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Use of Single Nucleotide Polymorphism Array Technology to Improve the Identification of Chromosomal Lesions in Leukemia

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Abstract: Acute leukemias are characterized by recurring chromosomal and genetic abnormalities that disrupt normal development and drive aberrant cell proliferation and survival. Identification of these abnormalities plays important role in diagnosis, risk assessment and patient classification. Until the last decade methods to detect these aberrations have included genome wide approaches, such as conventional cytogenetics, but with a low sensitivity (5-10%), or gene candidate approaches, such as fluorescent in situ hybridization, having a greater sensitivity but being limited to only known regions of the genome. Single nucleotide polymorphism (SNP) technology is a screening method that has revolutionized our way to find genetic alterations, enabling linkage and association studies between SNP genotype and disease as well as the identification of alterations in DNA content on a whole genome scale. The adoption of this approach for the study of lymphoid and myeloid leukemias contributed to the identification of novel genetic alterations, such as losses/gains/uniparental disomy not visible by cytogenetics and implicated in pathogenesis, improving risk assessment and patient classification and in some cases working as targets for tailored therapies. In this review, we reported recent advances obtained in the knowledge of the genomic complexity of chronic myeloid leukemia and acute leukemias thanks to the use of high-throughput technologies, such as SNP array.

Keywords: genetic lesions, leukemia, SNP array.

INTRODUCTION

A hallmark of leukemia is the presence of recurring chromosomal and genetic abnormalities that result in losses, amplifications, translocations, and inversions of DNA fragments or whole chromosome aneuploidies. Many of these anomalies are correlated with patient characteristics, such as age, white blood count cell, and immunophenotype. Identification of these anomalies has important implications for diagnosis and sub-classification, detecting minimal residual disease during follow-up, determining disease prognosis, and making appropriate therapeutic decisions [1-2]. The characterization of genetic rearrangements has led to the identification of genes (*e.g.*, *FLT3*, *NOTCH1, JAK2, TP53*) and fusion genes (*e.g., BCR-ABL1*) that have a key role in leukemogenesis and can be targeted for a tailored therapy. Therefore, there is a growing interest in identifying genetic subgroups that can be targeted to improve outcomes. The vast majority of recurrent chromosomal rearrangements associated with leukemia were originally identified by cytogenetic analysis, which is still the gold standard laboratory test since it provides in a single step a global analysis of abnormalities throughout the entire genome. However, conventional cytogenetics has some technical limitations: (a) the need for cellular proliferation, (b) the dependence on metaphases representative of the leukemia clone (traditionally a result of 2 abnormal metaphases of 20 tested is considered pathological), (c) the inability to detect small lesions (resolution of 1 to 10 Mb) [3], and (d) the difficulty in interpreting some variant translocations or complex rearrangements. The fluorescence in situ hybridization (FISH) technique overcomes some of the drawbacks of traditional cytogenetics because it does not require metaphases, can be used to map loci on specific chromosomes, can detect both structural chromosomal rearrangements and numerical chromosomal abnormalities, and can reveal cryptic abnormalities, such as small deletions. However, FISH can be applied only on candidate regions. Hence, a comprehensive screening for chromosomal aberrations cannot be carried out by FISH [4]. In the past decade, a sensitive genome-wide analysis of genetic aberrations has been enabled by the advent of comprehensive high-resolution genomic tools, such as molecular karyotyping using comparative genomic hybridization (CGH) and SNP microarray. Unlike conventional cytogenetics, these platforms are not dependent on the availability of mitotically dividing cells within the tumor tissue, as genomic undamaged DNA is used instead of metaphases. Although these techniques enable the detection of only unbalanced defects and do not distinguish between multiple large clones, they have greater resolution compared with classical cytogenetic methods. Moreover, SNP array has the advantage of simultaneous genotyping, enabling detection of copy number neutral loss of heterozygosity (CN-LOH), also referred to as somatic uniparental disomy (UPD) [5-7]. This review emphasizes the newest findings obtained from genome-wide analysis of genetic aberrations in chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML).

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GENOME-WIDE SNP ARRAY AND ITS IMPLICATIONS

Single Nucleotide Polymorphisms

Genotyping based on SNP array is a screening method that enables linkage and association studies between SNP genotype and disease [genome-wide association studies (GWAS)] as well as the identification of alterations in DNA content on a whole-genome scale. Detection of previously unknown deletions and duplications in human cancers revolutionized the understanding of genome complexity, allowing the identification of DNA variants that potentially contribute to disease, and uncovered the role of some molecular mechanisms that underlie disease onset or development. Therefore, this approach promotes advances in diagnosis and risk classification as well as the identification of new targets to achieve personalized therapy.

SNPs are DNA variants occurring in a large proportion of the human population (>1%). Each individual inherits one allele copy from each parent, so that the individual genotype at an SNP site is *AA*, *BB,* or *AB*. The Human Genome Project [8], the SNP Consortium [9], the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/index.html.en) [10], and, more recently, the 1000 Genomes Project [11] and the integrated map of genetic variation from 1092 human genomes [12] have collectively identified and catalogued approximately 15 million common DNA variants, mostly SNPs, providing a picture of the levels of genetic variation in humans at both the inter-individual and inter-population levels. Overall, approximately 8.4 million SNPs and ~840,000 insertions/deletions were described for the first time by the 1000 Genomes Project Consortium. Because SNPs are widespread and numerous across the entire genome, they represent suitable markers to study genomic alterations and identify specific loci of rearrangements.

Methodology

The two major SNP array manufacturers are Affymetrix, Inc. (Santa Clara, CA, USA) [13] and Illumina, Inc. (San Diego, CA, USA) [14]; both exploit the same biochemical principles but use different chemistries to identify copy number state (Fig. **1**). Fragmented and labeled target DNA is hybridized on arrays containing numerous immobilized oligonucleotide probes, each designed to contain a SNP site. Oligonucleotides are specific for each of the two SNP alleles, referred to as *A* and *B.* As a fluorescent signal is obtained for each allele at a given SNP site, hybridization intensity provides information about both SNP genotype (*A*, *B*, or *AB*) and copy number state (heterozygosis or homozygosis/hemizygosis due to hybridization of genomic DNA to both or one probe variant, respectively). Illumina uses a bead-based platform in which the whole genome is amplified and fragmented and then hybridized to an oligonucleotide bead array containing bead types specific for each allele in the SNP locus. Subsequent enzymatic extension (allele-specific primer or single base) and fluorescent staining allow allele discrimination. In the Affymetrix technology, DNA is digested and then amplified, labeled, and hybridized on a chip array.

Because the probes are localized at the SNPs' loci, the array's resolution is closely related to the density of the probes, hence also to the SNPs' distribution alongside each chromosome. SNPs are not evenly distributed in the genome, resulting in a non homogeneous coverage. To overcome this limit, Affymetrix, gradually improved array resolution. Genome-Wide Human SNP Array 6.0 version comprises more than 900,000 SNPs together with more than 940,000 non polymorphic markers to provide better coverage of all chromosome lengths, with a median intermarker distance over all 1.8 million markers of less than 700 bases. The recent high-density CytoScan HD Array includes 2.67 million markers for copy number analysis, approximately 750,000 SNP probes, and 1.9 million non polymorphic probes for comprehensive whole-genome coverage. The high-density SNP array enables analysis of regions identical-by-descent and enhanced detection of low-level mosaicism. CytoScan HD Array offers confident breakpoint determination (often with exon-level resolution) throughout the whole genome and highly accurate SNPs for parent-of-origin studies [15-16]. Using the high-resolution CytoScan HD Array with ChAS Software (www.affymetrix.com/chas), it is easy to distinguish between aberrations and artifacts. Another important feature of SNP array is the sensitivity, which depends on the number of cells harboring the specific genetic lesion required for SNP array detection. Therefore, only rearrangements that occur in large clones are detectable. Dilution studies revealed that the presence of 25% cells with uniform lesions may be detectable by Affymetrix GeneChip Human Mapping 250K [17].

After fluorescent signals are generated, samples are scanned and analyzed [18-19]. All arrays undergo brightness normalization to a baseline array to correct technological biases in probe intensity and to allow comparisons among them. Quality-control steps enable the identification of poor-quality samples. For these purposes, different algorithms have been developed over the years for specific arrays [20-22]. The output data can be generated using one of several software packages based on various bioanalytical principles, and both companies provide software for downstream assessment of the data [23].

SNP Array Analysis

To identify somatically acquired, cancer-specific lesions from patient-specific inherited copy number variations (CNVs) or segments of homozygosity, SNP genotypes of a tumor sample should be compared with appropriate normal DNA reference samples from each patient. The source of the matched normal genomic DNA may include buccal swab, long-lived T lymphocytes selected by flow cytometry from a bone marrow sample, or a skin biopsy, which can be taken at the site of the bone marrow aspirate. Swabs may not be the best choice for matched normal genomic DNA in patients with hematologic malignancies because may be contaminated with blood, which might introduce tumor DNA into the control sample. SNP array analysis with paired normal samples is recommended because it has been demonstrated to be more accurate than analysis with unpaired normal samples [24]. Moreover, using SNP array, clonal mosaicism

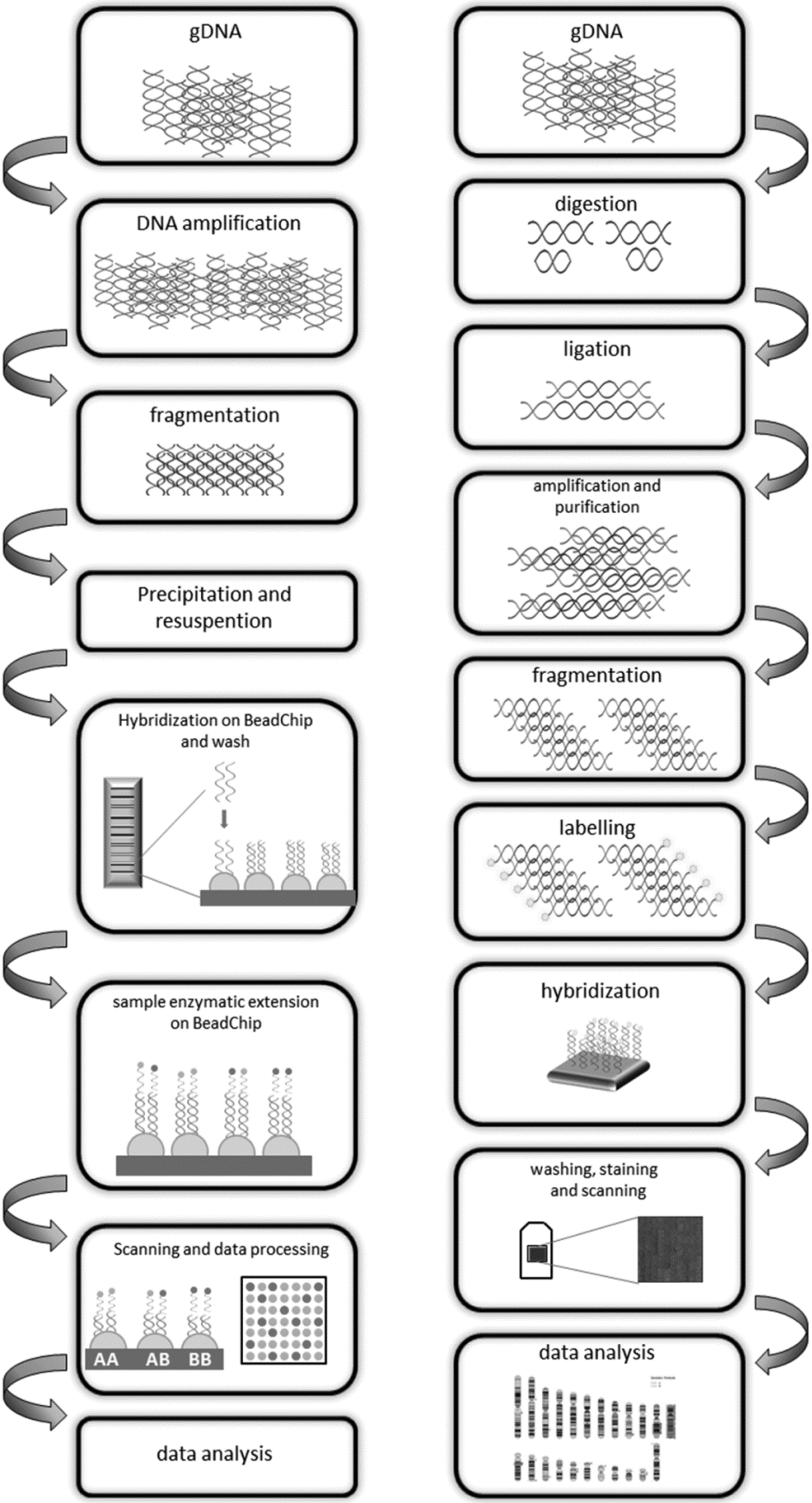

Fig. (1). Schematic view of SNP array analysis by Affymetrix (right) and Illumina (left).

for large chromosomal anomalies (duplications, deletions, and UPD) has recently been identified in cancer-free individuals. The frequency of detectable clonal mosaicism in peripheral blood was low (<0.5%) from birth until 50 years of age, after which it rapidly rose to 2% to 3% in the elderly. Many of the mosaic anomalies were characteristic of those found in hematologic malignancies and identify common deleted regions with genes previously associated with these cancers [25, 26]. This has important implications for the identification of somatic cancer alterations. The samples used as germline DNA should be carefully analyzed for detectable clonal mosaicism. Otherwise, comparisons between somatic cells (used as a surrogate for germline cells) and tumors may result in implausible somatic changes, and the results may be misinterpreted. SNP genotypes data are expressed as $\log_2$ ratio values (test to reference intensity ratio) and reflect either no copy number changes ($\log_2$ ratio of 0) or loss/gain (lower or higher $\log_2$ ratio values, respectively). Finally, results for each SNP locus are statistically modeled, and the copy number profile can be visualized along the entire chromosome [18, 27].

SNP Array Applications

Because SNP array detects the fluorescent signal generated by specific SNP probes' hybridization, an important feature of SNP array is to provide information about both genotype and copy number state, allowing identification of LOH and copy number aberrations (CNAs). LOH is defined as the loss of one allele at a given position, which is generally caused by deletions. The lost segment can be replaced by the same region of its homologous chromosome, resulting in a CN-LOH (or UPD), without any apparent copy number change, and can involve whole or partial chromosomes. Acquired UPD can occur during errors at meiosis, leading to the inheritance of regions derived from only one parent, while partial UPD occurs during clonal expansion of cancer cells (Fig. **2**). UPD can affect regions containing tumor suppressor genes or oncogenes with mutations, microlesions, or aberrantly methylated patterns that potentially confer positive selection of the neoplastic clone and influence cancer development. Because UPD detection depends on the identification of large regions of homozygosity, SNP arrays are excellent tools for this purpose. An important characteristic of the human genome is the presence of germline CNVs, defined as chromosomal segments (median length 150 Kb) that vary in number of copies between individuals and that represent a source of genetic diversity. These polymorphic variants are generally benign but can be associated with complex disease. CNVs have been identified in recent years through the new genome-wide technologies, including SNP array [28], and additional efforts are being made to classify them, such as the Database of Genomic Variants (DGV) [29].

Because of the complexity of the human genome, it is important to identify the proper genomic source to use as reference DNA. In contrast to other tools (*e.g.*, CGH array), SNP arrays do not require co-hybridization of reference and target DNA, so each sample is hybridized individually in a given array. Hence, hybridization results can be compared with normal but unrelated samples, such as reference genotyping data from the International HapMap Project or samples obtained from SNP array of DNA from unrelated donors. When unrelated samples are used, identification of CNAs or CN-LOH requires further analysis to interpret the data and requires excluding germline CNVs using predictive computational methods (*e.g.*, DGV database) and false CN-LOH using size-exclusion criteria. Nevertheless, the false discovery rate could remain significant [24]. Therefore, to avoid these mistakes, it is important to compare target DNA with a normal matched sample.

The main advantages of SNP array technology are its sensitivity, accuracy, resolution, and the ability to generate multiple data per sample in a whole genome–scale single experiment. Although molecular karyotyping provides significantly greater resolution than conventional cytogenetics, it is limited to identifying known genomic abnormalities in dividing cells. SNP array, however, requires a small amount of input material and is able to provide information about amplifications, deletions, and CN-LOH in all chromosomes. This approach was initially used for research purposes to discover novel disease-related aberrations. Several efforts have been made to standardize analysis for clinical and diagnostic purpose [7, 30]. However, SNP array remains limited in the detection of balanced rearrangements and inversions without DNA losses; therefore, FISH is still useful to interpret complex rearrangements or to distinguish tandem from dispersed duplications.

APPLICATIONS OF SNP ARRAY-BASED TECHNOLOGY IN LEUKEMIA

SNP Array-based Technology in Chronic Myeloid Leukemia

CML is genetically characterized by the presence of the reciprocal translocation t(9;22)(q34;q11), resulting in a *BCR-ABL1* gene fusion on the derivative chromosome 22. SNP arrays have been used in CML to detect cryptic submicroscopic genomic aberrations cooperating with *BCR-ABL1* in leukemogenesis and to elucidate whether characteristic genomic alterations are associated with tyrosine kinase inhibitor (TKI) resistance. The most common abnormal lesions detected by SNP arrays were submicroscopic 9q34 (10%), 22q11.2 (12%), or both (6%) deletions adjacent to the t(9;22) breakpoint. However, outcomes of treatment with imatinib were not significantly different among patients with these deletions ($p > 0.05$) [31-32], suggesting that imatinib could overcome the usual prognostic impact of clonal aberrations identified by SNP arrays. In addition to known TKI resistance-associated genomic lesions, such as duplication of the *BCR-ABL1* gene and trisomy 8, matched analysis of samples obtained at the time of diagnosis and at relapse identified recurrent submicroscopic alterations on chromosomes 1, 8, 9, 12, 16, 17, 19, and 22 [33-34]. Additional copy number changes are common in blast phase CML samples, compared with chronic phase samples, with losses occurring more frequently than gains [33]. One of the most common lesions was deletion in the immunoglobulin lambda constant 1 (*IGLC1*) locus on chromosome 22q11 [34]. Studies of the frequency of CN-LOH in CML patients have yielded different results. Huh *et al.,* reported CN-LOH in

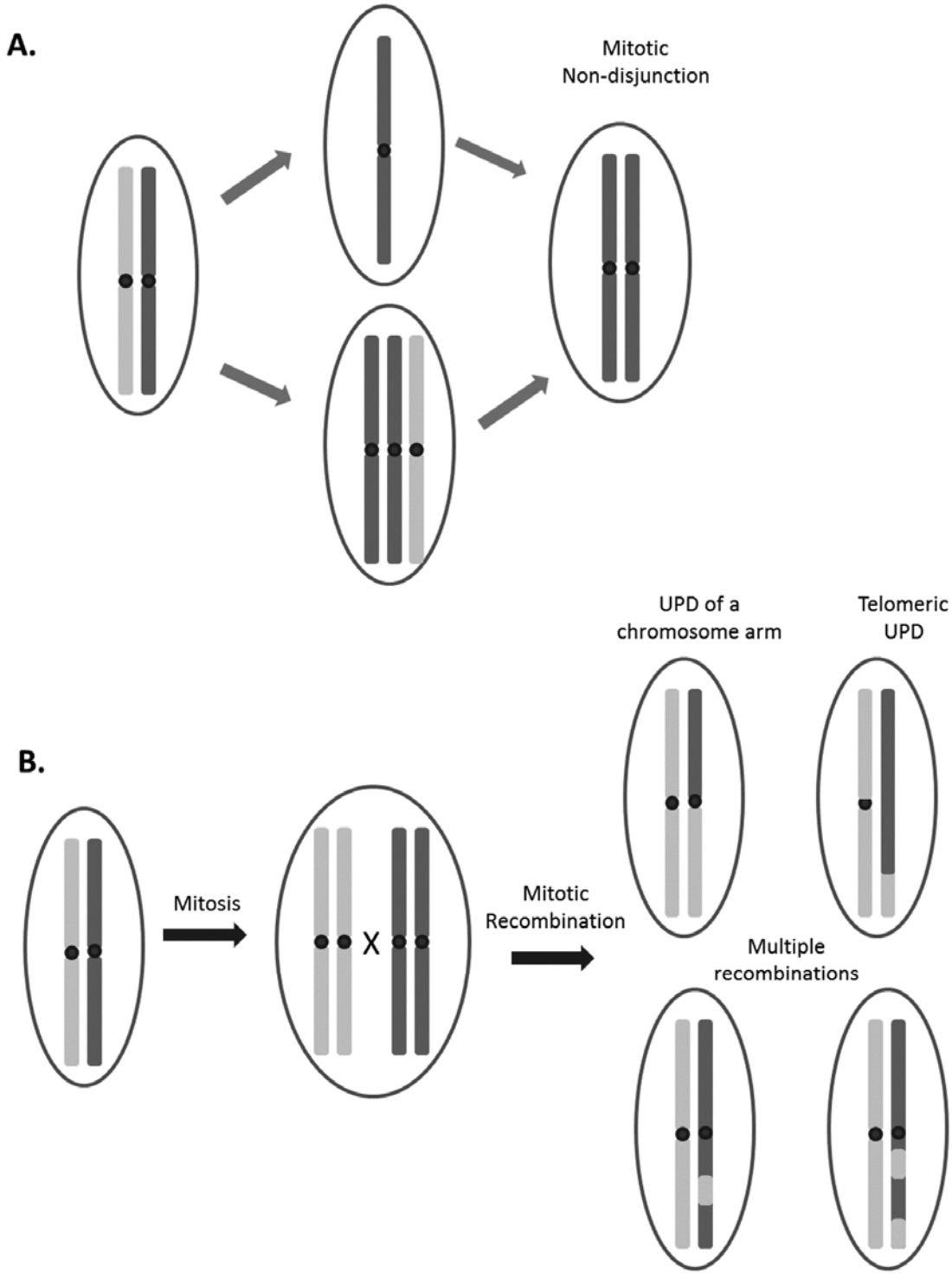

Fig. (2). Representation of mechanisms leading to uniparental disomy (UPD). **A**, Trisomy rescue by mitotic reduction of the single homologue in a trisomic cell; monosomic rescue by duplication of the normal homologue. **B**, UPD not involving a whole chromosome but only a part of it (segmental UPD), such as an arm or a telomeric region.

only two of 118 cases (1.7%) [31]. In contrast, studies including blast crisis and TKI-resistant CML reported CN-LOH in 11 of 45 and 8 of 41 patients [34]. This may be caused by the fact that genomic alterations are more prevalent in patients with advanced disease, such as TKI-resistant CML, compared with CML in chronic phase.

SNP Array-based Technology in Acute Lymphoblastic Leukemia

ALL represents a biologically and clinically heterogeneous group of lymphoid cell malignancies arising from genetic insults that block lymphoid differentiation and drive

Table 1. Some recurrent regions of genetic abnormalities in paediatric and adult ALL.

Genomic Location	Type of Alteration	Candidate Cancer Related Genes	Platform	Acute Leukemia Subtype	Frequency	References
5q33	Deletion	*EBF1*	50k, 500k	B-ALL	~ 6% pediatric B-ALL	[36, 38]
6q15-16	Deletion	*GRIK2, CASP8AP2, EPHA7*	CGH	T-ALL	~ 12% of T-ALL	[141-142]
6q23	Amplification	*c-MYB*	CGH, 100k, 500k	T-ALL	8-14% of pediatric T-ALL	[36, 38, 141]
7p12	Deletion	*IKZF1*	50k, 500k, SNP6.0	B-ALL	~ 80% of *BCR-ABL1* positive ALL; ~ 15% of pediatric B-ALL cases	[36, 48, 56-59]
9p13	Deletion	*PAX5*	50k, 500k, SNP6.0	B-ALL	~ 30% of pediatric and adult B-ALL	[38-39, 46, 48, 65]
9p21	Deletion	*CDKN2A/B*	50k, 500k, SNP6.0	B-ALL, T-ALL	~ 21%-36% pediatric B-ALL and nearly 50% of adult and adolescent B-ALL; 30-70% of pediatric and adult T-ALL	[36, 40, 64-66, 143-144]
10q23	Deletion	*PTEN*	CGH, 50k, 500k,	T-ALL	6-8% of T-ALL	[36, 145]
12p13	Deletion	*ETV6*	50k, 500k	B-ALL	~ 25% pediatric B-ALL	[36, 38]
13q14	Deletion	*RB1*	50k, 500k	B-ALL, T-ALL	~ 5% pediatric B-ALL; ~ 12% pediatric T-ALL	[36, 38]
18p11	Deletion	*PTPN2*	CGH, SNP6.0	T-ALL	6% of T-ALL	[146]
21q22	Amplification	*RUNX1*	50k, 500k	B-ALL	~ 3% pediatric B-ALL;	[36, 38]
Xp22; Yp11	*P2RY8-CRLF2* rearrangement	*CRLF2*	50k, 500k, SNP6.0	B-ALL	Up to16% of pediatric and adult B-ALL; > 50% Down-Syndrome ALL*	[69-72]
Xq26	Deletion	*PHF6*	CGH	T-ALL	16% of pediatric T-ALL cases; 38% of adult T-ALL cases*	[88]

* percentages include also cases with sequence mutations

aberrant cell proliferation and survival. Traditionally, ALL has been classified according to phenotype into precursor T-cell, precursor B-cell, and mature B-cell (Burkitt) ALL, which are then further classified according to recurrent cytogenetic abnormalities, including aneuploidy (hyperdiploidy or, less commonly, low hypodiploidy), chromosomal rearrangements, such as t(12;21) *ETV6-RUNX1*, t(1;19) *TCF3-PBX1*, t(9;22) *BCR-ABL1*, and rearrangement of mixed lineage leukemia (MLL) and the enhancer elements of the immunoglobulin heavy chain locus (*IGH@*) [35]. In the past decade, the advent of high-resolution genome-wide analyses of DNA CNAs and LOH have led to the detection of many novel genetic abnormalities, providing critical new insights into the pathogenesis of ALL and defining new prognostic models.

SNP Array-based Technology in B-Lineage ALL

Multiple studies have used SNP arrays to identify submicroscopic imbalances in ALL that were not visible with cytogenetic analysis, predominantly in children (Table **1**). These studies revolutionized the traditional ALL classification based on cytogenetics by identifying new subgroups. Genetic alterations are commonly less than one megabase (Mb) in size and in most cases target a single or few genes implicated in pathways with a key role in leukemogenesis, such as lymphoid development (*IKZF1, PAX5, EBF1, VPREB1*), cell cycle regulation and tumor suppression (*CDKN2A/CDKN2B, PTEN, BTG1, RB1*), lymphoid signaling (*BTLA, CD200, BLNK*), drug responsiveness (glucocorticoid receptor gene *NR3C1*), and DNA mismatch repair (*MTOR, HERC1, PRKCZ* and *PIK3C2B*) [36-42]. These alterations differ significantly in frequency and characteristics among specific ALL subtypes. *MLL*-rearranged leukemias harbor less than one CNA per case, suggesting that *MLL* is a potent oncogene that requires very few cooperating alterations to induce leukemia transformation. In contrast, *ETV6-RUNX1*- and *BCR-ABL1*-

rearranged leukemias harbor six to eight alterations per case that may occur years after the initial chromosome rearrangements, indicating that these additional postnatal genetic alterations are required to induce the full leukemic phenotype [36]. A higher number of CNAs (55; range, 51-63) has been observed in hyperdiploid ALL. In this disease, gain of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 has been shown to be even more characteristic of high hyperdiploid childhood ALL than previously believed, supporting the notion that these alterations comprise the primary genetic event in high hyperdiploid ALL. Moreover, additional sub-chromosomal imbalances have been observed, including microdeletions of *ETV6*, *CDKN2A*, *PAX5*, and possibly *PAN3* and constitutional and acquired changes of *ARID5B* [43]. A SNP array-based platform was recently used to investigate whether differences in survival between pediatric and adult ALL patients could be attributed, in part, to differences in genomic alterations. The study demonstrated that no unequivocal pattern of submicroscopic genomic alterations separates adult ALL from pediatric ALL. Therefore, in addition to different therapy regimens, differences in prognosis are probably based on genetic subgroups according to cytogenetically detectable lesions but not focal genomic copy number microlesions. The only two differences detected were deletions involving *ETV6* on chromosome 12 and alterations on chromosome 17; deletions of 12p13.2 (*ETV6*) were more common in non-pediatric ALL (30%) than in adult ALL (7%). Genomic changes of both deletion of 17p (*TP53*) and duplication of 17q (11% and 9%, respectively) were more frequent in adult ALL than in non-hyperdiploid pediatric ALL (2% and 1%, respectively) [44].

Genetic Alterations in B-Lymphoid Development Genes

Genetic abnormalities affecting genes involved in lymphoid development occur in more than two-thirds of cases. These alterations include deletions, focal amplifications, novel translocations, and point mutations involving transcriptional regulators of early development of B-cell lineage ALL (B-ALL), such as *IKZF1* (Ikaros family zinc finger 1), *PAX5* (paired box 5), *EBF1* (early B-cell factor), *LEF1* (lymphoid enhancer factor 1), and immunoglobulin family genes, such as *VPREB1* (pre-B lymphocyte gene 1).

The gene most frequently affected by genetic alterations is the transcription factor *PAX5* that plays a key role in B-cell commitment [45]. Alterations, including monoallelic deletion or focal amplifications, occur in nearly 30% of both children and adults with B-ALL and result in loss of Pax5 protein expression or in the production of a Pax5 isoform lacking the DNA binding domain and/or transcriptional regulatory domain [36, 39]. Inactivating point mutations in *PAX5* are also observed (7%-30%) as well as chromosomal translocations involving multiple partners, such as *ETV6*, *ENL*, *FOXP1, ZNF521, PML, C20ORF112, AUTS2, JAK2, POM121, HIPK1, DACH1, LOC392027, SLCO1B3, ASXL1,* and *KIF3B* [36, 46-47]. Alterations of *PAX5* have been shown to not influence treatment outcome [39, 48]. Genetic aberrations have been also identified in the DNA-binding zinc finger transcription factor *IKZF1* (Ikaros), which is crucial for early lymphoid development (Fig. **3**). Ikaros functions as a regulator of gene expression and chromatin remodelling in the development of the immune system and in hematopoietic differentiation. Data regarding the structure and function of Ikaros, the clinical relevance of genetic inactivation of *IKZF1*, and signal transduction pathways that regulate Ikaros function have been recently described by Payne KJ *et al.,* [49]. Deletion of a single *IKZF1* allele or mutations of a single copy of *IKZF1* were first detected in 15% of all cases of pediatric B-cell ALL and in more than 80% of Philadelphia chromosome–positive (Ph+) lymphoid leukemia cases, both de novo Ph+ ALL and chronic myeloid leukemia at progression to lymphoid blast crisis [37, 50-51]. In the majority of cases mutations functionally resulted in haploinsufficiency of the *IKZF1* gene, along with expression of a functionally inactive form of Ikaros, which could

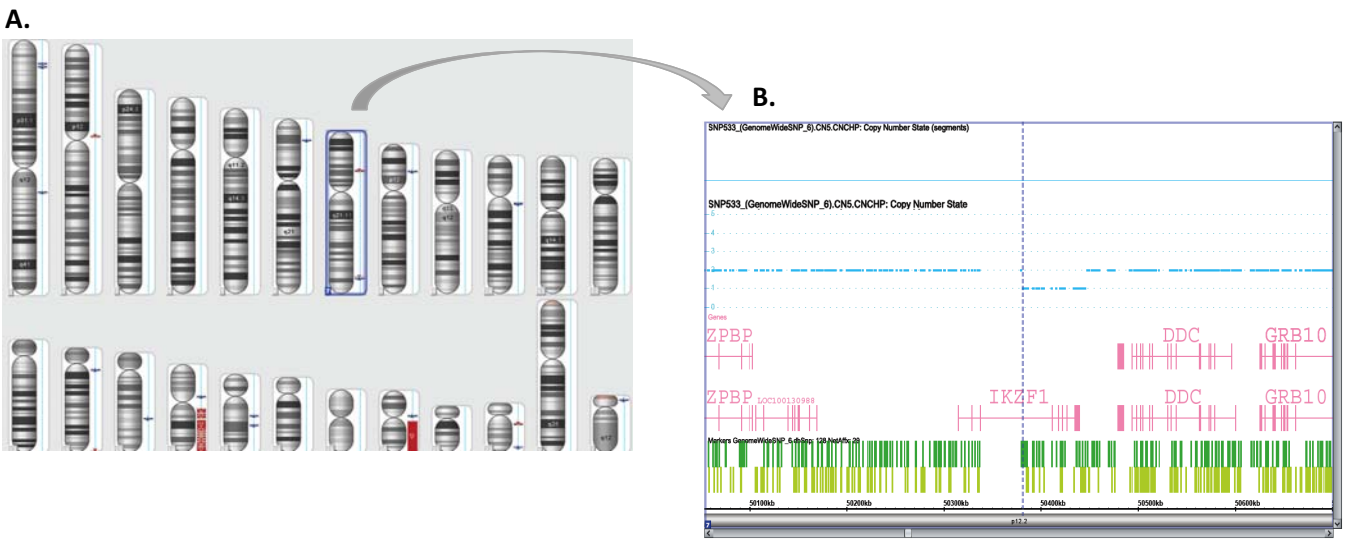

Fig. (3). A, Molecular carioview generated by Chromosome Analysis Suite (ChAS) Software (Affymetrix) of a B-ALL patient. Genomic gains are shown in blue, genomic losses in red. **B**, Deletion of IKZF1 involving only a subset of exons in a patient with B-ALL. The image was generated by ChAS Software (Affymetrix).

potentially act as a dominant-negative form. Expression of such dominant-negative isoform in hematopoietic progenitors impairs lymphoid development [52], and loss of *IKZF1* accelerates the onset of Ph+ ALL in a retroviral bone marrow transplant and transgenic models of this disease [53]. Moreover, we recently demonstrated that ALL patients with *IKZF1* deletions have a unique gene expression profile unrelated to *BCR-ABL1* rearrangement and characterized by down-regulation of genes required for B-cell lineage development and DNA repair upon response to DNA damage and up-regulation of genes involved in cell cycle/apoptosis, *JAK-STAT* signalling, and stem cell self-renewal [54]. The analysis of a twin pair concordant for ALL showed that in childhood Ph+ ALL, *BCR-ABL1* gene fusion can be a prenatal and possibly initiating genetic event, whereas deletion of *IKZF1* is a secondary and probable postnatal mutation that is associated with poor prognosis [55]. Several studies demonstrated that *IKZF1* deletions are significantly associated with an increased relapse rate and adverse events and are correlated with poor outcome in patients with Ph+ ALL [48, 56-57]. Alteration of *IKZF1* is also associated with poor outcome in *BCR-ABL1*-negative ALL [48, 56, 58-59], and this association is independent of commonly used risk stratification features, such as age, sex, white blood cell count, and level of minimal residual disease (MRD). *IKZF1* deletions and nonsense mutations identified at diagnosis are preserved at relapse [56] and may be used for highly sensitive MRD tests in addition to the repertoire of MRD markers currently available for monitoring MRD in ALL [60-61]. *BCR-ABL1*-negative cases with *IKZF1* deletions and poor prognosis were noted to have similar gene expression profiles as *BCR-ABL1*-positive ALL. This led to the definition of the *BCR-ABL1*-like subtype of B-cell ALL with haploinsufficiency of *IKZF1* or other transcriptional regulators [50, 62].

Genetic Alterations in Other Pathways

In addition to previously reported CNAs involving genes with a key role in lymphoid differentiation, genetic alterations targeting genes regulating cell cycle and tumor suppression, such as deletions involving the *CDKN2A/CDKN2B* genes [63], have been found in many studies. These deletions are present in 21% to 36% of pediatric B-ALL patients [64] and nearly 50% of adult and adolescent B-ALL patients [65-66]. In *BCR-ABL1*-positive ALL patients, *CDKN2A* and *CDKN2B* deletions have been observed at diagnosis in 29% and 25% of cases, respectively. Deletions were predominantly monoallelic, and in more than half of cases, the minimal overlapping region of the lost area on chromosome 9p included a large number of genes. The rate of *CDKN2A/ARF* loss was higher at relapse compared with diagnosis [40]. Whether this loss is associated with prognosis is still uncertain. Some studies reported a correlation of *CDKN2A/B* deletion with poor prognosis, whereas others showed no correlation [67]. Confirming previous findings from mice models [68], we demonstrated that deletions of *CDKN2A/B* are significantly associated with higher white blood cell count and poor outcome in terms of overall survival, disease-free survival, and cumulative incidence of relapse in adult patients with *BCR-ABL1*-positive ALL [40].

Studies using SNP arrays have also identified genetic lesions that define novel ALL subtypes characterized by rearrangement of *CRLF2,* the gene encoding the cytokine receptor-like 2 factor, also known as thymic stromal lymphopoietin receptor (*TSLPR*) [69-72]. Up to 50% of *BCR-ABL1*-like ALL harbors rearrangements of *CRLF2.* These may include a translocation of *CRLF2*, which is located at the pseudoautosomal region 1 (*PAR1*) of chromosome Xp/Yp, into the immunoglobulin heavy chain locus at chromosome 14q or a focal *PAR1* deletion proximal of *CRLF2* that results in a novel fusion *P2RY8-CRLF2* [69-72]. Less commonly, a missense mutation in exon 6, F232C, results in constitutive *CRLF2* dimerization [72]. All these events result in over-expression of full-length *CRLF2* on the surface of leukemic cells harboring the genetic alterations, providing a cell-surface marker amenable to detection by flow cytometry for clinical diagnostic purposes. Overall, aberrant expression of *CRLF2* was found in 12.5% to 15% of B-ALL that lacks typical chromosomal rearrangements, but was not over-expressed in B-ALL cases that have recurring rearrangements or in other lymphoid malignancies [69-72]. *CRLF2* alteration is seen at low rates (5%-7%) when all B-ALL cases are grouped together; however, it is seen in 50%-60% of Down syndrome–associated ALL, suggesting that *CRLF2* over-expression is especially relevant to tumorigenesis in patients with trisomy 21 [70-72]. In high-risk B-ALL, rearrangements of *CRLF2* are frequently found together with *IKZF1* alterations and activating mutations in *JAK1* and *JAK2*, most commonly at or near R683 in the pseudokinase domain of *JAK2* [69-71], and are associated with very poor outcome [73-74]. Therapeutically important, this JAK-STAT activation, in both experimental models or primary human leukemic cells, is impaired by the use of JAK inhibitors, [70] and a dose-finding phase I trial is ongoing to investigate the efficacy of a combination of JAK inhibitor and conventional chemotherapy in relapsed and refractory childhood tumors (COG ADVL1011). Recently, RNA-seq and whole-genome sequencing of 12 *BCR-ABL1*-like ALL cases showed that cryptic rearrangements, sequence variations, and submicroscopic structural variants are a hallmark of *CRLF2* wild-type *BCR-ABL1*-like ALL [75]. These include chimeric fusions encoding constitutively active tyrosine kinases (*EBF1-PDGFRB, NUP214-ABL1, STRN3-JAK2*, and *BCR-JAK2*); dysregulating cytokine receptors (*IGH@-EPOR*); activating complex sequence mutations in the transmembrane domain of IL7R, encoding the IL-7R chain; and deletions of *SH2B3* (LNK), which encodes a negative regulator of Jak2. Recurrence screening of large cohorts of patients with high-risk B-ALL has shown that several of these alterations (*NUP214-ABL1*, *EBF1-PDGFRB*, *IL7R* mutations, and *SH2B3* alterations) are recurrent in *BCR-ABL1*-like ALL [75].

Other genetic lesions associated with novel subtypes of ALL are intragenic deletions of *ERG* (v-ets erythroblastosis virus E26 oncogene homolog), a member of the erythroblast transformation-specific (ETS) family of transcription factors (Mullighan, C. G, ASH Annual Meeting Abstracts 2007). The ETS family is known to play important roles in several biological processes, such as development, differentiation, proliferation, apoptosis, migration, tissue remodeling, invasion, and angiogenesis in various cell types, including B

cells, endothelial cells, and fibroblasts as well as tumor cells [76]. The *ERG* deletions involve a subset of exons resulting in the expression of internally deleted *ERG* transcripts, with altered reading frames predicted to produce a prematurely truncated N-terminal protein fragment. Moreover, deletions are observed only in a novel ALL subtype of B-ALL with a characteristic gene expression signature [77], which lacks any previously identified cytogenetic or submicroscopic genetic alterations and has a high frequency (62%) of Ras signaling pathway mutations [78].

SNP Array-based Technology in T-Lineage ALL

Like B-ALL, T-cell lineage ALL (T-ALL) is also genetically heterogeneous and comprises multiple distinct subtypes defined by chromosomal rearrangements that commonly deregulate hematopoietic transcription factors, for instance by juxtaposition to regulatory elements of T-cell antigen receptor genes, or result in chimeric fusions, for example those involving MLL [79]. T-ALL is less common than B-ALL but has an inferior outcome [80]. The advent of genome-wide profiling of DNA CNAs by CGH and SNP array have led to the identification of additional novel genetic alterations targeting transcriptional regulators of lymphoid development, tumor suppression, and the cell cycle, providing critical new insights into the pathogenesis of T-ALL. These alterations include amplification associated with *NUP214-ABL1* rearrangement, [81] resulting in constitutive activation of the Abl1 kinase; cryptic deletion on chromosome 11 [del(11)(p12p13)] dysregulating *LMO2* [82]; focal deletions leading to dysregulated expression of *TAL1* [36]; rearrangements of the *LYL1* oncogene [83]; amplification of *MYB* [36]; deletions of *RB1* [36]; and deletion and sequence mutation of *PTEN* [36, 84], *FBXW7* [85] and *WT1* [86]. Moreover, the absence of biallelic TCR gamma (ABD) locus deletion, a characteristic of early thymocyte precursors before V(D)J recombination, has been demonstrated to be the most robust predictor of induction failure in children with T-ALL [87]. ABD T-ALL specimens are associated with specific gene expression characteristics, including over-expression of the *HOXA/MEIS1* cluster, *LYL1*, and *ERG,* and show increased expression of genes within the PI3K-AKT-mTOR and RAF-MEK-ERK signal transduction pathways [87].

Novel Alterations Identified by Next-Generation Sequencing in T-ALL

Knowledge of genetic alterations occurring in T-ALL has been improved by the use of next-generation sequencing (NGS). Exon capture and NGS of X chromosome genes has allowed the identification of inactivating mutations and deletions in the X-linked plant homeodomain finger 6 (*PHF6*) gene in childhood and adult T-ALL [88]. *PHF6* mutations were found nearly exclusively in T-ALL samples from male patients and were associated with leukemias driven by aberrant expression of the homeobox transcription factor oncogenes *TLX1* and *TLX3* [88]. Sequencing of the whole genome has provided a molecular characterization of early T-cell precursor (ETP) ALL, which comprises up to 15% of T-ALL and is associated with a high risk of treatment failure [89]. Activating mutations have been identified in genes regulating cytokine receptor and RAS signaling, such as *NRAS, KRAS, FLT3, IL7R, JAK3, JAK1, SH2B3* and *BRAF* (67%), inactivating lesions disrupting hematopoietic development, such as *GATA3, ETV6, RUNX1, IKZF1* and *EP300* (58%) and histone-modifying genes, such as *EZH2, EED, SUZ12*, *SETD2* and *EP300* (48%). Importantly, the gene expression profile of ETP ALL is similar to that of normal and myeloid leukemia hematopoietic stem cells, suggesting the possibility that myeloid-directed therapies might improve the poor prognosis of ETP ALL. Whole exome sequencing was recently performed in 67 T-ALL patients, including pediatric (n=30) and adult (n=37) patients and identified 15 (*NOTCH1, FBXW7, WT1, BCL11B, CNOT3, RPL10, RPL5, JAK3, PTEN, DNM2, ODZ2, PHF6, TET1, KDM6A, MAGEC3*) candidate driver genes. Among these, eight were known drivers in T-ALL, and seven were new. Mutations in *CNOT3* were present exclusively in adults, whereas *RPL10* mutations were mainly found in children. *RPL5* and *RPL10* are two genes encoding ribosomal proteins that occupy neighboring positions in the 60S ribosomal complex. *CNOT3* is part of the CCR4-NOT complex that regulates transcription initiation and elongation, mRNA degradation, and mRNA export. Additional screening in an independent confirmation cohort of 144 patients with T-ALL identified mutation frequencies of 3.8% (8/211) for *CNOT3* and 7.1% (15/211) for *RPL5* and *RPL10*. Functionally, *CNOT3* knockdown causes tumors in a sensitized Drosophila melanogaster model, whereas yeast and lymphoid cells expressing the *RPL10* Arg98Ser mutant showed a ribosome biogenesis defect [90]. Moreover, Ferrando *et al.,* [91] recently shed light on the basis of chemotherapy resistance by using NGS to systematically compare the genomes of cells from relapsed T-ALL tumors, paired pre-therapy tumor samples, and healthy patients. This approach resulted in the discovery of a mutation in the gene encoding the enzyme cytosolic 5′-nucleotidase II (*NT5C2*). *NT5C2* encodes a ubiquitous enzyme that has a role in the metabolism and inactivation of nucleoside analogue drugs, such as 6-mercaptopurine and 6-thioguanosine. Remarkably, the mutations identified in *NT5C2* resulted in as much as a 48-fold increase in *NT5C2* enzyme activity. These gain-of-function mutations were identified only in relapsed disease and conveyed resistance to 6-mercaptopurine and 6-thioguanine, but not to nelarabine or ara-G (guanine arabinoside) [91].

SNP Array-based Technology in Acute Myeloid Leukemia

AML is a clonal and heterogeneous disorder of hematopoietic blast cells that lose the ability to correctly differentiate and proliferate, causing bone marrow failure and ineffective production of mature myeloid lineage cells. In recent years, considerable progress has been made in understanding the molecular basis of AML to define new diagnostic and prognostic markers, and the recurrent cytogenetic aberrations together with the more recent identified molecular abnormalities have been included in the World Health Organization (WHO) classification of AML [92]. Chromosome rearrangements are detected in approximately 55% of adult AML and comprise translocations t(8;21) *RUNX1-RUNX1T1*, inv(16) or t(16;16) *CBFB-MYH11*, t(15;17) *PML-RARA*, t(9;11) *MLLT3-MLL*, and the new cytogenetic entities t(6;9) *DEK-NUP214*, inv(3)

or t(3;3) *RPN1-EVI1* and megakaryoblastic AML with t(1;22) *RBM15-MKL1*. Furthermore, new entities were defined by the presence of gene mutations in *NPM1*, *CEBPA*, and *FLT3* genes that impair hematopoietic differentiation or confer survival advantages [93]. Molecular abnormalities occur equally in the normal and complex karyotype subgroups affecting prognosis, while about 15% of cytogenetically normal AML are non mutated [92]. Nevertheless, the mechanisms of pathogenesis, development, and resistance are still far from being completely understood, and the clinical and biological heterogeneity of AML is only partially explained by chromosome aberrations and molecular mutations detectable with conventional cytogenetic or molecular approaches. Therefore, application of SNP array technology, with consequent identification of additional genetic alterations, improved AML classification, and prognostic classification has the ultimate aim of leading to effective and efficient therapy.

Uniparental Disomy

Because of the genotype potential of SNP array, studies have investigated the presence of UPD in AML, trying to correlate this phenomenon with clinical impact. The pathogenetic role of UPD in AML might be associated with the activation of oncogenes or the inactivation of tumor suppressor genes. Moreover, uncovering novel regions of UPD has the potential to identify previously unknown mutational targets, as demonstrated by the identification of *JAK2* V617F in myeloproliferative disorders (MPD) [94].

An early study conducted in adult patients with AML with diverse karyotypes revealed the presence of homozygous regions classified as interstitial, presumably derived from small deletions of genomic DNA, whole chromosomes and terminal, involving large telomeric regions [95]. Usually the total extent of UPD regions spans from 2 Mb to more than 100 Mb, depending on the resolution of the SNP array platform [95-99]. Analysis of UPD according to the karyotype risk classification revealed the highest frequency in AML with adverse risk cytogenetics and a relatively lower frequency in AML with favorable and intermediate-risk cytogenetics. Importantly, UPD occurred in approximately 15% to 20% of primary AML with normal karyotype compared with <10% of heterogeneous AML groups, and comparison with matched remission samples identified UPD somatic origin with restriction to the neoplastic clone [95-100]. Because of its frequency, especially in normal karyotype AML, UPD represents a possible approach to further classify the subgroup. In the largest screening of diagnosis AML samples with diverse range of karyotypes typical of the disease, acquired UPDs were observed in 17% of samples with a minimum genomic size of 7.17 Mb, and few samples had more than two acquired UPDs. The mechanisms that lead to segmental or whole chromosomal UPD are mitotic recombination or non-disjunction, respectively. Two important characteristics of UPDs in AML emerged from this study: the prevalent mitotic recombination origin (87%) and the nonrandom chromosomal distribution, with chromosomes 11 and 13 holding the highest number, suggesting that UPDs may preferentially target genes that are essential for proliferation and survival of hematopoietic progenitors [97]. In general, chromosomes involved at different levels in UPDs are 1p, 2p and 2q, 5q, 6p, 8q, 9p and 9q, 11p and 11q, 12q, whole chromosome 13 and 13q, 16p, 17p and 17q, 19p and 19q, 21q and Xq, and invariably the most frequently affected are 6p, 11p and 13q [95-104]. A further analysis revealed the presence of AML-related genes in most of these UPD regions, such as *JAK2* on 9p, *WT1* and *ATM* at 11p, *MLL* at 11q, *FLT3* at 13q, *CEBPA* at 19q, *RUNX1* at 21q. Other known cancer related genes possibly involved in these regions are *TP53* and *CDKN2A/B,* located at 17p and 9p, respectively, while no obvious candidate genes are located in the other loci. Acquired UPD give rise to homozygosity of a preexisting pathogenic mutation, thus the increased levels of mutation may confer an additional proliferative advantage to the homozygous clone. In accord with the implications of UPDs, clinically relevant homozygous mutations have been confirmed, at least in some cases, such as *WT1*, *FLT3*-internal tandem duplication (ITD), *CEBPA* [97, 105]. *WT1* homozygous mutations have functional significance and have been reported in AML. Different evidence demonstrated that the presence of a *WT1*-mutated clone confers drug resistance and failure to achieve complete remission, although its role in hematologic malignancies is not completely understood [106-108]. *FLT3*-ITD mutations are related to a worse clinical outcome, with a mutant allele dosage effect that results in poor prognosis or therapy failure [109]. UPD-dependent homozygosity of *FLT3*-ITD is common in AML [95, 97-98, 102, 105, 110]. Mutations in *CEBPA* genes represent an independent prognostic factor, conferring an improved outcome with lower relapse rate and improved survival [111]. Although a biallelic mutation is not a prerequisite for AML, it is important in development, as is shown in familial cases of AML in which a germline *CEBPA* mutation is followed after a long latency by a second mutation and subsequently develops into AML [112].

Due to the extent of the regions involved in UPDs, it is possible that more than one gene has the potential for pathogenetic mutations. Similarly, when no mutations are detected in cancer-related genes, it is possible that different and still unknown target genes are present. For example, in studies conducted in large cohorts of patients with myelo-dysplastic syndromes (MDS) and MDS/myeloproliferative neoplasms (MPN) overlap syndromes, including secondary AML (sAML) patients, screening of the high frequency UPD11q, UPD4q, and UPD7q led to the identification of the candidate genes c-*CBL, TET2*, and *EZH2,* respectively. Further analysis revealed the presence of novel missense, frameshift, and nonsense mutations, differentially distributed across the myeloid disorders, but generally prevailing in more aggressive subsets, as MDS/MPN evolved into AML [98, 113-115]. c-*CBL* is a member of E3 ubiquitin ligases that mediates proliferative signals through the ubiquitination of several tyrosine kinases, and its inactivation may lead to enhanced and prolonged signaling. Further screenings of the *Cbl* family demonstrated the presence of additional mutations in other members, such as *Cbl-b*, delineating a novel class of genes that confer a common mechanism of leukemogenesis, a typical phenotype, and a particularly poor prognosis [114]. Therefore delineation of common areas of UPD can facilitate the identification of pathogenic mutations and potentially explain clinical phenotype.

In relapsed AML, the number of acquired segmental UPDs is increased compared with diagnosis samples, ranging up to 40%, but lesions are comparable in extent, and the most common UPDs were observed on chromosome 13 (*FLT3*) and UPD19q (*CEBPA*). Interestingly, comparison of somatic *FLT3*-ITD mutation in diagnosis and relapsed samples can reveal the clonal evolution of the disease, confirming the correlation between mutant allele dosage and proliferation advantage and providing new markers for disease monitoring [110, 116]. Common regions of UPD are summarized in Table **2**.

Copy Number Alterations

SNP array analysis of AML usually revealed a lower number of CNAs compared with ALL, but sAML, evolved from MDS or MDS/MPD, is characterized by more lesions than primary AML (pAML), with a greater proportion of patients showing more than one defect [17, 104, 117] (Table **2**). Furthermore, in pAML the adverse-risk cytogenetics group is more affected by CNAs than the favorable and intermediate-risk groups, which show the same frequency of gains and losses, and alterations are equally distributed across all French-American-British (FAB) subtypes, with the exception of FAB-M7 acute megakaryoblastic leukemia, which contains significantly more CNAs per genome compared with all other subtypes. Generally, more than one CNA per genome occurs in more than 30% and 35% of primary and secondary AML, respectively, with gains occurring less frequently than losses [97, 99, 118]. The frequency of CNAs can differ in specific AML subtypes, such as in the cytogenetically normal AML (CN-AML) subgroup, where it occurs in approximately 50% of patients [100]. The most frequent deletions in whole AML subtypes are -7/del(7q), del(5q) , 17p (ranging from 5% to 20%), and the recurrent amplified regions (trisomies included) are 8q, 11q, 12p, 21q, and 13q (ranging from 5% to 15%, with the exception of chromosome 8 gains that reach 40%). The high-risk group comprises exclusively or at increased frequencies losses at 20q, 18q , 8p, 16q, 12p, 17p, 3p, and 3q and gains at 5p [97, 102]. The majority of CNAs involve known cancer-related genes, although some have not been previously implicated in AML. Examples of genes included in deleted regions are the tumor suppressors *TP53* (17p13), *CDKN2A/B* (9p21), *TET2* (4q24), *NF1* (17q11), the transcription factors *ETV6* (12p13), *CBFB* (16q22), *FOXP1* (3p14), *RUNX1* (21q22), and other tumor-related genes, including *FHIT* (3p14), *CTNNA1* (5q31), and *EZH2* (7q35). Genes involved in amplifications are the oncogenes *MYC* (*8q24*), *MLL* (11q23), and *ETS2* (21q22) [99, 101, 119]. How often these genes are affected can correlate with specific AML subtypes, as in the case of *NF1* deletions in the *CBFB–MYH11* AML subtype [120]. A preliminary identification of recurrent genomic lesions can be followed by a more detailed characterization, providing new insight into the leukemogenesis process and suggesting novel therapeutic approaches, such as *NF1* inactivation linked to the Ras pathway [121]. Novel genetic lesions that potentially play a role in tumorigenesis or disease progression are deletions of *RPS6KA2* (involved in cell growth control and differentiation), *TRPS1* (transcription factor), and gain of *PRKCH* (prostate cancer–related gene) [100, 119, 122]. Of note, *CUX1*, a DNA-binding protein involved in hematopoiesis, has been recently described as the candidate tumor suppressor gene associated to the common lesions on chromosome 7 [123]. SNP array approach allows to define more accurately the boundaries of known and common deletions to precisely identify the extent of the lesions, as in the case of del(20q) and 5q- AML [124, 125]. Interestingly, this approach also enables the uncovering of cryptic translocations caused by unbalanced rearrangements that carry focal deletions in the breakpoint regions, for example the *NUP98-NSD1* fusion transcript involving exon 12 in *NUP98* and exon 6 in *NSD1* and the cryptic rearrangements of bands 6q27 and 11q23, resulting in *MLL–MLLT4* gene fusions [99-100]. Finally, SNP array analysis of paired diagnosis and relapsed samples, enables the identification of shared lesions which potentially play a driver role in AML pathogenesis and delineate the persistence of not eradicated founder clones which after acquiring novel secondary aberrations give rise to the relapsed AML [126, 127].

SNP Array Analysis of Specific AML Subgroups

Acute promyelocytic leukemia (APL) is a favorable-prognosis AML subtype characterized by the translocation t(15;17), resulting in the fusion of the promyelocytic leukemia (*PML*) gene and retinoic acid receptor alpha (*RARA*) gene (*PML-RARA*) [128], and is recognized as a separate entity in the WHO classification [92]. SNP array of diagnosis APL showed a lower range (17%) of genome abnormalities compared with other myeloproliferative disorders, with the number of lesions in each sample ranging from one to four. Among these, 13% were UPDs occurring almost exclusively in regions 10q or 11p, with only one occurring in 19q. Deletions and duplication have been both identified in 15% of samples and spanning from 0.02 Mb to the whole chromosome arm. However, in some cases in which no recurrent altered region has been identified, results showed putative cancer genes involved in gains (*ERBB2* at 17p) or losses (*ETV6* at 12p and *TP53* at 17p) [129].

AML with t(8;21)(q22;q22) [*RUNX1-RUNX1T1*] and inv(16)(p13.1q22) or t(16;16)(p13.1;q22) [*CBFB-MYH11*] (core binding factor acute myeloid leukemia, CBF-AML) belong to a genetic entity recognized in the WHO classification (AML with recurrent genetic abnormalities) [92] and characterized by a relatively favorable prognosis. SNP array analysis in adult and pediatric samples identified a low rate of CN-LOH (3%-8%) and a low burden of CNAs (<30%) that correlate with a worse prognosis and a significant increase in rate of relapse, affecting survival [130-133]. In this AML group, regions of UPDs include chromosomes 11p and 11q, and 6p [130]. The majority of CNAs are adjacent to the breakpoints of the characteristic chromosomal rearrangements, suggesting a relative genomic stability. Other recurrent genomic lesions are 7q and 11p deletions, with a minimal deleted region containing *MLL3* and *WT1* genes, respectively, rare focal deletions, including *EZH2* (7q), *RAD21* (8q), *SUZ12* (17q) [131-132], and extended duplications and deletions that typically comprise more than 10 genes, including known cancer-related genes such as c-*MYC* [130].

Pediatric AML is an aggressive malignancy that accounts for approximately 20% of acute leukemias in children and adolescents [134]. In contrast to pediatric ALL, de novo

Table 2. Some recurrent regions of genetic abnormalities in acute myeloid leukemia.

Genomic Location	Type of Alteration	Candidate Cancer Related Genes	Platform	Acute Leukemia Subtype	Frequency	References
1p and 1q	UPD	*NRAS, c-MPL, TP73, FGR, EPHA2, EPHB2*	10k, 50k, 250k, 500k, SNP 6.0	pAML, sAML, CBF-AMLs	1p 1-1,5% of pAML and sAML, 6% of CN-AML; 1q 6% in sAML	[17, 97-98, 100-101, 131, 136]
4q	UPD	*TET2, c-KIT*	250k, SNP 6.0	pAML, sAML, CBF-AMLs	1-2% of pAML and sAML	[98, 113, 131]
6p	UPD	*PIM1, CDKN1A, DDR1*	10k, 50k, 250k, 500k, SNP 6.0	pAML, CBF-AMLs	2-6% of pAML including t(8;21) and CN-AML	[95, 98-101, 130, 136]
7q	UPD	*EZH2*	50k, 250k	pAML, sAML	1-3% of pAML and sAML	[17, 98, 102]
9p and 9q	UPD	*JAK2, CDKN2A/B*	10k, 250k	pAML, sAML	~3% of pAML and sAML including pediatric AML	[95, 98, 101, 135]
11p and 11q	UPD	*WT1, ATM, MLL, HRAS, c-CBL, CDKN1C, LMO2*	10k, 50k, 250k, 500k, SNP 6.0	pAML, sAML, APL, CBF-AMLs	11p 2-6% of pAML, sAML and APL; 11q 1-3% of pAML and sAML	[17, 95, 97, 99-100, 129-130, 135-136]
13q and whole 13	UPD	*FLT3, FLT1, RB1 BRCA2*	10k, 50k, 250k, 500k, SNP 6.0	pAML, sAML	2-6% of pAML and sAML including older patients	[95, 97-102, 135-136]
17p and q	UPD	*TP53*	10k, 250k	pAML, sAML	17p and 17q 1-3% of pAML and sAML	[17, 97-98]
19p and q	UPD	*CEBPA*	10k, 50k, 250k, 500k, SNP 6.0	pAML, APL, CBF-AMLs	19q 1,6% of pAML; 19p 1-3% in pAML	[95, 100-101, 131]
21q	UPD	*RUNX1*	10k, 250k	pAML, sAML	1-3% of pAM and sAML	[17, 95, 98, 101]
4q24	Deletion	*TET2*	50k, 250k	pAML, sAML	3% of CN-AML	[17, 101, 129]
5q/5q31	Deletion	*CTNNA1*	10k, 250k, SNP 6.0	pAML, sAML	3% of pAML, up to 10% of sAML	[17, 97, 99]
6q27	Deletion	*RPS6KA2, MLLT4*	250k, 500k	pAML, sAML	3% of pAML	[17, 100]
-7/del(7q)	Deletion	*EZH2, CUX1, MLL3*	10k, 50k, 250k	pAML, sAML, APL, CBF-AMLs	6-8% of pAML and t(8;21) AML; up to 15% of sAML	[17, 97, 123, 129-130]
8q21-24	Deletion	*TRPS1, c-MYC, RAD21*	250k, 500k, SNP 6.0	pAML, CBF-AMLs	3% of pAML and sAML, 8% of t(8;21) AML	[17, 100, 131]
9p12-21	Deletion	*CDKN2A/B*	250k, SNP 6.0	pAML, sAML, CBF-AMLs	3% of CN-AML. ~10% of sAML and t(8;21) AML	[17, 101, 131]
11p; 11q23-25	Deletion	*WT1, MLL*	250k, 500k	pAML, sAML, CBF-AMLs	3% of pAML	[17, 100]
12p/12p12-13	Deletion	*ETV6*	50k, 250k, 500k, SNP 6.0	pAML, sAML	3 to up 9% of pAML and sAML	[17, 99-101, 129]
16p13; 16q/16q22	Deletion	*MYH11; CBFB*	250k, SNP 6.0	sAML, CBF-AMLs	3-9% of sAML; ~15% of inv(16) AML	[17, 99, 131]
17p/17p13	Deletion	*TP53*	10k, 50k, 250k, SNP 6.0	pAML, APL	2% of pAML and APL, 6% of sAML	[17, 97, 99, 129]
17q/17q11	Deletion	*NF1, SUZ12*	250k, SNP 6.0	pAML, CBF-AMLs	3-6% of pAML, sAML and inv(16) AML	[17, 99, 101, 131]
21q21-22	Deletion	*RUNX1*	250k, SNP 6.0	pAML, sAML, CBF-AMLs	3% of CN-AML, 10% of t(8;21) AML	[17, 101, 131]

Table 2. contd….

Genomic Location	Type of Alteration	Candidate Cancer Related Genes	Platform	Acute Leukemia Subtype	Frequency	References
+4/4p and 4q	Amplification	*c-KIT*	250k	sAML, CBF-AMLs	3% of sAML and t(8;21) AML	[17, 130]
+8/ampl (8q)/8q24	Amplification	*MYC, CCDC26*	10k, 100k, 500k, 250k, SNP 6.0	pAML; sAML, CBF-AMLs	3-14% of pAML including pediatric AML	[17, 97, 99, 101, 130-131, 135]
11q/11q23	Amplification	*MLL*	10k, 250k, SNP 6.0	pAML, sAML, CBF-AMLs	2-5% of pAML and inv(16) AML	[17, 97, 99, 131]
+13/ampl (13q)	Amplification	*FLT3*	10k, 50k, 250k, SNP 6.0	pAML, sAML, APL, CBF-AMLs	2-5% of pAML, inv(16) AML and APL	[17, 97, 129, 131]
+15/15q21	Amplification	*PML*	50k, 250k	APL, CBF-AMLs	4% of APL and t(8;21) AML	[129-130]
+21/21q	Amplification	*ETS2*	10k, 50k, 250k, SNP 6.0	pAML, sAML, APL	1,5-6% of pAML and APL	[97, 99, 129]
+22/22q	Amplification	-	SNP 6.0	CBF-AMLs	14% of inv(16) AML	[131]

AML is characterized by a very low burden of genomic alterations, with 34% lacking any identifiable CNAs, including CN-LOH [135]. Analysis of more than 100 patients with various genetic subtypes identified somatic CN-LOH regions in only 13% of the entire cohort on chromosomes 13, 11p, 9p, 6p, 8q, 15q, 17q, and 21 that targeted genes previously shown to be involved in the pathogenesis of AML (*e.g.*, *FLT3*). A total of 207 CNAs were detected, with a mean of only 2.38 (range 0-45) gains or losses per sample. The frequency of CNAs was similar across the various AML genetic subtypes, with the exception of FAB-M7 acute megakaryoblastic leukemia, which had a higher number of lesions per patient (9.33). The majority of lesions were not recurrent, and statistical analysis revealed the presence of only 30 cancer-related genes, 21 of which had been previously implicated in AML. Finally, focal microdeletions occurred in targets of AML-associated chromosomal translocations, including *MYH11*, *CBF*, *MLL*, *NSD1*, *MLF1*, *ERG*, and *MYST4*, and the partners of chimeric transcripts *NUP98-NSD1* and *MLL*-MLLT4 [135].

Older patients, aged >60 years, have a worse prognosis compared with younger patients, with a higher incidence of high-risk cytogenetic changes, frequent antecedent hematologic disorders, and often poor performance status. SNP array analysis revealed increased genomic alterations, in particular CN-LOH (more than 46% compared with 25% in younger patients). As in pediatric and adult AML, the most common chromosomes involved are 13q, 1p, 6p and 11q [136].

Clinical Implications of SNP Array in AML

Taken together, results obtained from SNP array analysis provide contributions to risk assessment and improved patient classification based on clinical, morphological, and cytogenetic schemes. Generally, the presence of abnormal SNP array–detected lesions has an adverse impact on clinical outcome in both primary and secondary AML and is associated with disease progression [119]. Moreover, several acquired alterations have a specific role, such as *TP53* mutations and UPD17p, representing an independent prognostic factor [104, 118, 137]; aberrations involving chromosomes 5, 7, or 17p, which are closely related to leukemic transformation of MPN [138]; and UPD13q together with abnormal SNP lesions, which are related to an inferior outcome, lower remission rate, and inferior overall survival/event-free survival in normal cytogenetic AML [100, 139]. This approach can improve patient risk stratification in heterogeneous myeloid malignancies, including MDS, MDS/MDP and sAML, because it can detect additional alterations that contribute to the further delineation of subgroups with poorer overall and event-free survival [140].

CONCLUSIONS

The huge technological progress of the past decade has allowed a revolution in the way molecular experiments are performed. Two important players, improved resolution/ sensitivity and the ability to investigate the whole genome in a single step, have contributed to the identification of novel genetic alterations involved in the pathogenesis of CML, ALL, and AML, associated with acute leukemia subtypes, responsible for prognosis, and suitable for target therapies. SNP array technology, by overcoming the limits of standard cytogenetics, allows a global analysis of all CNAs and reveals the occurrence of LOH, which is missed by cytogenetics and CGH. Moreover, for diagnostic use, the SNP array–based approach could be of great importance in cases in which cells fail to divide, which is required for standard cytogenetics, and/or in cases that do not yield suitable material for karyotyping. Using this approach, a large number of candidate genes have been identified in both AML and ALL. However, this technique enables the detection of only unbalanced defects and does not allow for distinguishing between multiple large clones. Currently, NGS-based approaches are overcoming the limits of SNP array, allowing the comprehensive identification of all

sequence variations and structural rearrangements in leukemia. The challenges now are the availability of bioinformatic tools to analyze the data, the establishment of driver versus passenger mutations, and the development of novel target therapies.

CONFLICT OF INTEREST

The authors declare no conflicts of interest for this manuscript.

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ABBREVIATIONS

ABD	=	absence of biallelic TCR gamma
ALL	=	acute lymphoblastic leukemia
AML	=	acute myeloid leukemia
APL	=	acute promyelocytic leukemia
CGH	=	comparative genomic hybridization
Chas	=	chromosome Analysis Suite
CML	=	chronic myeloid leukemia
CN-AML	=	cytogenetically normal AML
CNAs	=	copy number aberrations
CN-LOH	=	copy number neutral loss of heterozygosity
CNVs	=	copy number variations
DGV	=	database of genomic variants
EBF1	=	early B-cell factor
ETS	=	erythroblast transformation-specific
FAB	=	french-american-british
FISH	=	fluorescence in situ hybridization
GWAS	=	genome-wide association studies
IGH@	=	immunoglobulin heavy chain
IGLC1	=	immunoglobulin lambda constant 1
IKZF1	=	Ikaros family zinc finger 1
ITD	=	internal tandem duplication
Kb	=	Kilobase
LEF1	=	lymphoid enhancer factor 1
Mb	=	Megabase
MDS	=	myelodysplastic syndromes
MLL	=	mixed lineage leukemia
MPD	=	myeloproliferative disorders
MPN	=	myeloproliferative neoplasms
NGS	=	next-generation sequencing
pAML	=	primary AML
PAX5	=	paired box 5
PML	=	promyelocytic leukemia
RARA	=	retinoic acid receptor alpha
sAML	=	secondary AML
SNP	=	single nucleotide polymorphism
T-ALL	=	T-cell lineage ALL
TKI	=	tyrosine kinase inhibitor
TSLPR	=	thymic stromal lymphopoietin receptor
UPD	=	uniparental disomy
VPREB1	=	pre-B lymphocyte gene 1

REFERENCES

[1] Grimwade, D.; Mrozek, K. Diagnostic and prognostic value of cytogenetics in acute myeloid leukemia. *Hematol. Oncol. Clin. North Am.* **2011**, *25* (6), 1135-61, vii.

[2] Iacobucci, I.; Papayannidis, C.; Lonetti, A.; Ferrari, A.; Baccarani, M.; Martinelli, G. Cytogenetic and molecular predictors of outcome in acute lymphocytic leukemia: recent developments. *Curr. Hematol. Malig. Rep.* **2012**, *7* (2), 133-143.

[3] Costantini, M.; Clay, O.; Federico, C.; Saccone, S.; Auletta, F.; Bernardi, G. Human chromosomal bands: nested structure, high-definition map and molecular basis. *Chromosoma* **2007**, *116* (1), 29-40.

[4] Najfeld, V. Diagnostic application of FISH to hematological malignancies. *Cancer Invest.* **2003**, *21* (5), 807-814.

[5] Maciejewski, J. P.; Mufti, G. J. Whole genome scanning as a cytogenetic tool in hematologic malignancies. *Blood* **2008**, *112* (4), 965-974.

[6] Maciejewski, J. P.; Tiu, R. V.; O'Keefe, C. Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. *Br. J. Haematol.* **2009**, *146* (5), 479-488.

[7] Simons, A.; Sikkema-Raddatz, B.; de Leeuw, N.; Konrad, N. C.; Hastings, R. J.; Schoumans, J. Genome-wide arrays in routine diagnostics of hematological malignancies. *Hum. Mutat.* **2012**, *33* (6), 941-948.

[8] 8. Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J. P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, N.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J. C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R. H.; Wilson, R. K.; Hillier, L. W.; McPherson, J. D.; Marra, M. A.; Mardis, E. R.; Fulton, L. A.; Chinwalla, A. T.; Pepin, K. H.; Gish, W. R.; Chissoe, S. L.; Wendl, M. C.; Delehaunty, K. D.; Miner, T. L.; Delehaunty, A.; Kramer, J. B.; Cook, L. L.; Fulton, R. S.; Johnson, D. L.; Minx, P. J.; Clifton, S. W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J. F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M.; Gibbs, R. A.; Muzny, D. M.; Scherer, S. E.; Bouck, J. B.; Sodergren, E. J.; Worley, K. C.; Rives, C. M.; Gorrell, J. H.; Metzker, M. L.;

Naylor, S. L.; Kucherlapati, R. S.; Nelson, D. L.; Weinstock, G. M.; Sakaki, Y.; Fujiyama, A.; Hattori, M.; Yada, T.; Toyoda, A.; Itoh, T.; Kawagoe, C.; Watanabe, H.; Totoki, Y.; Taylor, T.; Weissenbach, J.; Heilig, R.; Saurin, W.; Artiguenave, F.; Brottier, P.; Bruls, T.; Pelletier, E.; Robert, C.; Wincker, P.; Smith, D. R.; Doucette-Stamm, L.; Rubenfield, M.; Weinstock, K.; Lee, H. M.; Dubois, J.; Rosenthal, A.; Platzer, M.; Nyakatura, G.; Taudien, S.; Rump, A.; Yang, H.; Yu, J.; Wang, J.; Huang, G.; Gu, J.; Hood, L.; Rowen, L.; Madan, A.; Qin, S.; Davis, R. W.; Federspiel, N. A.; Abola, A. P.; Proctor, M. J.; Myers, R. M.; Schmutz, J.; Dickson, M.; Grimwood, J.; Cox, D. R.; Olson, M. V.; Kaul, R.; Shimizu, N.; Kawasaki, K.; Minoshima, S.; Evans, G. A.; Athanasiou, M.; Schultz, R.; Roe, B. A.; Chen, F.; Pan, H.; Ramser, J.; Lehrach, H.; Reinhardt, R.; McCombie, W. R.; de la Bastide, M.; Dedhia, N.; Blocker, H.; Hornischer, K.; Nordsiek, G.; Agarwala, R.; Aravind, L.; Bailey, J. A.; Bateman, A.; Batzoglou, S.; Birney, E.; Bork, P.; Brown, D. G.; Burge, C. B.; Cerutti, L.; Chen, H. C.; Church, D.; Clamp, M.; Copley, R. R.; Doerks, T.; Eddy, S. R.; Eichler, E. E.; Furey, T. S.; Galagan, J.; Gilbert, J. G.; Harmon, C.; Hayashizaki, Y.; Haussler, D.; Hermjakob, H.; Hokamp, K.; Jang, W.; Johnson, L. S.; Jones, T. A.; Kasif, S.; Kaspryzk, A.; Kennedy, S.; Kent, W. J.; Kitts, P.; Koonin, E. V.; Korf, I.; Kulp, D.; Lancet, D.; Lowe, T. M.; McLysaght, A.; Mikkelsen, T.; Moran, J. V.; Mulder, N.; Pollara, V. J.; Ponting, C. P.; Schuler, G.; Schultz, J.; Slater, G.; Smit, A. F.; Stupka, E.; Szustakowski, J.; Thierry-Mieg, D.; Thierry-Mieg, J.; Wagner, L.; Wallis, J.; Wheeler, R.; Williams, A.; Wolf, Y. I.; Wolfe, K. H.; Yang, S. P.; Yeh, R. F.; Collins, F.; Guyer, M. S.; Peterson, J.; Felsenfeld, A.; Wetterstrand, K. A.; Patrinos, A.; Morgan, M. J.; de Jong, P.; Catanese, J. J.; Osoegawa, K.; Shizuya, H.; Choi, S.; Chen, Y. J. Initial sequencing and analysis of the human genome. *Nature* **2001**, *409* (6822), 860-921.

[9] Sachidanandam, R.; Weissman, D.; Schmidt, S. C.; Kakol, J. M.; Stein, L. D.; Marth, G.; Sherry, S.; Mullikin, J. C.; Mortimore, B. J.; Willey, D. L.; Hunt, S. E.; Cole, C. G.; Coggill, P. C.; Rice, C. M.; Ning, Z.; Rogers, J.; Bentley, D. R.; Kwok, P. Y.; Mardis, E. R.; Yeh, R. T.; Schultz, B.; Cook, L.; Davenport, R.; Dante, M.; Fulton, L.; Hillier, L.; Waterston, R. H.; McPherson, J. D.; Gilman, B.; Schaffner, S.; Van Etten, W. J.; Reich, D.; Higgins, J.; Daly, M. J.; Blumenstiel, B.; Baldwin, J.; Stange-Thomann, N.; Zody, M. C.; Linton, L.; Lander, E. S.; Altshuler, D. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* **2001**, *409* (6822), 928-933.

[10] A haplotype map of the human genome. *Nature* **2005**, *437* (7063), 1299-1320.

[11] Abecasis, G. R.; Altshuler, D.; Auton, A.; Brooks, L. D.; Durbin, R. M.; Gibbs, R. A.; Hurles, M. E.; McVean, G. A. A map of human genome variation from population-scale sequencing. *Nature* **2010**, *467* (7319), 1061-1073.

[12] Abecasis, G. R.; Auton, A.; Brooks, L. D.; DePristo, M. A.; Durbin, R. M.; Handsaker, R. E.; Kang, H. M.; Marth, G. T.; McVean, G. A. An integrated map of genetic variation from 1,092 human genomes. *Nature* **2012**, *491* (7422), 56-65.

[13] Affymetrix (2007) Affymetrix Genome-Wide Human SNP Array 6.0 data sheet. Santa Clara (California). 2007.

[14] Illumina (2009) Genome-Wide DNA Analysis BeadChips Data Sheet. San Diego, California. 2009.

[15] Chen, W.; Yuan, L.; Cai, Y.; Chen, X.; Chi, Y.; Wei, P.; Zhou, X.; Shi, D. Identification of chromosomal copy number variations and novel candidate loci in hereditary nonpolyposis colorectal cancer with mismatch repair proficiency. *Genomics* **2013**, *102* (1), 27-34.

[16] Puiggros, A.; Puigdecanet, E.; Salido, M.; Ferrer, A.; Abella, E.; Gimeno, E.; Nonell, L.; Herranz, M. J.; Galvan, A. B.; Rodriguez-Rivera, M.; Melero, C.; Pairet, S.; Bellosillo, B.; Serrano, S.; Florensa, L.; Sole, F.; Espinet, B. Genomic arrays in chronic lymphocytic leukemia routine clinical practice: are we ready to substitute conventional cytogenetics and fluorescence in situ hybridization techniques? *Leuk. Lymphoma* **2012**, *54* (5), 986-995.

[17] Gondek, L. P.; Tiu, R.; O'Keefe, C. L.; Sekeres, M. A.; Theil, K. S.; Maciejewski, J. P. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. *Blood* **2008**, *111* (3), 1534-1542.

[18] Pounds, S.; Cheng, C.; Mullighan, C.; Raimondi, S. C.; Shurtleff, S.; Downing, J. R. Reference alignment of SNP microarray signals for copy number analysis of tumors. *Bioinformatics* **2009**, *25* (3), 315-321.

[19] Arteaga-Salas, J. M.; Zuzan, H.; Langdon, W. B.; Upton, G. J.; Harrison, A. P. An overview of image-processing methods for Affymetrix GeneChips. *Brief Bioinform.* **2008**, *9* (1), 25-33.

[20] Winchester, L.; Yau, C.; Ragoussis, J. Comparing CNV detection methods for SNP arrays. *Brief Funct. Genomic Proteomic* **2009**, *8* (5), 353-366.

[21] LaFramboise, T. Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. *Nucleic Acids Res.* **2009**, *37* (13), 4181-4193.

[22] de Andrade, M.; Atkinson, E. J.; Bamlet, W. R.; Matsumoto, M. E.; Maharjan, S.; Slager, S. L.; Vachon, C. M.; Cunningham, J. M.; Kardia, S. L. Evaluating the influence of quality control decisions and software algorithms on SNP calling for the affymetrix 6.0 SNP array platform. *Hum. Hered.* **2011**, *71* (4), 221-233.

[23] Schillert, A.; Ziegler, A. Genotype calling for the Affymetrix platform. *Methods Mol. Biol.* **2012**, *850*, 513-523.

[24] Heinrichs, S.; Li, C.; Look, A. T. SNP array analysis in hematologic malignancies: avoiding false discoveries. *Blood* **2010**, *115* (21), 4157-4161.

[25] Laurie, C. C.; Laurie, C. A.; Rice, K.; Doheny, K. F.; Zelnick, L. R.; McHugh, C. P.; Ling, H.; Hetrick, K. N.; Pugh, E. W.; Amos, C.; Wei, Q.; Wang, L. E.; Lee, J. E.; Barnes, K. C.; Hansel, N. N.; Mathias, R.; Daley, D.; Beaty, T. H.; Scott, A. F.; Ruczinski, I.; Scharpf, R. B.; Bierut, L. J.; Hartz, S. M.; Landi, M. T.; Freedman, N. D.; Goldin, L. R.; Ginsburg, D.; Li, J.; Desch, K. C.; Strom, S. S.; Blot, W. J.; Signorello, L. B.; Ingles, S. A.; Chanock, S. J.; Berndt, S. I.; Le Marchand, L.; Henderson, B. E.; Monroe, K. R.; Heit, J. A.; de Andrade, M.; Armasu, S. M.; Regnier, C.; Lowe, W. L.; Hayes, M. G.; Marazita, M. L.; Feingold, E.; Murray, J. C.; Melbye, M.; Feenstra, B.; Kang, J. H.; Wiggs, J. L.; Jarvik, G. P.; McDavid, A. N.; Seshan, V. E.; Mirel, D. B.; Crenshaw, A.; Sharopova, N.; Wise, A.; Shen, J.; Crosslin, D. R.; Levine, D. M.; Zheng, X.; Udren, J. I.; Bennett, S.; Nelson, S. C.; Gogarten, S. M.; Conomos, M. P.; Heagerty, P.; Manolio, T.; Pasquale, L. R.; Haiman, C. A.; Caporaso, N.; Weir, B. S. Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat. Genet.* **2012**, *44* (6), 642-650.

[26] Jacobs, K. B.; Yeager, M.; Zhou, W.; Wacholder, S.; Wang, Z.; Rodriguez-Santiago, B.; Hutchinson, A.; Deng, X.; Liu, C.; Horner, M. J.; Cullen, M.; Epstein, C. G.; Burdett, L.; Dean, M. C.; Chatterjee, N.; Sampson, J.; Chung, C. C.; Kovaks, J.; Gapstur, S. M.; Stevens, V. L.; Teras, L. T.; Gaudet, M. M.; Albanes, D.; Weinstein, S. J.; Virtamo, J.; Taylor, P. R.; Freedman, N. D.; Abnet, C. C.; Goldstein, A. M.; Hu, N.; Yu, K.; Yuan, J. M.; Liao, L.; Ding, T.; Qiao, Y. L.; Gao, Y. T.; Koh, W. P.; Xiang, Y. B.; Tang, Z. Z.; Fan, J. H.; Aldrich, M. C.; Amos, C.; Blot, W. J.; Bock, C. H.; Gillanders, E. M.; Harris, C. C.; Haiman, C. A.; Henderson, B. E.; Kolonel, L. N.; Le Marchand, L.; McNeill, L. H.; Rybicki, B. A.; Schwartz, A. G.; Signorello, L. B.; Spitz, M. R.; Wiencke, J. K.; Wrensch, M.; Wu, X.; Zanetti, K. A.; Ziegler, R. G.; Figueroa, J. D.; Garcia-Closas, M.; Malats, N.; Marenne, G.; Prokunina-Olsson, L.; Baris, D.; Schwenn, M.; Johnson, A.; Landi, M. T.; Goldin, L.; Consonni, D.; Bertazzi, P. A.; Rotunno, M.; Rajaraman, P.; Andersson, U.; Beane Freeman, L. E.; Berg, C. D.; Buring, J. E.; Butler, M. A.; Carreon, T.; Feychting, M.; Ahlbom, A.; Gaziano, J. M.; Giles, G. G.; Hallmans, G.; Hankinson, S. E.; Hartge, P.; Henriksson, R.; Inskip, P. D.; Johansen, C.; Landgren, A.; McKean-Cowdin, R.; Michaud, D. S.; Melin, B. S.; Peters, U.; Ruder, A. M.; Sesso, H. D.; Severi, G.; Shu, X. O.; Visvanathan, K.; White, E.; Wolk, A.; Zeleniuch-Jacquotte, A.; Zheng, W.; Silverman, D. T.; Kogevinas, M.; Gonzalez, J. R.; Villa, O.; Li, D.; Duell, E. J.; Risch, H. A.; Olson, S. H.; Kooperberg, C.; Wolpin, B. M.; Jiao, L.; Hassan, M.; Wheeler, W.; Arslan, A. A.; Bueno-de-Mesquita, H. B.; Fuchs, C. S.; Gallinger, S.; Gross, M. D.; Holly, E. A.; Klein, A. P.; LaCroix, A.; Mandelson, M. T.; Petersen, G.; Boutron-Ruault, M. C.; Bracci, P. M.; Canzian, F.; Chang, K.; Cotterchio, M.; Giovannucci, E. L.; Goggins, M.; Hoffman Bolton, J. A.; Jenab, M.; Khaw, K. T.; Krogh, V.; Kurtz, R. C.; McWilliams, R. R.; Mendelsohn, J. B.; Rabe, K. G.; Riboli, E.; Tjonneland, A.; Tobias, G. S.; Trichopoulos, D.; Elena, J. W.; Yu, H.; Amundadottir, L.; Stolzenberg-Solomon, R. Z.; Kraft, P.; Schumacher, F.; Stram, D.; Savage, S. A.; Mirabello, L.; Andrulis, I. L.; Wunder, J. S.; Patino Garcia, A.; Sierrasesumaga, L.; Barkauskas, D. A.; Gorlick, R. G.; Purdue, M.; Chow, W. H.; Moore, L. E.; Schwartz, K. L.; Davis, F. G.; Hsing, A. W.; Berndt, S. I.; Black, A.; Wentzensen, N.; Brinton, L. A.; Lissowska, J.;

Peplonska, B.; McGlynn, K. A.; Cook, M. B.; Graubard, B. I.; Kratz, C. P.; Greene, M. H.; Erickson, R. L.; Hunter, D. J.; Thomas, G.; Hoover, R. N.; Real, F. X.; Fraumeni, J. F., Jr.; Caporaso, N. E.; Tucker, M.; Rothman, N.; Perez-Jurado, L. A.; Chanock, S. J. Detectable clonal mosaicism and its relationship to aging and cancer. *Nat. Genet.* **2012**, *44* (6), 651-658.

[27] Gehlenborg, N.; O'Donoghue, S. I.; Baliga, N. S.; Goesmann, A.; Hibbs, M. A.; Kitano, H.; Kohlbacher, O.; Neuweger, H.; Schneider, R.; Tenenbaum, D.; Gavin, A. C. Visualization of omics data for systems biology. *Nat. Methods* **2010**, *7* (3 Suppl), S56-68.

[28] Zhang, F.; Gu, W.; Hurles, M. E.; Lupski, J. R. Copy number variation in human health, disease, and evolution. *Annu. Rev. Genomics Hum. Genet.* **2009**, *10*, 451-481.

[29] Iafrate, A. J.; Feuk, L.; Rivera, M. N.; Listewnik, M. L.; Donahoe, P. K.; Qi, Y.; Scherer, S. W.; Lee, C. Detection of large-scale variation in the human genome. *Nat. Genet.* **2004**, *36* (9), 949-951.

[30] Dougherty, M. J.; Wilmoth, D. M.; Tooke, L. S.; Shaikh, T. H.; Gai, X.; Hakonarson, H.; Biegel, J. A. Implementation of high resolution single nucleotide polymorphism array analysis as a clinical test for patients with hematologic malignancies. *Cancer Genet.* **2011**, *204* (1), 26-38.

[31] Huh, J.; Jung, C. W.; Kim, J. W.; Kim, H. J.; Kim, S. H.; Shin, M. G.; Kim, Y. K.; Suh, J. S.; Moon, J. H.; Sohn, S. K.; Nam, G. H.; Lee, J. E.; Kim, D. H. Genome-wide high density single-nucleotide polymorphism array-based karyotyping improves detection of clonal aberrations including der(9) deletion, but does not predict treatment outcomes after imatinib therapy in chronic myeloid leukemia. *Ann. Hematol.* **2011**, *90* (11), 1255-1264.

[32] Huh, J.; Jung, C. W.; Kim, H. J.; Kim, Y. K.; Moon, J. H.; Sohn, S. K.; Min, W. S.; Kim, D. H. Different characteristics identified by single nucleotide polymorphism array analysis in leukemia suggest the need for different application strategies depending on disease category. *Genes Chromosomes Cancer* **2013**, *52* (1), 44-55.

[33] Boultwood, J.; Perry, J.; Zaman, R.; Fernandez-Santamaria, C.; Littlewood, T.; Kusec, R.; Pellagatti, A.; Wang, L.; Clark, R. E.; Wainscoat, J. S. High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. *Leukemia* **2010**, *24* (6), 1139-1145.

[34] Nowak, D.; Ogawa, S.; Muschen, M.; Kato, M.; Kawamata, N.; Meixel, A.; Nowak, V.; Kim, H. S.; Kang, S.; Paquette, R.; Chang, M. S.; Thoennissen, N. H.; Mossner, M.; Hofmann, W. K.; Kohlmann, A.; Weiss, T.; Haferlach, T.; Haferlach, C.; Koeffler, H. P. SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations. *Blood* **2010**, *115* (5), 1049-1053.

[35] Pui, C. H.; Carroll, W. L.; Meshinchi, S.; Arceci, R. J. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *J. Clin. Oncol.* **2011**, *29* (5), 551-565.

[36] Mullighan, C. G.; Goorha, S.; Radtke, I.; Miller, C. B.; Coustan-Smith, E.; Dalton, J. D.; Girtman, K.; Mathew, S.; Ma, J.; Pounds, S. B.; Su, X.; Pui, C. H.; Relling, M. V.; Evans, W. E.; Shurtleff, S. A.; Downing, J. R. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **2007**, *446* (7137), 758-764.

[37] Iacobucci, I.; Storlazzi, C. T.; Cilloni, D.; Lonetti, A.; Ottaviani, E.; Soverini, S.; Astolfi, A.; Chiaretti, S.; Vitale, A.; Messa, F.; Impera, L.; Baldazzi, C.; D'Addabbo, P.; Papayannidis, C.; Lonoce, A.; Colarossi, S.; Vignetti, M.; Piccaluga, P. P.; Paolini, S.; Russo, D.; Pane, F.; Saglio, G.; Baccarani, M.; Foa, R.; Martinelli, G. Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). *Blood* **2009**, *114* (10), 2159-67.

[38] Kuiper, R. P.; Schoenmakers, E. F.; van Reijmersdal, S. V.; Hehir-Kwa, J. Y.; van Kessel, A. G.; van Leeuwen, F. N.; Hoogerbrugge, P. M. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia* **2007**, *21* (6), 1258-1266.

[39] Iacobucci, I.; Lonetti, A.; Paoloni, F.; Papayannidis, C.; Ferrari, A.; Storlazzi, C. T.; Vignetti, M.; Cilloni, D.; Messa, F.; Guadagnuolo, V.; Paolini, S.; Elia, L.; Messina, M.; Vitale, A.; Meloni, G.; Soverini, S.; Pane, F.; Baccarani, M.; Foa, R.; Martinelli, G. The PAX5 gene is frequently rearranged in BCR-ABL1-positive acute lymphoblastic leukemia but is not associated with outcome. A report on behalf of the GIMEMA Acute Leukemia Working Party. *Haematologica* **2010**, *95* (10), 1683-1690.

[40] Iacobucci, I.; Ferrari, A.; Lonetti, A.; Papayannidis, C.; Paoloni, F.; Trino, S.; Storlazzi, C. T.; Ottaviani, E.; Cattina, F.; Impera, L.; Abbenante, M. C.; Vignetti, M.; Vitale, A.; Potenza, L.; Paolini, S.; Soverini, S.; Pane, F.; Luppi, M.; Foa, R.; Baccarani, M.; Martinelli, G. CDKN2A/B alterations impair prognosis in adult BCR-ABL1-positive acute lymphoblastic leukemia patients. *Clin Cancer Res.* **2011**, *17* (23), 7413-7423.

[41] Trageser, D.; Iacobucci, I.; Nahar, R.; Duy, C.; von Levetzow, G.; Klemm, L.; Park, E.; Schuh, W.; Gruber, T.; Herzog, S.; Kim, Y. M.; Hofmann, W. K.; Li, A.; Storlazzi, C. T.; Jack, H. M.; Groffen, J.; Martinelli, G.; Heisterkamp, N.; Jumaa, H.; Muschen, M. Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. *J. Exp. Med.* **2009**, *206* (8), 1739-1753.

[42] Diouf, B.; Cheng, Q.; Krynetskaia, N. F.; Yang, W.; Cheok, M.; Pei, D.; Fan, Y.; Cheng, C.; Krynetskiy, E. Y.; Geng, H.; Chen, S.; Thierfelder, W. E.; Mullighan, C. G.; Downing, J. R.; Hsieh, P.; Pui, C. H.; Relling, M. V.; Evans, W. E. Somatic deletions of genes regulating MSH2 protein stability cause DNA mismatch repair deficiency and drug resistance in human leukemia cells. *Nat. Med.* **2011**, *17* (10), 1298-303.

[43] Paulsson, K.; Forestier, E.; Lilljebjorn, H.; Heldrup, J.; Behrendtz, M.; Young, B. D.; Johansson, B. Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (50), 21719-21724.

[44] Okamoto, R.; Ogawa, S.; Nowak, D.; Kawamata, N.; Akagi, T.; Kato, M.; Sanada, M.; Weiss, T.; Haferlach, C.; Dugas, M.; Ruckert, C.; Haferlach, T.; Koeffler, H. P. Genomic profiling of adult acute lymphoblastic leukemia by single nucleotide polymorphism oligonucleotide microarray and comparison to pediatric acute lymphoblastic leukemia. *Haematologica* **2010**, *95* (9), 1481-1488.

[45] Busslinger, M. Transcriptional control of early B cell development. *Annu. Rev. Immunol.* **2004**, *22*, 55-79.

[46] Familiades, J.; Bousquet, M.; Lafage-Pochitaloff, M.; Bene, M. C.; Beldjord, K.; De Vos, J.; Dastugue, N.; Coyaud, E.; Struski, S.; Quelen, C.; Prade-Houdellier, N.; Dobbelstein, S.; Cayuela, J. M.; Soulier, J.; Grardel, N.; Preudhomme, C.; Cave, H.; Blanchet, O.; Lheritier, V.; Delannoy, A.; Chalandon, Y.; Ifrah, N.; Pigneux, A.; Brousset, P.; Macintyre, E. A.; Huguet, F.; Dombret, H.; Broccardo, C.; Delabesse, E. PAX5 mutations occur frequently in adult B-cell progenitor acute lymphoblastic leukemia and PAX5 haploinsufficiency is associated with BCR-ABL1 and TCF3-PBX1 fusion genes: a GRAALL study. *Leukemia* **2009**, *23* (11), 1989-1998.

[47] Nebral, K.; Denk, D.; Attarbaschi, A.; Konig, M.; Mann, G.; Haas, O. A.; Strehl, S. Incidence and diversity of PAX5 fusion genes in childhood acute lymphoblastic leukemia. *Leukemia* **2009**, *23* (1), 134-143.

[48] Mullighan, C. G.; Su, X.; Zhang, J.; Radtke, I.; Phillips, L. A.; Miller, C. B.; Ma, J.; Liu, W.; Cheng, C.; Schulman, B. A.; Harvey, R. C.; Chen, I. M.; Clifford, R. J.; Carroll, W. L.; Reaman, G.; Bowman, W. P.; Devidas, M.; Gerhard, D. S.; Yang, W.; Relling, M. V.; Shurtleff, S. A.; Campana, D.; Borowitz, M. J.; Pui, C. H.; Smith, M.; Hunger, S. P.; Willman, C. L.; Downing, J. R. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N. Engl. J. Med.* **2009**, *360* (5), 470-480.

[49] Payne, K. J.; Dovat, S. Ikaros and tumor suppression in acute lymphoblastic leukemia. *Crit. Rev. Oncog.* **2011**, *16* (1-2), 3-12.

[50] Mullighan, C. G.; Miller, C. B.; Radtke, I.; Phillips, L. A.; Dalton, J.; Ma, J.; White, D.; Hughes, T. P.; Le Beau, M. M.; Pui, C. H.; Relling, M. V.; Shurtleff, S. A.; Downing, J. R., BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* **2008**, *453* (7191), 110-114.

[51] Iacobucci, I.; Lonetti, A.; Messa, F.; Cilloni, D.; Arruga, F.; Ottaviani, E.; Paolini, S.; Papayannidis, C.; Piccaluga, P. P.; Giannoulia, P.; Soverini, S.; Amabile, M.; Poerio, A.; Saglio, G.; Pane, F.; Berton, G.; Baruzzi, A.; Vitale, A.; Chiaretti, S.; Perini, G.; Foa, R.; Baccarani, M.; Martinelli, G. Expression of spliced oncogenic Ikaros isoforms in Philadelphia-positive acute lymphoblastic leukemia patients treated with tyrosine kinase inhibitors: implications for a new mechanism of resistance. *Blood* **2008**, *112* (9), 3847-3855.

[52] Tonnelle, C.; Dijon, M.; Moreau, T.; Garulli, C.; Bardin, F.; Chabannon, C. Stage specific over-expression of the dominant negative Ikaros 6 reveals distinct role of Ikaros throughout human B-cell differentiation. *Mol. Immunol.* **2009**, *46* (8-9), 1736-1743.

[53] Virely, C.; Moulin, S.; Cobaleda, C.; Lasgi, C.; Alberdi, A.; Soulier, J.; Sigaux, F.; Chan, S.; Kastner, P.; Ghysdael, J. Haploinsufficiency of the IKZF1 (IKAROS) tumor suppressor gene cooperates with BCR-ABL in a transgenic model of acute lymphoblastic leukemia. *Leukemia* **2010**, *24* (6), 1200-1204.

[54] Iacobucci, I.; Iraci, N.; Messina, M.; Lonetti, A.; Chiaretti, S.; Valli, E.; Ferrari, A.; Papayannidis, C.; Paoloni, F.; Vitale, A.; Storlazzi, C. T.; Ottaviani, E.; Guadagnuolo, V.; Durante, S.; Vignetti, M.; Soverini, S.; Pane, F.; Foa, R.; Baccarani, M.; Muschen, M.; Perini, G.; Martinelli, G. IKAROS deletions dictate a unique gene expression signature in patients with adult B-cell acute lymphoblastic leukemia. *PLoS One* **2012**, *7* (7), e40934.

[55] Cazzaniga, G.; van Delft, F. W.; Lo Nigro, L.; Ford, A. M.; Score, J.; Iacobucci, I.; Mirabile, E.; Taj, M.; Colman, S. M.; Biondi, A.; Greaves, M. Developmental origins and impact of BCR-ABL1 fusion and IKZF1 deletions in monozygotic twins with Ph+ acute lymphoblastic leukemia. *Blood* **2011**, *118* (20), 5559-5564.

[56] Kuiper, R. P.; Waanders, E.; van der Velden, V. H.; van Reijmersdal, S. V.; Venkatachalam, R.; Scheijen, B.; Sonneveld, E.; van Dongen, J. J.; Veerman, A. J.; van Leeuwen, F. N.; van Kessel, A. G.; Hoogerbrugge, P. M. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia* **2010**, *24* (7), 1258-1264.

[57] Martinelli, G.; Iacobucci, I.; Storlazzi, C. T.; Vignetti, M.; Paoloni, F.; Cilloni, D.; Soverini, S.; Vitale, A.; Chiaretti, S.; Cimino, G.; Papayannidis, C.; Paolini, S.; Elia, L.; Fazi, P.; Meloni, G.; Amadori, S.; Saglio, G.; Pane, F.; Baccarani, M.; Foa, R. IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. *J. Clin. Oncol.* **2009**, *27* (31), 5202-5207.

[58] Waanders, E.; van der Velden, V. H.; van der Schoot, C. E.; van Leeuwen, F. N.; van Reijmersdal, S. V.; de Haas, V.; Veerman, A. J.; van Kessel, A. G.; Hoogerbrugge, P. M.; Kuiper, R. P.; van Dongen, J. J. Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. *Leukemia* **2010**, *25* (2), 254-258.

[59] Yang, Y. L.; Hung, C. C.; Chen, J. S.; Lin, K. H.; Jou, S. T.; Hsiao, C. C.; Sheen, J. M.; Cheng, C. N.; Wu, K. H.; Lin, S. R.; Yu, S. L.; Chen, H. Y.; Lu, M. Y.; Wang, S. C.; Chang, H. H.; Lin, S. W.; Su, Y. N.; Lin, D. T. IKZF1 deletions predict a poor prognosis in children with B-cell progenitor acute lymphoblastic leukemia: a multicenter analysis in Taiwan. *Cancer Sci.* **2011**, *102* (10), 1874-1881.

[60] Venn, N. C.; van der Velden, V. H.; de Bie, M.; Waanders, E.; Giles, J. E.; Law, T.; Kuiper, R. P.; de Haas, V.; Mullighan, C. G.; Haber, M.; Marshall, G. M.; Md, N.; van Dongen, J. J.; Sutton, R. Highly sensitive MRD tests for ALL based on the IKZF1 Delta3-6 microdeletion. *Leukemia* **2011**, *26* (6), 1414-1416.

[61] Volejnikova, J.; Mejstrikova, E.; Dorge, P.; Meissner, B.; Zimmermannova, O.; Svojgr, K.; Stanulla, M.; Cario, G.; Schrappe, M.; Stary, J.; Hrusak, O.; Trka, J.; Fronkova, E. Ikaros (IKZF1) alterations and minimal residual disease at day 15 assessed by flow cytometry predict prognosis of childhood BCR/ABL-negative acute lymphoblastic leukemia. *Pediatr. Blood Cancer* **2012**, *60* (3), 420-427.

[62] Den Boer, M. L.; van Slegtenhorst, M.; De Menezes, R. X.; Cheok, M. H.; Buijs-Gladdines, J. G.; Peters, S. T.; Van Zutven, L. J.; Beverloo, H. B.; Van der Spek, P. J.; Escherich, G.; Horstmann, M. A.; Janka-Schaub, G. E.; Kamps, W. A.; Evans, W. E.; Pieters, R. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol.* **2009**, *10* (2), 125-134.

[63] Faderl, S.; Estrov, Z.; Kantarjian, H. M.; Thomas, D.; Cortes, J.; Manshouri, T.; Chan, C. C.; Hays, K. J.; Pierce, S.; Albitar, M. The incidence of chromosome 9p21 abnormalities and deletions of tumor suppressor genes p15(INK4b)/p16(INK4a)/p14(ARF) in patients with acute lymphoblastic leukemia. *Cytokines Cell Mol. Ther.* **1999**, *5* (3), 159-163.

[64] Sulong, S.; Moorman, A. V.; Irving, J. A.; Strefford, J. C.; Konn, Z. J.; Case, M. C.; Minto, L.; Barber, K. E.; Parker, H.; Wright, S. L.; Stewart, A. R.; Bailey, S.; Bown, N. P.; Hall, A. G.; Harrison, C. J. A comprehensive analysis of the CDKN2A gene in childhood acute lymphoblastic leukemia reveals genomic deletion, copy number neutral loss of heterozygosity, and association with specific cytogenetic subgroups. *Blood* **2009**, *113* (1), 100-107.

[65] Paulsson, K.; Cazier, J. B.; Macdougall, F.; Stevens, J.; Stasevich, I.; Vrcelj, N.; Chaplin, T.; Lillington, D. M.; Lister, T. A.; Young, B. D. Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: Unexpected similarities with pediatric disease. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (18), 6708-6713.

[66] Kim, M.; Yim, S. H.; Cho, N. S.; Kang, S. H.; Ko, D. H.; Oh, B.; Kim, T. Y.; Min, H. J.; She, C. J.; Kang, H. J.; Shin, H. Y.; Ahn, H. S.; Yoon, S. S.; Kim, B. K.; Shin, H. R.; Han, K. S.; Cho, H. I.; Lee, D. S. Homozygous deletion of CDKN2A (p16, p14) and CDKN2B (p15) genes is a poor prognostic factor in adult but not in childhood B-lineage acute lymphoblastic leukemia: a comparative deletion and hypermethylation study. *Cancer Genet. Cytogenet.* **2009**, *195* (1), 59-65.

[67] Heyman, M.; Rasool, O.; Borgonovo Brandter, L.; Liu, Y.; Grander, D.; Soderhall, S.; Gustavsson, G.; Einhorn, S. Prognostic importance of p15INK4B and p16INK4 gene inactivation in childhood acute lymphocytic leukemia. *J. Clin. Oncol.* **1996**, *14* (5), 1512-1520.

[68] Notta, F.; Mullighan, C. G.; Wang, J. C.; Poeppl, A.; Doulatov, S.; Phillips, L. A.; Ma, J.; Minden, M. D.; Downing, J. R.; Dick, J. E. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. *Nature* **2011**, *469* (7330), 362-367.

[69] Russell, L. J.; Capasso, M.; Vater, I.; Akasaka, T.; Bernard, O. A.; Calasanz, M. J.; Chandrasekaran, T.; Chapiro, E.; Gesk, S.; Griffiths, M.; Guttery, D. S.; Haferlach, C.; Harder, L.; Heidenreich, O.; Irving, J.; Kearney, L.; Nguyen-Khac, F.; Machado, L.; Minto, L.; Majid, A.; Moorman, A. V.; Morrison, H.; Rand, V.; Strefford, J. C.; Schwab, C.; Tonnies, H.; Dyer, M. J.; Siebert, R.; Harrison, C. J. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood* **2009**, *114* (13), 2688-2698.

[70] Mullighan, C. G.; Collins-Underwood, J. R.; Phillips, L. A.; Loudin, M. G.; Liu, W.; Zhang, J.; Ma, J.; Coustan-Smith, E.; Harvey, R. C.; Willman, C. L.; Mikhail, F. M.; Meyer, J.; Carroll, A. J.; Williams, R. T.; Cheng, J.; Heerema, N. A.; Basso, G.; Pession, A.; Pui, C. H.; Raimondi, S. C.; Hunger, S. P.; Downing, J. R.; Carroll, W. L.; Rabin, K. R. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat. Genet.* **2009**, *41* (11), 1243-1246.

[71] Hertzberg, L.; Vendramini, E.; Ganmore, I.; Cazzaniga, G.; Schmitz, M.; Chalker, J.; Shiloh, R.; Iacobucci, I.; Shochat, C.; Zeligson, S.; Cario, G.; Stanulla, M.; Strehl, S.; Russell, L. J.; Harrison, C. J.; Bornhauser, B.; Yoda, A.; Rechavi, G.; Bercovich, D.; Borkhardt, A.; Kempski, H.; te Kronnie, G.; Bourquin, J. P.; Domany, E.; Izraeli, S. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. *Blood* **2010**, *115* (5), 1006-1017.

[72] Yoda, A.; Yoda, Y.; Chiaretti, S.; Bar-Natan, M.; Mani, K.; Rodig, S. J.; West, N.; Xiao, Y.; Brown, J. R.; Mitsiades, C.; Sattler, M.; Kutok, J. L.; DeAngelo, D. J.; Wadleigh, M.; Piciocchi, A.; Dal Cin, P.; Bradner, J. E.; Griffin, J. D.; Anderson, K. C.; Stone, R. M.; Ritz, J.; Foa, R.; Aster, J. C.; Frank, D. A.; Weinstock, D. M. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (1), 252-257.

[73] Harvey, R. C.; Mullighan, C. G.; Chen, I. M.; Wharton, W.; Mikhail, F. M.; Carroll, A. J.; Kang, H.; Liu, W.; Dobbin, K. K.; Smith, M. A.; Carroll, W. L.; Devidas, M.; Bowman, W. P.; Camitta, B. M.; Reaman, G. H.; Hunger, S. P.; Downing, J. R.; Willman, C. L. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood* **2010**, *115* (26), 5312-5321.

[74] Cario, G.; Zimmermann, M.; Romey, R.; Gesk, S.; Vater, I.; Harbott, J.; Schrauder, A.; Moericke, A.; Izraeli, S.; Akasaka, T.; Dyer, M. J.; Siebert, R.; Schrappe, M.; Stanulla, M. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in

children treated according to the ALL-BFM 2000 protocol. *Blood* **2010**, *115* (26), 5393-5397.

[75] Roberts, K. G.; Morin, R. D.; Zhang, J.; Hirst, M.; Zhao, Y.; Su, X.; Chen, S. C.; Payne-Turner, D.; Churchman, M. L.; Harvey, R. C.; Chen, X.; Kasap, C.; Yan, C.; Becksfort, J.; Finney, R. P.; Teachey, D. T.; Maude, S. L.; Tse, K.; Moore, R.; Jones, S.; Mungall, K.; Birol, I.; Edmonson, M. N.; Hu, Y.; Buetow, K. E.; Chen, I. M.; Carroll, W. L.; Wei, L.; Ma, J.; Kleppe, M.; Levine, R. L.; Garcia-Manero, G.; Larsen, E.; Shah, N. P.; Devidas, M.; Reaman, G.; Smith, M.; Paugh, S. W.; Evans, W. E.; Grupp, S. A.; Jeha, S.; Pui, C. H.; Gerhard, D. S.; Downing, J. R.; Willman, C. L.; Loh, M.; Hunger, S. P.; Marra, M. A.; Mullighan, C. G., Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell* **2012**, *22* (2), 153-166.

[76] Shaikhibrahim, Z.; Wernert, N. ETS transcription factors and prostate cancer: the role of the family prototype ETS-1 (review). *Int. J. Oncol.* **2012**, *40* (6), 1748-1754.

[77] Yeoh, E. J.; Ross, M. E.; Shurtleff, S. A.; Williams, W. K.; Patel, D.; Mahfouz, R.; Behm, F. G.; Raimondi, S. C.; Relling, M. V.; Patel, A.; Cheng, C.; Campana, D.; Wilkins, D.; Zhou, X.; Li, J.; Liu, H.; Pui, C. H.; Evans, W. E.; Naeve, C.; Wong, L.; Downing, J. R. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* **2002**, *1* (2), 133-143.

[78] Harvey, R. C.; Mullighan, C. G.; Wang, X.; Dobbin, K. K.; Davidson, G. S.; Bedrick, E. J.; Chen, I. M.; Atlas, S. R.; Kang, H.; Ar, K.; Wilson, C. S.; Wharton, W.; Murphy, M.; Devidas, M.; Carroll, A. J.; Borowitz, M. J.; Bowman, W. P.; Downing, J. R.; Relling, M.; Yang, J.; Bhojwani, D.; Carroll, W. L.; Camitta, B.; Reaman, G. H.; Smith, M.; Hunger, S. P.; Willman, C. L. Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. *Blood* **2010**, *116* (23), 4874-4884.

[79] Graux, C.; Cools, J.; Michaux, L.; Vandenberghe, P.; Hagemeijer, A. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. *Leukemia* **2006**, *20* (9), 1496-1510.

[80] Aifantis, I.; Raetz, E.; Buonamici, S. Molecular pathogenesis of T-cell leukaemia and lymphoma. *Nat. Rev. Immunol.* **2008**, *8* (5), 380-390.

[81] Graux, C.; Cools, J.; Melotte, C.; Quentmeier, H.; Ferrando, A.; Levine, R.; Vermeesch, J. R.; Stul, M.; Dutta, B.; Boeckx, N.; Bosly, A.; Heimann, P.; Uyttebroeck, A.; Mentens, N.; Somers, R.; MacLeod, R. A.; Drexler, H. G.; Look, A. T.; Gilliland, D. G.; Michaux, L.; Vandenberghe, P.; Wlodarska, I.; Marynen, P.; Hagemeijer, A. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. *Nat. Genet.* **2004**, *36* (10), 1084-1089.

[82] Van Vlierberghe, P.; van Grotel, M.; Beverloo, H. B.; Lee, C.; Helgason, T.; Buijs-Gladdines, J.; Passier, M.; van Wering, E. R.; Veerman, A. J.; Kamps, W. A.; Meijerink, J. P.; Pieters, R. The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. *Blood* **2006**, *108* (10), 3520-3529.

[83] Homminga, I.; Vuerhard, M. J.; Langerak, A. W.; Buijs-Gladdines, J.; Pieters, R.; Meijerink, J. P., Characterization of a pediatric T-cell acute lymphoblastic leukemia patient with simultaneous LYL1 and LMO2 rearrangements. *Haematologica* **2012**, *97* (2), 258-261.

[84] Palomero, T.; Sulis, M. L.; Cortina, M.; Real, P. J.; Barnes, K.; Ciofani, M.; Caparros, E.; Buteau, J.; Brown, K.; Perkins, S. L.; Bhagat, G.; Agarwal, A. M.; Basso, G.; Castillo, M.; Nagase, S.; Cordon-Cardo, C.; Parsons, R.; Zuniga-Pflucker, J. C.; Dominguez, M.; Ferrando, A. A. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat. Med.* **2007**, *13* (10), 1203-1210.

[85] O'Neil, J.; Grim, J.; Strack, P.; Rao, S.; Tibbitts, D.; Winter, C.; Hardwick, J.; Welcker, M.; Meijerink, J. P.; Pieters, R.; Draetta, G.; Sears, R.; Clurman, B. E.; Look, A. T. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. *J. Exp. Med.* **2007**, *204* (8), 1813-1824.

[86] Tosello, V.; Mansour, M. R.; Barnes, K.; Paganin, M.; Sulis, M. L.; Jenkinson, S.; Allen, C. G.; Gale, R. E.; Linch, D. C.; Palomero, T.; Real, P.; Murty, V.; Yao, X.; Richards, S. M.; Goldstone, A.; Rowe, J.; Basso, G.; Wiernik, P. H.; Paietta, E.; Pieters, R.; Horstmann, M.; Meijerink, J. P.; Ferrando, A. A. WT1 mutations in T-ALL. *Blood* **2009**, *114* (5), 1038-1045.

[87] Gutierrez, A.; Dahlberg, S. E.; Neuberg, D. S.; Zhang, J.; Grebliunaite, R.; Sanda, T.; Protopopov, A.; Tosello, V.; Kutok, J.; Larson, R. S.; Borowitz, M. J.; Loh, M. L.; Ferrando, A. A.; Winter, S. S.; Mullighan, C. G.; Silverman, L. B.; Chin, L.; Hunger, S. P.; Sallan, S. E.; Look, A. T. Absence of biallelic TCRgamma deletion predicts early treatment failure in pediatric T-cell acute lymphoblastic leukemia. *J. Clin. Oncol.* **2010**, *28* (24), 3816-3823.

[88] Van Vlierberghe, P.; Palomero, T.; Khiabanian, H.; Van der Meulen, J.; Castillo, M.; Van Roy, N.; De Moerloose, B.; Philippe, J.; Gonzalez-Garcia, S.; Toribio, M. L.; Taghon, T.; Zuurbier, L.; Cauwelier, B.; Harrison, C. J.; Schwab, C.; Pisecker, M.; Strehl, S.; Langerak, A. W.; Gecz, J.; Sonneveld, E.; Pieters, R.; Paietta, E.; Rowe, J. M.; Wiernik, P. H.; Benoit, Y.; Soulier, J.; Poppe, B.; Yao, X.; Cordon-Cardo, C.; Meijerink, J.; Rabadan, R.; Speleman, F.; Ferrando, A. PHF6 mutations in T-cell acute lymphoblastic leukemia. *Nat. Genet.* **2010**, *42* (4), 338-342.

[89] Zhang, J.; Ding, L.; Holmfeldt, L.; Wu, G.; Heatley, S. L.; Payne-Turner, D.; Easton, J.; Chen, X.; Wang, J.; Rusch, M.; Lu, C.; Chen, S. C.; Wei, L.; Collins-Underwood, J. R.; Ma, J.; Roberts, K. G.; Pounds, S. B.; Ulyanov, A.; Becksfort, J.; Gupta, P.; Huether, R.; Kriwacki, R. W.; Parker, M.; McGoldrick, D. J.; Zhao, D.; Alford, D.; Espy, S.; Bobba, K. C.; Song, G.; Pei, D.; Cheng, C.; Roberts, S.; Barbato, M. I.; Campana, D.; Coustan-Smith, E.; Shurtleff, S. A.; Raimondi, S. C.; Kleppe, M.; Cools, J.; Shimano, K. A.; Hermiston, M. L.; Doulatov, S.; Eppert, K.; Laurenti, E.; Notta, F.; Dick, J. E.; Basso, G.; Hunger, S. P.; Loh, M. L.; Devidas, M.; Wood, B.; Winter, S.; Dunsmore, K. P.; Fulton, R. S.; Fulton, L. L.; Hong, X.; Harris, C. C.; Dooling, D. J.; Ochoa, K.; Johnson, K. J.; Obenauer, J. C.; Evans, W. E.; Pui, C. H.; Naeve, C. W.; Ley, T. J.; Mardis, E. R.; Wilson, R. K.; Downing, J. R.; Mullighan, C. G. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* **2012**, *481* (7380), 157-163.

[90] De Keersmaecker, K.; Atak, Z. K.; Li, N.; Vicente, C.; Patchett, S.; Girardi, T.; Gianfelici, V.; Geerdens, E.; Clappier, E.; Porcu, M.; Lahortiga, I.; Luca, R.; Yan, J.; Hulselmans, G.; Vranckx, H.; Vandepoel, R.; Sweron, B.; Jacobs, K.; Mentens, N.; Wlodarska, I.; Cauwelier, B.; Cloos, J.; Soulier, J.; Uyttebroeck, A.; Bagni, C.; Hassan, B. A.; Vandenberghe, P.; Johnson, A. W.; Aerts, S.; Cools, J. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. *Nat. Genet.* **2013**, *45* (2), 186-190.

[91] Tzoneva, G.; Perez-Garcia, A.; Carpenter, Z.; Khiabanian, H.; Tosello, V.; Allegretta, M.; Paietta, E.; Racevskis, J.; Rowe, J. M.; Tallman, M. S.; Paganin, M.; Basso, G.; Hof, J.; Kirschner-Schwabe, R.; Palomero, T.; Rabadan, R.; Ferrando, A. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nat. Med.* **2013**, *19* (3), 368-371.

[92] Dohner, H.; Estey, E. H.; Amadori, S.; Appelbaum, F. R.; Buchner, T.; Burnett, A. K.; Dombret, H.; Fenaux, P.; Grimwade, D.; Larson, R. A.; Lo-Coco, F.; Naoe, T.; Niederwieser, D.; Ossenkoppele, G. J.; Sanz, M. A.; Sierra, J.; Tallman, M. S.; Lowenberg, B.; Bloomfield, C. D. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* **2010**, *115* (3), 453-474.

[93] Vardiman, J. W.; Thiele, J.; Arber, D. A.; Brunning, R. D.; Borowitz, M. J.; Porwit, A.; Harris, N. L.; Le Beau, M. M.; Hellstrom-Lindberg, E.; Tefferi, A.; Bloomfield, C. D. 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.

[94] Baxter, E. J.; Scott, L. M.; Campbell, P. J.; East, C.; Fourouclas, N.; Swanton, S.; Vassiliou, G. S.; Bench, A. J.; Boyd, E. M.; Curtin, N.; Scott, M. A.; Erber, W. N.; Green, A. R. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **2005**, *365* (9464), 1054-1061.

[95] Raghavan, M.; Lillington, D. M.; Skoulakis, S.; Debernardi, S.; Chaplin, T.; Foot, N. J.; Lister, T. A.; Young, B. D. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. *Cancer Res.* **2005**, *65* (2), 375-378.

[96] Gorletta, T. A.; Gasparini, P.; D'Elios, M. M.; Trubia, M.; Pelicci, P. G.; Di Fiore, P. P. Frequent loss of heterozygosity without loss of genetic material in acute myeloid leukemia with a normal karyotype. *Genes Chromosomes Cancer* **2005**, *44* (3), 334-337.

[97] Gupta, M.; Raghavan, M.; Gale, R. E.; Chelala, C.; Allen, C.; Molloy, G.; Chaplin, T.; Linch, D. C.; Cazier, J. B.; Young, B. D. Novel regions of acquired uniparental disomy discovered in acute myeloid leukemia. *Genes Chromosomes Cancer* **2008**, *47* (9), 729-739.

[98] Dunbar, A. J.; Gondek, L. P.; O'Keefe, C. L.; Makishima, H.; Rataul, M. S.; Szpurka, H.; Sekeres, M. A.; Wang, X. F.; McDevitt, M. A.; Maciejewski, J. P. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. *Cancer Res.* **2008**, *68* (24), 10349-10357.

[99] Walter, M. J.; Payton, J. E.; Ries, R. E.; Shannon, W. D.; Deshmukh, H.; Zhao, Y.; Baty, J.; Heath, S.; Westervelt, P.; Watson, M. A.; Tomasson, M. H.; Nagarajan, R.; O'Gara, B. P.; Bloomfield, C. D.; Mrozek, K.; Selzer, R. R.; Richmond, T. A.; Kitzman, J.; Geoghegan, J.; Eis, P. S.; Maupin, R.; Fulton, R. S.; McLellan, M.; Wilson, R. K.; Mardis, E. R.; Link, D. C.; Graubert, T. A.; DiPersio, J. F.; Ley, T. J. Acquired copy number alterations in adult acute myeloid leukemia genomes. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (31), 12950-12955.

[100] Bullinger, L.; Kronke, J.; Schon, C.; Radtke, I.; Urlbauer, K.; Botzenhardt, U.; Gaidzik, V.; Cario, A.; Senger, C.; Schlenk, R. F.; Downing, J. R.; Holzmann, K.; Dohner, K.; Dohner, H. Identification of acquired copy number alterations and uniparental disomies in cytogenetically normal acute myeloid leukemia using high-resolution single-nucleotide polymorphism analysis. *Leukemia* **2010**, *24* (2), 438-449.

[101] Akagi, T.; Ogawa, S.; Dugas, M.; Kawamata, N.; Yamamoto, G.; Nannya, Y.; Sanada, M.; Miller, C. W.; Yung, A.; Schnittger, S.; Haferlach, T.; Haferlach, C.; Koeffler, H. P. Frequent genomic abnormalities in acute myeloid leukemia/myelodysplastic syndrome with normal karyotype. *Haematologica* **2009**, *94* (2), 213-223.

[102] Tyybakinoja, A.; Elonen, E.; Vauhkonen, H.; Saarela, J.; Knuutila, S. Single nucleotide polymorphism microarray analysis of karyotypically normal acute myeloid leukemia reveals frequent copy number neutral loss of heterozygosity. *Haematologica* **2008**, *93* (4), 631-632.

[103] Hahm, C.; Mun, Y. C.; Seong, C. M.; Chung, W. S.; Huh, J. Additional genomic aberrations identified by single nucleotide polymorphism array-based karyotyping in an acute myeloid leukemia case with isolated del(20q) abnormality. *Ann. Lab Med.* **2012**, *32* (6), 445-449.

[104] Milosevic, J. D.; Puda, A.; Malcovati, L.; Berg, T.; Hofbauer, M.; Stukalov, A.; Klampfl, T.; Harutyunyan, A. S.; Gisslinger, H.; Gisslinger, B.; Burjanivova, T.; Rumi, E.; Pietra, D.; Elena, C.; Vannucchi, A. M.; Doubek, M.; Dvorakova, D.; Robesova, B.; Wieser, R.; Koller, E.; Suvajdzic, N.; Tomin, D.; Tosic, N.; Colinge, J.; Racil, Z.; Steurer, M.; Pavlovic, S.; Cazzola, M.; Kralovics, R. Clinical significance of genetic aberrations in secondary acute myeloid leukemia. *Am. J. Hematol.* **2012**, *87* (11), 1010-1016.

[105] Fitzgibbon, J.; Smith, L. L.; Raghavan, M.; Smith, M. L.; Debernardi, S.; Skoulakis, S.; Lillington, D.; Lister, T. A.; Young, B. D. Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. *Cancer Res.* **2005**, *65* (20), 9152-9154.

[106] Summers, K.; Stevens, J.; Kakkas, I.; Smith, M.; Smith, L. L.; Macdougall, F.; Cavenagh, J.; Bonnet, D.; Young, B. D.; Lister, T. A.; Fitzgibbon, J. Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. *Leukemia* **2007**, *21* (3), 550-1; author reply 552.

[107] King-Underwood, L.; Pritchard-Jones, K. Wilms' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance. *Blood* **1998**, *91* (8), 2961-2968.

[108] Nyvold, C. G.; Stentoft, J.; Braendstrup, K.; Melsvik, D.; Moestrup, S. K.; Juhl-Christensen, C.; Hasle, H.; Hokland, P. Wilms' tumor 1 mutation accumulated during therapy in acute myeloid leukemia: Biological and clinical implications. *Leukemia* **2006**, *20* (11), 2051-2054.

[109] Kottaridis, P. D.; Gale, R. E.; Frew, M. E.; Harrison, G.; Langabeer, S. E.; Belton, A. A.; Walker, H.; Wheatley, K.; Bowen, D. T.; Burnett, A. K.; Goldstone, A. H.; Linch, D. C. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* **2001**, *98* (6), 1752-1759.

[110] Bungaro, S.; Raghavan, M.; Dell'Oro, M. G.; Paolucci, P.; Young, B. D.; Biondi, A.; Cazzaniga, G. Assessment of submicroscopic genetic lesions by single nucleotide polymorphism arrays in a child with acute myeloid leukemia and FLT3-internal tandem duplication. *Haematologica* **2006**, *91* (7), 998-1000.

[111] Ho, P. A.; Alonzo, T. A.; Gerbing, R. B.; Pollard, J.; Stirewalt, D. L.; Hurwitz, C.; Heerema, N. A.; Hirsch, B.; Raimondi, S. C.; Lange, B.; Franklin, J. L.; Radich, J. P.; Meshinchi, S. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. *Blood* **2009**, *113* (26), 6558-6566.

[112] Smith, M. L.; Cavenagh, J. D.; Lister, T. A.; Fitzgibbon, J. Mutation of CEBPA in familial acute myeloid leukemia. *N. Engl. J. Med.* **2004**, *351* (23), 2403-2407.

[113] Jankowska, A. M.; Szpurka, H.; Tiu, R. V.; Makishima, H.; Afable, M.; Huh, J.; O'Keefe, C. L.; Ganetzky, R.; McDevitt, M. A.; Maciejewski, J. P. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood* **2009**, *113* (25), 6403-6410.

[114] Makishima, H.; Cazzolli, H.; Szpurka, H.; Dunbar, A.; Tiu, R.; Huh, J.; Muramatsu, H.; O'Keefe, C.; Hsi, E.; Paquette, R. L.; Kojima, S.; List, A. F.; Sekeres, M. A.; McDevitt, M. A.; Maciejewski, J. P. Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. *J. Clin. Oncol* .**2009**, *27* (36), 6109-6116.

[115] Ernst, T.; Chase, A. J.; Score, J.; Hidalgo-Curtis, C. E.; Bryant, C.; Jones, A. V.; Waghorn, K.; Zoi, K.; Ross, F. M.; Reiter, A.; Hochhaus, A.; Drexler, H. G.; Duncombe, A.; Cervantes, F.; Oscier, D.; Boultwood, J.; Grand, F. H.; Cross, N. C. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat. Genet.* **2010**, *42* (8), 722-726.

[116] Raghavan, M.; Smith, L. L.; Lillington, D. M.; Chaplin, T.; Kakkas, I.; Molloy, G.; Chelala, C.; Cazier, J. B.; Cavenagh, J. D.; Fitzgibbon, J.; Lister, T. A.; Young, B. D. Segmental uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia. *Blood* **2008**, *112* (3), 814-821.

[117] Gondek, L. P.; Dunbar, A. J.; Szpurka, H.; McDevitt, M. A.; Maciejewski, J. P. SNP array karyotyping allows for the detection of uniparental disomy and cryptic chromosomal abnormalities in MDS/MPD-U and MPD. *PLoS One* **2007**, *2* (11), e1225.

[118] Tiu, R. V.; Gondek, L. P.; O'Keefe, C. L.; Huh, J.; Sekeres, M. A.; Elson, P.; McDevitt, M. A.; Wang, X. F.; Levis, M. J.; Karp, J. E.; Advani, A. S.; Maciejewski, J. P. New lesions detected by single nucleotide polymorphism array-based chromosomal analysis have important clinical impact in acute myeloid leukemia. *J. Clin. Oncol.* **2009**, *27* (31), 5219-5226.

[119] Klampfl, T.; Harutyunyan, A.; Berg, T.; Gisslinger, B.; Schalling, M.; Bagienski, K.; Olcaydu, D.; Passamonti, F.; Rumi, E.; Pietra, D.; Jager, R.; Pieri, L.; Guglielmelli, P.; Iacobucci, I.; Martinelli, G.; Cazzola, M.; Vannucchi, A. M.; Gisslinger, H.; Kralovics, R. Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression. *Blood* **2011**, *118* (1), 167-176.

[120] Haferlach, C.; Dicker, F.; Kohlmann, A.; Schindela, S.; Weiss, T.; Kern, W.; Schnittger, S.; Haferlach, T. AML with CBFB-MYH11 rearrangement demonstrate RAS pathway alterations in 92% of all cases including a high frequency of NF1 deletions. *Leukemia* **2010**, *24* (5), 1065-1069.

[121] Parkin, B.; Ouillette, P.; Wang, Y.; Liu, Y.; Wright, W.; Roulston, D.; Purkayastha, A.; Dressel, A.; Karp, J.; Bockenstedt, P.; Al-Zoubi, A.; Talpaz, M.; Kujawski, L.; Shedden, K.; Shakhan, S.; Li, C.; Erba, H.; Malek, S. N. NF1 inactivation in adult acute myelogenous leukemia. *Clin. Cancer Res.* **2010**, *16* (16), 4135-4147.

[122] Thoennissen, N. H.; Lasho, T.; Thoennissen, G. B.; Ogawa, S.; Tefferi, A.; Koeffler, H. P. Novel CUX1 missense mutation in association with 7q- at leukemic transformation of MPN. *Am. J. Hematol.* **2011**, *86* (8), 703-705.

[123] McNerney, M. E.; Brown, C. D.; Wang, X.; Bartom, E. T.; Karmakar, S.; Bandlamudi, C.; Yu, S.; Ko, J.; Sandall, B. P.; Stricker, T.; Anastasi, J.; Grossman, R. L.; Cunningham, J. M.; Le Beau, M. M.; White, K. P. CUX1 is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. *Blood* **2013**, *121* (6), 975-983.

[124] Huh, J.; Tiu, R. V.; Gondek, L. P.; O'Keefe, C. L.; Jasek, M.; Makishima, H.; Jankowska, A. M.; Jiang, Y.; Verma, A.; Theil, K. S.; McDevitt, M. A.; Maciejewski, J. P. Characterization of chromosome arm 20q abnormalities in myeloid malignancies using genome-wide single nucleotide polymorphism array analysis. *Genes Chromosomes Cancer* **2010**, *49* (4), 390-399.

[125] Jerez, A.; Gondek, L. P.; Jankowska, A. M.; Makishima, H.; Przychodzen, B.; Tiu, R. V.; O'Keefe, C. L.; Mohamedali, A. M.; Batista, D.; Sekeres, M. A.; McDevitt, M. A.; Mufti, G. J.; Maciejewski, J. P. Topography, clinical, and genomic correlates of 5q myeloid malignancies revisited. *J. Clin. Oncol.* **2012**, *30* (12), 1343-1349.

[126] Parkin, B.; Ouillette, P.; Li, Y.; Keller, J.; Lam, C.; Roulston, D.; Li, C.; Shedden, K.; Malek, S. N. Clonal evolution and devolution after chemotherapy in adult acute myelogenous leukemia. *Blood* **2013**, *121* (2), 369-377.

[127] Kronke, J.; Bullinger, L.; Teleanu, V.; Tschurtz, F.; Gaidzik, V. I.; Kuhn, M. W.; Rucker, F. G.; Holzmann, K.; Paschka, P.; Kapp-Schworer, S.; Spath, D.; Kindler, T.; Schittenhelm, M.; Krauter, J.; Ganser, A.; Gohring, G.; Schlegelberger, B.; Schlenk, R. F.; Dohner, H.; Dohner, K. Clonal evolution in relapsed NPM1 mutated acute myeloid leukemia. *Blood* **2013**, *122* (1),100-108.

[128] de The, H.; Lavau, C.; Marchio, A.; Chomienne, C.; Degos, L.; Dejean, A. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* **1991**, *66* (4), 675-684.

[129] Akagi, T.; Shih, L. Y.; Kato, M.; Kawamata, N.; Yamamoto, G.; Sanada, M.; Okamoto, R.; Miller, C. W.; Liang, D. C.; Ogawa, S.; Koeffler, H. P. Hidden abnormalities and novel classification of t(15;17) acute promyelocytic leukemia (APL) based on genomic alterations. *Blood* **2009**, *113* (8), 1741-1748.

[130] Akagi, T.; Shih, L. Y.; Ogawa, S.; Gerss, J.; Moore, S. R.; Schreck, R.; Kawamata, N.; Liang, D. C.; Sanada, M.; Nannya, Y.; Deneberg, S.; Zachariadis, V.; Nordgren, A.; Song, J. H.; Dugas, M.; Lehmann, S.; Koeffler, H. P. Single nucleotide polymorphism genomic arrays analysis of t(8;21) acute myeloid leukemia cells. *Haematologica* **2009**, *94* (9), 1301-1306.

[131] Kuhn, M. W.; Radtke, I.; Bullinger, L.; Goorha, S.; Cheng, J.; Edelmann, J.; Gohlke, J.; Su, X.; Paschka, P.; Pounds, S.; Krauter, J.; Ganser, A.; Quessar, A.; Ribeiro, R.; Gaidzik, V. I.; Shurtleff, S.; Kronke, J.; Holzmann, K.; Ma, J.; Schlenk, R. F.; Rubnitz, J. E.; Dohner, K.; Dohner, H.; Downing, J. R. High-resolution genomic profiling of adult and pediatric core-binding factor acute myeloid leukemia reveals new recurrent genomic alterations. *Blood* **2012**, *119* (10), e67-75.

[132] Huh, J.; Kim, H. J.; Jung, C. W.; Kim, S. H.; Kim, Y. K.; Shin, M. G.; Moon, J. H.; Sohn, S. K.; Lee, W. S.; Won, J. H.; Mun, Y. C.; Kim, H.; Park, J.; Min, W. S.; Kim, D. H. A genome-wide single-nucleotide polymorphism-array can improve the prognostic stratification of the core binding factor acute myeloid leukemia. *Am. J. Hematol.* **2012**, *87* (10), 961-968.

[133] Costa, A. R.; Vasudevan, A.; Krepischi, A.; Rosenberg, C.; Chauffaille Mde, L. Single-nucleotide polymorphism-array improves detection rate of genomic alterations in core-binding factor leukemia. *Med. Oncol.* **2013**, *30* (2), 579.

[134] Rubnitz, J. E. How I treat pediatric acute myeloid leukemia. *Blood* **2012**, *119* (25), 5980-5988.

[135] Radtke, I.; Mullighan, C. G.; Ishii, M.; Su, X.; Cheng, J.; Ma, J.; Ganti, R.; Cai, Z.; Goorha, S.; Pounds, S. B.; Cao, X.; Obert, C.; Armstrong, J.; Zhang, J.; Song, G.; Ribeiro, R. C.; Rubnitz, J. E.; Raimondi, S. C.; Shurtleff, S. A.; Downing, J. R., Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (31), 12944-12949.

[136] Koren-Michowitz, M.; Sato-Otsubo, A.; Nagler, A.; Haferlach, T.; Ogawa, S.; Koeffler, H. P. Older patients with normal karyotype acute myeloid leukemia have a higher rate of genomic changes compared to young patients as determined by SNP array analysis. *Leuk. Res.* **2012**, *36* (4), 467-473.

[137] Parkin, B.; Erba, H.; Ouillette, P.; Roulston, D.; Purkayastha, A.; Karp, J.; Talpaz, M.; Kujawski, L.; Shakhan, S.; Li, C.; Shedden, K.; Malek, S. N. Acquired genomic copy number aberrations and survival in adult acute myelogenous leukemia. *Blood* **2010**, *116* (23), 4958-4967.

[138] Rumi, E.; Harutyunyan, A.; Elena, C.; Pietra, D.; Klampfl, T.; Bagienski, K.; Berg, T.; Casetti, I.; Pascutto, C.; Passamonti, F.; Kralovics, R.; Cazzola, M. Identification of genomic aberrations associated with disease transformation by means of high-resolution SNP array analysis in patients with myeloproliferative neoplasm. *Am. J. Hematol.* **2011**, *86* (12), 974-979.

[139] Yi, J. H.; Huh, J.; Kim, H. J.; Kim, S. H.; Kim, Y. K.; Sohn, S. K.; Moon, J. H.; Kim, K. H.; Won, J. H.; Mun, Y. C.; Kim, H.; Park, J.; Jung, C. W.; Kim, D. H. Adverse prognostic impact of abnormal lesions detected by genome-wide single nucleotide polymorphism array-based karyotyping analysis in acute myeloid leukemia with normal karyotype. *J. Clin. Oncol.* **2011**, *29* (35), 4702-4708.

[140] Tiu, R. V.; Gondek, L. P.; O'Keefe, C. L.; Elson, P.; Huh, J.; Mohamedali, A.; Kulasekararaj, A.; Advani, A. S.; Paquette, R.; List, A. F.; Sekeres, M. A.; McDevitt, M. A.; Mufti, G. J.; Maciejewski, J. P. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. *Blood* **2011**, *117* (17), 4552-4560.

[141] Remke, M.; Pfister, S.; Kox, C.; Toedt, G.; Becker, N.; Benner, A.; Werft, W.; Breit, S.; Liu, S.; Engel, F.; Wittmann, A.; Zimmermann, M.; Stanulla, M.; Schrappe, M.; Ludwig, W. D.; Bartram, C. R.; Radlwimmer, B.; Muckenthaler, M. U.; Lichter, P.; Kulozik, A. E. High-resolution genomic profiling of childhood T-ALL reveals frequent copy-number alterations affecting the TGF-beta and PI3K-AKT pathways and deletions at 6q15-16.1 as a genomic marker for unfavorable early treatment response. *Blood* **2009**, *114* (5), 1053-1062.

[142] Lopez-Nieva, P.; Vaquero, C.; Fernandez-Navarro, P.; Gonzalez-Sanchez, L.; Villa-Morales, M.; Santos, J.; Esteller, M.; Fernandez-Piqueras, J. EPHA7, a new target gene for 6q deletion in T-cell lymphoblastic lymphomas. *Carcinogenesis* **2012**, *33* (2), 452-458.

[143] Mullighan, C. G.; Williams, R. T.; Downing, J. R.; Sherr, C. J. Failure of CDKN2A/B (INK4A/B-ARF)-mediated tumor suppression and resistance to targeted therapy in acute lymphoblastic leukemia induced by BCR-ABL. *Genes Dev.* **2008**, *22* (11), 1411-14115.

[144] Yamada, Y.; Kamihira, S. Inactivation of tumor suppressor genes and the progression of adult T-cell leukemia-lymphoma. *Leuk. Lymphoma* **2005**, *46* (11), 1553-1559.

[145] Gutierrez, A.; Sanda, T.; Grebliunaite, R.; Carracedo, A.; Salmena, L.; Ahn, Y.; Dahlberg, S.; Neuberg, D.; Moreau, L. A.; Winter, S. S.; Larson, R.; Zhang, J.; Protopopov, A.; Chin, L.; Pandolfi, P. P.; Silverman, L. B.; Hunger, S. P.; Sallan, S. E.; Look, A. T. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood* **2009**, *114* (3), 647-650.

[146] Kleppe, M.; Soulier, J.; Asnafi, V.; Mentens, N.; Hornakova, T.; Knoops, L.; Constantinescu, S.; Sigaux, F.; Meijerink, J. P.; Vandenberghe, P.; Tartaglia, M.; Foa, R.; Macintyre, E.; Haferlach, T.; Cools, J. PTPN2 negatively regulates oncogenic JAK1 in T-cell acute lymphoblastic leukemia. *Blood* **2011**, *117* (26), 7090-7098.

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