF1* (14%) (28). Despite recent therapeutic advances, Ph+ ALL is still an adverse subtype with a 4-year EFS of 84% (29).

BCR::ABL1 Fusion-like (Ph-like)

Ph-like ALLs are leukemias characterized by gene expression profiles and phenotypic features similar to those of Ph+ ALL but lack the *BCR::ABL1* fusion. The prevalence varies from ~12% in children to 20 – 27% in adults. Ph-like ALL occurs in all age groups and is associated with poor prognosis, especially due to treatment failure, resulting in high rates of MRD positivity. The 5-year EFS is reported at 59.5%, compared to an average of 84.4% in other B-ALL cases (30).

A wide spectrum of genetic alterations has been described in Ph-like B-ALL cases including translocations, cryptic gene rearrangements, sequence mutations and copy number changes. The majority of these alterations lead to constitutively active kinase or cytokine receptor signaling, and many of them have been shown to be druggable with a variety of TKIs (Table 2) (31, 32). Founder mutations may be classified into four groups:

(i) JAK/STAT alterations including mutations activating cytokine receptors (e.g., *CRLF2*); rearrangements or gene fusions hijacking cytokine receptor expression (e.g., cryptic *EPOR* rearrangements, *IGH::CRLF2* and, *P2RY8::CRLF2*) (Figure 3); gene fusions and/or mutations activating kinases (e.g., *JAK1*) (33, 34). Approximately half of Ph-like ALL cases exhibit deregulated CRFL2 expression, with many of these cases showing alterations in the Janus kinases JAK1 or JAK2 (29).

TABLE 2 **Genetic alterations in Ph-like B-ALL**

Genetic Alterations	Genes Involved	Fusion Partner Genes	Incidence	Targeted Therapy
Deletions of B-cell developmental genes	*IKZF1*	N/A	70–80%	N/A
	PAX5	N/A	30%	N/A

(Continued)

TABLE 2 Genetic alterations in Ph-like B-ALL (*Continued*)

Genetic Alterations	Genes Involved	Fusion Partner Genes	Incidence	Targeted Therapy
Kinase Classes				
JAK-STAT	*CRLF2*	*IGH, P2RY8*	Children, 24.1% Adolescents (16–21 y), 32% Young adults (21–39 y), 14.6% Adults (40–86 y), 11.2%	JAK inhibitors PI3K/mTOR inhibitors
	JAK2	*ATF7IP, BCR, EBF1, ETV6, GOLGA5, HMBOX1, OFD1, PAX5, PCM1, PPFIBP1, RFX3, SMU1, SNX29, SSBP2, STRN3, TERF2, TPR, USP25, ZNF274, ZBTB46,*		JAK inhibitors PI3K/mTOR inhibitors
	EPOR	*IGH, IGK, LAIR1, THADA*		JAK inhibitors
	IL2RB	*MYH9*		JAK inhibitors
	TYK2	*MYB, SMARCA4, ZNF340*		TYK2 inhibitors
	JAK1, JAK3, IL7R, SH2B3	N/A		JAK inhibitors
ABL	*ABL1*	*CENPC, ETV6, FOXP1, LSM14, NUP214, NUP153, RCSD1, ANBP2, SNX2, SFPQ, SPTAN1, ZMIZ1*	Children, 16.7% Adolescents (16–21 y), 9% Young adults (21–39 y), 10.4% Adults (40–86 y), 9.2%	Dasatinib
	ABL2	*PAG1, RCSD1, ZC3HAV1*		Dasatinib
	CSF1R	*MEF2D, SSBP2, TBL1XR1*		Dasatinib
	LYN	NCOR1, GATAD2A		Dasatinib
	PDGFRA	FIP1L1		Dasatinib
	PDGFRB	ATF7IP, EBF1, ETV6, SSBP2, TNIP1, ZEB2, ZMYND8		Dasatinib
RAS pathway	*KRAS, NRAS, PTNP11, CBL1, NF1, BRAF*	N/A	4%	MEK Inhibitors
Rare fusions	*NTRK3*	*ETV6*	Children, 2.8% Adolescents (16–21 y), 3% Young adults (21–39 y), 5.2% Adults (40–86 y), 3.1%	TKI
	FLT3	*ZMYM2*		FLT3 inhibitors
	FGFR1	*BCR*		FGFR inhibitors
	BLNK	*DNIT*		unknown
	PT2KB	*KDM6A, STAG2, TMEM2*		FAK inhibitors

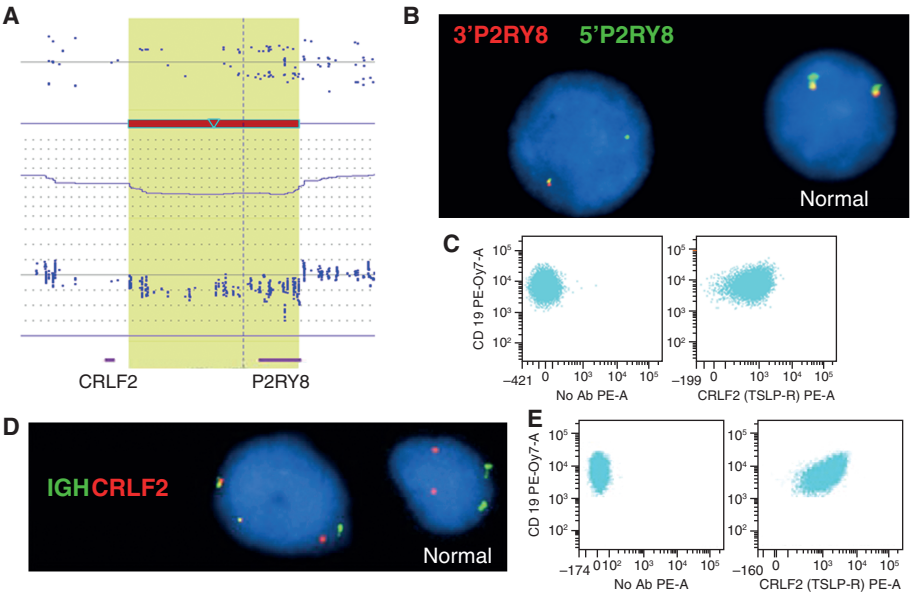

Figure 3. Cytogenetic and flow cytometry findings of B- ALL with *CRLF2* rearrangement. **A,** SNP-Array analysis shows *CRLF2-P2RY8* fusion. **B,** FISH analysis using *P2RY8* break-apart probes demonstrates 3'*P2RY8* deletion. **C,** Flow cytometry study shows CRLF2 expression. **D,** FISH analysis using *IGH* and *CRLF2* fusion probes demonstrates *IGH-CRLF2* fusion. **E,** Flow cytometry study shows CRLF2 expression. A, B and C from one case; D and E from another case.

(ii) fusions involving ABL-class genes (e.g., *ABL1*)
(iii) mutations activating Ras signaling (*NRAS, KRAS, PTPN11*)
(iv) less common fusions (e.g., *FLT3*) with a growing number due to the application of sequencing techniques (33, 34).

Deletions involving B-cell developmental genes are common in Ph-like B-ALL. Similar to Ph+ ALL, *IKZF1* alterations are also a hallmark of Ph-like ALL, occurring in 70–80% of Ph-like B-ALL and conferring a poor prognostic outcome (34). *PAX5* is another gene frequently altered, occurring in ~30% of Ph-Like B-ALL cases. *IKZF1* and *PAX5* alterations often occur together (35).

KMT2A Rearrangements

KMT2A (formerly called *MLL*) gene has more than 90 translocation partners and is rearranged in greater than 80% of infant B-ALL (2) and 4 – 9% of adult B-ALL (29). In B-ALL, the most common partner is *AFF1* (formerly named *AF4*) at 4q21. The *KMT2A::AFF1* fusion [t(4;11)(q21;q23)] (Figure 4) is estimated to be present in 50% of infants with *KMT2A*-rearranged (*KMT2A*-r) B-ALL. The second most common fusion is *KMT2A::MLLT3* (*AF9*) resulting from t(9;11)(p22;q23) followed by *KMT2A::MLLT1* (*ENL*) originating from t(11;19)(q23;p13.3). Fusions with *KMT2A* breakpoint in intron 11 are reported to have a poorer outcome.

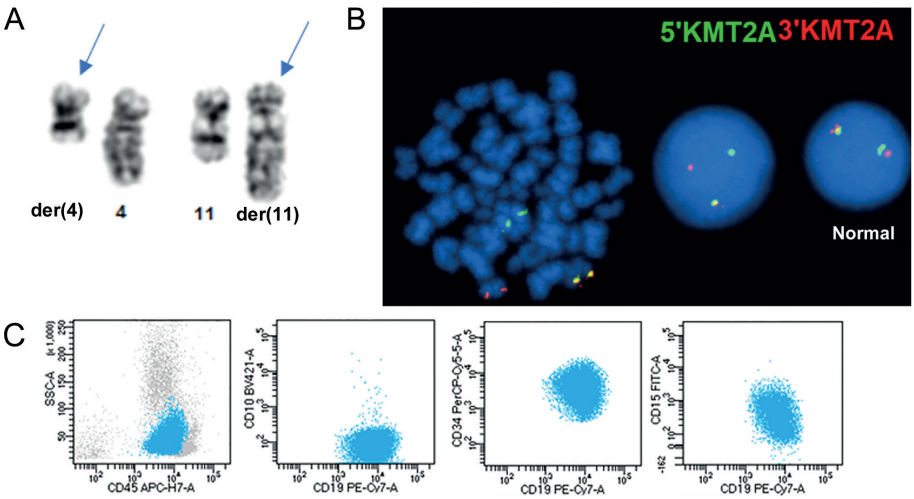

Figure 4. Cytogenetic and flow cytometry findings of B-ALL with *KMT2A* rearrangement. **A,** Conventional cytogenetic study shows t(4;11)(q21;q23). **B,** FISH analysis using *KMT2A* break-apart probes demonstrates *KMT2A* rearrangement. **C,** Flow cytometry study shows Pro-B-ALL immunophenotype and aberrant expression of CD15.

Other major partner genes in infant ALL patients include *MLLT10* (*AF10*), *MLLT6* (*AF17*), and *MLLT4* (*AF6*) (36). The immunophenotype associated with *KMT2A*-r B-ALL includes expression of CD19, lack of CD10, and co-expression of myeloid markers such as CD15, CD33, and CD68 (Figure 4C).

KMT2A-driven leukemias are aggressive and have a very poor prognosis in infants. The estimated 5-year EFS is 30 – 40% (37). Activating mutations in tyrosine kinase-PI3K-RAS signaling pathway components can be detected in 47% of cases *(38)*. The poor prognosis is associated, in some cases, with cooperating mutations in *FLT3, NF1* and *KRAS*. The prognosis is better for children older than 1 year.

TCF3 Rearrangement

TCF3 (also called *E2A*) has two major translocation partners, *PBX1* and *HLF*. *TCF3::PBX1* fusion results from t(1;19)(q23;p13); both the balanced and unbalanced variants of this translocation are found in 6% of pediatric B-ALL and 1 – 3% of adult B-ALL (29). B-ALL patients with *TCF3::PBX1* fusion usually have a pre-B immunophenotype that expresses cytoplasmic immunoglobulin μ heavy chain. It has an intermediate prognosis, reported at a 5-year EFS of 84% (29), the result of considerable improvement due to modern intensive, CNS-directed therapy.

TCF3::HLF fusion results from t(17;19)(q22;p13.3), occurring in less than 1% of childhood B-ALL and rarely in adults (39). *TCF3::HLF* is associated with a particularly poor prognosis (40). There are two types of *TCF3::HLF* fusion with same intron 3 breakpoint in *HLF* gene, but different breakpoints in *TCF3*. Type I rearrangement has breakpoint in intron 13 of *TCF3* associated with disseminated

intravascular coagulation (DIC) while type II has breakpoint in intron 12 of *TCF3* associated with hypercalcemia (41). Other genetic abnormalities, frequently associated with *TCF3::HLF* fusion, include *PAX5* and *VPREB1* deletions and aberrations in the RAS pathway genes (42).

IGH::IL3 Fusion - t(5;14)

IgH::IL3 fusion is seen in less than 1% of B-ALL and represents an aggressive subtype with poor outcomes (43). It results from t(5;14)(q31.1; q32.3) and leads to overexpression of *IL3* as a result of the juxtaposition of *IL3* gene to the potent *IGH* enhancer. These cases are rare and poorly characterized but are observed predominantly in males and the adolescent/young adult (AYA) age group. Patients with this translocation clinically present with reactive hypereosinophilia and lack of peripheral blasts.

Intrachromosomal amplification of chromosome 21 (iAMP21)

iAMP21 presents in approximately 2% of pediatric patients with B-ALL and is associated with worse prognosis when treated with a low-intensity National Cancer Institute (NCI) standard-risk (SR) regimen. It is extremely rare in adult B-ALL, and its prognostic effect in adults is unclear (44). Patients with iAMP21 are usually older children or adolescents with a common/pre-B immunophenotype, and generally have a low WBC. Individuals carrying constitutional Robertsonian translocation der(15;21)(q10;q10) and trisomy 21 have a 2700-fold and 10–12-fold increased risk, respectively, of developing B-ALL with iAMP21 compared to children without these genetic anomalies (6).

The iAMP21 chromosome is a single abnormal chromosome 21 resulting from Breakage–Fusion–Bridge (BFB) cycles followed by chromothripsis, thus containing multiple regions of gain, amplification, inversion, and deletion (Figure 5). It is defined as the amplification of the 5.1-Mb common region containing genes mapping to the Down Syndrome Critical Region (DSCR), *RUNX1* and *miR-802*, with the presence of three or more extra copies of *RUNX1* on a single abnormal chromosome 21 (a total of five or more *RUNX1* signals per cell) by FISH (37). However, a recent study has shown that approximately 9% of iAMP21 B-ALL failed to meet the FISH definition, but amplifications were confirmed by microarray, indicating the importance of incorporation of microarray into testing strategy (45). Common secondary abnormalities in B-ALL with iAMP21 include gain of chromosomes X, 10, or 14; monosomy 7/deletion of 7q; deletions of 11q including the *ATM* and *KMT2A* genes; as well as abnormalities affecting *IKZF1, CDKN2A, PAX5, ETV6*, and *RB1*. More than 60% of iAMP21-ALL patients have a mutation in genes related to the RAS signaling pathway, and 20% of patients have a *P2RY8/CRLF2* gene fusion (46).

Dic(9;20)

The dic(9;20) is a rare aberration seen in 2% of children and 1% of adults with B-ALL. Both favorable and poor prognoses associated with dic(9;20) have been reported. Relapses in these patients are fairly common; however, treatment after

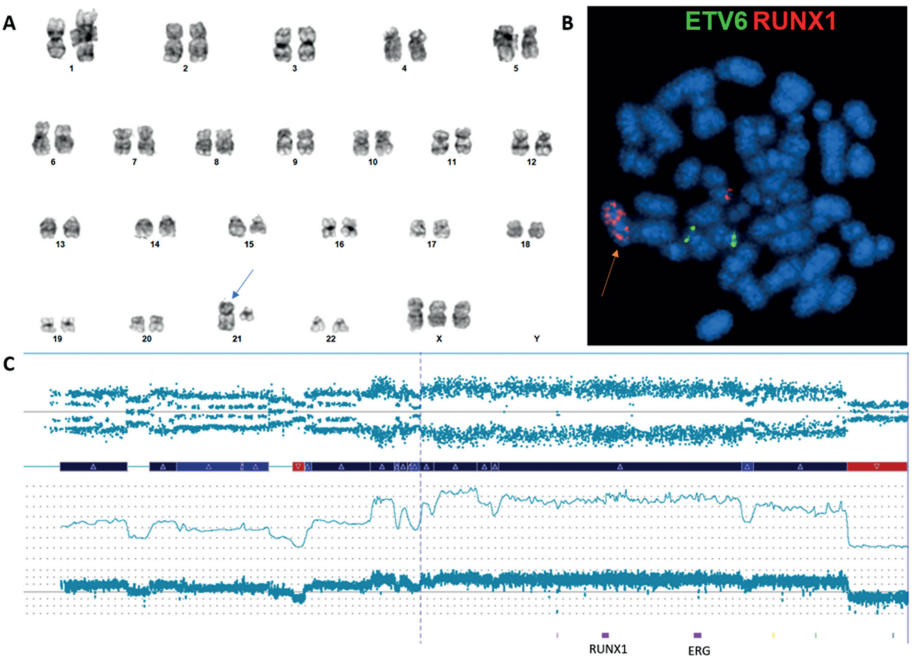

Figure 5. Intrachromosomal amplification of chromosome 21 (iAMP21). A, A big marker chromosome in the position of chromosome 21 with absence of a normal chromosome 21. **B,** Metaphase FISH shows the marker chromosome contains multiple copies of *RUNX1* probes (red) consistent with *RUNX1* amplification. **C,** Microarray shows complex structural abnormalities including amplification, gains, and deletions (across an approximately 11 Mb region).

relapse is often successful. Additional studies are needed to accurately define the prognostic value of dic(9;20) (6). The dic(9;20) arises from the fusion of chromosomes 9p and 20q resulting in the loss of 9p and 20q material, which masquerades as monosomy 20. The breakpoints on 9p target *PAX5*, and the breakpoints on 20q target *ASXL1*.

Other significant gene alterations

CRLF2 deregulation rearrangements, resulting in overexpression of its gene product, thymic stromal lymphopoietin receptor (TSLPR), are seen in ~5% of pediatric B-ALL, ~5% of adult B-ALL, ~50% of Down syndrome-associated B-ALL, and ~50% of Ph-like B-ALL cases (29). *CRLF2* has two main translocation partners, *IGH* and *P2RY8* (Figure 3), and both translocations are associated with poor prognosis. Rarely, activating mutations can also result in *CRLF2* overexpression. The *CRLF2::P2RY8* fusion is caused by interstitial deletions within the pseudoautosomal region (PAR1) located at Xp22.3 or Yp11.3 which bring *CRLF2* to the *P2RY8* promoter. *CRLF2* rearrangements have been associated with *IKZF1* deletion and activation of the JAK-STAT, ERK and mTOR/PI3K pathways with 50% of cases harboring mutations in *JAK* family genes. The concomitance of *CRLF2*

overexpression and *JAK2* mutations is associated with inferior outcomes (47). Although the rearrangements of *CRLF2* are the most common alterations in Ph-like ALL., approximately 5–10% of *CRLF2*-rearranged B-ALL cases are not Ph-like ALL. *P2RY8::CRLF2* fusion is also often a secondary lesion in leukemias with iAMP21, hyperdiploidy, or dic(9;20) (48).

DUX4 rearrangement is a newly identified subtype seen in 4–7% of pediatric B-ALL cases with a slightly higher incidence in AYA patients and rarely seen in adult B-ALL (6). *DUX4*-rearranged B-ALL has a unique immunophenotype (CD2 and CD371 positive) with CD371 expression being pathognomonic of this leukemia (49). The most frequent translocation partner is *IgH*. The fusion results in truncation of the highly conserved C terminus of *DUX4*. This truncated form binds *ERG* (ETS-related gene), coding for a C-terminal ERG protein fragment that is a dominant-negative inhibitor of wild-type ERG function, thus contributing to leukemogenesis (49). Other partners, such as *ERG* and *ZNF384*, have also been reported (25). Interestingly, more than 50% of patients within this group harbor intragenic deletions of *ERG*, and *ERG* deletions occur exclusively in this subgroup.

DUX4 rearrangement is associated with excellent prognosis, with an 8-year EFS and 8-year OS of 86.4% and 95.6%, respectively (50). The presence of *ERG* deletion in these rearrangements is reported to neutralize the bad prognostic effect of *IKZF1* alterations (51).

ZNF384 rearrangement is a new subtype present in 3–5% of pediatric and 3–8% of adult patients with B-ALL. Overall, its prognostic impact is intermediate, but varies with different partner genes. The *EP300::ZNF384* fusion is reported to be associated with a better outcome than *TCF3::ZNF384* fusion. Patients with *ZNF384* fusions share a characteristic immunophenotype of negative or weak CD10 expression and aberrant expression of myeloid antigens CD13/33 (52). More than 10 partner genes have been identified with *EP300, TCF3, TAF15*, and *CREBBP* being the most common. Alterations in *NRAS* and *FLT3* occur in 60% of cases (53). In addition, deletions in lymphoid regulator genes including *LEF1, EBF1, CDKN2A, FBXW7*, and *ETV6* have also been detected in *ZNF384*-rearranged B-ALL.

MEF2D rearrangement is present in 1–4% of pediatric B-ALL (usually older children and adolescents) and 1% of adult patients. It is associated with high WBC and classified as an intermediate to high risk factor. Patients with the *MEF2D* fusion gene have an immunophenotype of low or no CD10 expression and high CD38 expression. The most commonly associated fusion partners are *BCL9* and *HNRNPUL1*. Additional genetic alterations observed in this group include deletions in *IKZF1* and a significantly higher prevalence of *CDKN2A/CDKN2B* deletions (6, 42).

NUTM1 rearrangement is a rare subtype present in 5–7% of all infants B-ALL (21.7% of non-*KMT2A*-rearranged infant cases) and 1% in children, with no report in adults. The current limited data suggests *NUTM1* rearrangement is a favorable prognostic factor in B-ALL. Reported partner genes include *ACIN1, CUX1, BRD9*, and *ZNF618* (54).

IGH rearrangements, although individually rare, are present in approximately 5% of B-ALL cases, forming part of the B-other-ALL subgroup. *IGH* rearrangements are detected in all age groups with peak incidence in AYA. Collectively, they have been associated with an adverse outcome in adults, although they did not

represent an independent prognostic factor in children and adolescents (51, 55). In addition to the previously mentioned *IL3, CRLF2,* and *DUX4*, there are other reported partners, for example, *MYC* and *CEBPA* (56). Most karyotypes are relatively simple and near-diploid, with many having cooperating deletions of *CDKN2A* and/or *PAX5* (51).

IKZF1 alterations are present in approximately 15% of pediatric and 40–50% of adult patients with B-ALL and are highly prevalent in *Ph*+ (~85%) and *Ph*-like (~70%) subtypes (47). It is also over- represented in Down syndrome-ALL and patients with other features of high-risk disease, but rarely detected in cases with *TCF3*-rearrangements (3%) and *ETV6::RUNX1* fusion (3%). It ranges from 15-20% among other subtypes such as hyperdiploid and B-ALL, NOS (6). Various studies have found *IKZF1* aberrations to have a negative prognostic impact, manifesting as resistance to TKI therapy, high level of MRD, poor survival and increased frequency of relapse (57, 58). *IKZF1* plays a key role in hematopoiesis, differentiation, and proliferation of all lymphoid lineages, especially in the activation and development of B cells. Deletions are responsible for up to 90% of cases with *IKZF1* alterations, with the rest being point mutations (59). Deletions of the *IKZF1* gene which result in haploinsufficiency constitute up to 55% of B-ALL with *IKZF1* deletions. Focal exons 4–7 deletions affecting the DNA-binding domain comprise 33% of *IKZF1* deletions, and exert a dominant–negative effect over the unaffected allele, resulting in loss of the tumor suppressor function attributed to wildtype *IKZF1* (60). Exons 4–7 deletions lead to more severe phenotype than haploinsufficiency in B-ALL patients. The adverse prognosis normally associated with *IKZF1* deletion is abrogated by the presence of *ERG* deletions (61). Point mutations resulting in loss-of-function of *IKZF1* are described in up to 10% of *Ph*+ and ~1% of non-*Ph*+ cases without *IKZF1* deletions, with a similar impact on outcome (57). Specifically, *IKZF1* p.Asn159Tyr (N159Y) mutation has been considered as a distinct subtype in B-ALL with unique expression profile. In addition, an increasing number of cases with fusion transcripts involving *IKZF1* have been described (*IKZF1::PRDM16, IKZF1::NUMT1, IKZF1::ETV6, IKZF1::CDK2, IKZF1::ZEB2, IKZF1::SETD5, IKZF1::STIM2*) (5). A new group of B-ALL, called *IKZF1*plus, has been defined recently. *IKZF1*plus is characterized by co-existence of *IKZF1* deletions with deletions of *CDKN2A, CDKN2B* or *PAX5* or the PAR1 region in the absence of *ERG* deletion. This group confers the most unfavorable outcome in MRD-positive patients with childhood B-ALL (62).

PAX5 alterations are present in ~30% of B-ALL cases (63). Deletion and mutations have been considered to be secondary events because they are present in many subtypes of B-ALL. However, two categories of distinct alterations in the *PAX5* gene have been identified as drivers: the *PAX5* p.Pro80Arg (PAX5 P80R) point mutation and the *PAX5*-altered (PAX5alt), which have different gene expression profiles and are now considered distinct genetic subtypes (5). The PAX5 P80R subtype presents in 3–4% of pediatric and 4% of adults B-ALL and is associated with intermediate prognosis. The PAX5 P80R mutation is accompanied by inactivation of the second PAX5 allele (biallelic events) through deletion or a second mutation (homozygous or compound heterozygous) or loss of heterozygosity, in all reported cases. This mutation also frequently co-occurs with biallelic *CDKN2A/B* deletion and mutations in the RAS or JAK-STAT pathways as well as *FLT3, BRAF* and *PIK3CA* (5, 64). The PAX5alt subtype is present in 7.4% of B-ALL and confers an intermediate prognosis in children treated with intensive

chemotherapy, but poor prognosis in adults (5). This subtype contains diverse *PAX5* alterations, including rearrangements with partner genes, sequence mutations and focal/intragenic amplifications. More than 20 fusion partners have been reported with *ETV6* being the most common. The PAX5alt subtype commonly has codeletion of the *IKZF1* and *CDKN2A/B* genes, giving rise to the poorer outcome of *IKZF1*plus subtype (62).

CDKN2A deletions are found in 15–35% of children and 30–45% of adults with B-ALL, and commonly involve both *CDKN2A* and *CDKN2B* as well as *PAX5* due to their co-location on chromosome 9p. The deletions are more frequently found in *Ph+* and *Ph*-like ALL than in *ETV6::RUNX1* and hyperdiploid ALL (48). *CDKN2A/B* losses have been reported to be associated with an inferior outcome in adults, but do not appear to affect the outcome in pediatric B-ALL (65).

MYC, BCL2 and/or *BCL6* rearrangements are known to be associated with B-cell non-Hodgkin's lymphomas. In B-ALL, translocations of *MYC, BCL2* and/or *BCL6* with immunoglobulin genes (*IGH/ IGL/IGK*) form a rare subgroup, which is seen predominantly in adults and is associated with a poor prognosis (66).

RB1, BTG1, EBF1 and other genes involved in cell cycle control, lymphoid development, signaling, or tumor suppressor genes, are frequently altered in B-ALL. *RB1* gene deletions are present in 2–4% of children with B-ALL. It is frequently deleted (39%) in children with iAMP21 and low hypodiploidy. *BTG1* gene deletions are seen in up to 10% of children with B-ALL and are known to be clustered with *ETV6::RUNX1* fusion (15%). *EBF1* deletions were present in 6% of B-ALL and were enriched in *Ph*-like cases (15%), but absent in *KMT2A*-rearranged and *TCF3::PBX1* fusion cases. Patients carrying *EBF1* deletion tend to have higher MRD levels compared with the cases without *EBF1* deletion. Other genes, which have been reported to be deleted in B-ALL, include *ABL1, CASP8AP2, CD200/ BTLA, MLLT3, IKZF2, NF1, PHF6, PTEN, PTPN2, TBL1XR1, TP53*, and *VPREB1*. Deletions in *VPREB1, RB1, IKZF2,* and *TBL1XR1* can be biallelic (60).

IMMUNOPHENOTYPIC BIOMARKERS

As above-mentioned, genetic biomarkers are critically important for prognostic prediction and WHO classification of B-ALL. The prognostic value provided by immunophenotyping is most likely attributed to its prediction of certain cytogenetic/molecular subtypes. B-ALL can be subclassified as pro-B-ALL (CD10–), common ALL (CD10+) and pre-B-ALL (cytoplasmic IgM+) based on the developmental stage demonstrated by immunophenotyping (67). Pro-B-ALL is commonly seen in B-ALL with *KMT2A* rearrangement, which is also commonly positive for CD15 or other myeloid markers (Figure 4 C). Pre-B-ALL is commonly seen in B-ALLs with *TCF3::PBX1* and *MEF2D* fusions (68). Surface CD371 expression is specifically associated with *DUX4* rearrangement. CD25 expression is commonly seen in Ph+ B-ALL. Expression of *CRLF2* gene product (TSLPR) assessed by FCM (Figure 3, C and E) can serve as a screening test for *CRLF2* gene rearrangement and the identification of Ph-like B-ALL (69). Approximately 80% of our CRLF2+ cases identified by FCM had *CRLF2* gene rearrangements identified by cytogenetic study and/or next generation sequencing (data not published). Besides these

associations, some antigens have been studied and showed data indicating their roles as independent prognostic biomarkers. In addition, some leukemia cell surface antigens are the targets of recently developed immunotherapies and are important predictors of the response to these treatments.

CD45

CD45, also known as leukocyte common antigen, is a receptor type protein tyrosine phosphatase expressed in all leukocytes and most hematopoietic precursors. Most B-ALL cases express low level CD45. There have been a few studies demonstrating the adverse prognostic impact of high CD45 expression on pediatric ALL (70–72). The early Pediatric Oncology Group (POG) study on 1231 pediatric B-ALL cases showed that high CD45 intensity (>75th percentile) was associated with a worse EFS, and the association was not related to other known poor prognostic factors such as NCI risk group, ploidy, and unfavorable translocations (72). A Japanese study conducted several years later confirmed the negative impact of high CD45 intensity on EFS only in the NCI HR pediatric B-ALL patients (70). Later, a German team also found a shorter EFS in high-CD45 group due to a higher cumulative incidence of relapse (CIR). And they also found that high CD45 expression was associated with NCI HR, presence of unfavorable genetic biomarkers, and poor prednisone response (71).

CD20

CD20 is a B-lymphocyte-specific membrane protein, which plays a role in B-cell development, differentiation, and activation. It is expressed by most B lymphocytes including intermediate and late-stage B-cell precursors, naïve B cells and memory B cells. Approximately 40–50% of B-ALL cases show CD20 expression, which is often dim and variable. The prognostic impact of CD20 on pediatric B-ALL is contradictory among different studies (72, 73), which questions the role of CD20 as a prognostic biomarker in pediatric B-ALL. In contrast, the studies in adult B-ALL patients consistently demonstrated that CD20 positivity was generally associated with an inferior outcome (74, 75). As a commonly used target by immunotherapy for B-cell malignancy, the expression of CD20 in adult B-ALL provides a therapeutic option for using Rituximab (anti-CD20 monoclonal antibody). A randomized clinical study has demonstrated that the addition of Rituximab to the B-ALL-chemotherapy protocol can improve the outcome for younger adults with CD20+ Ph-negative B-ALL (76).

Antigens targeted by immunotherapy

Novel therapies such as monoclonal antibodies or chimeric antigen receptor transfused T cells (CART) have shown significant promise in the management of relapsed/refractory B- ALL (1, 2). The most common targeted surface antigens for B-ALL treatment are CD19 and CD22. The expression of these antigens in leukemic cells is a prerequisite for the success of these treatments. Moreover, the expression level or intensity of these antigens may also predict the therapeutic response.

CD19 is a diagnostic biomarker for B-ALL and is present in greater than 90% of all cases. Blinatumomab is a bispecific T cell engager (BiTE) monoclonal antibody directed against CD19 and CD3. By binding to CD19+ leukemic cells and CD3+ T cells simultaneously, it induces antibody-dependent cellular cytotoxicity against the leukemic cells, thereby eliminating the CD19+ blasts. The efficacy of blinatumomab in relapsed/refractory B-ALL is well established, with superiority over high dose chemotherapy in children and young adults. Tisagenlecleucel (Kymriah), a CD19 CART cell treatment, is the most common CART cell therapy in B-ALL. It has shown remarkable efficacy for relapsed/refractory B-ALL in children and adults, including relapsed CNS disease. Antigen density was demonstrated as a major factor influencing the activity of CART cells, and low surface CD19 density pre-CART-19 treatment was associated with poor response (77, 78).

CD22 is an inhibitory B-cell co-receptor, which is positive in more than 90% of B-ALL cases. Inotuzumab ozogamicin (IO) is a monoclonal anti-CD22 antibody attached to calicheamicin. Initial phase 2 and phase 3 studies have demonstrated the superiority of IO over standard chemotherapy in relapsed/refractory B-ALL patients (79). CD22 CART therapy and dual CD19/CD22 CART therapy are also at different stages of clinical development.

Others

CD36 is a membrane glycoprotein present on monocytes, macrophages, platelets, erythroblasts, adipocytes, and some epithelia. It is a scavenger receptor involved in many physiologic functions. A one-center retrospective study has shown the negative impact of CD36 expression on the outcome of pediatric B-ALL cases. In this study, 5-year EFS and OS of the NCI-SR patients were significantly worse in CD36+ group compared with CD36- group (80). CD34 is a transmembrane glycoprotein expressed on lymphohematopoietic progenitor cells. A large proportion of B-ALL cases demonstrate CD34 expression, which is commonly partial or variable. A recently published study (81) demonstrated high CD34 expression as a predictor of poor induction therapy response. The CD34+ cases were approximately 6.5 times more likely to have a positive MRD result at the end of induction compared with CD34- cases. Further studies with increased number of patients are needed to confirm these results.

RESPONSE TO CHEMOTHERAPY AND MEASURABLE RESIDUAL DISEASE

Early response to therapy is an independent prognostic factor in pediatric B-ALL, and patients with a slower early response are more likely to have an adverse event than patients with a more rapid early response. It has been traditionally assessed by morphologic evaluation of BM and peripheral blood (PB). The response to induction treatment has been categorized based on lymphoblast count in BM: M1, <5%; M2, 5 to <25%; and M3, ≥25%. Complete remission (CR) is defined as M1 BM at the end of induction, absence of leukemic blasts in PB and cerebrospinal

fluid (CSF), and no evidence of local disease. Although the vast majority of pediatric B-ALL cases achieve CR based on current treatment protocols, a significant proportion of the cases will relapse. Clearly, it is not enough to use therapeutic response for risk prediction based on morphology alone.

Measurable residual disease (MRD), also known as minimal residual disease, refers to the presence of a small number of malignant cells in leukemia patients during or after treatment. MRD is under the detection limit of morphology, and is usually detected by FCM and/or molecular methods. MRD is an in-depth measure of the therapeutic response, and it has been demonstrated as the strongest independent predictor of relapse and survival outcome. The patients with undetectable MRD or good MRD response consistently demonstrate a lower risk of relapse and better survival outcomes compared with similarly treated patients with positive MRD or poor MRD response. Please see the MRD chapter for more information (82).

OTHER BIOMARKERS

Initial WBC, a measure of extramedullary leukemia burden, has been one of the historical strongest risk factors. It has been used for NCI risk stratification (83) in pediatric B-ALL. The two groups of patients with NCI-SR (WBC <50,00/uL and age 1–9 years) or NCI-HR (all others) had significantly different 4-year EFS (80% vs. 60%) (83). NCI risk stratification is currently still in use to guide risk-adapted treatment for pediatric B-ALL. A study involving 2666 ALL patients from five Nordic countries showed that WBC was not associated with the risk of an event for B-ALL or T-ALL in patients with day 29 MRD (MRDd29) <0.1%. In contrast, for patients with MRDd29 ≥ 0.1% and <5%, the 5-year EFS for patients with WBC ≥ 100,000/uL was significantly shorter than that of patients with WBC <100,000/uL (84). Other blood cell counts such as platelet and neutrophil counts may also have some prognostic value in certain patient groups, however, their independent prognostic values are in question and should be further studied.

Initial CSF finding (CNS involvement) has also been found to have an impact on the outcome of pediatric B-ALL patients. A COG study published in 2017 demonstrated significantly lower 5-year EFS and OS rates in the CNS2 group (76% and 86.8%) and CNS3 group (76% and 82.1%) than those in the CNS1 (no blasts) group (85% and 92.7%), regardless of NCI risks (85). These findings indicate that the presence of blasts in CSF, regardless of CSF cell count, is an independent predictor of adverse outcome in pediatric B-ALL patients.

CONCLUSION

There have been many prognostic and predictive biomarkers identified in B-ALL. With these biomarkers, B-ALL cases can be categorized into a risk group and treated accordingly. This risk-adapted treatment has led to the current very high curable rate in de novo pediatric ALL cases. However, a significant proportion of B-ALL cases will relapse, and the prognosis of relapsed cases is dismal.

Recent advances in sequencing technology and integrated analyses of large-scale data have allowed the discovery of many new genetic biomarkers, which show promise to improve the accuracy of risk stratification, identify new genetic/molecular defects associated with potential therapeutic targets, and eventually improve the overall clinical outcome.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

Copyright and Permission Statement: The authors confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder(s), and all original sources have been appropriately acknowledged or referenced.

REFERENCES

1. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017;7(6):e577. https://doi.org/10.1038/bcj.2017.53
2. Malard F, Mohty M. Acute lymphoblastic leukaemia. Lancet. 2020;395(10230):1146–62. https://doi.org/10.1016/S0140-6736(19)33018-1
3. Escalante-Bautista D, Haydeé RV, Cerecedo D. Novel Aspects of Leukemia Pharmacogenomics. In: Li W. editor. Leukemia. Brisbane (AU): Exon Publications. https://doi.org/10.36255/exon-publications-leukemia-pharmacogenomics
4. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720–48. https://doi.org/10.1038/s41375-022-01620-2
5. Gu Z, Churchman ML, Roberts KG, Moore I, Zhou X, Nakitandwe J, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. Nat Genet. 2019;51(2):296–307. https://doi.org/10.1038/s41588-018-0315-5
6. Lejman M, Chalupnik A, Chilimoniuk Z, Dobosz M. Genetic Biomarkers and Their Clinical Implications in B-Cell Acute Lymphoblastic Leukemia in Children. Int J Mol Sci. 2022;23(5). https://doi.org/10.3390/ijms23052755
7. Sutcliffe MJ, Shuster JJ, Sather HN, Camitta BM, Pullen J, Schultz KR, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. Leukemia. 2005;19(5):734–40. https://doi.org/10.1038/sj.leu.2403673
8. Ramos-Muntada M, Trincado JL, Blanco J, Bueno C, Rodriguez-Cortez VC, Bataller A, et al. Clonal heterogeneity and rates of specific chromosome gains are risk predictors in childhood high-hyperdiploid B-cell acute lymphoblastic leukemia. Mol Oncol. 2022. https://doi.org/10.1002/1878-0261.13276/v2/response1
9. Pui CH, Raimondi SC, Williams DL. Isochromosome 17q in childhood acute lymphoblastic leukemia: an adverse cytogenetic feature in association with hyperdiploidy? Leukemia. 1988;2(4):222–5.
10. El Ashry MS, Elsayed GM, Madney Y, Arafah O, Allam RM, Rasekh EO. Duplication 1q is highly correlated with poor prognosis in high hyperdiploid pediatric B-acute lymphoblastic leukemia. Int J Lab Hematol. 2021;43(2):235–43. https://doi.org/10.1111/ijlh.13369
11. Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2009;48(8):637–60. https://doi.org/10.1002/gcc.20671
12. Chen C, Bartenhagen C, Gombert M, Okpanyi V, Binder V, Rottgers S, et al. Next-generation-sequencing of recurrent childhood high hyperdiploid acute lymphoblastic leukemia reveals

mutations typically associated with high risk patients. Leuk Res. 2015;39(9):990–1001. https://doi.org/10.1016/j.leukres.2015.06.005
13. Studd JB, Cornish AJ, Hoang PH, Law P, Kinnersley B, Houlston R. Cancer drivers and clonal dynamics in acute lymphoblastic leukaemia subtypes. Blood Cancer J. 2021;11(11):177. https://doi.org/10.1038/s41408-021-00570-9
14. Groeneveld-Krentz S, Schroeder MP, Reiter M, Pogodzinski MJ, Pimentel-Gutierrez HJ, Vagkopoulou R, et al. Aneuploidy in children with relapsed B-cell precursor acute lymphoblastic leukaemia: clinical importance of detecting a hypodiploid origin of relapse. Br J Haematol. 2019;185(2):266–83. https://doi.org/10.1111/bjh.15770
15. Pui CH, Rebora P, Schrappe M, Attarbaschi A, Baruchel A, Basso G, et al. Outcome of Children With Hypodiploid Acute Lymphoblastic Leukemia: A Retrospective Multinational Study. J Clin Oncol. 2019;37(10):770–9. https://doi.org/10.1200/JCO.18.00822
16. Harrison CJ, Moorman AV, Broadfield ZJ, Cheung KL, Harris RL, Reza Jalali G, et al. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. Br J Haematol. 2004;125(5):552–9. https://doi.org/10.1111/j.1365-2141.2004.04948.x
17. Molina O, Bataller A, Thampi N, Ribera J, Granada I, Velasco P, et al. Near-Haploidy and Low-Hypodiploidy in B-Cell Acute Lymphoblastic Leukemia: When Less Is Too Much. Cancers (Basel). 2021;14(1). https://doi.org/10.3390/cancers14010032
18. Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45(3):242–52. https://doi.org/10.1038/ng.2532
19. Creasey T, Enshaei A, Nebral K, Schwab C, Watts K, Cuthbert G, et al. Single nucleotide polymorphism array-based signature of low hypodiploidy in acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2021;60(9):604–15. https://doi.org/10.1002/gcc.22956
20. Muhlbacher V, Zenger M, Schnittger S, Weissmann S, Kunze F, Kohlmann A, et al. Acute lymphoblastic leukemia with low hypodiploid/near triploid karyotype is a specific clinical entity and exhibits a very high TP53 mutation frequency of 93%. Genes Chromosomes Cancer. 2014;53(6):524–36. https://doi.org/10.1002/gcc.22163
21. Odero MD, Carlson K, Calasanz MJ, Lahortiga I, Chinwalla V, Rowley JD. Identification of new translocations involving ETV6 in hematologic malignancies by fluorescence in situ hybridization and spectral karyotyping. Genes Chromosomes Cancer. 2001;31(2):134–42. https://doi.org/10.1002/gcc.1127
22. Schafer D, Olsen M, Lahnemann D, Stanulla M, Slany R, Schmiegelow K, et al. Five percent of healthy newborns have an ETV6-RUNX1 fusion as revealed by DNA-based GIPFEL screening. Blood. 2018;131(7):821–6. https://doi.org/10.1182/blood-2017-09-808402
23. Sun C, Chang L, Zhu X. Pathogenesis of ETV6/RUNX1-positive childhood acute lymphoblastic leukemia and mechanisms underlying its relapse. Oncotarget. 2017;8(21):35445–59. https://doi.org/10.18632/oncotarget.16367
24. Enshaei A, Schwab CJ, Konn ZJ, Mitchell CD, Kinsey SE, Wade R, et al. Long-term follow-up of ETV6-RUNX1 ALL reveals that NCI risk, rather than secondary genetic abnormalities, is the key risk factor. Leukemia. 2013;27(11):2256–9. https://doi.org/10.1038/leu.2013.136
25. Lilljebjorn H, Henningsson R, Hyrenius-Wittsten A, Olsson L, Orsmark-Pietras C, von Palffy S, et al. Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukaemia. Nat Commun. 2016;7:11790. https://doi.org/10.1038/ncomms11790
26. Lee SHR, Li Z, Tai ST, Oh BLZ, Yeoh AEJ. Genetic Alterations in Childhood Acute Lymphoblastic Leukemia: Interactions with Clinical Features and Treatment Response. Cancers (Basel). 2021;13(16). https://doi.org/10.3390/cancers13164068
27. Kurzrock R, Kantarjian HM, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Ann Intern Med. 2003;138(10):819–30. https://doi.org/10.7326/0003-4819-138-10-200305200-00010
28. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453(7191):110–4. https://doi.org/10.1038/nature06866

29. Ghazavi F, Lammens T, Van Roy N, Poppe B, Speleman F, Benoit Y, et al. Molecular basis and clinical significance of genetic aberrations in B-cell precursor acute lymphoblastic leukemia. Exp Hematol. 2015;43(8):640–53. https://doi.org/10.1016/j.exphem.2015.05.015
30. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10(2):125–34. https://doi.org/10.1016/S1470-2045(08)70339-5
31. Plotka A, Lewandowski K. BCR/ABL1-Like Acute Lymphoblastic Leukemia: From Diagnostic Approaches to Molecularly Targeted Therapy. Acta Haematol. 2022;145(2):122–31. https://doi.org/10.1159/000519782
32. Tasian SK, Loh ML, Hunger SP. Philadelphia chromosome-like acute lymphoblastic leukemia. Blood. 2017;130(19):2064–72. https://doi.org/10.1182/blood-2017-06-743252
33. Yadav V, Ganesan P, Veeramani R, Kumar VD. Philadelphia-Like Acute Lymphoblastic Leukemia: A Systematic Review. Clin Lymphoma Myeloma Leuk. 2021;21(1):e57-e65. https://doi.org/10.1016/j.clml.2020.08.011
34. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15. https://doi.org/10.1056/NEJMoa1403088
35. Ou Z, Sherer M, Casey J, Bakos HA, Vitullo K, Hu J, et al. The Genomic Landscape of PAX5, IKZF1, and CDKN2A/B Alterations in B-Cell Precursor Acute Lymphoblastic Leukemia. Cytogenet Genome Res. 2016;150(3-4):242–52. https://doi.org/10.1159/000456572
36. Emerenciano M, Meyer C, Mansur MB, Marschalek R, Pombo-de-Oliveira MS, Brazilian Collaborative Study Group of Infant Acute L. The distribution of MLL breakpoints correlates with outcome in infant acute leukaemia. Br J Haematol. 2013;161(2):224–36. https://doi.org/10.1111/bjh.12250
37. Zou X, Calame K. Signaling pathways activated by oncogenic forms of Abl tyrosine kinase. The Journal of biological chemistry. 1999;274(26):18141–4. https://doi.org/10.1074/jbc.274.26.18141
38. Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330–7. https://doi.org/10.1038/ng.3230
39. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405. https://doi.org/10.1182/blood-2016-03-643544
40. Fischer U, Forster M, Rinaldi A, Risch T, Sungalee S, Warnatz HJ, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Nat Genet. 2015;47(9):1020–9. https://doi.org/10.1038/ng.3362
41. Hunger SP, Devaraj PE, Foroni L, Secker-Walker LM, Cleary ML. Two types of genomic rearrangements create alternative E2A-HLF fusion proteins in t(17;19)-ALL. Blood. 1994;83(10):2970–7. https://doi.org/10.1182/blood.V83.10.2970.2970
42. Inaba H, Pui CH. Advances in the Diagnosis and Treatment of Pediatric Acute Lymphoblastic Leukemia. J Clin Med. 2021;10(9). https://doi.org/10.3390/jcm10091926
43. McClure BJ, Heatley SL, Rehn J, Breen J, Sutton R, Hughes TP, et al. High-risk B-cell acute lymphoblastic leukaemia presenting with hypereosinophilia and acquiring a novel PAX5 fusion on relapse. Br J Haematol. 2020;191(2):301–4. https://doi.org/10.1111/bjh.17002
44. Moorman AV. New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. Haematologica. 2016;101(4):407–16. https://doi.org/10.3324/haematol.2015.141101
45. Koleilat A, Smadbeck JB, Zepeda-Mendoza CJ, Williamson CM, Pitel BA, Golden CL, et al. Characterization of unusual iAMP21 B-lymphoblastic leukemia (iAMP21-ALL) from the Mayo Clinic and Children's Oncology Group. Genes Chromosomes Cancer. 2022.
46. Harrison CJ. Blood Spotlight on iAMP21 acute lymphoblastic leukemia (ALL), a high-risk pediatric disease. Blood. 2015;125(9):1383–6. https://doi.org/10.1182/blood-2014-08-569228
47. Yeung DTO, Osborn MP, White DL. B-cell acute lymphoblastic leukaemia: recent discoveries in molecular pathology, their prognostic significance, and a review of the current classification. Br J Haematol. 2022;197(1):13–27. https://doi.org/10.1111/bjh.17879

48. Schwab CJ, Chilton L, Morrison H, Jones L, Al-Shehhi H, Erhorn A, et al. Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. Haematologica. 2013;98(7):1081–8. https://doi.org/10.3324/haematol.2013.085175
49. Schinnerl D, Mejstrikova E, Schumich A, Zaliova M, Fortschegger K, Nebral K, et al. CD371 cell surface expression: a unique feature of DUX4-rearranged acute lymphoblastic leukemia. Haematologica. 2019;104(8):e352-e5. https://doi.org/10.3324/haematol.2018.214353
50. Aberuyi N, Rahgozar S, Ghodousi ES, Ghaedi K. Drug Resistance Biomarkers and Their Clinical Applications in Childhood Acute Lymphoblastic Leukemia. Front Oncol. 2019;9:1496. https://doi.org/10.3389/fonc.2019.01496
51. Schwab C, Harrison CJ. Advances in B-cell Precursor Acute Lymphoblastic Leukemia Genomics. Hemasphere. 2018;2(4):e53. https://doi.org/10.1097/HS9.0000000000000053
52. Hirabayashi S, Ohki K, Nakabayashi K, Ichikawa H, Momozawa Y, Okamura K, et al. ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. Haematologica. 2017;102(1):118–29. https://doi.org/10.3324/haematol.2016.151035
53. Li J, Dai Y, Wu L, Zhang M, Ouyang W, Huang J, et al. Emerging molecular subtypes and therapeutic targets in B-cell precursor acute lymphoblastic leukemia. Front Med. 2021;15(3):347–71. https://doi.org/10.1007/s11684-020-0821-6
54. Boer JM, Valsecchi MG, Hormann FM, Antic Z, Zaliova M, Schwab C, et al. Favorable outcome of NUTM1-rearranged infant and pediatric B cell precursor acute lymphoblastic leukemia in a collaborative international study. Leukemia. 2021;35(10):2978–82. https://doi.org/10.1038/s41375-021-01333-y
55. Russell LJ, Enshaei A, Jones L, Erhorn A, Masic D, Bentley H, et al. IGH@ translocations are prevalent in teenagers and young adults with acute lymphoblastic leukemia and are associated with a poor outcome. J Clin Oncol. 2014;32(14):1453–62. https://doi.org/10.1200/JCO.2013.51.3242
56. Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R, et al. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007;109(8):3451–61. https://doi.org/10.1182/blood-2006-08-041012
57. Stanulla M, Cave H, Moorman AV. IKZF1 deletions in pediatric acute lymphoblastic leukemia: still a poor prognostic marker? Blood. 2020;135(4):252–60. https://doi.org/10.1182/blood.2019000813
58. Marke R, van Leeuwen FN, Scheijen B. The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia. Haematologica. 2018;103(4):565–74. https://doi.org/10.3324/haematol.2017.185603
59. Mullighan CG. The molecular genetic makeup of acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2012;2012:389–96. https://doi.org/10.1182/asheducation.V2012.1.389.3798360
60. Agarwal M, Seth R, Chatterjee T. Recent Advances in Molecular Diagnosis and Prognosis of Childhood B Cell Lineage Acute Lymphoblastic Leukemia (B-ALL). Indian J Hematol Blood Transfus. 2021;37(1):10–20. https://doi.org/10.1007/s12288-020-01295-8
61. Zaliova M, Potuckova E, Hovorkova L, Musilova A, Winkowska L, Fiser K, et al. ERG deletions in childhood acute lymphoblastic leukemia with DUX4 rearrangements are mostly polyclonal, prognostically relevant and their detection rate strongly depends on screening method sensitivity. Haematologica. 2019;104(7):1407–16. https://doi.org/10.3324/haematol.2018.204487
62. Stanulla M, Dagdan E, Zaliova M, Moricke A, Palmi C, Cazzaniga G, et al. IKZF1(plus) Defines a New Minimal Residual Disease-Dependent Very-Poor Prognostic Profile in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia. J Clin Oncol. 2018;36(12):1240–9. https://doi.org/10.1200/JCO.2017.74.3617
63. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446(7137):758–64. https://doi.org/10.1038/nature05690
64. Bastian L, Schroeder MP, Eckert C, Schlee C, Tanchez JO, Kampf S, et al. PAX5 biallelic genomic alterations define a novel subgroup of B-cell precursor acute lymphoblastic leukemia. Leukemia. 2019;33(8):1895–909. https://doi.org/10.1038/s41375-019-0430-z

65. Gonzalez-Gil C, Ribera J, Ribera JM, Genesca E. The Yin and Yang-Like Clinical Implications of the CDKN2A/ARF/CDKN2B Gene Cluster in Acute Lymphoblastic Leukemia. Genes (Basel). 2021;12(1). https://doi.org/10.3390/genes12010079
66. Stratmann JA, von Rose AB, Koschade S, Wendelin K, Kohler F, Heinsch M, et al. Clinical and genetic characterization of de novo double-hit B cell precursor leukemia/lymphoma. Ann Hematol. 2019;98(3):647–56. https://doi.org/10.1007/s00277-018-03590-x
67. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
68. Ohki K, Takahashi H, Fukushima T, Nanmoku T, Kusano S, Mori M, et al. Impact of immunophenotypic characteristics on genetic subgrouping in childhood acute lymphoblastic leukemia: Tokyo Children's Cancer Study Group (TCCSG) study L04–16. Genes Chromosomes Cancer. 2020;59(10):551–61. https://doi.org/10.1002/gcc.22858
69. Konoplev S, Lu X, Konopleva M, Jain N, Ouyang J, Goswami M, et al. CRLF2-Positive B-Cell Acute Lymphoblastic Leukemia in Adult Patients: A Single-Institution Experience. American journal of clinical pathology. 2017;147(4):357–63. https://doi.org/10.1093/ajcp/aqx005
70. Nakamura A, Tsurusawa M, Kato A, Taga T, Hatae Y, Miyake M, et al. Prognostic impact of CD45 antigen expression in high-risk, childhood B-cell precursor acute lymphoblastic leukemia. Leuk Lymphoma. 2001;42(3):393–8. https://doi.org/10.3109/10428190109064596
71. Cario G, Rhein P, Mitlohner R, Zimmermann M, Bandapalli OR, Romey R, et al. High CD45 surface expression determines relapse risk in children with precursor B-cell and T-cell acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol. Haematologica. 2014;99(1):103–10. https://doi.org/10.3324/haematol.2013.090225
72. Borowitz MJ, Shuster J, Carroll AJ, Nash M, Look AT, Camitta B, et al. Prognostic significance of fluorescence intensity of surface marker expression in childhood B-precursor acute lymphoblastic leukemia. A Pediatric Oncology Group Study. Blood. 1997;89(11):3960–6. https://doi.org/10.1182/blood.V89.11.3960
73. Jeha S, Behm F, Pei D, Sandlund JT, Ribeiro RC, Razzouk BI, et al. Prognostic significance of CD20 expression in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2006;108(10):3302–4. https://doi.org/10.1182/blood-2006-04-016709
74. Thomas DA, O'Brien S, Jorgensen JL, Cortes J, Faderl S, Garcia-Manero G, et al. Prognostic significance of CD20 expression in adults with de novo precursor B-lineage acute lymphoblastic leukemia. Blood. 2009;113(25):6330–7. https://doi.org/10.1182/blood-2008-04-151860
75. Maury S, Huguet F, Leguay T, Lacombe F, Maynadie M, Girard S, et al. Adverse prognostic significance of CD20 expression in adults with Philadelphia chromosome-negative B-cell precursor acute lymphoblastic leukemia. Haematologica. 2010;95(2):324–8. https://doi.org/10.3324/haematol.2009.010306
76. Maury S, Chevret S, Thomas X, Heim D, Leguay T, Huguet F, et al. Rituximab in B-Lineage Adult Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(11):1044–53. https://doi.org/10.1056/NEJMoa1605085
77. Spiegel JY, Patel S, Muffly L, Hossain NM, Oak J, Baird JH, et al. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nat Med. 2021;27(8):1419–31. https://doi.org/10.1038/s41591-021-01436-0
78. Majzner RG, Rietberg SP, Sotillo E, Dong R, Vachharajani VT, Labanieh L, et al. Tuning the Antigen Density Requirement for CAR T-cell Activity. Cancer Discov. 2020;10(5):702–23. https://doi.org/10.1158/2159-8290.CD-19-0945
79. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740–53. https://doi.org/10.1056/NEJMoa1509277
80. Newton JG, Horan JT, Newman S, Rossi MR, Ketterling RP, Park SI. CD36-positive B-lymphoblasts Predict Poor Outcome in Children With B-lymphoblastic Leukemia. Pediatric and developmental pathology: the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society. 2017;20(3):224–31. https://doi.org/10.1177/1093526616688753

81. Modvig S, Wernersson R, Obro NF, Olsen LR, Christensen C, Rosthoj S, et al. High CD34 surface expression in BCP-ALL predicts poor induction therapy response and is associated with altered expression of genes related to cell migration and adhesion. Mol Oncol. 2022;16(10):2015–30. https://doi.org/10.1002/1878-0261.13207
82. Li W. Measurable Residual Disease Testing in Acute Leukemia: Technology and Clinical Significance. In: Li W. editor. Leukemia. Brisbane (AU): Exon Publications. https://doi.org/10.36255/exon-publications-leukemia-measurable-residual-disease
83. Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14(1):18–24. https://doi.org/10.1200/JCO.1996.14.1.18
84. Vaitkeviciene G, Forestier E, Hellebostad M, Heyman M, Jonsson OG, Lahteenmaki PM, et al. High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies. Eur J Haematol. 2011;86(1):38–46. https://doi.org/10.1111/j.1600-0609.2010.01522.x
85. Winick N, Devidas M, Chen S, Maloney K, Larsen E, Mattano L, et al. Impact of Initial CSF Findings on Outcome Among Patients With National Cancer Institute Standard- and High-Risk B-Cell Acute Lymphoblastic Leukemia: A Report From the Children's Oncology Group. J Clin Oncol. 2017;35(22):2527–34. https://doi.org/10.1200/JCO.2016.71.4774

Index

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

DOI: https://doi.org/10.36255/exon-publications-leukemia.index

Leukemia

Weijie Li, MD, PHD
Editor

Weijie Li, MD, PhD, is an Associate Professor of Pathology and Laboratory Medicine at the University of Missouri-Kansas City School of Medicine and the Director of Hematopathology and Flow Cytometry at Children's Mercy Hospital, Kansas City, USA. He is a physician board-certified in anatomic pathology, clinical pathology, and hematopathology. He has clinical expertise in the diagnosis of leukemias/lymphomas and other hematolymphoid diseases, and laboratory monitoring of the therapeutic response of these diseases, especially in acute leukemias. He is actively involved in teaching and research. He is the principal investigator or co-investigator of multiple funded research projects. His research is focused on diagnostic and prognostic markers of leukemia/lymphoma, minimal residual disease detection of acute leukemia, flow cytometry study of hematolymphoid malignancy, and the deregulation of signaling pathway in pediatric leukemia/lymphoma. He has published extensively in these areas and served as a reviewer for multiple journals in these fields.

ISBN 978-0-645-33207-0

9 780645 332070

eXon PUBLICATIONS