Principles and Practice

Michał Witt
Małgorzata Dawidowska
Tomasz Szczepański *Editors*

Molecular Aspects of Hematologic Malignancies

Diagnostic Tools and Clinical Applications

Springer

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Michał Witt · Małgorzata Dawidowska
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Editors
Michał Witt
International Institute of Molecular and Cell Biology
Warsaw
Poland

and

Department of Molecular and Clinical Genetics
Institute of Human Genetics
Polish Academy of Sciences
Poznań
Poland

Małgorzata Dawidowska
Department of Molecular and Clinical Genetics
Institute of Human Genetics
Polish Academy of Sciences
Poznań
Poland

Tomasz Szczepański
Department of Pediatric Hematology and Oncology
Medical University of Silesia
Zabrze
Poland

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Preface

In recent years, there has been rapid development in molecular techniques, allowing for precise and highly sensitive support of existing traditional methods used in hemato-oncology. The most frequent applications of molecular techniques in hematology include the identification of fusion genes resulting from chromosomal translocations, the detection of minimal residual disease, and the analysis of chimerism following allogeneic hematopoietic stem cell transplantation. They provide a methodological basis for the widening of horizons in hematology toward molecular problems, significantly modernizing basic research and allowing fundamental questions to be tackled relating to hematopoietic stem cell biology, the process of leukemogenesis, the response to therapy, etc. These methods are also quickly becoming an essential component of modern diagnostic and therapeutic programs, revolutionizing clinical hemato-oncology toward more targeted treatment. At the moment it is hard to imagine effective care for patients subjected to transplantation or to other forms of therapy consistent with modern criteria, without the systematic monitoring of molecular markers of disease, giving usually the most precise insight into the prognostic dilemma. This results in the widespread creation of molecular hematology laboratories performing analyses exclusively for the needs of relevant clinical units and creates the necessity for collaboration between hematology clinical units and existing molecular genetic labs. This entails understanding and collaboration between specialists from these two quite different fields.

This monograph is neither another handbook of clinical hemato-oncology nor an exclusive practical laboratory guide. Descriptions of selected hematological malignancies (Part I), diagnostic procedures (Part II), and various aspects of hematopoietic stem cell transplantation (Part III) focus on their molecular aspects and in most of the cases correspond to a set of relevant laboratory protocols as their counterparts (Part IV), giving, together with appropriate references to a clinical realm, a bigger picture of the problem. Such a layout of the text proved

appropriate in our previous publications on the topic, while the smooth blend of clinical and molecular problems perfectly matches the realities of medicine today.

M. Witt
M. Dawidowska
T. Szczepański

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Chapter 1
Molecular Biology of Acute Lymphoblastic Leukemia

Małgorzata Dawidowska, Monika D. Kraszewska, Katarzyna Derwich and Tomasz Szczepański

Abstract Acute lymphoblastic leukemia (ALL) is the most common but also the most successfully treated malignancy in children. Current cure rates of approximately 85 % have been reached through multi-agent therapeutic regimens and particularly through risk-stratification enabling therapy individualization. Nevertheless, relapse is still the main cause of treatment failure. Therefore, the main effort is now focused on improving the outcome of high risk ALL subtypes, i.e., Ph + ALL, infant ALL, ALL with *MLL* gene rearrangements, hypodiploid ALL, some T-ALL subsets, recurrent and refractory leukemia. Recent research using advanced molecular techniques, in particular microarray-based genomic gene expression profiling (GEP) and high resolution single nucleotide polymorphism (SNP) microarray approaches, resulted in the identification of novel genetic factors with a potential impact on ALL classification and treatment. The main goal is now

M. Dawidowska (✉) · M. D. Kraszewska
Department of Molecular and Clinical Genetics, Institute of Human Genetics, Polish Academy of Sciences, Strzeszyńska 32, 60-479 Poznań, Poland
e-mail: ma.dawidowska@wp.pl

M. D. Kraszewska
e-mail: m.kraszewska@gmail.com

K. Derwich
Department of Pediatric Oncology, Hematology and Transplantology, Poznań University of Medical Sciences, Szpitalna 27/33, 60-572 Poznań, Poland
e-mail: kderwich@poczta.onet.pl

T. Szczepański
Department of Pediatric Hematology and Oncology, Medical University of Silesia, 3 Maja 13/15, 41-800 Zabrze, Poland
e-mail: szczep57@poczta.onet.pl

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to translate these findings on ALL blast biology and those on pharmacogenetics of patient response to therapy into improved diagnostics, prognostic classification, and treatment of this malignancy.

1.1 Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children accounting for approximately 30 % of all childhood neoplasms, with peak incidence at the age of 3–4 years (Kaatsch 2010). In adults ALL is less common, accounting for approximately 20 % of adults leukemias, with two incidence peaks—before the age of 30 and after the age of 70 (Piccaluga et al. 2007).

According to the revised World Health Organization (WHO) classification, two major ALL subtypes are distinguished: B lymphoblastic leukemia/lymphoma, traditionally termed B-cell precursor ALL (BCP-ALL) and T lymphoblastic leukemia/lymphoma (T-ALL). These subtypes are characterized by neoplastic outgrowth of lymphoid progenitor cells, within B or T lineage, respectively, which may occur at any stage of lymphoid differentiation, and results in a substantial heterogeneity of the disease (Vardiman et al. 2009).

With current protocols, based on a multi-agent chemotherapy, approximately 80 % of patients reach long-term remission; however, relapse still remains the main cause of treatment failure. Rapid development of molecular technologies in the recent years has enabled significant advances in the understanding of ALL biology. It is now evident that several disease subtypes can be distinguished within BCP-ALL and T-ALL, characterized by specific genetic abnormalities, distinct biology of leukemic blasts, and thus different prognosis (Pui et al. 2011). Therefore, the current challenge is to implement the recent findings on the biology of ALL cells, in combination with those on host-related pharmacogenetics of treatment response, to further improve prognostic classification, treatment individualization, and enable development of novel more targeted therapeutic strategies.

1.2 Etiopathogenesis

ALL is perceived as a complex disease, caused by a combination of genetic and environmental factors, although its etiology is still not fully elucidated. It is known that conditions characterized by chromosomal instability (ataxia-telangiectasia, Fanconi anemia, Nijmegen breakage syndrome), and those related to constitutional chromosomal abnormalities (Down syndrome) are associated with higher ALL prevalence. However, these syndromes are the cause of leukemia in only up to 5 % of ALL patients (Schrappe 2003; Houlston 2010).

The commonly accepted hypothesis states that at least two genetic aberrations (two hits) must take place for neoplastic transformation of a single cell, followed by uncontrolled proliferation of the leukemic clone (Knudson 1971). In most children, the first hit presumably takes place in their prenatal life, the second, is

most probably caused by environmental exposition to toxins, ionizing radiation, or viral infections and triggers the disease onset (Greaves 1999). The suspected infectious etiology of ALL has been addressed by two hypotheses; one suggesting existence of a virus directly involved in pathogenesis of ALL and another implying that ALL might be the consequence of an abnormal immune response to infection. Of these two, the latter seems more plausible (Greaves 2006).

Since most environmental factors studied so far failed to provide strong epidemiological evidence for their causative role in ALL, it is probable that susceptibility to ALL upon exposure to these factors might be determined by genetic polymorphism. A candidate gene approach, focused on a limited number of ALL functionally related genes, revealed a positive association of increased risk of ALL with polymorphism in several genes (e.g., *MTHFR*, methylenetetrahydrofolate reductase or *GSTM1*, glutathione S-transferase mu-1); however, these results have not been consistent (Houlston 2010). The more recent research, based on genome-wide association study (GWAS) approach, enabling analysis of hundreds of thousands of SNPs (single nucleotide polymorphisms), with no prior knowledge on their possible involvement in pathogenesis, revealed that polymorphic variants of three genes contribute to higher leukemia prevalence in children: *IKZF1* (IKAROS; early lymphoid development transcription factor), *ARID5B* (AT-rich interactive domain 5B; B-lineage commitment transcription factor), and *CEBPE* (CAAT/enhancer-binding protein, epsilon; T-cell differentiation transcription factor) (Houlston 2010; Mullighan 2010; Prasad et al. 2010). It is noteworthy that, ALL risk alleles identified through the candidate gene strategy have not been confirmed by GWAS.

Results of GWAS analyses indicate that common genetic variants of multiple genes, most probably involved in regulation of lymphocyte differentiation, contribute to increased risk of ALL development. The impact of a single loci is low, and individual susceptibility to ALL likely depends on the cumulative effect of variation in multiple genes (low-penetrance susceptibility alleles) (Houlston 2010). Some genetic variants are specifically associated with increased ALL risk in particular populations, e.g., 657del5 *NBN* (nibrin; encoding a component of a protein complex crucial for response to DNA damage) gene mutation in Polish population (Pastorczak et al. 2011).

1.3 Classification of ALL and Risk Factors

1.3.1 Morphological Versus Immunophenotypic Classification of ALL

Diagnosis of ALL is based on the presence of at least 25 % of lymphoblasts in bone marrow along with clinical symptoms suggestive of leukemic infiltrations. Based on lymphoblast morphology, according to the guidelines of French–American–British (FAB) classification, three types of lymphoblasts were distinguished:

Table 1.1 Immunophenotypic classification of BCP-ALL (Bene et al. 1995)

Leukemia subtype	CD10	cyIgμ	smIg[a]
B–I (pro-B) ALL	−	−	−
B-II (common) ALL	+	−	−
B-III (pre-B) ALL	±	+	−

cyIg cytoplasmic immunoglobulin, μ heavy chain protein, *sIg* surface immunoglobulin
[a] In a small subset of BCP-ALL, expression of surface membrane Ig is observed together with the surrogate light chains. Such subset is called transitional pre-B-ALL and is associated with favorable outcome

Table 1.2 Immunophenotypic classification of T-ALL (Bene et al. 1995)

Leukemia subtype	cy CD3	sm CD3	CD7	CD2	CD5	CD4	CD8	CD1a	TCR$\alpha\beta$	TCR$\gamma\delta$
T-I (pro-T) ALL	+	−	+	−	−	−	−	−	−	−
T-II (pre-T) ALL	+	−	+	+	+	−	−	−	−	−
T-III (cortical-T) ALL	+	±	+	+	+	+	+	+	−	−
T-IVa (α/β + mature-T) ALL	+	+	+	+	+	±	±	−	+	−
T-IVb (γ/δ + mature-T) ALL	+	+	+	+	+	±	±	−	−	+

TCR$\alpha\beta$ $\alpha\beta$ T cell receptor, *TCR$\gamma\delta$* $\gamma\delta$ T cell receptor

L1—observed in 85 % of cases,
L2—observed in 10–15 % of cases,
L3—observed in 1–3 % of cases.

The L3 cell morphology is typically associated with surface expression of immunoglobulins (Ig) (normally observed only in mature B cells) and translocations typical for Burkitt Lymphoma: t(8;14), t(2;8) or t(8;22). Historically, this type of leukemia was classified as B-ALL. Currently, according to recent WHO recommendations, it is included as a subtype of Burkitt Lymphoma/Leukemia. It should be noted that FAB classification is no longer advocated as the sole basis for ALL diagnosis due to its limited clinical and prognostic value.

Based on flow cytometric immunophenotypic assessment of bone marrow two basic ALL types are distinguished: BCP-ALL (80–85 % of cases) and T-ALL (15–20 %), which are further divided, according to current recommendations of the European Group for the Immunological Characterization of Leukemias (EGIL), into several subtypes listed in Tables 1.1 and 1.2. The immunophenotype of blast cells reflects the differentiation stage, at which the neoplastic transformation occurred (maturation arrest), which might be complemented by the analysis of rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes, further discussed in Sect. 1.5.1.