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

Ilaria Iacobucci1,* , Annalisa Lonetti2 , Cristina Papayannidis1 and Giovanni Martinelli1

1Institute of Hematology “L. e A. Seràgnoli”, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy; 2Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

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 cyogenetic 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).
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 (heterozygosity or homozygosity/hemizygosity 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 log2 ratio values (test to reference intensity ratio) and reflect either no copy number changes (log2 ratio of 0) or loss/gain (lower or higher log2 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
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...
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.

### 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           | \(^{GRK2, CASP8AP2, EPHA7}\)   | 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      | \(^{RUNXI}\)                    | 50k, 500k| B-ALL                  | ~ 3% pediatric B-ALL; | [36, 38]|
| Xp22; Yp11       | \(^{P2R18-CRLF2}\) rearrangement | \(^{CRLF2}\)                   | 50k, 500k, SNP6.0| B-ALL                | Up to 16% 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
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 subchromosomal 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

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**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-ABLI, 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-ABLI, 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 intragene 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 Ab1 kinase; cryptic deletion on chromosome 11 [del(11)(q12p13)] 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 protein 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, DNMT2, 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. RPL3 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 RPL10Arg98Ser 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) 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 UPD 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, 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), RUNXI (21q22), and other tumor-related genes, including FHIT (3p14), CTNNAL (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) [RUNXI–RUNXIT1] 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 6q [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, c-MYC, CTNNA1, 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 BRC42          | 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 1-3% 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] |
| 5q5q31 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; 16q16q22 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] |
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, 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, NDUFA9, MELF1, 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

| Abbreviation | Definition |
|--------------|------------|
| 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 |
| CN-LOH | copy number neutral loss of heterozygosity |
| CNVs | copy number variations |
| DGV | database of genomic variants |
| EBF1 | early B-cell factor |
| ETS | erythroleukemia 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 |

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