Arch. Immunol. Ther. Exp. (2011) 59:61–68
DOI 10.1007/s00005-010-0110-1

REVIEW

Relapse of Acute Lymphoblastic Leukemia in Children in the Context of Microarray Analyses

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Received: 19 April 2010 / Accepted: 19 August 2010 / Published online: 19 January 2011

Abstract Over the last four decades the treatment of patients with newly diagnosed childhood acute lymphoblastic leukemia (ALL) has improved remarkably. However, still about 20% of children with ALL relapse despite risk-adapted polychemotherapy. The prognosis of relapsed ALL is relatively poor, even with modern aggressive chemotherapy. Identification of the biological and genetic mechanisms contributing to recurrence in patients with ALL is critical for the development of effective therapeutic strategies to treat refractory leukemic patients. Allogeneic hematopoietic stem-cell transplantation is the treatment of choice for many children with relapsed ALL. The gene expression profile obtained by microarray technology could provide important determinants of the drug response and clinical outcome in childhood ALL. Incorporation of the data on expression levels of newly identified genes into existing strategies of risk stratification might improve clinical management. Current microarray data show correlation of in vitro drug resistance with significant patterns of gene expression and explain clinical differences between early and late relapse. Genes involved in cell proliferation, self-renewal and differentiation, protein biosynthesis, carbohydrate metabolism, and DNA replication and repair are usually among those highly expressed in relapsed lymphoblasts. Current status and future perspectives of microarray data on gene expression and drug resistance profile in relapsed pediatric ALL are discussed in this review.

Keywords Gene expression profile · Copy number abnormalities · Microarray · Children · Acute lymphoblastic leukemia · Relapse

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, with an incidence of 30.9 cases/million children/year (Gaynon et al. 1998; Pui and Jeha 2007). With contemporary treatment based on systemic combination chemotherapy and specific preventive therapy of the central nervous system (CNS), about 98% of children with ALL experience complete remission (Holleman et al. 2004; Pui et al. 2001, 2004; Pui and Evans 1998). This improved outcome is also related to stratification of individual patients into risk groups based on prognostic factors incorporating age, immunophenotype, white blood cell (WBC) count, CNS involvement, response to therapy and genetic findings. However, still about 20–25% of newly diagnosed patients subsequently experience leukemic recurrence, with bone marrow (BM) being the most common site of disease relapse. Pediatric relapsed ALL is a more frequent disease than non-Hodgkin lymphoma, Wilms tumor, Hodgkin disease or acute myeloid leukemia (Gaynon et al. 1998).

Relapsed ALL

Relapse of ALL can be defined as the reappearance of leukemic lymphoblasts in any compartment following achievement of complete remission. Recurrence of leukemia can occur any time during therapy or after completion of treatment. Most relapses occur in patients without well established unfavorable prognostic markers at diagnosis. Before the era of hematopoietic stem-cell transplantation (HSCT), outcome in relapse was poor and the long-term survival rate was below 10% of patients with early marrow relapses and 50% of those with late relapses (Bailey et al. 2008; Bhojwani et al. 2006).

The underlying pathways that lead to relapse in ALL are multifactorial. There are several possible mechanisms of relapse, related to minimal residual disease and drug resistance: persistence of blasts in "sanctuary sites" such as testes or CNS, protected by anatomic barriers causing inadequate access of chemotherapy; persistence of leukemic cells in G0 phase due to intrinsic properties or changes of environmental conditions, causing lack of response to chemotherapy; primary resistance of the leukemic cells present at diagnosis and secondary resistance of blasts after exposure to the frontline treatment. Mechanisms of drug resistance protect leukemic cells from apoptosis.

Leukemia relapse is the most frequent cause of treatment failure in childhood ALL (Coustan-Smith et al. 2004; Rivera et al. 2005; Rizzari et al. 2004). The survival of patients who relapse despite intensive multi-agent chemotherapy is still not satisfactory (Gaynon et al. 1998). While the majority of children with recurrent ALL attain a second remission, prognosis is generally unfavorable, particularly for those with BM relapse following a short initial remission duration (Einsiedel et al. 2005; Gaynon et al. 1998). Two-thirds of relapsing patients attain a second remission, but the 5-year survival probability is only 10–40% (Eckert et al. 2001; Einsiedel et al. 2005), depending on time and site of relapse. Relapse during or soon after the completion of treatment is considered more unfavorable than relapse a year or later after treatment cessation. Relapsed ALL continues to make a major contribution to the morbidity and mortality of childhood cancer (Gaynon et al. 1998). Treatment protocols also include the issue of relapse site. Aggressive chemotherapy and radiation treatment, often followed by BM transplantation (BMT), are used to treat relapse of childhood leukemia.

Relapses of ALL usually occur in BM, the CNS and testes. Combined relapses are also frequently observed. BM is the most common site of relapse and nearly 75% of recurrences involve the marrow. Very early relapse is diagnosed when it occurs <18 months after initial diagnosis and <6 months after cessation of first-line therapy. Early relapse is diagnosed when it occurs >18 months after initial diagnosis and <6 months after cessation of first-line therapy. Late relapse is diagnosed when it occurs >6 months after cessation of first-line therapy (even if <18 months after initial diagnosis, in rare, unusual cases) (Einsiedel et al. 2005).

An isolated BM relapse is diagnosed with ≥25% lymphoblasts among nucleated cells in the BM and without evidence of leukemia at extramedullary sites (Table 1), while it is often proposed that the presence of leukemic lymphoblasts at levels lower than 25% in BM after complete remission constitutes a criterion for diagnosis of relapse. In children with proven leukemia at extramedullary sites, a combined relapse is diagnosed with marrow involvement of ≥5% lymphoblasts. Accordingly, isolated extramedullary relapses are those with clinically overt extramedullary manifestation of leukemia and less than 5% marrow infiltration. CNS relapse is diagnosed in the case of at least five leukocytes per microliter of colony-stimulating factor and the unequivocal presence of lymphoblasts. Testicular relapse should be confirmed by open-wedge biopsy. Involvement of any other extramedullary site has to be confirmed histologically (Einsiedel et al. 2005).

Clinical and Cellular Parameters of ALL Relapse

Cellular drug resistance is thought to be an important cause of the poor prognosis for children with relapsed or refractory ALL. Relapsed ALL is significantly more resistant to glucocorticoids, L-asparaginase, anthracyclines, and thiopurines, but not to vinca alkaloids, cytarabine, ifosfamide, and epipodophyllotoxins. Relapsed ALL cells express the highest level of resistance to glucocorticoids. In vitro drug resistance is also related to the clinical response to chemotherapy in relapsed/refractory childhood ALL (Klumper et al. 1995).

Table 1 Site of relapse

Bone marrow		<5% blasts	5–25% blasts	≥25% blasts
Extramedullary relapse	No	No relapse	Further study required[a]	Isolated BM relapse
	Yes	Isolated extramedullary relapse	Combined BM relapse	

[a] It is often proposed that the presence of leukemic lymphoblasts at levels lower than 25% in bone marrow (BM) after complete remission constitutes a criterion for diagnosis of relapse

Prognosis after relapse is poor. The outcome is better for combined marrow and isolated CNS, testicular, or other extramedullary relapse in comparison to isolated BM relapse (especially with T-immunophenotype). Better treatment responses are usually related to longer remission lengths of initial disease. Later relapse is more favorable than earlier relapse (Bailey et al. 2008; Borgmann et al. 2003) (Table 2).

In the non-minimal residual disease (non-MRD) strategy of the German Berlin-Frankfurt-Munster protocol-2002, the intermediate risk group, as shown in Table 2, is subdivided into subgroups A–D (Table 3).

According to this non-MRD strategy, patients in subgroup A are qualified for chemotherapy or matched sibling donor HSCT (MFD-HSCT), in subgroup B to MFD-HSCT or matched unrelated donor HSCT (MUD-HSCT) or chemotherapy, in subgroup C to MFD-BMT, MUD-BMT, haploidentical HSCT or autologous HSCT, and in subgroup D to chemotherapy or to autologous HSCT. Significantly better cure rates for combined recurrences in comparison to isolated BM relapse could reflect differences in disease biology and result from therapeutic craniospinal irradiation (Beesley et al. 2005).

A major challenge is to identify patients with an increased risk of recurrence. Detecting such a group gives an opportunity to use a more efficient treatment strategy (Andersson et al. 2007). Leukemic relapse is caused by surviving drug-resistant cells. These cells could arise from a selection of a sub-population of cells with intrinsic resistance to therapy, or after the acquisition of resistance during therapy. To minimize the side effects and to employ more effective therapy regimens, ALL treatment is tailored based on stratification and prognostic risk factors of relapse. Current predictive accuracy of these factors is not optimal with standard clinical and biological features. Frequently, therapy failure occurs in patients with favorable prognostic features at diagnosis (Carroll et al. 2006). All studies on pediatric ALL are aimed at identifying patients at high risk of relapse.

The prognosis for patients with relapsed ALL depends on the time and site of relapse as well as immunophenotype (Gaynon et al. 1998; Uderzo et al. 2001). These factors are crucial predictors of second remission, event-free survival, and overall survival after ALL recurrence. Further prognostic factors (e.g. peripheral blood cell count, cytogenetic aberrations) could be useful in identifying patients requiring more intensive therapy. The identification of leukemic cell features is essential for treatment stratification in clinical practice. Currently, the most important risk factors predictive for an inferior outcome are a short duration of first remission, a higher initial leukocyte count, an isolated BM relapse, T-cell immunophenotype, and the presence of

Table 2 BFM and UKALL relapse risk group assignment for ALL

Time of relapse	Site of relapse		
	Extramedullary relapse	Combined BM and extramedullary relapse	Marrow relapse
Precursor B-cell ALL			
Very early relapse (<18 months from diagnosis)	Intermediate	High	High
Early relapse (>18 months from diagnosis and <6 months from completion of therapy)	Intermediate	Intermediate	High
Late relapse (>6 months from completion of therapy)	Standard	Intermediate	Intermediate
T-cell ALL			
Very early relapse (<18 months from diagnosis)	Intermediate	High	High
Early relapse (>18 months from diagnosis and <6 months from completion of therapy)	Intermediate	High	High
Late relapse (>6 months from completion of therapy)	Standard	High	High

BFM German Berlin-Frankfurt-Munster protocol, *UKALL* United Kingdom ALL protocol

Table 3 Stratification of intermediate risk group in non-MRD strategy

Site	Isolated BM	Combined BM relapse		Isolated extramedullary relapse
Time	Late	Late	Early	Early/very early
<1/μl PBC	A	A	B	D
1 to <10,000/μl	B			
≥10,000/μl	C			
BCR-ABL	C	C	C	

PBC peripheral blast count, *BCR-ABL* gene rearrangement

the translocation t(9;22) [*BCR-ABL* gene rearrangement] (Chessells et al. 2003; Einsiedel et al. 2005; Rivera et al. 2005). The presence of the *TEL-AML1* fusion gene at the time of first B-lineage ALL relapse could be related to better outcome (Rivera et al. 2005; Uderzo et al. 2001).

Duration of first remission it is the most significant predictor of outcome after either BM or isolated extramedullary relapse of standard risk ALL (Gaynon et al. 1998; Malempati et al. 2007). Patients with early relapse have less chances of attaining a second remission and have shorter remission than those with later relapses (≥36 months from diagnosis) (Bhojwani et al. 2006). Prognosis for long-term survival after very early relapse is poor, with a less than 10–20% probability of long-term survival using chemotherapy alone (Buchanan et al. 2000; Gaynon et al. 1998; Henze et al. 1991; Schroeder et al. 1995; Wheeler et al. 1998). In large studies, 30–40% of patients with late relapse achieved long-term disease-free survival (Buhrer et al. 1996; Rivera et al. 1996; Sadowitz et al. 1993).

Site of relapse BM relapses have a worse prognosis than other sites (Buchanan et al. 2000; Gaynon et al. 1998). Patients with combined BM relapse have better prognosis than do those with isolated recurrence occurring in the BM (Buchanan et al. 2000; Gaynon et al. 1998; Henze et al. 1991; Schroeder et al. 1995; Wheeler et al. 1998), while patients with extramedullary relapse have superior outcome.

Immunophenotype dismal outcomes have been observed at relapse in patients with a T-cell phenotype, with survival rates less than 20% (Abshire et al. 1992; Bhojwani et al. 2006; Schroeder et al. 1995).

MRD status has prognostic significance in patients with late relapsing disease; if patients have a high level of MRD after induction and induction consolidation the probability of relapsing is very high (Eckert et al. 2001). Patients whose MRD rapidly improves are likely to have a very good outcome with chemotherapy (Eckert et al. 2001).

WBC count Initial WBC count <20,000/μl, peripheral blast cell count <10,000/μl, and age 2–10 years suggested favorable prognosis; WBC count ≥50,000/μl, age ≥10 years, and male sex suggested unfavorable prognosis (Abshire et al. 1992; Gaynon et al. 1998).

Presence of *TEL-AML1* and *BCR-ABL* and other new molecular parameters need clarifying as to their value and to be integrated together with the well-known and validated clinical factors into a revised model of risk prediction (Einsiedel et al. 2005). Patients whose leukemic cells carry 11q23 abnormalities, or Ph-positive ALL, tend to relapse early and to have a worse prognosis (Chessells et al. 2003).

Isolated CNS, testes, or other extramedullary relapse has a relatively good prognosis, while combined marrow and extramedullary relapse, an intermediate prognosis, and isolated BM relapse carry the worst prognosis (Bailey et al. 2008). The prognosis is significantly better for patients with combined BM recurrence, compared to patients with isolated BM disease.

Results of Therapy

Event-free-survival (EFS) after BM relapse treated with chemotherapy is usually in the range 1–9% after very early relapse, 10–35% after early relapse and 33–71% after late relapse (Bailey et al. 2008; Gaynon 2005; Nguyen et al. 2008). Three-year EFS after CNS or testes relapse is 60% (Gaynon 2005) (Table 4). There is evidence for the superiority of allo-HSCT from an HLA-identical sibling in patients with BM and combined relapses. Long-term EFS after MFD-HSCT for very early and early relapse is in the range 40–62% (Bleakley et al. 2002; Borgmann et al. 2003; Saarinen-Pihkala et al. 2006; Uderzo et al. 1995).

Analysis of Genetic and Gene Expression Profiles at ALL Relapse

Gene expression profiling performed by microarray technology has enabled the identification and measurement of thousands of genes in a single experiment. Expression profiling of leukemic blasts using microarray technology rapidly emerged as a powerful tool to reveal multiple gene expression signatures associated with pediatric acute leukemia, including potential prognosis and outcome (Bhojwani et al. 2008; Flotho et al. 2007) or drug resistance markers (Cheok et al. 2003; Holleman et al. 2004; Lugthart et al. 2005; Sorich et al. 2008). Genome-wide studies are a useful method of classification of different cytogenetic subtypes of ALL, early prediction of response to treatment, overall outcome, the final effect and adverse effects. As shown in numerous studies on the progression of disease (from initial diagnosis to the appearance of recurrence),

Table 4 Survival after relapse

Site of relapse	Time of relapse	3-year survival (%)
Marrow relapse (isolated or combined)	Very early	5–13
	Early	10–35
	Late	33–57
CNS relapse (isolated)	Very early	49
	Early	73
	Late	77
Testes relapse (isolated)	Very early	50
	Early	57
	Late	70

dynamic changes occur in cell genetics (Mullighan et al. 2008; Yang et al. 2008).

The current strategy of molecular studies is to identify a small group of genes responsible for leukemic relapse. However, it is not clear whether molecular signatures will show a significant relationship with leukemic progression or relapse. Several study groups have examined specific gene expression profiles of refractory leukemia in comparison to the gene expression profile in de novo leukemia. These data provide new insights into the genomic basis of drug resistance, patient differences in drug response or outcome in relapsed ALL.

Many relapses occur in standard and intermediate risk groups, while some patients in the high-risk group are unnecessarily exposed to the toxic effects of very intensive therapy. Thus it is important to improve characterization of risk groups defined on the basis of traditional prognostic factors. Studies focusing on the possibility of predicting the effectiveness of therapy based on changes in gene expression profiles have shown that one can identify groups of patients who are able to achieve complete remission and those with high risk of treatment failure. These two groups of patients were identified irrespective of the immunophenotype and the presence of specific cytogenetic changes (Andersson et al. 2007; Willenbrock et al. 2004). Kang et al. developed a 38-gene classifier, allowing stratification of patients into two groups with different risk of recurrence in B-precursor ALL (Kang et al. 2010). The results of these studies can lead to the progressive improvement of the classification of risk, and enable identification of pediatric patients who respond well to current therapy and those who may require advanced treatment programs.

In relapsed ALL cells, major functional classes of genes have been identified on the basis of ontological analyses. Comparison of the genetic profiles of cells from patients at diagnosis and relapse (particularly early) revealed that the most significant changes occur in the expression levels of genes involved in cell cycle regulation, DNA repair, apoptosis (Bhojwani et al. 2006), oncogenesis, drug resistance and metastasis (Beesley et al. 2005). The vast majority of genes were upregulated at relapse. In addition, samples from patients with very early relapse showed a significant increase in the percentage of S and G2-M phase cells and this correlated well with the expression level of cell cycle genes (Kirschner-Schwabe et al. 2006). Comparison of transcriptome leukemic cells at different times of recurrence has led to the identification of several potential cellular pathways. The main patterns of variations between early and late relapses of leukemia were related to genes that play a role in cell cycle regulation (Bhojwani et al. 2006), signal transduction pathways, cell proliferation, DNA repair and cell survival (Carroll et al. 2006; Staal et al. 2003). Early relapse samples were more likely to be similar to their respective diagnostic sample, while they noted greater divergence in gene expression patterns among late relapse patients (Bhojwani et al. 2006).

The contribution of individual genes to treatment failure and disease progression is largely unknown. Molecular alterations identified in recurrent ALL include p53 mutations, increased cyclin D1 and *BAX* expression, *DHFR* gene amplification and deletions at the *INK4A/ARF* and *TEL* chromosomal loci (Carter et al. 2001a, b; Goker et al. 1995; Maloney et al. 1999; Zhu et al. 1999; Zuna et al. 2004). These data are still insufficient to understand the exact mechanism of relapse (Beesley et al. 2005). Many of the selected genes based on gene expression profiles are already known to be involved in the development of cancer (Bhojwani et al. 2006). Thus, they constitute attractive targets for future therapies. Comparison of de novo and relapse samples revealed genes (e.g., survivin, topoisomerase 2A and cyclin B1) and pathways that may be potential targets at relapse (Carroll et al. 2006). Comparative analysis of the gene expression profile of blasts of patients with B-precursor ALL who relapsed led to the selection of just six genes overexpressed at relapse (*SH3 BP5*, *TOSO*, *Smad3*, *CD79a* (*MB1*), *RASGRP2*, *WASF1bv*; Table 5) (Staal et al. 2003). These six genes are potentially new biomarkers for drug targets. Beesley et al. identified a set of genes overexpressed in either de novo or relapsed ALL in each lineage, and proposed the possible existence of common mechanisms of relapse (Beesley et al. 2005). The pattern of gene expression was characterized by the presence of genes involved primarily in oncogenesis, drug resistance and metastasis (e.g. *BSG*, *GRP58*, *LAMP*, *PBX2*, *FUS*, *GTF2F1*, *RoXaN*, *PIP5K1A*), while no significant change in the level of expression of genes involved in classical multidrug resistance pathways was found. Similarly, studies performed by Mullighan et al. (2009) showed that many of the new genetic alterations at relapse involve genes previously implicated in treatment failure (e.g., *IKZF1* and IKAROS family members, *CDKN2A/B*) (Kang et al. 2010). In microarray studies, no differences in the expression of multidrug resistance markers (e.g. *MDR-1*) or genes involved in xenobiotic metabolism (e.g. *P450*, *GSH* transferases) were found (Staal et al. 2003). On the other hand, genes involved in apoptosis have frequently changed their expression at relapse. Apoptosis pathways are related to drug resistance, and are usually disturbed in relapsed ALL, leading to different tumor biology and the development of therapy-refractory disease in ALL relapse in comparison with the disease at the time of initial diagnosis. Relapse in childhood ALL is associated with a decrease of the *BAX/BCL-2* ratio and loss of spontaneous caspase-3 processing in vivo (Prokop et al. 2000). Many genes are involved in regulation of drug resistance. *MSH6* deletion at relapse is associated with drug resistance (Yang et al.

Table 5 Potential markers of relapsed ALL

Genes	Cellular function	Changes at relapse
BRCA2, CDC kinases, TOP2A, KIF 11 and *RAD51*	Cell cycle regulation, DNA replication and repair, molecular transport, cellular assembly and protein trafficking, ubiquitination, B-lymphocyte signaling, B-cell development	Upregulation (Staal et al. 2010)
CCNB2, LIMS1, TEGT, CACYBP,GNB1, WWOX and *PPIH*	Cell cycle, amino acid and purine metabolism	Upregulation (Staal et al. 2010)
DEFA1-3, α-defensin1-3	Genes encoding α-defensins, which are part of a family of anti-microbial peptides; play a role in antigen driven immune response and in anti-tumor immunity	Upregulation (Te Kronnie et al. 2006)
SH3 BP5	The Btk-associated SH3-domain containing protein	Upregulation (Staal et al. 2003)
TOSO	Antiapoptotic gene	Upregulation (Staal et al. 2003)
Smad3	TGF-β responsive transcription factor	Upregulation (Staal et al. 2003)
CD79a (Mb1)	The immunoglobulin-associated gene	Upregulation (Staal et al. 2003)
RASGRP2	The Ras guanyl releasing protein	Upregulation (Staal et al. 2003)
WASF1bv	A member of the Wiskott Aldrich syndrome protein, a gene family that is involved in actin filament reorganization and changes in cell shape	Downregulation (Staal et al. 2003)
CDCA8, Aurora kinase B, MAD2L1, PTTG1, Spc25, TPX2, NUSAP1	Genes for proteins involved in mitosis	Upregulation (Kirschner-Schwabe et al. 2006)
KIFC1, KIF23, KIF11, KNSL7	Members of the kinesin superfamily of mitotic motor proteins involved in spindle function and cytokinesis	Upregulation (Kirschner-Schwabe et al. 2006)

2008). *MSH6* is a critical component of the cellular mismatch repair machinery and is implicated in tumorigenesis and resistance to thiopurines and DNA alkylating agents (Guo et al. 2004). *MSH6* expression was significantly lower in leukemic blasts resistant to mercaptopurine than in sensitive cells (Yang et al. 2008).

Several studies concerning the cell biology, genetics and pathogenesis of leukemia relapse were published recently. Analyses of single nucleotide polymorphism (SNP) arrays in T-cell leukemia confirmed that both the original and its recurrence have a common genetic change; however, the cells of relapsed ALL do not share the polymorphisms specific to the diagnosis. Probably the cases of recurrence emerged from an ancestral clone (Tosello et al. 2009). Similarly, a study of the SNP array profiling of over 60 paired diagnosis-relapse childhood ALL samples showed that the diagnosis and relapse samples have different patterns of genomic copy number abnormalities (Mullighan et al. 2008). These data indicate that the progenitor cells for relapse are ancestral to the primary leukemia cells. It was also found that genomic abnormalities (cellular signaling pathways) contributing to ALL relapse are promoted in the course of treatment. In studies using human 500 K SNP arrays, molecular evolution of relapsed childhood B-precursor ALL has been shown, particularly with frequent *EBF1*, *MSH6*, *IKZF1*, *CDKN2A* and *CDKN2B* gene deletions (Yang et al. 2008). A significant association between changes in *MSH6* expression and cell sensitivity to glucocorticoids and mercaptopurine at relapse was confirmed. Microarray studies indicate that the mechanism of the development of relapse is the consequence of the selection or emergence of cells with different expression of genes involved in pathways responsible for regulation B-cell signaling, development, cell cycle, cellular division and DNA metabolism (Table 5) (Staal et al. 2010).

Genome-wide studies clearly indicate that relapse is also a consequence of changes in the regulation of many metabolic pathways. These changes are associated primarily with different expression of genes involved in cell cycle regulation, cell division, signaling pathways, protein biosynthesis, carbohydrate metabolism, development and activation of lymphoblasts (Staal et al. 2010).

The knowledge gained through genomic analysis helps to identify the key cellular pathways that lead to treatment failure and development of recurrence. Identification of a group of patients who are expected to be at high risk of relapse may indicate a need for more tailored, individualized therapy. Further study of the genome and transcriptome of

leukemic cells supplemented by the analysis of gene polymorphism, identification of miRNAs, and analyses of methylation status and proteome-level changes may help in the future to better understand the mechanism of recurrence and to improve diagnosis and treatment of childhood ALL (Heinrichs et al. 2010; Staal et al. 2010).

Conclusions and Areas for Future Research

Common changes that contribute to development of drug resistance and malignant transformation are usually found in expression of genes at ALL relapse. Genes involved in cell proliferation, self-renewal and differentiation and DNA replication/repair are usually among those highly expressed in relapsed versus newly diagnosed blasts. The results of gene expression studies provide evidence that improved outcome prediction and risk classification can be achieved in pediatric ALL. The application of gene expression classifiers will soon allow for the prospective identification of a significant subgroup of ALL patients with little chance for cure on contemporary chemotherapeutic regimens. Further analysis of these expression profiles, coupled with other comprehensive genomic studies, will hopefully lead to the identification of novel targets and more effective therapies for these children.

Acknowledgments This study was supported by Grant MNiSW No. N407 078 32/2964.

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