Genomic Characterization of Acute Leukemias

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Abstract
Over the past two decades, hematologic malignancies have been extensively evaluated due to the introduction of powerful technologies, such as conventional karyotyping, FISH analysis, gene and microRNA expression profiling, array comparative genomic hybridization and SNP arrays, and next-generation sequencing (including whole-exome sequencing (WES) and RNA-seq). These analyses have allowed for the refinement of the mechanisms underlying the leukemic transformation in several oncohematologic disorders and, more importantly, they have permitted the definition of novel prognostic algorithms aimed at stratifying patients at the onset of disease and, consequently, treating them in the most appropriate manner. Furthermore, the identification of specific molecular markers is opening the door to targeted and personalized medicine. The most important findings on novel acquisitions in the context of acute lymphoblastic leukemia of both B and T lineage and de novo acute myeloid leukemia are described in this review.

Key Words
Acute leukemias · Genomic aberrations · Next-generation sequencing

Introduction
Over the past two decades, hematologic malignancies have been extensively evaluated due to the introduction of powerful technologies, such as conventional karyotyping, FISH analysis, gene and microRNA expression profiling, array comparative genomic hybridization (a-CGH) and SNP arrays, and, very recently, next-generation sequencing [NGS; including whole-exome sequencing (WES) and RNA-seq]. In detail, cytogenetics first allowed the discovery and classification of recurrent chromosomal translocations, while gene and microRNA expression profiling has defined the deregulation of a set of transcripts in a given subgroup, and a-CGH and SNP arrays have permitted the identification of cryptic changes in the genome. Finally, WES and more generally NGS are revealing a large set of point mutations; the roles of some of these have already been elucidated, while others require further investigation.

Prompt recognition of these lesions is already permitting the definition of novel prognostic algorithms aimed at stratifying patients at the onset of disease; examples include BCR/ABL-like, iAMP21, hypodiploid acute lymphoblastic leukemia (ALL) and TP53 disrupted subgroups in B-lineage ALL (B-ALL), the early T-cell pre-
curator (ETP) subset in T-lineage ALL (T-ALL), and the stratification of chromosomally normal karyotypes (CN) in acute myeloid leukemia (AML). Furthermore, it should result in the most appropriate treatment for these subgroups in the future. Also, experimental models have established that primary cytogenetic abnormalities alone, though they play a pivotal role in leukemogenesis, are insufficient to induce leukemia – with few exceptions – and additional genetic alterations are required. In fact, comprehensive sequencing efforts have revealed that most leukemia cases harbor multiple mutations that sequentially occurred in a single cell lineage to generate a dominant leukemic clone. Mutations that confer a selective growth advantage to the leukemic cells are called ‘drivers’, while those that confer no selective growth advantage are called ‘passengers’. While the definitions of driver and passenger mutations are relatively well established, further experimental studies are required to determine the role of most of the recently identified mutations. Furthermore, deep characterization of cancer genomes has provided insight into clonal evolution and allowed the identification of subclones using acquired mutations as genetic markers. Clonal progression is an evolutionary process in which mutations provide genetic diversity within a cell lineage and selection drives the evolutionary process in which mutations provide genetic advantages to the leukemic cells. This review focuses on novel acquisitions in the context of acute ALL of both B and T lineages and de novo AML. The identification of specific molecular markers will likely open the door to targeted and personalized medicine, at least in cases in which a targetable lesion with pathogenic significance is recognized.

**Acute Lymphoblastic Leukemia**

*Introduction*

ALL is a malignant disorder that originates from hemopoietic precursors of B-cell (80–85%) or T-cell (20–25%) derivation; the acquisition of a series of genetic aberrations leads to impaired maturation, with arrest of the differentiation process and abnormal proliferation. As a consequence, the accumulation of leukemic cells occurs in both the bone marrow, where it suppresses the physiologic hemopoiesis, and at extramedullary sites.

ALL is the most common neoplasm in childhood, with the highest peak of incidence occurring in children between 2 and 5 years of age, whereas it is rather rare in adulthood. In fact, according to the US SEER, the patient ages at diagnosis are as follows: younger than 20 years, approximately 60.3%; 20–34 years, 10.3%; 35–44 years, 5.9%; 45–54 years, 6.7%; 55–64 years, 6.1%; 65–74 years, 5.0%; 75–84 years, 4.0%, and 85 years or older, 1.7%. In addition to the different incidences of the disease and possible causes [1], outcomes vary profoundly between children and adults. In fact, to date, the majority of pediatric patients are considered curable, while the prognosis of adults is still extremely poor, with only 40% of individuals free of leukemia in the long term. Cytogenetics has long been used for diagnosis, risk stratification, and therapeutic implications; however, up to a quarter of children and a higher proportion of adults lack recurrent aberrations. Consequently, there is a need to improve the molecular dissection of subtypes, identifying genetic alterations that predict the risk of treatment failure and developing novel and targeted therapies.

**Historic Genetic Defects in B-Cell ALL**

A set of genetic lesions, mostly represented by translocations and including *BCR/ABL*, *ETV6/RUNX1*, *E2A/PBX1*, and *MLL* rearrangements, have been well recognized in B-ALL and represent 'historical' aberrations. The *BCR/ABL* rearrangement t(9;22)(q34;q11) (Ph chromosome) [2] represents the hallmark of chronic myeloid leukemia (CML) and can also be detected in ALL. It induces constitutive activation of the ABL kinase, which in turns activates the mitogenic signaling pathways and induces altered cellular adhesion, inhibition of apoptosis, and proteasomal degradation of physiologically important cellular proteins, ultimately contributing to tumor growth and proliferation [3, 4]. At least 3 different fusion proteins have been detected, i.e. p210 (prevalently found in CML), p190 (detected in roughly 50% of adult Ph+ ALL patients and in the majority of child Ph+ ALL patients) [5], and the rare p230 protein (reported in chronic neutrophilic leukemia and seldom observed in about 1% of classic Ph+ CML cases) [6]. The frequency of *BCR/ABL* is very low in childhood ALL, while it starts to increase during adolescence and reaches more than 50% among the elderly [7]. The prognosis of these cases was dismal until the introduction of tyrosine kinase inhibitors; their use in clinical practice has profoundly changed the clinical management of these patients [8–13].

In contrast, *ETV6/RUNX1* is more frequent in children and virtually disappears with age progression [14].
It originates from t(12;21)(p13;q22) which creates a fusion gene including the 5’ portion of ETV6, a member of the ETS family of transcription factor genes, and almost the entire coding region of the transcription factor RUNX1 which encodes the α subunit of the core-binding factor, a master regulator of the formation of hematopoietic stem cells [15, 16]. As a result, the chimeric ETV6/RUNX1 transcription factor retains an essential protein-protein interaction domain of ETV6 and the DNA-binding and transcriptional regulatory sequences of RUNX1 [15, 16]. The most prominent effect of the ETV6/RUNX1 fusion protein is the inhibition of the transcriptional activity that is normally initiated when RUNX1 binds to a DNA region termed the core-enhanced sequence [17]. Clinically, children with ETV6/RUNX1 rearrangement usually have excellent outcomes.

The MLL gene can be disrupted in leukemias of B- and T-cell origin as well as in myeloid leukemias, and so far about 100 partners have been recognized [18, 19]. The MLL fusion proteins have a dominant gain-of-function effect that enhances their transcriptional activity. These alterations mainly disrupt the normal pattern of expression of HOX genes, causing a change in the self-renewal and growth properties of hematopoietic stem cells and committed progenitors, thus eventually leading to leukemia [20, 21]. Among the HOX genes known to play a predominant function in these processes, HOXA7 and HOXA9 play a pivotal role [22]. MLL rearrangements represent the most frequent aberrations in infants (children aged <1 year), in whom they are detected in more than 90% of cases, whereas they are very rare in children and adults (2–5%). In all cases, MLL rearrangements are associated with a very unfavorable outcome.

Finally, E2A/PBX1 rearrangement arises from t(1;19) (q23;p13), initially described in 1984 [23], and is strongly associated with a pre-B immunophenotype as blasts usually express cytoplasmic immunoglobulins. Its frequency is similar in children and adults, i.e. around 2–7%. This translocation juxtaposes the E2A gene on chromosome 19 with the PBX1 gene on chromosome 1 to form the E2A/PBX1 fusion gene [24]; the resulting protein induces cell differentiation arrest and tumor formation, most probably because of a reduction of the levels of wild-type E2A [25] which eventually induce deregulation of lymphoid cell maturation and proliferation; furthermore, E2A/PBX1 itself induces the transcription activation of target genes.

Overall, the identification of these molecular aberrations has been of pivotal importance for the establishment of a prognostic algorithm; ETV6/RUNX1 is associated with a very favorable outcome and MLL rearrangements as well as BCR/ABL aberrations are associated with a poorer outcome [26], while E2A/PBX1 is still controversial, although it is likely that patients harboring this aberration can benefit from intensive regimens [27, 28].

Novel Genetic Acquisitions in B-Cell ALL and Definition of High-Risk Subsets: BCR/ABL1-Like, iAMP21, Hypodiploid ALL, and TP53-Disrupted Cases

The introduction of powerful technologies, such as gene expression profiling (GEP) first, SNP array analysis later, and currently NGS, has allowed a better definition of the molecular scenario of B-ALL.

The first report focusing on GEP revealed that the transcriptional profiling of ALL is different from that of AML [29]. However, the most important study, carried out by the St. Jude Children’s Research Hospital group, highlighted specific gene expression signatures for all of the known subgroups of pediatric ALL, i.e. T-ALL, hyperdiploid cases with more than 50 chromosomes, and E2A/PBX1, BCR/ABL, ETV6/RUNX1, and MLL rearrangements [30, 31]. Similar results were also reported by us and others in adult cohorts [32–34], indicating that at the transcriptional level there are no major differences between adult and pediatric cohorts, at least in the presence of specific aberrations. In adult B-ALL, GEP also showed that a set of cases without major molecular aberrations tend to cluster with BCR/ABL+ cases, hence identifying the subgroup ‘BCR/ABL-like’ that might be regarded as different and might benefit from aggressive treatment strategies [33, 34].

More recently, the introduction of SNP array technology further defined the spectrum of genetic lesions [35–39]; overall, this approach has shown that in ALL the number of copy number alterations is rather high, with the deletions always outnumbering the gains. Lesions frequently affect fundamental pathways, such as B-cell differentiation, tumor suppression, the cell cycle, and apoptosis [36]. Interestingly, the number of copy number alterations varies according to the molecular subgroup, i.e. MLL rearrangements are characterized by very few additional lesions while BCR/ABL and ETV6/RUNX1 are associated with a mean number of 6 alterations. Taken together, these results further confirm that MLL itself is able to induce a leukemic transformation [40] whereas BCR/ABL and ETV6/RUNX1 may require supplementary hits.

Furthermore, this study has revealed that IKZF1, encoding for the transcription factor Ikaros and playing a
pivotal role in lymphoid development [41], is frequently disrupted in ALL, particularly in BCR/ABL+ cases, where it is deleted in both adult and pediatric cohorts in roughly 80% of cases, making it the most frequent aberration associated with BCR/ABL. The deletion of IKZF1 has functional consequences because it impairs B-lymphoid maturation and pre-B cell receptor signaling and accelerates leukemogenesis in a BCR/ABL+ murine model [42–45]. Importantly, IKZF1 deletions are important predictors of a poor outcome in Ph+ ALL regardless of age, and they currently represent the hallmark of high-risk leukemias [46–49]. In fact, in children, IKZF1 can be deleted also in non-Ph+ ALL [49, 50] (as in adult B-ALL) without major molecular aberrations and is correlated with a poor prognosis. More importantly, its deletion recognizes a subgroup with inferior event-free survival and disease-free survival also in non-high-risk childhood ALL, and it has been proposed as a useful marker for monitoring minimal residual disease [50].

Together with IKZF1, another recently recognized lesion is represented by rearrangements involving CRLF2, a cytokine type I receptor known to play a pivotal role in dendritic development, the T-cell response, allergic inflammation, and the proliferation of normal and leukemic B cells [51, 52]. CRLF2 is located in the pseudo-autosomal region (PAR1) of chromosomes X and Y; rearrangements involving this transcript lead to its overexpression and can be of two types: either a rearrangement that involves CRLF2 and the Ig heavy-chain locus (IGH-CRLF2) or an interstitial PAR1 deletion that juxtaposes intron 1 of P2RY8 to the coding region of CRLF2 itself. Interestingly, the latter rearrangement is frequently detected in roughly 50% of individuals with Down syndrome who have ALL [53, 54]. CRLF2 alterations are coupled with the presence of JAK mutations (JAK1 or JAK2) or IL7R, thus suggesting that these events together contribute to leukemogenesis. Moreover, they are frequently detected in IKZF1-deleted Ph− ALL patients. Overall, CRLF2 impairment has been detected in 5–10% of cases without molecular lesions in both adult and pediatric cohorts [55–58]. Its presence correlates with a poor outcome; more importantly, because it is correlated with activation of the Jak-Stat pathway, it might be of particular relevance in a therapeutic algorithm, as patients with such lesions might benefit from treatment with JAK inhibitors.

Moreover, the integration of genome-wide technologies has allowed refinement of the prognostic subgroups. In fact, as previously mentioned, the BCR/ABL-like subgroup has been identified in both pediatric and adult populations; it accounts for 17 and 25% of cases, respectively, and Den Boer et al. [59] elegantly showed that children have a poor outcome and genetic lesions similar to those observed in BCR/ABL+ patients (i.e. IKZF1 deletions and CRLF2 rearrangements). Similarly, Harvey et al. [57] performed a GEP analysis in a large set of high-risk ALL patients and showed (via unsupervised analysis) the presence of 8 subgroups that differed from each other in terms of outcomes and associated deletions. In line with Den Boer et al. [59], cases with poorer outcomes were characterized by IKZF1 deletions [60]. More recently, NGS has partly clarified the biological determinants of the similarity observed between high-risk ALL and BCR/ABL-like cases and true BCR/ABL+ cases: BCR/ABL-like cases are in fact characterized by mutations and/or rearrangement-activating tyrosine kinases, i.e. IGH-CRLF2, NUP214-ABL1 rearrangements, in-frame fusions of EBF1-PDGFRB, BCR-JAK2 or STRN3-JAK2, and cryptic IGH-EPOR rearrangements; therefore, a prominent involvement of receptor tyrosine kinases can be detected. Importantly, primary cells from patients with such lesions, if treated with tyrosine kinase inhibitors, can respond in vitro, thus paving the way for new therapeutic options in such cases [61].

Another lesion which has been recognized and analyzed in detail via SNP array is represented by iAMP21 [62–66]; it is a rare aberration (2% of pediatric ALL cases) and was initially described as being characterized by multiple copies of the RUNX1 gene, indeed located on chromosome 21. Initially, it was possible to define a common region of amplification of 33.192 and 39.796 Mb on chromosome 21 (which includes RUNX1) that was later redefined to a 5.11-Mb region [66]. In at least 2 studies, survival analysis of patients harboring such lesion has indicated that they have an increased risk of relapse [62, 65]. Subsequent SNP array analyses allowed the definition of recurrent abnormalities affecting genes in key pathways, such as IKZF1, CDKN2A/B, PAX5, ETV6, and RB1. An analysis of clonal architecture indicated that these lesions, together with P2RY8-CRLF2 aberrations, are events secondary to chromosome 21 rearrangements. Patients’ outcomes were reconfirmed to be poor if they were treated with standard therapy [64], thus rendering it another suitable marker of a poor prognosis.

Childhood hypodiploid ALL has also been widely investigated [67], mostly in view of the poorer outcomes associated with this subset. Holmfeldt et al. [67] evaluated the copy number, transcriptional profile, and WES of 124 hypodiploid cases and showed that the degree of aneuploidy varied widely among patients, ranging from near haploid (24–31 chromosomes) to low hypodiploid
(32–39 chromosomes) and near diploid (44–45 chromosomes). These patients can be easily discriminated by their transcriptional profile. Finally, WES proved that lesions involving receptor tyrosine kinases and RAS signaling (i.e. NRAS, KRAS, FLT3, and NFI) can be detected in up to 70% of near-haploid patients, whereas low-hypodiploid cases are characterized by lesions involving members of the Ikaros family, particularly IKZF2, and by TP53 disruptions that can be identified in 91.2% of these cases. As for the latter lesion, it is worth mentioning that, while in childhood hypodiploid ALL TP53 mutations could be detected also in germline material, in adult hypodiploid ALL they were found only on tumor DNA, thus favoring the hypothesis that in children they can be inherited.

TP53 disruptions have recently been the focus of several studies [68–70] in both childhood and adult ALL. Their role in tumorigenesis is well recognized and mutations/deletions have been extensively described in solid cancers [71, 72] as well as in hematologic malignancies, particularly in AML and in chronic lymphocytic leukemia. In AML, the overall incidence of TP53 disruption is around 7% and it increases significantly in cases with a complex karyotype [73, 74], while in chronic lymphocytic leukemia TP53 mutations/deletions are detected in 3–40% of cases according to the stage of the disease [75–77]. Indeed, in both AML and chronic lymphocytic leukemia, TP53 deregulation is one of the most important predictors of short survival and chemoresistances [73–77]. In 2011, Hof et al. [68] showed that TP53 lesions, which can be either mutations or deletions, could be detected in 6.4 and 11.1% of relapsed B-ALL and T-ALL cases, respectively. The authors also showed that, by sample backtracking, the same alterations could be detected in up to 70% of near-haploid patients, whereas low-hypodiploid cases are characterized by lesions involving members of the Ikaros family, particularly IKZF2, and by TP53 disruptions that can be identified in 91.2% of these cases. As for the latter lesion, it is worth mentioning that, while in childhood hypodiploid ALL TP53 mutations could be detected also in germline material, in adult hypodiploid ALL they were found only on tumor DNA, thus favoring the hypothesis that in children they can be inherited.

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er in ALL whose deregulation should be further investigated.

In relapsed ALL, by resequencing, Mullighan et al. [78] performed a paired diagnosis-relapse comparison and identified CREBBP mutations in about 18% of childhood relapsed cases. CREBBP and its paralog EP300 (p300) are transcriptional coactivators that are involved in hematopoiesis [79]. Functional consequences of CREBBP mutations include a reduced acetylation and an impaired expression of glucocorticoid-receptor-responsive genes. Overall, their presence in relapsed samples and their role in the regulation of glucocorticoid-responsive genes suggest that these alterations may influence the response to therapy and relapse; they also suggest that therapeutic approaches directed towards acetylation may be useful in high-risk ALL.

Furthermore, Meyer et al. [80] recently reported that NT5C2 mutations can occur in roughly 10% of childhood B-ALL cases; importantly, these mutations confer increased enzymatic activity to the NT5C2 protein, which normally dephosphorylates nucleoside analogs. As a final consequence, this leads to a higher resistance to nucleoside analogs and chemoresistance. Thus, it appears that a small set of mutations that could confer resistance to chemotherapy are specific to relapsed samples. Whether a subclone harboring the mutation(s) was already present at diagnosis or the mutation was selected by the therapeutic regimen administered will likely be clarified in the near future.

A summary of the findings and their relative incidences in children and adults is presented in table 1.

**Recurrent Lesions in T-Cell ALL**

Until a decade ago, little was known about the biology of T-cell ALL (T-ALL). Well-recognized aberrations involved the T-cell receptor (TCR) that was juxtaposed with different fusion partners. In fact, aberrations involving the 14q11 (TCRA/D) and 7q34 (TCRB) regions could be detected in 35% of patients [81]. They juxtapose enhancer or promoter elements of the TCR genes to transcription factor genes such as LMO1, LMO2, TAL1, and TLX1, resulting in the overexpression of these oncogenic transcription factors and a consequent deregulation of hematopoiesis. Chromosomal deletions can occur and result in gene rearrangements such as TLX3-BCL11B in roughly 20% of patients and SIL-TAL1 in 10–25% of patients [82] or in focal gene deletions such as CDKN2A and CDKN2B loci on chromosome 9p. Moreover, chromosomal rearrangements can also lead to in-frame fusion genes encoding chimeric proteins with oncogenic properties such as...
The improvement of cytogenetic assays, mutational analysis, GEP, and the integration of these techniques has nowadays largely permitted a recapitulation of the genomic complexity of T-ALL (table 2).

**Table 1. Overview of the most frequent and significant lesions occurring in B-ALL**

| Gene(s) involved | Functional consequences | Frequency | Clinical relevance |
|------------------|------------------------|-----------|-------------------|
| Translocations | | | |
| t(9;22)(q34;q11) | BCR-ABL | ABL constitutive activation, activation of mitogenic pathways, cellular adhesion deregulation | ~5–10% | Up to 50% | Poor outcome |
| t(4;11)(q21;q23) | MLL-AF4 | Disruption of HOX gene expression and the self-renewal properties of hemopoietic progenitors | 3–5%, >90% in infants | 5–10% | Poor outcome |
| t(12;21)(p13;q22) | ETV6-RUNX1 | Transcriptional activity inhibition | 20–30% | <1% | Favorable outcome |
| t(1;19)(q23;p13) | E2A-PBX1 | Cell differentiation deregulation | ~5% | ~5% | Not clearly established |
| Other types of lesions | | | |
| Focal deletions, rarely mutations | IKZF1, 7p13-p11.1 | Deregulation of lymphoid differentiation | 15%, >80% in BCR-ABL+, ~30% in HR BCR-ABL- | 7%, >80% in BCR-ABL+ | Poor outcome |
| Rearrangements, interstitial Par1 deletion, mutations | CRLF2, Xp22.3, Yp11.3 | Together with JAK mutations, constitutive JAK-STAT activation | 5–10%, 50% in DS-ALL | 5–10% | Poor outcome |
| Mutations | JAK1, 1p32.3-p31.3, JAK2, 9p24 | Constitutive JAK-STAT activation | ~10% in HR BCR-ABL+, 18–35% in DS-ALL | – | Associated with CRLF2, IKZF1, poor outcome |
| Focal deletions, mutations | CREBBP, 16p13.3, EP300, 22q13.2 | Impaired histone acetylation and transcriptional regulation | 18% in relapsed ALL | – | Increased incidence at relapse, association with glucocorticoid resistance |
| Focal deletions, mutations | NT5C2, 10q24.32 | Increased dephosphorylation of nucleoside analogs | 10% in relapsed ALL (also in T-ALL) | – | Identified only at relapse |
| Intrachromosomal amplification of chromosome 21 | RUNX1, 21q22.3 | Multiple copies of the RUNX1 gene, possibly secondary events | 2% | – | Poor outcome |
| TP53 disruption | TP53, 17p13.1 | Mutations and/or deletions | 90% in hypodiploid ALL, 6–11% in relapsed childhood ALL (also in T-ALL) | 8% in ALL at onset of disease (also in T-ALL) | Poor outcome |

HR = High-risk; DS-ALL = Down syndrome ALL.

**PICALM-MLLT10** in 8% of patients, *NUP214-ABL1* fusion formed on episomes, *EML-ABL1, SET-NUP214* fusion, and *MLL* gene rearrangements to numerous different partners [83–86].

The improvement of cytogenetic assays, mutational analysis, GEP, and the integration of these techniques has nowadays largely permitted a recapitulation of the genomic complexity of T-ALL (table 2). Via GEP, Ferrando et al. [87] showed the presence of several subgroups, each associated with the overexpression of known oncogenes. Among these clusters, it is interesting to notice that it was possible to define a novel subgroup which clustered tightly to *TLXI* (*HOX11*) and was characterized by the overexpression of *TLX3*. Furthermore, GEP showed that several lesions, including *MLL* rearrangements, inv(7)(p15q34) involving the *TCRB* and the *HOXA* gene locus (7p15), t(10;11)(p13;q23) that

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**Table 2.**

| Gene(s) involved | Functional consequences | Frequency | Clinical relevance |
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| Translocations | | | |
| t(9;22)(q34;q11) | BCR-ABL | ABL constitutive activation, activation of mitogenic pathways, cellular adhesion deregulation | ~5–10% | Up to 50% | Poor outcome |
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HR = High-risk; DS-ALL = Down syndrome ALL.
result in **PICALM-MLLT10** rearrangement, and del(9) (q34.11q34.13) that leads to **SET-NUP214**, all induced the overexpression of **HOXA** genes, thus indicating that different genetic lesions activate the same pathway [88–91]. However, one of the most important and recent contributions of GEP, also in combination with immunophenotyping analysis, is represented by the recognition of a subgroup that accounts for about 10% of cases, defined either as early T-cell precursor (ETP-ALL) [92] or myeloid-like [93], detected in both pediatric and adult cohorts (see below).

**ETP/Myeloid-Like Leukemias**

ETP/myeloid-like leukemias appear as a distinct subset of T-ALL and have been described in both pediatric and adult cohorts with a similar incidence [92, 93]. In the pediatric setting, Coustan-Smith et al. [92] showed that at the immunophenotypic level these patients display an early T-cell phenotype and coexpress at least one myeloid marker and at the transcriptional level these children have a stem-cell like profile. More importantly, ETP children are characterized by very poor outcomes. Our group [93], by performing an unsupervised analysis of 52 adult patients with T-ALL, identified a subset of cases characterized by the overexpression of a large number of myeloid transcription factors, including **CEBPA**, **CEBPB**, and **CEBPD**. Furthermore, these cases also expressed miR-223, a microRNA that is involved in the myeloid differentiation process [94]. Similar to the observations in children, also in our cohort myeloid-like patients appeared to have unfavorable outcomes, as the majority was refractory to induction chemotherapy.

These cases were recently further evaluated by sequencing and WES; Van Vlierberghe et al. [95] in fact showed that ETP cases are indeed characterized by an immature transcriptional profile and, more importantly, by a wide spectrum of gene mutations usually detected in AML, such as **IDH1, IDH2, DNMT3A, FLT3, and NRAS**. Furthermore, a prominent role has been suggested for **ETV6** because mutations of this gene were detected exclusively in such subgroups.

In line with these findings, Zhang et al. [96] reported that ETP cases are characterized by several intrachromosomal translocations, deletions, and insertions. Among the mutations and/or translocations identified, it was possible to confirm the involvement of **ETV6** because mutations of this gene were detected exclusively in such subgroups.

**Table 2. Summary of recurrent genetic lesions in T-ALL**

| Gene(s) involved | Functional consequences | Frequency | Clinical relevance |
|-----------------|-------------------------|-----------|-------------------|
| Translocation of TCR with various oncogenes | **LMO1, LMO2, TAL1, TLX1, and TLX3** | Hemopoiesis deregulation, impairment of differentiation | ~35% | No impact |
| Del(1)(p32) | **SIL-TAL1** | Impairment of differentiation | ~10% | Not clearly established |
| 9p deletion | **CDKN2A and CDKN2B** | Loss of cell proliferation control | 20–30% | <1% |
| 11q23 rearrangements | **MLL with various partners** | Disruption of HOX gene expression and the self-renewal properties of hemopoietic progenitors | ~5% | Poor outcome |
| (t(9;9)(q34;q34)) | **NUP214-ABL** | ABL constitutive activation | 6% | No impact |
| (t(9;14)(q34;q32)) | **EML1-ABL** | ABL constitutive activation | 1% | No impact |

In ETP/Myeloid-Like Leukemias, mutations in **FBW7** gene, located on chromosome 4q31.3, is a component of the ubiquitin ligase complex and is involved in the degradation of MYC, cyclin E, and particularly **NOTCH1** [97, 98]. Mutations of this gene are de-
detected in 8–16% of cases [98, 99]. In the presence of a mutation, the protein either fails to bind to its target proteins (NOTCH1) or binds to its targets but fails to tag them for degradation (MYC); in both instances, this results in a prolongation of the targets’ half-life.

FBW7 mutations have been widely investigated in clinical trials, usually in combination with NOTCH1 mutations. Because they are concurrent with an elevated intracellular NOTCH1 activity and the overexpression of its downstream targets, they ultimately result in deregulated cell cycle control and tumor development. Their role in outcome prediction is still controversial. In fact, Asnafi et al. [100] analyzed adult patients and concomitantly evaluated NOTCH1 and FBW7 mutations and reported an association with a favorable outcome. Similar results were recently reported by the GRAALL study [101] which suggests that NOTCH1/FBW7-mutated patients have particularly favorable outcomes, especially when treated with intensified chemotherapeutic regimens. In contrast, Park et al. [102] did not report any significant impact of FBW7 on the pediatric ALL-97 protocol, in line with the results obtained in adult patients enrolled into the MRC UKALLXII/ECOG E2993 clinical trial [103]. Finally, Kox et al. [104], who analyzed separately the role of FBW7 mutations, reported that FBW7 mutations are indeed associated with an early minimal residual disease response that is lost at later time points.

BCL11B is a transcription factor that has an important role in normal T-cell development and is usually highly expressed at almost all stages of T-cell differentiation, with the exception of ETP cells. In murine thymocytes, BCL11B inactivation leads to developmental arrest at the DN2-DN3 stage, the acquisition of NK-like features, and aberrant self-renewal activity [105]. More recent findings have also indicated that BCL11B can be deregulated in T-ALL as a consequence of mutations that are prevalent in 16% of TLX1-overexpressing patients [108]. De Keersmaecker et al. [108] proposed a mechanistic mouse model in which TLX1 directly downregulates

| Table 3. Gene mutations in T-ALL |
|---------------------------------|
| Gene(s) involved | Gene position | Functional consequences | Frequency | Clinical relevance |
| NOTCH1 | 9q34.3 | Impairment of differentiation and proliferation | 60–70% | Overall favorable outcome |
| FBW7 | 4q31.3 | Arrest of differentiation and aberrant self-renewal activity | ~10% – 10–20% | Usually evaluated in combination with NOTCH1 |
| BCL11B | 14q32.2 | Loss of cell proliferation control | 9% – 10% | Not defined |
| JAK1 | 1p32.3-p31.3 | Cytokine growth independence, resistance to dexamethasone-induced apoptosis, and JAK signaling activation | 2% – 7–18% | Unfavorable outcome |
| PTPN2 | 18p11.3-p11.2 | Negative regulator of tyrosine kinases | 6% – 6% | No impact |
| IL7R | 5p13 | Lymphoid development | 5% – 16% | No impact |
| PHF6 | Xq26.3 | Putative tumor suppressor | 18–38% | No impact |
| ETV6 | 12p13 | Various, including signaling, developmental arrest, and histone modification | Detected in ETP leukemia | Unfavorable, as per subgroup of the disease |
| IDH1 | 2q33.3 | | | |
| IDH2 | 15q26.1 | | | |
| DNMT3A | 2q33.3 | | | |
| FLT3 | 13q12 | | | |
| NRAS | 1p13.2 | | | |
| JAK3 | 19p13.1 | | | |
| IKZF1 | 7p13-p11.1 | | | |
| CNOT3 | 19q13.4 | Presumed tumor suppressor | 8% – 8% | Not known |
| RPL5 and RPL10 | 1p22.1 | Ribosomal activity impairment | 8% – 8% | Not known |
| NTSC2 | 10q24.32 | Increased dephosphorylation of nucleoside analogs | 19% of relapsed ALL cases | Identified only at relapse |
the expression of CHEKI together with additional mitotic control genes and induces loss of the mitotic checkpoint in nontransformed preleukemic thymocytes. This phenomenon, in turn, induces the accumulation of several mutations, including BCL11B. More recently, it has also been shown that mutations or deletions can occur in about 2–9% of T-ALL cases, not being limited to the presence of TLX1 overexpression [109, 110].

JAK1 encodes a cytoplasmic tyrosine kinase and plays a role in lymphoid cell precursor proliferation, survival, and differentiation. The first report focusing on this topic showed that JAK1 mutations can be identified mostly in adult patients (18%), whereas they are rare in childhood T-ALL (2%). From a functional point of view, 3 mutations (A634D, R724H, and R879C) are able to promote JAK1 gain of function and confer interleukin (IL)-3-independent growth in Ba/F3 cells and/or IL-9-independent resistance to dexamethasone-induced apoptosis in the T-cell lymphoma BW5147 cell line. In line with this, primary T-ALL cells harboring JAK1 mutations display a gene expression signature characterized by the transcriptional up-regulation of genes positively controlled by JAK signaling. More importantly, the presence of JAK1 mutations correlates with a poor response to induction therapy and the overall prognosis [111]. While similar results were reported by Jeong et al. [112], the French GRAALL group could not confirm either the relatively high incidence (3–7% in the French study) or the association with a poorer outcome [113], thus suggesting that other events might contribute to the impact of JAK1 mutations on the prognosis.

PTPN2 is a tyrosine phosphatase located on chromosome 18p11.3-11.2, it is engaged in a cell cycle-dependent manner, and it is considered a negative regulator of tyrosine kinases. PTPN2 was recently found to be deleted in 6% of T-ALL patients, especially within subgroups overexpressing TLX1 T-ALL or in NUP214-ABL+ patients [114]. Recent evidence indicates that PTPN2 can be lost also in JAK1-mutated T-ALL, where it increases the transforming capability of JAK1 mutations, thus overall inducing a more resistant phenotype when these cells are treated with a JAK inhibitor [115].

IL7Ralpha, located at 5p13, encodes for the alpha chain of the IL-7 receptor and is required for lymphoid development [116, 117]. This gene can be mutated in 9% of pediatric T-ALL cases [118, 119], as well as in 6% of B-ALL cases overexpressing CRLF2 [118].

In T-ALL, mutations lead to a gain of function, with consequent constitutive JAK1 activation and enhancement of cell cycle progression. Overall, these mutations are more frequently detected in cases that, by GEP, fall into the HOXA cluster and are not associated with JAK1 and PTEN mutations, and there is no significant difference in their distribution between NOTCH1-mutated and wild-type cases. There is no association between IL7Ralpha mutational status and clinical outcome [119].

PHF6 mutations were recently described by Van Vlierbergh et al. [120]. This gene, previously known to be involved in the Börjeson-Forssman-Lehmann syndrome [121], is located at Xq26, encodes for a plant homeodomain factor (PHD), regulates gene expression, is phosphorylated in a cell cycle-dependent manner, and has ubiquitous expression. Mutations of PHF6 were initially reported to be almost exclusively found in males, associated with TLX1 and TLX3 overexpression but not with NOTCH1, FBW7, or PTEN [120], and were detected more frequently in adults than in children (38 vs. 16%). However, a more recent report [122] showed contradicting results. In fact, mutations were found at a much lower frequency (18% of adults vs. 5.4% of children) and were not associated with male gender, whereas there was a significant association with NOTCH1 and JAK1 mutations, as well as with SET-NUP214 rearrangement. Both studies showed that there was no significant correlation between the presence of PHF6 mutations and outcomes [120, 122].

Mutations in the PTPRC gene, located on chromosomes in bands 1q31-q32 and encoding for the protein tyrosine phosphatase CD45, have been described; they induce loss of function, are usually detected in combination with activating mutations of IL7Ralpha, JAK1, or LCK, and are associated with the downregulation of CD45 expression [123].

NGS has recently permitted the identification of novel putative lesions in T-ALL. CNOT3 mutations have been recurrently (roughly in 8% of cases) detected in adult patients with newly diagnosed T-ALL. CNOT3 is presumed to be a tumor suppressor, and in the Drosophila eye cancer model its downmodulation leads to a drastic increase in the incidence of tumors. In contrast, mutations of the RPL5 and RPL10 genes have been detected mostly in children (8.2%) and have been shown to impair ribosomal activity. Both types of mutations are not associated with any specific molecular subgroup [124]. Furthermore, similar to observations in relapsed B-ALL, in relapsed T-ALL the group of Ferrando recently showed the acquisition at relapse of a recurrent mutation affecting the NT5C2 gene, with an incidence of 19% of individuals [125]. The mutation appears to be acquired at relapse and, overall, to be more frequent in T-ALL.
**Notch1 Mutations in T-ALL**

Notch1 receptors (Notch1–4) play a pivotal role in the tuning differentiation and proliferation of both physiological and leukemic T cells. Their activation is dependent upon ligands expressed from neighboring cells, that include Jagged 1–2 and Delta-like 1, 3, and 4 (DLL1, DLL3, and DLL4) [126].

From a structural point of view, the mature form of Notch contains intracellular and extracellular portions which are associated by a noncovalent, extracellular, and Ca++-dependent bond in the HD (heterodimerization) domain. The extracellular portion contains the epidermal growth factor-like repeats, the LIN12/Notch1 cysteine-rich repeats, and the HD domain. The epidermal growth factor-like repeats are required for binding with Notch ligands; the LIN12/Notch1 cysteine-rich repeats prevent receptor activation in the absence of the appropriate ligands, while the proteolytic cleavage upon ligand binding occurs in the HD domain. The intracellular portion contains the RAM domain involved in Notch-dependent activation, and at the C-terminal is the PEST (proline, glutamic acid, serine, and threonine) domain; the latter is involved in the proteosomal degradation of Notch itself, since it has a sequence required for polyubiquitination [127, 128].

Notch activation requires 2 cleavages, at both extracellular and intracellular sites, operated by 2 distinct proteases, i.e. the ADAM/TACE metalloprotease and the γ-secretase, respectively. Upon cleavage, the Notch intracellular portion (ICN) migrates into the nucleus, displacing the transcriptional repressor complex (CSL), recruits several coactivators, and eventually activates the transcription of downstream targets, including proliferation (MYC, CDKN1A, CDKN1B, and CCND1) and receptor genes (CCR4, CCR8, CXCR6, CCR6, CCR7 IL-6, IL-8, VEGFR, and IL7R) [129–133].

The first evidence of NOTCH1 involvement in T-ALL came from the description of t(7;9)(q34;q34.3), detected in about 1% of cases, which creates a truncated form of NOTCH1 by juxtaposing it to TCRB [134]. Importantly, it was shown that retrovirus-mediated expression of the resulting truncated form induced leukemia in irradiated mice, thus indicating that Notch1 aberrant forms might be causative of leukemia [135].

Besides the rare translocation mentioned above, NOTCH1 mutations can be detected in about 60–70% of T-ALL patients [136, 137] and are mostly located in the HD domain or the PEST domain and in the extracellular juxtamembrane region (JME). HD domain mutations weaken the binding of the 2 subunits and favor ligand-independent activation, whereas PEST domain mutations are usually small deletions/insertions that generate premature stop codons which, in turn, lead to a decreased proteosomal degradation and eventually to an increased half-life of NOTCH1 itself. In all cases, the final result is an augmented Notch1 pathway activity.

Given the high incidence of NOTCH1 mutations and their key role in differentiation and proliferation, several studies have attempted to correlate their presence with outcomes, with unequivocal results. Overall, a number of studies showed that the presence of NOTCH1 mutations, evaluated mostly in pediatric cohorts, was significantly correlated with a good prednisone response and favorable minimal residual disease levels independently of gender, age, white blood cell count, and T-cell immunophenotype and were associated with an excellent prognosis [136, 137]. However, it must be kept in mind that the interaction with other lesions, such as FBW7, makes the scenario more complex and it is likely that the presence of either NOTCH1 or FBW mutations, rather than NOTCH1 only, may identify a subgroup with a favorable prognosis, as suggested by several authors [100, 101, 103, 104], especially if intensive treatments are administered.

Since NOTCH1 activation is driven by cleavage of the receptor, blockage of this process would theoretically provide the molecular basis for therapeutic intervention. γ-Secretase inhibitors (GSIs), which directly inhibit NOTCH1 cleavage, were initially developed for Alzheimer’s disease [138] and have shown activity in T-ALL in vitro [136, 139]. However, their use presents some important limitations: first, because NOTCH1 is regulated and interacts with other intracellular mediators, particularly c-Myc and PTEN, their efficacy is somehow limited by the concomitant deregulations of these transcripts, which induce a resistance to GSIs [140, 141]; second, the use of these compound has been limited by their important gastrointestinal toxicity. More recently, it has been shown that the combined use of GSIs and glucocorticoids can improve the antileukemic effects of GSIs and reduce gut toxicity in vivo, thus suggesting that their use is feasible on such a schedule [142].

**De novo AML**

**Introduction**

AML represents one the most frequent leukemias in adults (25%), with a median age at diagnosis of 67 years [143, 144]. While the majority of patients aged less than
60 years achieve complete remission (CR), the overall long-term survival rates continue to be poor, i.e. around 30–40% [145, 146]. The prognosis is even poorer for patients with high-risk AML, where the CR rate is less than 40% and survival rates are below 10% [146]. From a biological point of view, it is represented by a clonal hematopoietic disorder resulting from genetic alterations in hematopoietic stem cells. These alterations disrupt normal differentiation and/or cause excessive proliferation of abnormal immature leukemic blasts. As the disease progresses, blast cells accumulate in the bone marrow, blood, and organs and interfere with the production of normal blood cells.

In recent years, technologic progress has allowed better definition of the lesions underlying the malignant transformation and permitted a more precise elucidation of the heterogeneity of the disease. As proof of principle, the WHO classification now includes several subsets of AML, indeed defined on the basis of their genomic characteristics and characterized by specific morphologic and prognostic features [147]. In this section, the most important findings that have arisen from cytogenetic analyses and SNP arrays, gene and microRNA profiling, gene mutations, and WES will be described.

Cytogenetic Aberrations in AML

Cytogenetic abnormalities can be detected in approximately 50–60% of newly diagnosed AML patients and are usually represented by nonrandom chromosomal translocations that often result in gene rearrangements [147], monosomies, or deletions of part or all of chromosome 5 or 7 (–5/–7 AML) and trisomy 8 [148]. Chromosomal abnormalities also include balanced translocations between chromosomes 15 and 17 (t(15;17)) and chromosomes 8 and 21 (t(8;21)) and inversions such as inv(16) and lesions involving the long arm of chromosome 11 (11q) [149]. The t(15;17) translocation is always associated with acute promyelocytic leukemia (APL) and leads to expression of the PML-RARα oncofusion gene in hematopoietic myeloid cells. The PML-RARα protein acts as a transcriptional repressor that interferes with gene expression programs involved in differentiation, apoptosis, and self-renewal [150].

Following the understanding of the underlying molecular mechanisms, patients have been treated with all-trans retinoic acid (ATRA) as part of remission induction. This has had a profound clinical impact both in adults and in children [151–155], since ATRA alters the corepressor activity, bypasses the differentiation block of the leukemic cells, and eventually induces differentiation of the myeloid lineage [156] with very successful results. As a matter of fact, it was recently shown that the combination of arsenic trioxide and ATRA without chemotherapy can induce a 100% CR and a 97% 2-year event-free survival in low-risk APL patients, thus making it a milestone in the treatment of leukemia patients without chemotherapy [157].

The t(8;21) translocation results in the AML1-ETO oncofusion protein. AML1 (RUNX1), a DNA-binding transcription factor, is a master regulator of the formation of hematopoietic stem cells [15, 16], while ETO encodes a protein harboring transcriptional repressor activities [158]. The fusion protein AML1-ETO is suggested to function as a transcriptional repressor that blocks AML1-dependent transactivation. This aberration is associated with a relatively favorable outcome [159].

Inv(16) leads to a CBFβ-MYH11 rearrangement that is found in approximately 8% of AML cases. It fuses the first 165 amino acids of the core-binding factor-β (CBFβ) to the C-terminal coiled-coil region of a smooth muscle myosin heavy chain (MYH11). The CBFβ-MYH11 fusion protein is suggested to cooperate with AML1 to repress transcription [160].

Finally, MLL (11q23) rearrangements are implicated in different types of acute leukemias and so far about 100 partners have been recognized [18, 19]. The MLL fusion proteins have a dominant gain-of-function effect that enhances the transcriptional activity. In general, the prognosis of patients with MLL translocations is poor [161]. Beyond these recurrent translocations, a recent study evaluating more than 100 AML patients using SNP array technology showed that the rate of acquired chromosomal copy number changes and the loss of heterozygosity was variable among cases. More importantly, using multivariate analysis, the authors found that the presence of ≥2 genomic lesions doubled the risk of death when controlling for age- and karyotype-based risk, thus confirming the impact of genomic complexity on outcomes. Finally, as expected, the negative prognostic impact of TP53 mutations, or TP53 mutations plus 17p-loss of heterozygosity, was confirmed [162].

Gene Mutations in AML and the Contribution of NGS

Mutations of important genes have now been recognized in AML and can be categorized into at least two groups: class I mutations that activate signal transduction and class II mutations that impair differentiation. The identification of such mutations has been particularly useful in the prognostic stratification of AML cases with CN-AML.
Among class I mutations, KIT mutations, mostly frequently reported in inv(16) and t(8;21), are very rare in other AML subtypes [143] and are usually associated with an unfavorable outcome.

FLT3 mutations can be of two types: internal tandem duplication (ITD) and tyrosine kinase domain mutations (TKD). FLT3-ITD are found in approximately 20% of unselected cases of AML and mainly cluster in the juxta-membrane domain, whereas TKD are represented by point mutations, small insertions, or deletions, mainly at codons 835 and 836, in 5–10% of AML cases. FLT3-ITD in CN-AML are usually associated with a dismal outcome [163–165]; clinical trials based on the use of FLT3 inhibitors are ongoing.

Similarly, among class II mutations, CEBPA mutations are predominantly found in CN-AML [166] and can be of two types: nonsense mutations affecting the N-terminal region that result in a truncated CEBPA isoform with dominant-negative properties, or in-frame mutations in the C-terminal basic region in CEBPA proteins that result in decreased DNA binding or dimerization activity. Such mutations can be biallelic. From a prognostic point of view, they have been associated with a relatively favorable outcome [164–166]; however, recent findings indicate that only double CEBPA mutations predict this favorable outcome, as also corroborated by a discrete gene-expression signature of double mutated cases [167].

NPM1 mutations, located in exon 12, induce an abnormal cytoplasmic localization of the NPM1 protein [168]. They are usually found in one third of adult cases of AML, making them the most frequent mutations in AML [169], and are associated with a normal karyotype in 85% of cases. NPM1 mutations are mutually exclusive of other AML recurrent chromosomal translocations but can be associated with secondary chromosome abnormalities, such as trisomy 8, trisomy 4, and del(9q), and additional gene mutations, most frequently in the FLT3 and IDH1 genes [170–173].

In CN-AML, NPM1 mutations have an important prognostic impact, mostly when they are not concomitant with FLT3-ITD; in fact, they are associated with the achievement of CR and a favorable outcome [171, 172]. For this reason, AML with mutated NPM1 without FLT3-ITD was recently allocated to the genetic favorable-risk category of AML [174]. Furthermore, given the presence of specific gene and microRNA expression signatures [175–177], they were incorporated as provisional entities in the 2008 WHO classification of AML [147].

The introduction of WES has made it possible to identify a potential third class of mutations in AML that induces impairment of epigenetic regulation [178]. The first of such genes identified by WES is represented by IDH1/IDH2 [179]. This gene encodes for a protein that has a significant role in cytoplasmic NADPH production. Since mutations have been reported also in gliomas [180, 181], it has been postulated that IDH1 might function as a tumor suppressor gene. IDH1/IDH2 mutations were first reported in more than 15% of adult CN-AML patients and this frequency was later confirmed by other studies [173, 179, 182]. Similar results were also observed in pediatric cohorts [183]. Furthermore, they are more frequently detected in patients without FLT3-ITD and with NPM1 mutations. In this subset of patients, at least 2 independent studies have shown an impact on disease-free survival and overall survival [173, 183], whereas a third study, carried out by Wagner et al. [184], highlighted a role for the IDH1 SNP rs11554137 polymorphism. Finally, from a functional point of view, both IDH1 and IDH2 mutations appear to induce hypermethylation [185].

Another mutated gene in AML is DNMT3A, a transcript with methyltransferase activity that catalyzes the methylation of cytosine residues of CpG dinucleotides. DNMT3A mutations are represented mostly by nonsense, frameshift, and missense mutations throughout the open-reading frame, with the most recurrent hotspot being a missense mutation at amino acid R882. They are found in approximately 20–22% of adults with de novo AML [186] and are associated with intermediate-risk AML, where they also correlate with inferior outcomes [186, 187]. In contrast, DNMT3A mutations appear to be rare in children [188, 189].

BCOR (BCL6 corepressor) mutations were initially identified by WES in a CN-AML patient lacking NPM1, CEBPA, FLT3-ITD, IDH1, and MLL-PTD mutations. Extended analysis in a large cohort of patients (>500 cases) showed that BCOR mutations are relatively recurrent (17.1%) in CN-AML patients without NPM1, CEBPA, FLT3-ITD, and IDH1 mutations and MLL-PTD, whereas they are rarely detected in unselected CN-AML (3.8%) and virtually absent in the other subgroups [190]. The mutations identified are similar to those reported in ocu-lofaciocardiodental genetic syndrome [191], scattered across the whole coding sequence and associated with decreased BCOR mRNA and absence of the protein. Furthermore, they are frequently associated with DNMT3A mutations and tend to be associated with a reduced overall survival.

Finally, TET2 mutations, which play a pivotal role in DNA demethylation, are detected mostly in myelodys-
plastic syndromes and myeloproliferative disorders and are highly recurrent in chronic myelomonocytic leukemias, in which they have been found to be associated with significant monocytosis and poor outcomes [192–195]. While the impact on the prognosis is controversial, it is interesting to highlight that TET2 mutations appear to be mutually exclusive with IDH1 mutations. This is in line with the role of such mutations; both have been shown to induce a hypermethylated state [185] and thus act on the same pathway.

To gain a more comprehensive overview of the genomic complexity of AML, the genome of an M3 (i.e. promyelocytic leukemia) patient was compared to that of a patient with CN-AML [196]. This approach led to the identification of a few mutations that are likely to be an initiating event in CN-AML and comprise the above-mentioned genes NPM1, DNMT3A, and IDH1. More in general [196, 197], a wide analysis of the AML genome has permitted the drawing of an AML model that can be recapitulated in the following steps: (1) the number of mutations required for leukemia initiation is very small; (2) these mutations usually occur in a hemopoietic stem cell that has already accumulated, with age progression, several random mutations; (3) a number of cooperating mutations (1–5) will then determine the overt transformation in AML; (4) mutations usually occur in genes relevant for pathogenesis, i.e. transcription factor fusions (18% of cases), NPM1 (27%), tumor suppressor genes (16%), DNA-methylation-related genes (44%), signaling genes (59%), chromatin-modifying genes (30%), myeloid transcription factor genes (22%), cohesion complex genes (13%), and spliceosome complex genes (14%), and (5) finally, the acquisition of additional mutations will give rise to subclones that might be responsible for relapse.

**Gene and MicroRNA Expression Profiling in AML**

Similar to observations in ALL, GEP was initially used to stratify patients by known chromosomal translocations. This approach clearly showed that AML with t(8;21)/RUNX1-RUNXIT1 and inv(16)/CBFB-MYH11 could be easily discriminated from other cytogenetic subgroups [198–200]. Similarly, it was shown that cases with a complex karyotype had a peculiar profile characterized by the upregulation of genes with a role in DNA repair such as RAD21 [201, 202]. GEP studies have also been applied to identify transcriptional profiles associated with emerging mutations. NPM1-mutated AMLs were found to be associated with the overexpression of distinct HOX cluster genes and genes involved in apoptosis [175, 176]. Similarly, CEBPA mutations have been associated with distinct GEPs that include downregulation of HOXA and HOXB cluster genes and upregulation of erythroid-specific genes, including GATA1 and EPOR [203]. Wouters et al. [204] revealed the presence of a subset of AML that did not harbor CEBPA mutations but had a similar transcriptional profile. These cases were characterized by CEBPA silencing through promoter hypermethylation and phenotypically showed the aberrant expression of T-cell genes, among which was CD7. Furthermore, these cases harbored NOTCH1 mutations, suggesting the presence of a mixed myeloid-lymphoid commitment. Subsequently, the same group showed the presence of a distinct signature for CEBPA double-mutated cases; this signature was not evident in CEBPA (single-mut) and is associated with favorable outcomes [167]. As for FLT3, Neben et al. [205] showed that GEP is able to discriminate FLT3-ITD from FLT3-TKD mutations. The discriminating set included genes involved in cell cycle control, gene transcription, and signal transduction.

Gene expression signatures have also been linked to a high expression of specific genes. Langer et al. [206] identified a high BAALC signature consisting of the overexpression of genes involved in drug resistance and stem cell markers. The same group [207] showed high expression levels of BAALC, CD200, and ABCB1 in patients overexpressing MNI. Finally, GEP has been used to define prognostic classifiers, mostly in karyotypically normal patients (CN-AML). Bullinger et al. [199] and Valk et al. [200] identified clusters associated with a different outcome. In particular, one cluster was characterized by the overexpression of several transcriptional regulators such as GATA2, and the second was characterized by the involvement of genes playing a role in leukocyte differentiation and the immune response. These results, validated by Radmacher et al. [208], confirm the prognostic value of this approach.

MicroRNAs have been extensively evaluated in AML; the first study showed the ability to distinguish AML from ALL on the basis of 21 microRNAs, of which 4 – let-7b, miR-128a, miR-128b, and miR-223 – were the most discriminative [209]. Furthermore, microRNA expression profiling has been shown to be able to distinguish different cytogenetic subtypes of AML: MLL rearrangements are characterized by a high expression of the miR-17–92 polycistrionic microRNA cluster, as well as miR-196b, with the latter located between the homeobox (HOX) A9 and HOXA10 genes at 7p15 [210]. APL displays high expression levels of microRNAs localized at chromosome band 14q32, while the downregulation of miR-133a in patients with t(8;21) has been described [211, 212].
Distinctive microRNA profiles were correlated to several mutations: NPM1 mutations display upregulation of miR-10a, miR-10b, and miR-196a and downregulation of miR-204 and miR-128a, predicted to target the HOX genes [177]. FLT3-ITD mutations have been reported to be associated with miR-155 upregulation which, in turn, interferes with SHP1, ultimately leading to leukemic expansion [213], while CEBPA mutations are characterized by the upregulation of members of the miR-181 family in CN-AML [165].

Finally, microRNA expression was correlated with outcomes; low expression levels of let7b and miR-9 were detected in patients classified into the favorable-risk group [212], whereas among the microRNAs likely to be associated with unfavorable outcomes it is worth mentioning the overexpression of miR-20a, miR-25, miR-191, miR-199a, and miR-199b [214]. A more recent study identified, within CN-AML belonging to the molecular high-risk group (i.e. FLT3-ITD mutations and wild-type NPM1), a set of microRNAs discriminative of the outcome. Among these, an increased expression of miR-181a and miR-181b was again associated with outcomes [215].

Concluding Remarks

Overall, the genomic era has permitted the redefinition of the molecular basis and the distinct mechanisms that lead to leukemogenic transformation. In B-ALL, beyond the well-recognized molecular rearrangements, i.e. BCR/ABL ETV6/RUNX1, E2A/PBX1, and MLL, it is to possible to identify a BCR/ABL-like subset, lesions of IKZF1, CRLF2, and of genes involved in lymphocyte development and differentiation. Furthermore, it is possible to refine the current knowledge of additional subsets, such as iAMP21 and hypodiploid ALL, and to recognize novel lesions (TP53 and tyrosine kinases) that might be relevant for the ALL pathogenesis. Similarly, the constellation of lesions in T-ALL now includes the presence of several mutations, such as NOTCH1, FBW7, BCL11B, JAK1, PTPN2, IL7Ra, and PHF6, and the recognition of a grey zone between T-ALL and AML. Importantly, in both B- and T-ALL, emerging mutations are identified at relapse only, thus leading to the hypothesis that these might be either responsible for the reoccurrence of disease or, alternatively, selected by the therapy itself.

Finally, in AML a major contribution has been made by gene and microRNA expression profiling, as well as by the mutational screening of several transcripts. The integration of these approaches has allowed the subdivision of patients into prognostic subgroups, to redefine the current WHO classification, and it has proven particularly useful in the dissection of cases with a normal karyotype. Moreover, the integration of this information has improved our overall understanding of the AML pathogenesis, in which very few mutations are required for leukemia initiation, a few others determine an overt leukemic transformation, and the acquisition of additional mutations will give rise to relapse. This model is likely to be applicable to other oncohematologic disorders. Furthermore, the deeper characterization of cancer genomes and the identification of additional subclonal mutations are not just of importance for understanding tumor evolution but may be also clinically important to shift the target of therapy and include both the dominant diagnostic clone and a potentially minor relapse clone.

While the above mentioned findings have a pivotal biological relevance, they have not yet been applied in the current therapeutic strategies, which are still based on the administration of polychemotherapeutic regimens. Favorable examples obtained in the past years include APL, CML, and partly Ph+ ALL. In APL, the clinical use of ATRA in combination with As2O3 (arsenite) has caused a revolution in the outcome of this AML subset; similar results have been obtained with the use of tyrosine kinase inhibitors in CML and, to a lesser, extent in Ph+ ALL.

The identification of novel lesions will probably make it possible in the near future to obtain other encouraging examples and design personalized therapeutic strategies where biological acquisitions are translated into the clinical setting. Drug targets should be validated in large groups of patients and therapies directed against these lesions should be used, mostly likely in combination with more standard approaches, in the patient’s treatment regimen when possible. These results suggest that few targeted inhibitors directed toward recurrent lesions may be applied to different therapeutic approaches in order to reduce the toxicity and maximize the efficacy.

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