Review

Medical Principles and Practice

Med Princ Pract 2014;23:487–506
DOI: 10.1159/000362793

Received: November 3, 2013
Accepted: April 10, 2014
Published online: June 20, 2014

Genomic Characterization of Acute Leukemias

Sabina Chiaretti Valentina Gianfelici Giulia Ceglie Robin Foà

Division of Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Rome, Italy

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

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 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.

© 2014 S. Karger AG, Basel

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-

KARGER

E-Mail karger@karger.com
www.karger.com/mpp

© 2014 S. Karger AG, Basel
1011–7571/14/0236–0487$39.50/0

Karger Open access

This is an Open Access article licensed under the terms of the Creative Commons Attribution-NonCommercial 3.0 Unported license (CC BY-NC) (www.karger.com/OA-license), applicable to the online version of the article only. Distribution permitted for non-commercial purposes only.

Prof. Sabina Chiaretti
Division of Hematology, Department of Cellular Biotechnologies and Hematology
Sapienza University of Rome
Via Benevento 6, IT–00161 Rome (Italy)
E-Mail chiaretti@bce.uniroma1.it

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

cursor (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 expansion of more fit subclones; this model is likely to be applicable to most cancers. The identification of subclonal lesions is not just important for our understanding of leukemia biology but it also has implications for therapy.

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].

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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 technique has revealed that *IKZF1,* encoding for the transcription factor *Ikaros* and playing a

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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 pseudoautosomal 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

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

(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 *NF1*) 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 chemorefractoriness [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 a minority of cases also at diagnosis. Furthermore, it was shown that *TP53* disruption correlates with reduced event-free and overall survivals. Similar findings were also reported by Krentz et al. [69], who again showed that *TP53* disruptions in childhood relapsed ALL occurred in 11% of patients. On the other hand, the authors showed that *TP53* lesions harbor a prognostic significance only in intermediate-risk patients and not in cases that are already classified as high risk. In newly diagnosed adult ALL, our group has shown that *TP53* mutations identified in 8.2% of cases are more frequent in T-ALL than in B-ALL (11.1 vs. 6.4%) and are preferentially identified in patients without molecular aberrations, where they are detected in 14% of cases [70]. Finally, they are associated with refractoriness to chemotherapy. Taken together, these results point to *TP53* as a 'novel' marker 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 hemopoiesis. 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

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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

	Gene(s) involved	Functional consequences	Frequency		Clinical relevance
			children	adults	
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 *TLX1 (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

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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

	Gene(s) involved	Functional consequences	Frequency		Clinical relevance
			children	adults	
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%	5–10%	Not clearly established
9p deletion	*CDKN2A* and *CDKN2B*	Loss of cell proliferation control	20–30%	<1%	No impact
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

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, FLT3,* and *NRAS*.

Thus, this subgroup clearly emerges as a grey zone between AML and T-ALL, is detected in all age cohorts, presents a peculiar phenotypic and transcriptional profile and several additional lesions, and, most importantly, defines a subset of patients with a poor prognosis. In line with these findings, the use of myeloid-directed therapies appears to be an appealing approach.

Gene Mutations in T-ALL

An important contribution to the understanding of T-ALL has been made by the mutational screening of a set of transcripts which might play a pivotal role in T-ALL leukemogenesis (table 3). Beyond *NOTCH1* mutations, which will be described in detail, other recurrent lesions are represented by *FBW7, BCL11B, JAK1, PTPN2, IL7R,* and *PHF6*.

The *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-

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

Table 3. Gene mutations in T-ALL

Gene(s) involved	Gene position	Functional consequences	Frequency		Clinical relevance
			children	adults	
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%	–	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%	–	No impact
IL7R	5p13	Lymphoid development	6%	–	No impact
PHF6	Xq26.3	Putative tumor suppressor	5–16%	18–38%	No impact
ETV6 *IDH1* *IDH2* *DNMT3A* *FLT3* *NRAS* *JAK3* *IKZF1*	12p13 2q33.3 15q26.1 2q33.3 13q12 1p13.2 19p13.1 7p13-p11.1	Various, including signaling, developmental arrest, and histone modification	Detected in ETP leukemia		Unfavorable, as per subgroup of the disease
CNOT3	19q13.4	Presumed tumor suppressor	–	8%	Not known
RPL5 and *RPL10*	1p22.1 Xq28	Ribosomal activity impairment	8%	–	Not known
NT5C2	10q24.32	Increased dephosphorylation of nucleoside analogs	19% of relapsed ALL cases		Identified only at relapse

tected 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]. *BCL11B* can be involved in leukemogenesis through either an inv(14) or a recurrent cryptic t(5;14)(q35;q32) [106, 107]. 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

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

the expression of *CHEK1* 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 upregulation 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 Vlierberghe 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.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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, displaces 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

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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 juxtamembrane 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 oculofaciocardiodental 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-

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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 abovementioned 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-RUNX1T1* 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 *MN1.* 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* polycistronic 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].

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

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, IL7Rα,* 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 As_2O_3 (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.

References

1 Inaba H, Greaves M, Mullighan CG: Acute lymphoblastic leukemia. Lancet 2013;381:1943–1955.
2 Nowell PC, Hungerford DA: A minute chromosome in human chronic granulocytic leukemia. Science 1960;132:1497–1499.
3 Sattler M, Griffin JD: Molecular mechanisms of transformation by the BCR-ABL oncogene. Semin Hematol 2003;40:4–10.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

4 Melo JV, Deininger MW: Biology of chronic myelogenous leukemia – signaling pathways of initiation and transformation. Hematol Oncol Clin North Am 2004;18:545–568, vii–viii.
5 Faderl S, Garcia-Manero G, Thomas DA, et al: Philadelphia chromosome-positive acute lymphoblastic leukemia – current concepts and future perspectives. Rev Clin Exp Hematol 2002;6:142–160.
6 Pane F, Frigeri F, Sindona M, et al: Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood 1996;88:2410–2414.
7 Chiaretti S, Vitale A, Cazzaniga G, et al: Clinico-biologic features of 5,202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age-cohorts. Haematologica 2013;98:1702–1710.
8 Vignetti M, Fazi P, Cimino G, et al: Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. Blood 2007;109:3676–3678.
9 Ottmann O, Dombret H, Martinelli G, et al: Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood 2007:110:2309–2315.
10 Schultz KR, Bowman WP, Aledo A, et al: Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a Children's Oncology Group study. J Clin Oncol 2009;27:5175–5181.
11 Ravandi F, Kebriaei P: Philadelphia chromosome-positive acute lymphoblastic leukemia. Hematol Oncol Clin North Am 2009;23:1043–1063.
12 Gruber F, Mustjoki S, Porkka K: Impact of tyrosine kinase inhibitors on patient outcomes in Philadelphia chromosome-positive acute lymphoblastic leukaemia. Br J Haematol 2009;145:581–597.
13 Foà R, Vitale A, Vignetti M, et al; GIMEMA Acute Leukemia Working Party: Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2011;118:6521–6528.
14 Harrison CJ: Cytogenetics of paediatric and adolescent acute lymphoblastic leukaemia. Br J Haematol 2009;144:147–156.
15 Loh ML, Rubnitz JE: TEL/AML1-positive pediatric leukemia: prognostic significance and therapeutic approaches. Curr Opin Hematol 2002;9:345–352.
16 Speck NA, Gilliland DG: Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer 2002;2:502–513.
17 Hiebert SW, Sun W, Davis JN, et al: The t(12;21) translocation converts AML-1B from an activator to a repressor of transcription. Mol Cell Biol 1996;16:1349–1355.
18 Meyer C, Kowarz E, Hofmann J, et al: New insights to the MLL recombinome of acute leukemias. Leukemia 2009;23:1490–1499.
19 Meyer C, Hofmann J, Burmeister T, et al: The MLL recombinome of acute leukemias in 2013. Leukemia 2013;27:2165–2176.
20 Corral J, Lavenir I, Impey H, et al: An MII-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell 1996;85:853–861.
21 Dobson CL, Warren AJ, Pannell R, et al: The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. EMBO J 1999;18:3564–3574.
22 Ayton PM, Cleary ML: Transformation of myeloid progenitors by MLL oncoproteins is dependent on *Hoxa7* and *Hoxa9*. Genes Dev 2003;17:2298–2307.
23 Carroll AJ, Crist WM, Parmley RT, et al: Pre-B cell leukemia associated with chromosome translocation 1;19. Blood 1984;63:721–724.
24 Hunger SP: Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia: clinical features and molecular pathogenesis. Blood 1996;87:1211–1224.
25 Aspland S, Bendall HH, Murre C: The role of E2A-PBX1 in leukemogenesis. Oncogene 2001;20:5708–5711.
26 Pui CH, Evans WE: Treatment of acute lymphoblastic leukemia. N Engl J Med 2006;354:166–1678.
27 Foa R, Vitale A, Mancini M, et al: E2A-PBX1 fusion in adult acute lymphoblastic leukaemia: biological and clinical features. Br J Haematol 2003;120:484–487.
28 Burmeister T, Gökbuget N, Schwartz S, et al: Clinical features and prognostic implications of TCF3-PBX1 and ETV6-RUNX1 in adult acute lymphoblastic leukemia. Haematologica 2010;95:241–246.
29 Golub TR, Slonim DK, Tamayo P, et al: Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–537.
30 Yeoh EJ, Ross ME, Shurtleff SA, et al: Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133–143.
31 Ross ME, Zhou X, Song G, et al: Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood 2003;102:2951–2959.
32 Kohlmann A, Schoch C, Schnittger S, et al: Pediatric acute lymphoblastic leukemia (ALL) gene expression signatures classify an independent cohort of adult ALL patients. Leukemia 2004;18:63–71.
33 Chiaretti S, Li X, Gentleman R, et al: Gene expression profiles of B-lineage adult acute lymphocytic leukemia reveal genetic patterns that identify lineage derivation and distinct mechanisms of transformation. Clin Cancer Res 2005;11:7209–7219.
34 Haferlach T, Kohlmann A, Schnittger S, et al: Global approach to the diagnosis of leukemia using gene expression profiling. Blood 2005;106:1189–1198.
35 Irving JA, Bloodworth L, Bown NP, et al: Loss of heterozygosity in childhood acute lymphoblastic leukemia detected by genome-wide microarray single nucleotide polymorphism analysis. Cancer Res 2005;65:3053–3058.
36 Mullighan CG, Goorha S, Radtke I, et al: Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446:758–764.
37 Paulsson K, Cazier JB, Macdougall F, et al: Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: unexpected similarities with pediatric disease. Proc Natl Acad Sci USA 2008;105:6708–6713.
38 Kawamata N, Ogawa S, Zimmermann M, et al: Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. Blood 2008;111:776–784.
39 Kuiper RP, Schoenmakers EF, van Reijmersdal SV, et al: High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. Leukemia 2007;21:1258–1266.
40 Pui CH, Robison LL, Look AT: Acute lymphoblastic leukemia. Lancet 2008;371:1030–1043.
41 Georgopoulos K, Bigby M, Wang JH, et al: The Ikaros gene is required for the development of all lymphoid lineages. Cell 1994;79:143–156.
42 Tonnelle C, Bardin F, Maroc C, et al: Forced expression of the Ikaros 6 isoform in human placental blood CD34(+) cells impairs their ability to differentiate toward the B-lymphoid lineage. Blood 2001;98:2673–2680.
43 Tonnelle C, Dijon M, Moreau T, et al: Stage specific over-expression of the dominant negative Ikaros 6 reveals distinct role of Ikaros throughout human B-cell differentiation. Mol Immunol 2009;46:1736–1743.
44 Trageser D, Iacobucci I, Nahar R, et al: Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. J Exp Med 2009;206:1739–1753.
45 Collins-Underwood JR, Miller CB, Downing JR, et al: Ikzf1 haploinsufficiency contributes to the pathogenesis of BCR-ABL1 positive acute lymphoblastic leukemia (abstract 678). Blood 2009;114:283.
46 Mullighan CG, Miller CB, Radtke I, et al: BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature 2008;453:110–114.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

47 Iacobucci I, Storlazzi CT, Cilloni D, et al: Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). Blood 2009;114:2159–2167.

48 Martinelli G, Iacobucci I, Storlazzi CT, et al: IKZF1 (Ikaros) deletions in BCRABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. J Clin Oncol 2009; 27:5202–5207.

49 Mullighan CG, Su X, Zhang J, et al; Children's Oncology Group: Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 2009;360:470–480.

50 Kuiper RP, Waanders E, van der Velden VH, et al: IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia 2010;24:1258–1264.

51 Levin SD, Koelling RM, Friend SL, et al: Thymic stromal lymphopoietin: a cytokine that promotes the development of IgM+ B cells in vitro and signals via a novel mechanism. J Immunol 1999;162:677–683.

52 Brown VI, Hulitt J, Fish J, et al: Thymic stromal-derived lymphopoietin induces proliferation of pre-B leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7Ralpha signaling. Cancer Res 2007; 67:9963–9970.

53 Mullighan CG, Collins-Underwood JR, Phillips LA, et al: Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet 2009;41:1243–1246.

54 Hertzberg L, Vendramini E, Ganmore I, et al: Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood 2010;115: 1006–1117.

55 Russell LJ, Capasso M, Vater I, et al: Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood 2009;114:2688–2698.

56 Cario G, Zimmermann M, Romey R, et al: Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood 2010; 115:5393–5397.

57 Harvey RC, Mullighan CG, Chen IM, et al: Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood 2010;115:5312–5321.

58 Yoda A, Yoda Y, Chiaretti S, et al: Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci USA 2010;107:252–257.

59 Den Boer ML, van Slegtenhorst M, De Menezes RX, et al: A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 2009;10:125–134.

60 Harvey RC, Mullighan CG, Wang X, et al: Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. Blood 2010;116:4874–4884.

61 Roberts KG, Morin RD, Zhang J, et al: Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell 2012;22: 153–166.

62 Robinson HM, Broadfield ZJ, Cheung KL, et al: Amplification of AML1 in acute lymphoblastic leukemia is associated with a poor outcome. Leukemia 2003;17:2249–2250.

63 Harewood L, Robinson H, Harris R, et al: Amplification of AML1 on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases. Leukemia 2003;17:547–553.

64 Strefford JC, van Delft FW, Robinson HM, et al: Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. Proc Natl Acad Sci USA 2006;103:8167–8172.

65 Moorman AV, Richards SM, Robinson HM, et al; UK Medical Research Council (MRC)/ National Cancer Research Institute (NCRI) Childhood Leukaemia Working Party (CLWP): Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood 2007;109:2327–2330.

66 Rand V, Parker H, Russell LJ, et al: Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. Blood 2011;117:6848–6855.

67 Holmfeldt L, Wei L, Diaz-Flores E, et al: The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet 2013;45:242–252.

68 Hof J, Krentz S, van Schewick, et al: Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol 2011;29:3185–3193.

69 Krentz S, Hof J, Mendioroz A, et al: Prognostic value of genetic alterations in children with first bone marrow relapse of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia 2013;27:295–304.

70 Chiaretti S, Brugnoletti F, Tavolaro S, et al: TP53 mutations are frequent in adult acute lymphoblastic leukemia cases negative for recurrent fusion genes and correlate with poor response to induction therapy. Haematologica 2013;98:e59–e61.

71 Aas T, Børresen AL, Geisler S, et al: Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat Med 1996;2:811–814.

72 Poeta ML, Manola J, Goldwasser MA, et al: TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med 2007;357:2552–2561.

73 Fenaux P, Preudhomme C, Quiquandon I, et al: Mutations of the P53 gene in acute myeloid leukaemia. Br J Hematol 1992;80:178–183.

74 Haferlach C, Dicker F, Herholz H, et al: Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. Leukemia 2008;22:1539–1541.

75 Cordone I, Masi S, Mauro FR, et al: p53 expression in B-cell chronic lymphocytic leukemia: a marker of disease progression and poor prognosis. Blood 1998;91:4342–4349.

76 Rossi D, Cerri M, Deambrogi C, et al: The prognostic value of TP53 mutations in chronic lymphocytic leukemia is independent of Del17p13: implications for overall survival and chemorefractoriness. Clin Cancer Res 2009;15:995–1004.

77 Zenz T, Eichhorst B, Busch R, et al: TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol 2010;28:4473–4479.

78 Mullighan CG, Zhang J, Kasper LH, et al: CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature 2011;471:235–239.

79 Goodman RH, Smolik S: CBP/p300 in cell growth, transformation, and development. Genes Dev 2000;14:1553–1577.

80 Meyer JA, Wang J, Hogan LE, et al: Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet 2013;45:290–294.

81 Cauwelier B, Dastugue N, Cools J, et al: Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCRbeta locus rearrangements and putative new T-cell oncogenes. Leukemia 2006;20:1238–1244.

82 Janssen JW, Ludwig WD, Sterry W, et al: SIL-TAL1 deletion in T-cell acute lymphoblastic leukemia. Leukemia 1993;7:1204–1210.

83 Szczepanski T, Harrison CJ, van Dongen JJ: Genetic aberrations in paediatric acute leukaemias and implications for management of patients. Lancet Oncol 2010;11:880–889.

84 Graux C, Cools J, Melotte C, et al: Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet 2004;36:1084–1089.

85 Chiaretti S, Tavolaro S, Ghia EM, et al: Characterization of ABL1 expression in adult T-cell acute lymphoblastic leukemia by oligonucleotide arrays analysis. Haematologica 2007;92:619–626.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

86 De Keersmaecker K, Graux C, Odero MD, et al: Fusion of EML1 to ABL1 in T-cell acute lymphoblastic leukemia with cryptic t(9;14)(q34;q32). Blood 2005;105:4849–4852.

87 Ferrando AA, Neuberg DS, Staunton J, et al: Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002;1:75–87.

88 Ferrando AA, Armstrong SA, Neuberg DS, et al: Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. Blood 2003;102:262–268.

89 Speleman F, Cauwelier B, Dastugue N, et al: A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias. Leukemia 2005;19:358–366.

90 Dik WA, Brahim W, Braun C, et al: CALM-AF10+ T-ALL expression profiles are characterized by overexpression of HOXA and BMI1 oncogenes. Leukemia 2005;19:1948–1957.

91 Van Vlierberghe P, van Grotel M, Tchinda J, et al: The recurrent SET-NUP214 fusion as a new HOXA activation mechanism in pediatric T-cell acute lymphoblastic leukemia. Blood 2008;111:4668–4680.

92 Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol 2009;10:147–156.

93 Chiaretti S, Messina M, Tavolaro S, et al: Gene expression profiling identifies a subset of adult T-cell acute lymphoblastic leukemia with myeloid-like gene features and over-expression of miR-223. Haematologica 2010;95:1114–1121.

94 Fazi F, Rosa A, Fatica A, et al: A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis. Cell 2005;123:819–831.

95 Van Vlierberghe P, Ambesi-Impiombato A, Perez-Garcia A, et al: ETV6 mutations in early immature human T cell leukemias. J Exp Med 2011;208:2571–2579.

96 Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012;481:157–163.

97 Demarest RM, Ratti F, Capobianco AJ: It's T-ALL about Notch. Oncogene 2008;27:5082–5091.

98 O'Neil J, Grim J, Strack P, et al: FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med 2007;204:1813–1824.

99 Thompson BJ, Buonamici S, Sulis ML, et al: The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med 2007;204:1825–1835.

100 Asnafi V, Buzyn A, Le Noir S, et al: NOTCH1/FBXW7 mutation identifies a large subgroup with favourable outcome in adult T-cell acute lymphoblastic leukemia (TALL): a GRAALL study. Blood 2009;113:3918–3924.

101 Ben Abdelali R, Asnafi V, Leguay T, et al; Group for Research on Adult Acute Lymphoblastic Leukemia: Pediatric-inspired intensified therapy of adult T-ALL reveals the favorable outcome of NOTCH1/FBXW7 mutations, but not of low ERG/BAALC expression: a GRAALL study. Blood 2011;118:5099–5107.

102 Park MJ, Tak T, Oda M, et al: FBXW7 and NOTCH1 mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma. Br J Haematol 2009;145:198–206.

103 Mansour MR, Sulis ML, Duke V, et al: Prognostic implications of NOTCH1 and FBXW7 mutations in adults with T-cell acute lymphoblastic leukemia treated on the MRC UKALLXII/ECOG E2993 protocol. J Clin Oncol 2009;27:4352–4356.

104 Kox C, Zimmermann M, Stanulla M, et al: The favorable effect of activating NOTCH1 receptor mutations on long-term outcome in T-ALL patients treated on the ALL-BFM 2000 protocol can be separated from FBXW7 loss of function. Leukemia 2010;24:2005–2013.

105 Liu P, Li P, Burke S: Critical roles of Bcl11b in T-cell development and maintenance of T-cell identity. Immunol Rev 2010;238:138–149.

106 Przybylski GK, Dik WA, Wanzeck J, et al: Disruption of the BCL11B gene through inv(14)(q11.2q32.31) results in the expression of BCL11B-TRDC fusion transcripts and is associated with the absence of wild-type BCL11B transcripts in T-ALL. Leukemia 2005;19:201–208.

107 Bernard OA, Busson-LeConiat M, Ballerini P, et al: A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia 2001;15:1495–1504.

108 De Keersmaecker K, Real PJ, Gatta GD, et al: The TLX1 oncogene drives aneuploidy in T cell transformation. Nat Med 2010;16:1321–1327.

109 Gutierrez A, Kentsis A, Sanda T, et al: The BCL11B tumor suppressor is mutated across the major molecular subtypes of T-cell acute lymphoblastic leukemia. Blood 2011;118:4169–4173.

110 Kraszewska MD, Dawidowska M, Szczepański T, et al: T-cell acute lymphoblastic leukaemia: recent molecular biology findings. Br J Haematol 2012;156:303–315.

111 Flex E, Petrangeli V, Stella L, et al: Somatically acquired Jak1 mutation in adult acute lymphoblastic leukemia. J Exp Med 2008;205:751–758.

112 Jeong EG, Kim MS, Nam HK, et al: Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. Clin Cancer Res 2008;14:3716–3721.

113 Asnafi V, Le Noir S, Lhermitte L, et al: JAK1 mutations are not frequent events in adult T-ALL: a GRAALL study. Br J Haematol 2010;148:178–179.

114 Kleppe M, Lahortiga I, El Chaar T, et al: Deletion of the protein tyrosine phosphatase gene PTPN2 in T-cell acute lymphoblastic leukemia. Nat Genet 2010;42:530–535.

115 Kleppe M, Soulier J, Asnafi V, et al: PTPN2 negatively regulates oncogenic JAK1 in T-cell acute lymphoblastic leukemia. Blood 2011;117:7090–7098.

116 Peschon JJ, Morrissey PJ, Grabstein KH, et al: Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;180:1955–1960.

117 Puel A, Ziegler SF, Buckley RH, et al: Defective IL7R expression in T(–)B(+)NK(+) severe combined immunodeficiency. Nat Genet 1998;20:394–397.

118 Shochat C, Tal N, Bandapalli OR, et al: Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med 2011;208:1333.

119 Zenatti PP, Ribeiro D, Li W, et al: Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet 2011;43:932–939.

120 Van Vlierberghe P, Palomero T, Khiabanian H, et al: PHF6 mutations in T-cell acute lymphoblastic leukemia. Nat Genet 2010;42:338–342.

121 Lower KM, Turner G, Kerr BA, et al: Mutations in PHF6 are associated with Börjeson-Forssman-Lehmann syndrome. Nat Genet 2002;32:661–665.

122 Wang Q, Qiu H, Jiang H, et al: Mutations of PHF6 are associated with mutations of NOTCH1, JAK1 and rearrangement of SET-NUP214 in T-cell acute lymphoblastic leukemia. Haematologica 2011;96:1808–1814.

123 Porcu M, Kleppe M, Gianfelici V, et al: Mutation of the receptor tyrosine phosphatase PTPRC (CD45) in T-cell acute lymphoblastic leukemia. Blood 2012;119:4476–4479.

124 De Keersmaecker K, Atak ZK, Li N, et al: Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 2013;45:186–190.

125 Tzoneva G, Perez-Garcia A, Carpenter Z, et al: Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat Med 2013;19:368–371.

126 Mirandola L, Comi P, Cobos E, et al: Notching from T-cell to B-cell lymphoid malignancies. Cancer Lett 2011;308:1–13.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

127 Gordon WR, Roy M, Vardar-Ulu D, et al: Structure of the Notch1-negative regulatory region: implications for normal activation and pathogenic signaling in T-ALL. Blood 2009;113:4381–4390.
128 Kelly DF, Lake RJ, Middelkoop TC, et al: Molecular structure and dimeric organization of the Notch extracellular domain as revealed by electron microscopy. PLoS One 2010;5:e10532.
129 Artavanis-Tsakonas S, Rand MD, Lake RJ: Notch signaling: cell fate control and signal integration in development. Science 1999; 284:770–776.
130 Lai EC: Notch signaling: control of cell communication and cell fate. Development 2004;131:965–973.
131 Maerki S, Ceredig R, Rolink A: Induction of chemokine receptor expression during early stages of T cell development. Immunol Lett 2006;104:110–117.
132 Buonamici S, Trimarchi T, Ruocco MG, et al: CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. Nature 2009;459:1000–1004.
133 Garcia-Peydro M, de Yebenes VG, Toribio ML: Notch1 and IL-7 receptor interplay maintains proliferation of human thymic progenitors while suppressing non-T cell fates. J Immunol 2006;177:3711–3720.
134 Ellisen LW, Bird J, West DC, et al: TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991;66:649–661.
135 Pear WS, Aster JC, Scott ML, et al: Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. J Exp Med 1996;183: 2283–2291.
136 Weng AP, Ferrando AA, Lee W, et al: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269–271.
137 Breit S, Stanulla M, Flohr T, et al: Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. Blood 2006;108:1151–1157.
138 Selkoe D, Kopan R: Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. Annu Rev Neurosci 2003;26:565–597.
139 De Keersmaecker K, Lahortiga I, Mentens N, et al: In vitro validation of gamma-secretase inhibitors alone or in combination with other anti-cancer drugs for the treatment of T-cell acute lymphoblastic leukemia. Haematologica 2008;93:533–542.
140 Palomero T, Lim WK, Odom DT, et al: NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci USA 2006;103:18261–18266.
141 Palomero T, Sulis ML, Cortina M, et al: Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med 2007;13:1203–1210.
142 Real PJ, Tosello V, Palomero T, et al: Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. Nat Med 2009;15:50–58.
143 Kumar CC: Genetic abnormalities and challenges in the treatment of acute myeloid leukemia. Genes Cancer 2011;2:95–107.
144 Thomas X: Chemotherapy of acute leukemias in adults. Exp Opin Pharmacother 2009;10:221–237.
145 Farag SS, Ruppert AS, Mrózek K, et al: Outcome of induction and postremission therapy in younger adults with acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. J Clin Oncol 2005; 23:482–483.
146 Klepin HD, Balducci L: Acute myelogenous leukemia in older adults. Oncologist 2009; 14:222–232.
147 Vardiman JW, Thiele J, Arber DA, et al: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937–951.
148 Martens JHA, Stunnenberg HG: The molecular signature of oncofusion proteins in acute myeloid leukemia. FEBS Lett 2010; 584:2662–2669.
149 Byrd JC, Mrózek K, Dodge RK, et al; Cancer and Leukemia Group B (CALGB 8461): Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002; 100:4325–4336.
150 de The H, Chomienne C, Lanotte M, et al: The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor α gene to a novel transcribed locus. Nature 1990;347:358–361.
151 Mrozek K, Radmacher MD, Bloomfield CD, et al: Molecular signatures in acute myeloid leukemia. Curr Opin Hematol 2009;16:64–69.
152 Tallman MS, Andersen JW, Schiffer CA, et al: All-*trans*-retinoic acid in acute promyelocytic leukemia. N Engl J Med 1997:337: 1639–1646.
153 Avvisati G, Lo Coco F, Diverio D, et al: AIDA (all-*trans* retinoic acid + idarubicin) in newly diagnosed acute promyelocytic leukemia: a Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) pilot study. Blood 1996;88:1390–1398.
154 Testi AM, Biondi A, Lo Coco F, et al: GIMEMA-AIEOPAIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. Blood 2005;106:447–453.
155 Avvisati G, Lo-Coco F, Paoloni FP, et al; GIMEMA, AIEOP, and EORTC Cooperative Groups: AIDA 0493 protocol for newly diagnosed acute promyelocytic leukemia: very long-term results and role of maintenance. Blood 2011;117:4716–4725.
156 Licht JD: Reconstructing a disease: what essential features of the retinoic acid receptor fusion oncoproteins generate acute promyelocytic leukemia? Cancer Cell 2006;9:73–74.
157 Lo-Coco F, Avvisati G, Vignetti M, et al: Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med 2013; 369:111–121.
158 Davis JN, McGhee L, Meyers S: The ETO (MTG8) gene family. Gene 2003;303:1–10.
159 Kolitz JE, George SL, Dodge RK, et al; Cancer and Leukemia Group B: Dose escalation studies of cytarabine, daunorubicin, and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia younger than 60 years: final induction results of Cancer and Leukemia Group B study 9621. J Clin Oncol 2004;22:4290–4301.
160 Lutterbach B, Hiebert SW: Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. Gene 2000;245:223–235.
161 Eguchi M, Eguchi-Ishimae M, Greaves M: Molecular pathogenesis of MLL-associated leukemias. Int J Hematol 2005;82:9–20.
162 Parkin B, Erba H, Ouillette P, et al: Acquired genomic copy number aberrations and survival in adult acute myelogenous leukemia. Blood 2010;116:4958–4967.
163 Fröhling S, Scholl C, Gilliland DG, et al: Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol 2005;23:6285–6295.
164 Schlenk RF, Döhner K, Krauter J, et al; German-Austrian Acute Myeloid Leukemia Study Group: Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358: 1909–1918.
165 Marcucci G, Haferlach T, Döhner H: Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol 2011;29:475–486.
166 Koschmieder S, Halmos B, Levantini E, et al: Dysregulation of the C/EBPalpha differentiation pathway in human cancer. J Clin Oncol 2009;27:619–628.
167 Wouters BJ, Löwenberg B, Erpelinck-Verschueren CA, et al: Double *CEBPA* mutations, but not single *CEBPA* mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood 2009;113:3088–3091.
168 Falini B, Nicoletti I, Martelli MF, et al: Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc + AML): biologic and clinical features. Blood 2007; 109:874–885.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

169 Falini B, Mecucci C, Tiacci E, et al; GIMEMA Acute Leukemia Working Party: Cytoplasmic nucleophosmin in acute myelogenous leukemi with a normal karyotype. N Engl J Med 2005;352:254–266.

170 Schnittger S, Schoch C, Kern W, et al: Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood 2005;106:3733–3739.

171 Döhner K, Döhner H: Molecular characterization of acute myeloid leukemia. Haematologica 2008;93:976–982.

172 Mrózek K, Marcucci G, Paschka P, et al: Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 2007;109:431–448.

173 Marcucci G, Maharry K, Wu YZ, et al: IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B Study. J Clin Oncol 2010;28:2348–2355.

174 Döhner H, Estey EH, Amadori S, et al; European LeukemiaNet: Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010;115:453–474.

175 Verhaak RG, Goudswaard CS, van Putten W, et al: Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood 2005;106:3747–3754.

176 Mullighan CG, Kennedy A, Zhou X, et al: Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. Leukemia 2007;21:2000–2009.

177 Garzon R, Garofalo M, Martelli MP, et al: Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci USA 2008;105:3945–3950.

178 Dombret H: Gene mutation and AML pathogenesis. Blood 2011;118:5366–5367.

179 Mardis ER, Ding L, Dooling DJ, et al: Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 2009;361:1058–1066.

180 Sanson M, Marie Y, Paris S, et al: Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol 2009;27:4150–4154.

181 Yan H, Parsons DW, Jin G, et al: IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–773.

182 Paschka P, Schlenk RF, Gaidzik VI, et al: IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication J Clin Oncol 2010;28:3636–3643.

183 Andersson AK, Miller DW, Lynch JA, et al: IDH1 and IDH2 mutations in pediatric acute leukemia. Leukemia 2011;25:1570–1577.

184 Wagner K, Damm F, Göhring G, et al: Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. J Clin Oncol 2010;28:2356–2364.

185 Figueroa ME, Abdel-Wahab O, Lu C, et al: Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 2010;18:553–567.

186 Ley TJ, Ding L, Walter MJ, et al: DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010;363:2424–2433.

187 Thol F, Damm F, Lüdeking A, et al: Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. J Clin Oncol 2011;29:2889–2896.

188 Thol F, Heuser M, Damm F, et al: DNMT3A mutations are rare in childhood acute myeloid leukemia. Haematologica 2011;96:1238–1240.

189 Paganin M, Pigazzi M, Bresolin S, et al: A methyltransferase 3a hot-spot locus is not mutated in pediatric patients affected by acute myeloid or T-cell acute lymphoblastic leukemia: an Italian study. Haematologica 2011;96:1886–1887.

190 Grossmann V, Tiacci E, Holmes AB, et al: Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. Blood 2011;118:6153–6163.

191 Ng D, Thakker N, Corcoran CM, et al: Oculofaciocardiodental and Lenz microphthalmia syndromes result from distinct classes of mutations in BCOR. Nat Genet 2004;36:411–416.

192 Delhommeau F, Dupont S, Della Valle V, et al: Mutation in TET2 in myeloid cancers. N Engl J Med 2009;360:2289–2301.

193 Abdel-Wahab O, Mullally A, Hedvat C, et al: Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood 2009;114:144–147.

194 Tefferi A, Pardanani A, Lim KH, et al: TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia 2009;23:905–911.

195 Kosmider O, Gelsi-Boyer V, Ciudad M, et al; Groupe Francophone des Myélodysplasies: TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica 2009;94:1676–1681.

196 Welch JS, Ley TJ, Link DC, et al: The origin and evolution of mutations in acute myeloid leukemia. Cell 2012;150:264–278.

197 Cancer Genome Atlas Research Network: Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059–2074.

198 Schoch C, Kohlmann A, Schnittger S, et al: Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. Proc Natl Acad Sci USA 2002;99:10008–10013.

199 Bullinger L, Döhner K, Bair E, et al: Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. N Engl J Med 2004;350:1605–1616.

200 Valk PJ, Verhaak RG, Beijen MA, et al: Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 2004;350:1617–1628.

201 Schoch C, Kern W, Kohlmann A, et al: Acute myeloid leukemia with a complex aberrant karyotype is a distinct biological entity characterized by genomic imbalances and a specific gene expression profile. Genes Chromosomes Cancer 2005;43:227–238.

202 Rücker FG, Bullinger L, Schwaenen C, et al: Disclosure of candidate genes in acute myeloid leukemia with complex karyotypes using microarray-based molecular characterization. J Clin Oncol 2006;24:3887–3894.

203 Marcucci G, Maharry K, Radmacher MD: Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. J Clin Oncol 2008;26:5078–5087.

204 Wouters BJ, Jordà MA, Keeshan K, et al: Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood 2007;110:3706–3714.

205 Neben K, Schnittger S, Brors B: Distinct gene expression patterns associated with FLT3- and NRAS-activating mutations in acute myeloid leukemia with normal karyotype. Oncogene 2005;24:1580–1588.

206 Langer C, Radmacher MD, Ruppert AS, et al; Cancer and Leukemia Group B (CALGB): High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a Cancer and Leukemia Group B (CALGB) study. Blood 2008;111:5371–5379.

207 Langer C, Marcucci G, Holland KB, et al: Prognostic importance of MN1 transcript levels, and biologic insights from MN1-associated gene and microRNA expression signatures in cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol 2009;27:3198–3204.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM

208 Radmacher MD, Marcucci G, Ruppert AS, et al; Cancer and Leukemia Group B: Independent confirmation of a prognostic gene expression signature in adult acute myeloid leukemia with a normal karyotype: a Cancer and Leukemia Group B study. Blood 2006; 108:1677–1683.
209 Mi S, Lu J, Sun M, et al: RNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci USA 2007;104: 19971–19976.
210 Mi S, Li Z, Chen P, et al: Aberrant overexpression and function of the miR-17–92 cluster in MLL-rearranged acute leukemia. Proc Natl Acad Sci USA 2010;107:3710–3715.
211 Li Z, Lu J, Sun M, et al: Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. Proc Natl Acad Sci USA 2008;105:15535–15540.
212 Dixon-McIver A, East P, Mein CA, et al: Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. PLoS One 2008;14;3:e2141.
213 O'Connell RM, Chaudhuri AA, Rao DS, et al: Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci USA 2009;106:7113–7118.
214 Garzon R, Volinia S, Liu CG, et al.: MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood 2008;111:3183–3189.
215 Marcucci G, Radmacher MD, Maharry K, et al.: MicroRNA expression in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1919–1928.

Downloaded by:
41.235.68.206 - 2/6/2015 9:36:54 AM