Leukemia (2008) 22, 915–931

www.nature.com/leu

REVIEW

Cooperating gene mutations in acute myeloid leukemia: a review of the literature

A Renneville[1,4], C Roumier[1,4], V Biggio[1], O Nibourel[1], N Boissel[2], P Fenaux[3] and C Preudhomme[1]

[1]Laboratoire d'Hématologie A, INSERM U837-Institut de Recherche sur le Cancer, CHU of Lille, North-West Canceropole, France; [2]Service d'Hématologie Adulte, Hôpital Saint-Louis, Paris, France and [3]Service d'Hématologie Clinique, Hôpital Avicenne, Bobigny, France

Acute myeloid leukemia (AML) is a heterogeneous group of neoplastic disorders with great variability in clinical course and response to therapy, as well as in the genetic and molecular basis of the pathology. Major advances in the understanding of leukemogenesis have been made by the characterization and the study of acquired cytogenetic abnormalities, particularly reciprocal translocations observed in AML. Besides these major cytogenetic abnormalities, gene mutations also constitute key events in AML pathogenesis. In this review, we describe the contribution of known gene mutations to the understanding of AML pathogenesis and their clinical significance. To gain more insight in this understanding, we clustered these alterations in three groups: (1) mutations affecting genes that contribute to cell proliferation (*FLT3, c-KIT, RAS, protein tyrosine standard phosphatase nonreceptor 11*); (2) mutations affecting genes involved in myeloid differentiation (*AML1* and *CEBPA*) and (3) mutations affecting genes implicated in cell cycle regulation or apoptosis (*P53, NPM1*). This nonexhaustive review aims to show how gene mutations interact with each other, how they contribute to refine prognosis and how they can be useful for risk-adapted therapeutic management of AML patients.

Leukemia (2008) **22,** 915–931; doi:10.1038/leu.2008.19; published online 21 February 2008
Keywords: acute myeloid leukaemia; mutations; proliferation; differentiation; prognosis; review

Introduction

Acute myeloid leukemia (AML) is a phenotypically and genetically heterogeneous but aggressive disease. Major progress has been made to understand mechanisms of leukemogenesis in AML. One of the most relevant hypothesis is the implication of a primitive hematopoietic stem cell or progenitor cell capable of transformation in a so-called leukemic stem cell (LSC), which keeps the ability to self-renewal. This LSC comes from the accumulation of genomic alterations affecting proliferation, cell death and the perturbation of genes involved in the carefully regulated pathways of hematopoietic differentiation. These key oncogenic events are often divided into two classes according to the two-hit model hypothesis of leukemogenesis, in which class I mutations (such as FLT3-ITD) confer a proliferation or survival advantage to blast cells, while class II mutations block myeloid differentiation and give self-renewability.[1] Such events have now been observed in different types of AML. Differentiation blocks can occur by alteration at specific stages, like mutations of *CEBPA* gene or of the core-binding factor (CBF) complex genes, including *RUNX1*, which results in different subtypes of AML.

The two-hit model of leukemogenesis, combining an activating lesion of tyrosine kinase pathways with an event blocking myeloid differentiation, appears very interesting not only to modelize *in vitro* leukemogenesis, but also to screen molecular events in AML patients, as observed by the frequent association of an FLT3-ITD mutation with a fusion transcript like *AML1-ETO* or *PML-RARA*.[1] Over the past years, besides deregulation of myeloid differentiation resulting from the expression of chimeric proteins, numerous gene mutations have been reported in different types of AML.

In this article, we reviewed, in AML, mutations of genes that contribute to cell proliferation by alteration of tyrosine kinase signaling (*FLT3*, *c-KIT*, *RAS*…) or tyrosine phosphatase (*PTPN11*) pathways, mutations of genes involved in myeloid differentiation (*AML1*, *CEBPA*) and in the regulation of cell cycle and apoptosis (*NPM1*, *P53*).

Gene mutations inducing a proliferative advantage

FLT3

The FMS-like tyrosine kinase 3 (*FLT3*) gene, located on chromosome 13q12, encodes a membrane-bound receptor tyrosine kinase (RTK) that belongs to the RTK subclass III family, characterized by five immunoglobulin-like extracellular domains, a single transmembrane domain, a juxtamembrane domain (JMD) and an intracellular domain consisting of two protein tyrosine kinase (PTK) domains linked by a kinase-insert domain. Other members of the RTK subclass III include macrophage colony-stimulating factor (M-CSF) receptor, c-KIT and the receptors for platelet-derived growth factors A and B (PDGFRA and PDGFRB).[2] FLT3 is normally expressed by myeloid and lymphoid progenitor cells and expression is lost as hematopoietic cells differentiate. FLT3 plays an important role in the proliferation, differentiation and survival of multipotent stem cells[3] and is overexpressed at the RNA and protein level in AML blasts.[4] FLT3 is one of the most frequent somatic alterations in AML, found in 25–45% of all AML patients. *FLT3* mutations consist of two major types. The most common type is an intern tandem duplication (ITD) in exons 14 and 15 (previously known as exons 11 and 12) that map to the JMD, seen in 25–35% of adult and 12% of childhood AML.[5–16] These ITD, and deletion or insertion/deletion mutations affecting the JMD, are sometimes called length mutations (LM) in the literature. Here, we use the term ITD to refer to all JMD mutations.[13–16] The length of the duplicated JMD region varies from 3 to 400 nucleotides but, despite this heterogeneity, the resultant transcripts are always in-frame.[17] The second most

Correspondence: Professor C Preudhomme, Unité INSERM 837 and Laboratoire d'Hématologie A, Centre de Biologie-Pathologie, CHU of Lille, Boulevard du Pr Leclercq, Nord, Lille 59037, France.
E-mail: cpreudhomme@chru-lille.fr
[4]These authors contributed equally to this work.
Received 25 May 2007; revised 5 January 2008; accepted 18 January 2008; published online 21 February 2008

common type of *FLT3* mutation is a missense point mutation in exon 20, within the activation loop (AL) of the tyrosine kinase domain (TKD), found in 5–10% of AML patients. This mutation typically involves codons D835 and/or I836, and less frequently N841 or Y842.[18] Several point mutations in the JMD have also recently been described in some AML cases. Their prevalence seems to be lower than 1% in all AML subtypes and has not yet been established in cytogenetically normal AML (CN-AML).[19,20] Lastly, a novel gain-of-function mutation (K663Q), located not in the AL but in the N-terminal part of TKD, has been recently identified in AML.[21] Interestingly, a recent study stressed the importance of functional studies, since among the different *FLT3* mutations reported outside the JMD and the TKD, some were not associated with kinase activation, and did not contribute to leukemogenesis.[22] Most affected AML patients have only one type of *FLT3* mutation, but some patients have both an ITD and TKD of *FLT3*.

FLT3 mutations lead to constitutive phosphorylation of the receptor in the absence of FLT3 ligand and consecutive activation of the downstream signaling pathways such as phosphatidylinositol 3 kinase/AKT, Ras/mitogen-activated protein kinase (MAPK) and Janus kinase 2 (JAK2)/STAT5.[20,23,24] However, there is increasing evidence suggesting that the downstream cellular responses to FLT3-ITD and FLT3-TKD mutation are not equivalent. In murine bone marrow transplantation models as well as in transgenic mice, FLT3-ITD induces a myeloproliferative disease that closely resembles human chronic myelomonocytic leukemia, and FLT3-TKD an oligoclonal lymphoid disorder with longest latency, but neither FLT3-ITD nor FLT3-TDK mutations induce AML.[23–25] These findings support the idea that *FLT3* alterations are not sufficient to cause AML. Furthermore, the fact that *FLT3* mutations can be acquired or lost at relapse or during disease progression is in accordance with the hypothesis that *FLT3* mutations are secondary events in leukemogenesis.[3,26]

The frequency of FLT3-ITD mutations varies dramatically across cytogenetically and molecularly defined subsets of AML. FLT3-ITD has a particularly high incidence in CN-AML and t(15;17)-positive AML (30–35% of cases in each group), and in AML with t(6;9).[7,27–29] FLT3-ITD-positive patients are more often diagnosed with *de novo* than secondary AML.[7,8] Both FLT3-ITD and -TKD mutations have been strongly associated with high white blood cell (WBC) count and high bone marrow blast percentages at presentation.[5,7,9,10,30] A positive correlation between *MLL* (mixed lineage leukemia) intragenic abnormalities (MLL partial tandem duplication (MLL-PTD) or DNA double-strand breakage within the *MLL* breakpoint cluster region induced by topoisomerase II inhibitors) and *FLT3* mutations (ITD and TKD) has been reported.[31] In contrast, there is an almost mutually exclusive occurrence of *FLT3* and *RAS* mutations in AML samples.[32,33]

Many studies have shown that FLT3-ITD has a negative impact on outcome in both adult and pediatric AML patients. FLT3-ITD confers a poor prognosis due to an increased relapse rate, reduced disease-free survival (DFS) and overall survival (OS), while the rate of complete remission (CR) is not significantly affected.[34] Several studies restricted to CN-AML have demonstrated that patients harboring at least one allele with FLT3-ITD had a significantly shorter CR duration (CRD), and OS than patients without FLT3-ITD. Moreover, FLT3-ITD has been found to be an independent prognostic factor for OS, and CRD in CN-AML.[5,7,35] However, in other cytogenetic subsets including t(15;17) AML, FLT3-ITD appears not to be an independent prognostic factor.[36,37]

The molecular characteristics of FLT3-ITD are extremely variable among patients. FLT3-ITDs are found in the heterozygous state, but they are sometimes found in a homozygous state after loss of the normal *FLT3* allele. A particularly bad prognostic subgroup consists of patients who have lost the wild-type (WT) allele of *FLT3*.[5] Among intermediate-risk AML, patients with low allelic ratio quantified by Genescan analysis (mutated/WT *FLT3* < 0.8) appear to have survival comparable to AML patients without FLT3-ITD mutations. The absence of *FLT3* WT allele likely results from acquired uniparental disomy, which could be either partial, due to somatic recombination between homologous chromosomes occurring after FLT3-ITD had been generated, or could involve loss of the entire chromosome 13 with the WT allele and subsequent duplication of the remaining homologous chromosome 13 with FLT3-ITD.[38,39] Another explanation to the variation of allelic ratios lies in the fact that FLT3-ITD may occur only in a subclone of malignant cells and not in LSC. Differences in the size of the duplicated insert may also explain some of the prognostic variability of FLT3-ITDs. A recent study found that patients with larger duplicated segment (over 40 nucleotides) had worse DFS and OS than patients with smaller ITDs.[40] However, this finding was not confirmed by others[41,42] and needs to be tested on larger cohorts of patients.

In contrast to FLT3-ITD, the prognostic impact of TKD point mutations of *FLT3* is still controversial.[7,30,34,43] According to a recent study, the impact of FLT3-TKD mutations on OS when including all mutant-positive patients was not significant, but patients with high-level mutations (more than 25% mutant) had significantly improved outcome.[30] Further studies are however necessary to determine the clinical relevance of FLT3-TKD, especially in relation with other molecular prognostic factors.

FLT3 mutational status may change between diagnosis and relapse, with about 9% (4–27%) of patients losing their FLT3-ITD mutation at relapse, whereas approximately 6% acquire FLT3-ITD mutation on this occasion. Moreover, in about 6% of FLT3-ITD-positive patients, the length or number of FLT3-ITD change at relapse.[26,44–46] The situation is very different for FLT3-TDK mutations, which are lost at relapse in more than 50% of cases.[47] This again strongly suggests that *FLT3* alterations are secondary genetic events, occurring in a subclone and not in the LSC. In addition to be a prognostic marker, FLT3-ITD is a potential target for minimal residual disease (MRD) monitoring and for the development of novel treatments. However, the use of FLT3-ITD as MRD marker is limited by its relative instability during follow-up, as discussed later in this review.

c-KIT

The *Kit* proto-oncogene, located on chromosome band 4q12, encodes a transmembrane glycoprotein, which is a member of the type III RTK family, and whose ligand is stem cell factor (SCF).[48,49] SCF binding promotes c-KIT dimerization and transphosphorylation that leads to activation of downstream signaling pathways involved in proliferation, differentiation, migration and survival, particularly of hematopoietic stem cells. Ligand-independent activation of c-KIT can be caused by different types of mutations that have been reported in AML and also in other human malignancies (including mast cell disorders, gastrointestinal stromal tumors, and testicular germ cell tumors).[50] Gain-of-function mutations may affect either the extracellular portion of c-KIT receptor, believed to play a role in the dimerization (in-frame insertion/deletions of exon 8 that all result in loss of the acid aspartic residue at amino acid 419), or the JMD (ITD of exon 11), or the structure of the AL in the TKD,

such as the substitution of a single amino acid in exon 17 (mostly mutation D816V, and less commonly other mutations affecting codon 816 (D816Y/H/F/I), 821, 822 or 823).[51–54] Codon 816 *c-KIT* mutations were shown to induce constitutive activation of PI3K and downstream of PI3K, Jnk1 and Jnk2, as well as STAT3 and upregulation of STAT3 downstream targets, such as BLCXL and MYC.[55,56] *c-KIT* mutations have been identified with a particularly high incidence in specific cytogenetic subsets of AML: CBF AML and AML with trisomy 4 (as either the sole cytogenetic aberration or associated with t(8;21)), and are generally not observed in other AML subtypes, such as AML with t(15;17) or with complex karyotype. Likewise, *c-KIT* mutations are not equally distributed among FAB subtypes and are mainly found in M1, M4, M4eo and particularly in M2, since nearly 70% of *c-KIT*-mutated AML patients are classified as M2.[51–53,57–59] The overall frequency of *c-KIT* D816 and exon 8 mutations in AML is approximately 2% (33/1940)[58] and 6–8%,[51–59] respectively. In contrast, *c-KIT* D816 and exon 8 mutations have been reported with an average incidence of 16 and 12% among AML patients with t(8;21), and 13 and 22% among AML patients with inv(16), respectively.[51–53,57–63] Recently, a complex *c-KIT* ITD involving exon 11 and exon 12 was identified in 4/60 (7%) of childhood AML.[64] The incidence of *c-KIT* D816 mutations was found not to significantly differ between *de novo* AML, AML secondary to myelodysplactic syndromes (MDS) and therapy-related AML (t-AML).[58] In AML with t(8;21), the presence of *c-KIT* mutations is associated with a higher WBC count at diagnosis.[60,61] Some authors described a negative association between *c-KIT* and *FLT3* mutations, but both alterations are not mutually exclusive.[58,60] Functional redundancy is a potential reason for the fact that mutations of these two RTK are rarely found to co-exist in the same patient. This supports the findings that activating mutations of *c-KIT* and *FLT3* are associated with distinct cytogenetic AML subgroups, respectively CBF AML and AML with normal karyotype and t(15;17).[53,58–60,62] In CBF AML, *c-KIT* D816 and exon 8 mutations appear to predict a higher incidence of relapse.[52,58,60–63] In some studies, *c-KIT* exon 17 mutations were also significantly associated with shorter CRD and OS in adult and pediatric AML with t(8;21).[58,60–63] According to Paschka *et al.*,[63] both exon 17 and exon 8 mutations appear to adversely affect OS in AML with inv(16). Overall, these data indicate that *c-KIT* mutations have a significant negative impact on the outcome of CBF AML, especially D816 mutation in AML with t(8;21). Detection of *c-KIT* mutations in CBF AML is important not only for outcome prediction, but also for therapeutic implications since *c-KIT*-activating mutations may represent a target for tyrosine kinase inhibitors (TKI).

RAS

RAS oncogenes encode a family of guanine nucleotide-binding proteins that regulate signal transduction on binding to a variety of membrane receptors, including c-KIT and FLT3, and play an important role in proliferation, differentiation and apoptosis processes. RAS proteins normally exist in an equilibrium between active (GTP-bound) and inactive (GDP-bound) states.[65,66] There are three functional *RAS* genes: N-(from a neuroblastoma cell line), K-(Kirsten) and H-(Harvey) *RAS*, each containing four exons. Mutations in *N-* or *K-RAS* occur nearly exclusively by one base change in codons 12, 13 or 61, which abrogate intrinsic RAS GTPase activity and thus confer constitutive activation of RAS proteins and downstream effectors, such as RAF and MAPK/ERK kinases.[67]

Point mutations in *N-* and *K-RAS* genes are found in approximately 10–15 and 5% of all AML patients, respectively. Codons most frequently mutated are *N-RAS* codon 12 (43%), mostly resulting in changes from glycine to asparagine, *N-RAS* codon 13 (21%) and *K-RAS* codon 12 (21%).[68,69] All of the mutated patients were found heterozygous for the mutation. In MDS studies, *RAS* mutations have been found to be significantly associated to progression from MDS to AML.[70,71] In AML, *RAS* mutations frequency does not seem to vary significantly with age, sex, initial leukocytosis, WHO (World Health Organization) performance status and *de novo* versus secondary or t-AML.[68,69,72,73] The subgroup of AML with inv(16)/t(16;16) and inv(3)/t(3;3) shows a significantly higher frequency of *RAS* mutations than in other groups, approximately 35 and 27%, respectively, with *N-RAS* codon 61 being significantly more frequently involved than in other patients.[59,60,68,69] By contrast, *RAS* mutations are significantly less common in AML with t(15;17) (2%) and with complex aberrant karyotypes (1.5%). *RAS* mutations are relatively overrepresented in FAB type M4eo/inv(16),[68,69] and, in some studies, in M4 AML.[59,74] *RAS* mutations and FLT3-ITD were found to co-exist in only 16 of 815 (2%) patients analyzed for the two mutations, indicating a significant negative association between them.[32,68] The rarity of *RAS* mutations in *FLT3* mutants, and *vice versa*, is in line with the two-hit model of AML pathogenesis. Contrary to MDS studies,[70,71] most large studies found no significant prognostic impact of *RAS* mutations for OS, CR rate and CRD in AML.[32,59,60,68,69,73–75] In 107 patients for which *N-RAS* mutations status was studied both at diagnosis and at relapse, findings were similar in 102/107 (95%) patients at diagnosis and at relapse. However, one patient gained a mutation at relapse whereas four out of eight *RAS*-mutated patients had no more mutation at relapse, indicating that *N-RAS* mutations are not good markers for follow-up control of residual disease after treatment[69] and arguing against a role for *RAS* mutations as primary leukemogenic events.

PTPN11

The *PTPN11* gene (protein tyrosine standard phosphatase nonreceptor 11), located on chromosome 12q24, encodes a cytoplasmic protein tyrosine phosphatase (PTP) called SHP-2. The SHP-2 protein has three functional domains: two tandem SH2 (Src homology 2) domains at the N-terminal (N-SH2 and C-SH2) that both positively regulate SHP-2 activity and a single catalytic (PTP) domain at the C terminus. The N-SH2 domain basically interacts with the PTP domain and blocks the catalytic site. After binding the phosphotyrosyl substrate, the N-SH2 domain undergoes a conformational change that weakens the autoinhibiting intramolecular interaction, making the catalytic site available to substrate, thereby activating the phosphatase. The substrates upon which SHP-2 acts are not known with certainty.

SHP-2 participates in signal transduction downstream of growth factor, cytokines, hormones and cell adhesion molecules. SHP-2 is highly expressed in hematopoietic cells and is implicated in the response to KIT-ligand, interleukin-3 (IL-3), IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF) and EPO. The regulatory function of SHP-2 is complex, but, in most circumstances, it plays a positive role in transducing signals, which is mediated, at least in part, through the Ras/MAP kinase cascade.[76]

Germ-line mutations in *PTPN11* are seen in more than 50% of cases of Noonan's syndrome, an autosomal dominant disorder characterized by facial dysmorphy, skeletal malformations and a

wide spectrum of heart defects.[77] Somatic mutations in *PTPN11* are found in about 35% of juvenile myelomonocytic leukemia (JMML) cases. JMML patients without *PTPN11* mutations have either *RAS* mutations or homozygous neurofibromatosis-type 1 gene deletion or inactivation, illustrating the important role of activated RAS in this disease.[76] With the exception of two trinucleotide deletions described in patients with Noonan syndrome, all the *PTPN11* mutations reported so far have been missense substitutions that result in gain-of-function of SHP-2 catalytic activity. The vast majority of mutations cluster in the interacting portions of the N-SH2 domain and the PTP domains involved in switching the protein between its inactive and active conformations. Like in other myeloid malignancies, >80% of the mutations identified in AML are localized in exon 3, with lesions altering codons 72 and 76 accounting together for nearly 50% of all mutations reported in AML.[76] The role of SHP-2 in AML pathogenesis remains to be fully determined. However, genetic evidence supports the hypothesis that *PTPN11* mutations contribute to leukemia growth by deregulating the RAS pathway. The existing data do not permit to determine if somatic SHP-2 mutations can initiate AML or represent a secondary event, acquired during disease evolution.[78]

Approximately 4.4% (31/702) of pediatric AML cases show *PTPN11* mutations.[79–81] The prevalence of *PTPN11* mutations among adult AML patients seems to be lower since only 9 (2.6%) mutations were observed in nearly 340 adult patients with secondary or *de novo* AML.[76,82–84] In pediatric AML, the probability of achieving CR, CRD and OS was found similar in patients with and without *PTPN11* mutations, but the power to detect significant differences was diminished by the low frequency of mutations.[79,81] The prognostic impact of *PTPN11* mutations in adult AML has not been evaluated. One report found that *PTPN11* mutations occurred preferentially in AML with FAB M5 morphology (12 versus 1.5% in non-FAB M5),[76] but this finding was not confirmed by Goemans *et al*.[80] Within the FAB-M5 group, no significant association with any clinical variable was found. Similarly, the presence of *PTPN11* mutations was not related to any specific-gene rearrangement or cytogenetic risk group.[79] In 7/32 (22%) pediatric AML patients with *PTPN11* mutations, *RAS* and/or *FLT3* lesions were found to co-exist.[79–81]

Other mutated genes

JAK2 is a cytoplasmic tyrosine kinase involved in signaling pathways by members of the single chain receptors (for example, EPOR, TPOR), the IL-3 receptor family (IL-3R, IL-5R and GM-CSF-R), the Gp130 receptor family and the class II receptor cytokine family. JAK2 plays a key role in signal transduction initiated by multiple growth factor-receptors and cytokines required in hematopoiesis.[85,86] The recurrent JAK2V617F mutation causes replacement of a key valine residue by phenylalanine in the pseudo-kinase domain JH2 that has an inhibitory role on the kinase domain JH1. The mutant enzyme had enhanced kinase activity and leads to hyperactivation in downstream signaling components, STAT5, PI3K/Akt, ERKs. JAK2V617F mutation is present in more than 90% of polycythemia vera (PV) cases, in approximately 50% of essential thrombocythemia (ET) and myelofibrosis with myeloid metaplasia (MMM) cases and in about 70% of patients with AML following myeloproliferative disorders. By contrast, JAK2V617F mutation is a rare event in *de novo* AML patients reported in only 1.6% (25/1546) of analysed cases.[87–97] JAK2V617F mutations seem to cluster in AML with karyotypic aberrations. An incidence of 3.6% was found in CBF AML.[97] Recent data suggest that JAK2V617F mutation may cooperate as class I mutation in leukemogenesis with t(8;21)/*AML1-ETO*, especially in therapy-related t(8;21)/*AML1-ETO*-positive AML.[98] Besides, one case of *de novo* AML with a JAK2K607N mutation has been described.[94] Two other *JAK2*-activating mutations, JAK2T875N and JAK2A572V, have been identified in megakaryoblastic leukemia cell lines.[99] *JAK2* mutations affecting exon 12 have recently been discovered in patients with JAK2V617F-negative PV or idiopathic erythrocytosis, but the existence of this type of mutation in AML has not been evaluated.[100]

Recently, gain-of-function mutations in the transmembrane domain of the thrombopoietin receptor (*MPL*), MPLW515L and MPLW515K, have been described in approximately 5% of MMM, 1% of ET and 2% (3/126) of AML cases.[96,101] Among AML patients, the 3 *MPL* mutants reported to date had AML following MMM. Expression of MPL515 mutant allele confers cytokine-independent growth and thrombopoietin hypersensitivity, and results in constitutive phosphorylation of JAK2, STAT3, STAT5, AKT, and ERK. *MPL* mutations may occur concurrently with the JAK2V617F mutation, suggesting that these mutations may have functional complementation in myeloid disorders.[96,101]

Inactivating mutations of c-CBL and CBL-b ubiquitin ligases, which are important regulators of RTK signaling, have recently been reported in AML. In particular, these mutations can upregulate the expression of FLT3 protein, suggesting a novel mechanism of FLT3 deregulation and leukemic transformation in AML. The prevalence and clinical significance of these mutations in AML remains to be determined.[102,103]

Mutations that impair myeloid differentiation

AML1

The human *AML1* gene (also named CBFA2 or RUNX1), located in 21q22 chromosomal band, encodes for one of the two subunits forming the human CBF.[104–106] Expression of RUNX1 is controlled by two distinct promoters P1 and P2. During mouse embryogenesis the spatio-temporal expression of *RUNX1* is developmentally regulated by alternative use of the two mutually distinct promoters, P1 and P2. These promoters, differentially regulated, allow the transcription of mRNAs with variant 5′-UTRs and the translation of structurally different protein isoforms showing the non-redundant function of P1 and P2.[107] AML1 protein contains, in its N-terminal part, a highly evolutionary conserved domain of 128 amino acids called RUNT domain, responsible for both heterodimerization with the β subunit of CBF and for DNA binding on the PEBP2β sequence and a transactivation domain in C-terminal part.[108–110] *AML1* encodes three alternative spliced forms (AML1a, AMl1b and AML1c) which differ mainly in the C-terminal domain.[111] These three isoforms have different and sometimes antagonist cellular functions. For example, AML1b can release myeloid cells from the AML1a-mediated differentiation block.[112] AML1a can potentiate stem or progenitor cell engraftment, whereas AML1b has opposite effects. The expression of each isoform appears to be time dependent during embryogenesis but also in adult hematopoiesis.[113] AML1 is normally expressed in all hematopoietic lineages and acts to regulate the expression of various genes specific to hematopoiesis, including *M-CSF* receptor, *IL-3*, myeloperoxydase and *TCRβ* genes.[114–117] Mice lacking *AML1* or *CBFβ* have no fetal liver hematopoiesis, showing that the heterodimeric complex CBF is essential for definitive hematopoiesis of all lineages.[118–121]

AML1 is one of the genes most frequently deregulated in leukemia mainly through chromosomal translocations, point mutations and amplifications. Chromosomal translocations involving *AML1* gene occur in different leukemia subtypes, leading to the formation of fusion genes encoding for chimeric proteins, including AML1-ETO (t(8;21)) in AML, AML1-ETV6 (t(12;21)) in childhood acute lymphoblastic leukemia and less often AML1-MDS1 (t(3;21)) in MDS and blastic phase of chronic myeloid leukemia (CML), or other rare translocations.[122,123] Other mechanisms of *AML1* inactivation have been reported in hematological malignancies, through point mutations, in AML and in MDS.[124–129] Both germ-line and acquired *AML1* mutations have been reported in hematological malignancies. Germ-line mutations of the *AML1* gene have initially been reported by Song *et al*. in very rare cases of familial platelet disorder with predisposition to AML (FPD/AML).[130] Affected FPD/AML individuals had thrombocytopenia with platelet functional defects. In all cases, mutations were monoallelic and resulted in haploinsufficiency. Thus, loss of one *AML1* allele appears sufficient for propensity to develop leukemia. Consistent with this observation, mouse embryos with hemizygosity for the mutant *AML1* allele have an increased number of hematopoietic stem cells associated to a decrease in committed progenitors, suggesting that haploinsufficiency of *AML1* affects maturation and proliferation of hematopoietic cells.[131]

On the other hand, acquired *AML1* mutations are observed in 6–10% of sporadic AML and are found at similar frequency in adult and pediatric AML. Mutations are equally distributed between missense and nonsense mutations (resulting in truncated protein) in *de novo* AML. They occur mainly in the RUNT domain, located in the N-terminal part of the protein, and impair normal AML1 functions through the loss of transactivation and/or heterodimerization property. C-terminal mutations are seen at lower frequency and almost exclusively in MDS or AML following MDS, and seem to be associated with poor prognosis.[132] A high frequency (40%) of N-terminal *AML1* mutation has also been observed in t-AML and t-MDS.[132] Some monoallelic mutations may act in a dominant-negative manner through inhibition of the effects the remaining WT AML1. This hypothesis was confirmed by Osato *et al*. and Imai *et al*.,[124,126] who demonstrated that some *AML1* gene missense mutation co-transfected with WT *AML1* abolished transactivation of the M-CSF receptor promoter. Moreover, Imai *et al*.[126] demonstrated that this dominant-negative effect was due to the fact that the mutated protein did not bind DNA but had enhanced capacity to bind CBFβ and, by sequestering it, inhibited action of WT AML1, as observed for chimeric products of t(3;21) or t(8;21) translocations. *AML1* point mutations predominate in M0 FAB subtype, where they are seen in 15–50% of cases and often biallelic mutations, inducing a total loss of function of *AML1* gene.[125,128] Acquired *AML1* mutations observed in other FAB subtypes are often monoallelic and often associated with acquired trisomy 21 or other cytogenetic abnormalities, such as trisomy 13, or with *FLT3*, *P53* or *N-RAS* mutations.[133,134] Interestingly, in patients with constitutional trisomy 21 (that is, Down syndrome), who have a propensity to develop AML, no *AML1* mutations have been found. Copy number variation of the *AML1* gene could be presompted to participate to leukemogenesis both by increase or defect. Perhaps, gene dosage plays a role on P1 or P2 promoters recruitment or on the isoforms splicing. Those findings suggest that monoallelic mutations (except dominant-negative ones) are probably not sufficient to induce leukemia and that a second event, such as cytogenetic abnormalities or class I mutations affecting *FLT3* or *RAS* genes is necessary.

CEBPA

The *CEBPA* gene, located on chromosome 19q13.1 band, belongs to the CCAAT/enhancer-binding protein family involved in the balance between cell proliferation and terminal differentiation. The *CEBPA* gene encodes a transcription factor playing a crucial role during differentiation of various cell types including hematopoietic cells. In hematopoiesis, CEBPA plays a pivotal role in early stages of myeloid differentiation and is particularly expressed in myelomonocytic cells.[135–137] CEBPA acts in multiple ways: by down regulation of c-MYC expression allowing differentiation, by upregulation of the expression of granulocytic lineage-specific genes, and by synergistic action with other key genes involved in myeloid development, including CBF complex genes or PU.1.[138–140] In addition to specific DNA binding, CEBPA could act by protein–protein interaction. The main partners of these interactions are p21, CDK2, CDK4 and E2F. CEBPA protein inhibits cell proliferation by activating transcription of p21/waf1, by stabilizating p21, by inhibiting CDK2 and CDK4.[141–143] The CEBPA protein is a 42-kDa protein with four main domains: in the C-terminal part are a leucine zipper (bZIP) domain mediating homo- or heterodimerization and the DNA-binding domain, a basic positively charged domain able to interact with specific DNA sequences; in the N-terminal part of the protein are located two regulatory and transactivating domains, TAD1 and TAD2. The expression of *CEBPA* begins with the commitment of the myeloid lineage precursors and is upregulated during granulocytic differentiation. *CEBPA*-defective mice have no mature granulocytes, whereas cells of the other lineages are not affected.[144] In addition, CEBPA expression could block monocytic differentiation.[145]

The implication of CEBPA in leukemogenesis has now been confirmed by many experiments. Pabst *et al*. found that events leading to the loss of CEBPA function facilitate leukemogenesis by blocking granulocytic differentiation in AML.[146–149] Three mechanisms of *CEBPA* inactivation have been reported: (1) downregulation of *CEBPA* expression consecutive to the *AML1-ETO* fusion transcript in t(8,21) leukemia cells, interestingly conditional expression of *CEBPA* in these cells is sufficient to trigger granulocytic differentiation;[150] recently, Wouters *et al*. reported that the *CEBPA* gene could also be silenced by promoter hypermethylation in a specific subtype of AML that phenotypically showed aberrant expression of T-cell genes such as CD7 or enhanced expression of TRIB2, suggesting aberrantly activated NOTCH signaling.[151] (2) Suppression of CEBPA function through inhibition of the translation of CEBPA mRNA by interaction with hnRNPE2, which is induced by BCR-ABL;[152] this mechanism could explain transition of chronic phase to myeloid blast crisis in CML by blocking myeloid differentiation and (3) other genetic abnormalities affecting *CEBPA* gene, particularly mutations, which have been reported in hematological malignancies, mainly in AML.[153]

Acquired point mutations of the *CEBPA* gene have been reported in 89 of 965 (9%) AML patients in 7 studies including 12 M0-AML, 159 M1-AML, 203 M2-AML, 44 M3-AML, 101 M4-AML, 98 M5-AML, 8 M6-AML, 4 M7-AML and 15 secondary AML and 321 AML for which FAB or WHO classification was not mentioned.[146,153–160] *CEBPA* mutations can be divided into two main groups: N-terminal mutations that lead to increased translation of the alternative 30-kDa form with dominant-negative activity on the full-length 42-kDa protein; and C-terminal mutations that result in deficient DNA-binding and/or homodimerization activities. Some patients present a single *CEBPA* mutation, while some others have multiple alterations resulting from biallelic mutations or from several mutations on the same allele.[153,155,159]

In those studies, *CEBPA* gene mutations were frequent in M1, M2 and M4-AML (respectively, 19, 16 and 11% of mutated cases). The strong correlation between M1 and M2 FAB subtypes and *CEBPA* mutations (77% of AML with *CEBPA* mutation belong to these subtypes) supports the critical role of *CEBPA* gene in the intermediate stages of granulocytic differentiation. Patients with *CEBPA* mutations have distinct immunophenotypic features with a significantly higher frequency of the co-expression of CD7, CD34, HLA-DR and CD15.[160] All but one of the reported patients with *CEBPA* mutations belonged to the 'intermediate' subgroup of the MRC cytogenetic prognostic classification, whereas no mutation was observed in the 'favorable' prognostic subgroup (that is, with translocation involving CBF genes or t(15;17)) and only one in the 'adverse' prognostic subgroup (complex karyotype: more than three abnormalities, del(5q)/-5, -7, 3q abnormalities), CBF AML were never associated with *CEBPA* mutations, showing that the above described mechanisms (1) and (3) that block differentiation of neutrophil granulocytes through CEBPA loss of function are mutually exclusive.

Four studies totaling 71 mutated cases versus 670 unmutated patients have evaluated the correlation between *CEBPA* mutations and other disease characteristics and prognosis.[153,154,157–160] No correlation was observed between *CEBPA* mutations and age, sex, WBC count, circulating blast count and platelet count. In the four studies, the CR rate was not significantly different in patients with or without *CEBPA* mutations. Nevertheless, in all but one of these studies, a correlation was found between *CEBPA* mutations and longer CRD or longer OS, reinforcing the association between event inducing loss of CEBPA function and favorable response to treatment.[153,155,157,159] Clinical data strongly support very favorable results with chemotherapy regimens containing high-dose AraC in AML patients with CEBPA loss of function. Analysis of cooperating mutations showed no difference in the incidence of FLT3 or MLL alterations between *CEBPA*-mutated and *CEBPA* nonmutated patients.[157,159] To note, germ-line mutation of *CEBPA* gene have been described in 10 cases of familial AML corresponding to 3 different families.[161–163] All members affected by AML had a M1 or M2 FAB subtypes, as frequently observed in patients with somatic *CEBPA* mutations. Germ-line *CEBPA* mutations appear to be fully penetrant, since all mutated patients developed AML, and cause autosomal dominant inheritance of predisposition to AML.[162] Within the three families reported, all members affected with AML harbored a germ-line *CEBPA* mutation that prevents the expression of the 42-kDa protein and results in truncated isoform with dominant-negative activity. At time of diagnosis, somatic C-terminal mutations of *CEBPA* gene were observed in the blast cells of the 10 patients, whereas 1 of the 5 remaining patients had an acquired trisomy 21.[161–163] This observation supports that germ-line mutations in *CEBPA* predispose to AML and indicates that additional acquired *CEBPA* somatic mutations contribute to CEBPA loss of function, perhaps as a second event in the leukemogenesis.

Other mutated genes

Wilms' tumor 1 (*WT1*) gene, located at chromosome band 11p13, encodes a zinc-finger DNA-binding protein. Depending on the cellular context, it can be involved in transcriptional activation or repression. WT1 has a functional duality since it may act either as a tumor suppressor gene or as an oncogene. The precise role of WT1 in hematopoiesis and its contribution to leukemogenesis are still uncertain, although it has been suggested that impairment of WT1 protein function could promote stem cell proliferation and induce a differentiation block of hematopoietic cells.[164,165] *WT1* gene is highly expressed in various types of leukemias, particularly in AML, and therefore can be used for MRD monitoring in AML patients. Mutations in *WT1* gene in AML were first reported in 1998 by King-Underwood *et al*.[166] in about 10–15% of AML cases. A recent study restricted to CN-AML confirmed this incidence and the association of *WT1* mutations with failure to achieve CR with standard chemotherapy, in agreement with the results of the initial study.[167] However, the poor prognostic impact of *WT1* mutations needs to be evaluated in larger cohorts of AML patients.

PU.1 is a hematopoietic-specific Ets-family transcription factor required for normal blood cell development. It regulates a large number of target genes, including crucial myeloid genes, such as the granulocyte colony-stimulating factor (*G-CSF*) receptor, the *M-CSF* receptor and the *GM-CSF* receptor. To our knowledge, screening for *PU.1* mutations has been performed in four main studies, including altogether 262 AML patients.[168–171] *PU.1* mutations were found in only 9 of those 262 AML patients (all in the study by Mueller *et al*.[168]), showing that *PU.1* mutations rarely contribute to AML pathogenesis.

Mutations of genes involved in cell cycle and apoptosis

NPM1

Nucleophosmin (NPM1, also known as nucleolar phosphoprotein B23 or numatrin) is a ubiquitously expressed nucleolar phosphoprotein that continuously shuttles between the nucleus and cytoplasm with predominant nucleolar localization.[172] NPM1 is a multifunctional phosphoprotein to which both tumor suppressor and oncogenic functions have been attributed, depending on gene dosage, expression levels, interacting partners and compartmentalization. NPM1 is a molecular chaperone that prevents protein aggregation in the nucleolus and regulates the assembly and transport of preribosomal particles through the nuclear membrane and DNA polymerase-α activity.[173] It is a target of CDK2/cyclin E in centrosome duplication.[174] NPM1 is also involved in cell cycle progression, response to stress and oncogenic stimuli and regulation of the alternate reading frame protein (ARF)-p53 tumor suppressor pathway at multiple levels[175–177] and is required for the stabilization and the proper nucleolar localization of p14-ARF.[178]

The *NPM1* gene, located at 5q35, is a partner in the chromosomal translocations of leukemias and lymphomas that result in fusion proteins containing only the NPM1 N-terminal region. A t(2;5)(p23;q35) translocation is observed in anaplastic large cell lymphomas and results in the chimeric fusion of *NPM1* to *ALK*.[179] The *NPM1* gene also rearranges exceptionally with the retinoic acid receptor-α (*RARα*) in acute promyelocytic leukemia with variant t(5;17) translocation and with *MLF1* in rare cases of AML and myelodysplasia with t(3;5)(q25.1;q34) translocation.[180,181]

More recently, mutations in exon 12 of the gene encoding NPM1 have been described in AML.[182] More than 95% of the mutations consist of a 4-bp insertion at position 960. Mutation A, due to a TCTG duplication, accounts for 70–80% of adult cases and mutation B and D together for 15–20%. About 40 different variant mutations have been identified to date. All cases are heterozygous for the mutation and retain the WT allele.[6,11,12,182–184] Despite the heterogeneity of *NPM1* mutations, all variants cause frameshifts in the region encoding the C-terminal region of the NPM1 protein. As a consequence,

abnormal proteins have acquired, at the C terminus, an additional nuclear export signal motif and have lost both tryptophan residues 290 and 288 (or only tryptophan 290), which determine nucleolar localization. This molecular mechanism is responsible for the aberrant cytoplasmic localization of NPM1-mutated protein, which can easily be detected by immunohistochemistry. The cytoplasmic localization of NPM1-mutated protein is probably critical for its putative role in leukemogenesis. *NPM1* mutations have been found nearly exclusively in *de novo* AML with an incidence of approximately 30% in adults, and not in secondary AML, thus becoming the most frequent genetic lesions in adult *de novo* AML. *NPM1* mutations have been found to occur in an age-dependent fashion with significantly higher incidence in older compared to younger adults.[182,184] Their incidence is however much lower in children than in adults (about 2–6%).[185,186] *NPM1* mutations are associated with all morphological FAB subtypes (except M3) with highest frequency in M4/M5 AML. *NPM1* mutations are significantly associated with higher initial WBC count, higher BM blast count as well as higher platelet count, female gender and low CD34 expression levels on blasts.[12] Except for five cases reported, no *NPM1* mutations have been reported in AML with recurrent translocations t(8;21), inv(16) and t(15;17).[12,187] In cases with complex karyotype or diverse cytogenetic abnormalities, *NPM1* mutations are also rare.[12] *NPM1* mutations occur predominantly in CN-AML: about 50% of CN-AML are *NPM1* mutated and 85% of *NPM1*-mutated AML are CN-AML.[187] *NPM1* mutations are significantly correlated with FLT3-ITD, FLT3-TKD mutations and in some series with a decreased prevalence of *CEBPA* mutations and MLL-PTD.[6,11,12,182–184] The frequency of *c-KIT* and *N-RAS* mutations does not differ in mutated and WT *NPM1* AML.[6,187] Within CN-AML, *NPM1*-mutated AML show a distinct gene-expression profile (GEP) characterized by upregulation of genes involved in stem-cell maintenance including an overexpression of *HOX* genes (*HOXA, HOXB*).[184,188] Patients with intermediate cytogenetic risk AML *NPM1*-mutated/FLT3-ITD negative have a significantly better response to induction therapy, better EFS and better OS than those without NPM1 mutation. Indeed, *NPM1* mutations predict favorable outcome in CN-AML only in the absence of FLT3-ITD.[6,12,182–184] Döhner *et al*.[171] found that *NPM1*-mutated/FLT3-ITD-negative patients had a 5 years survival probability of approximately 60%, comparable to those of patients with either CBF AML or CN-AML with *CEBPA* mutations. In a donor versus no donor comparison, NPM1-mutated/FLT3-ITD-negative patients did not benefit from allogenic stem cell transplantation.[183] *NPM1* mutations seem to be relatively stable during disease evolution. Of the 32 cases sequentially studied and published so far, patients who had *NPM1* mutation at diagnosis all still carried the same mutation at relapse, except 3 who lost the mutation (but this change was associated with the emergence of new cytogenetic abnormalities in 2 of them). Besides, in all *NPM1* WT patients studied at diagnosis, none acquired *NPM1* mutation at relapse. Clearly, the stability of *NPM1* mutations during follow-up needs to be confirmed in a large cohort of paired AML patients who relapse.[11,186,187,189]

TP53 gene

The *TP53* gene is a tumor suppressor gene located in 17p13 that acts as the 'guardian of the genome'.[190,191] Many diverse cellular events, including DNA damage and hypoxia, activate the *TP53* gene. The P53 protein functions as a transcription factor, regulating downstream genes involved in cell cycle arrest, DNA repair and programmed cell death.[191–193] Loss of P53 function confers genomic instability, impaired apoptosis and diminished cell cycle restraint.[194–199] In AML, *P53* can be inactivated by deletion or point mutations, which are generally missense mutations, involving almost exclusively exons 4–8 of the gene.[200] *P53* gene mutations are found in less than 10% of *de novo* AML.[200,201] In *de novo* AML, single-base substitutions at G-C pairs represent the most frequent mutations, but, compared with t-AML and other hematological malignancies or solid tumors, the mutational spectrum does not show a unique pattern.[202,203] *P53* mutations are generally associated to 17p deletion, generally through unbalanced translocations such as t(5;17), therefore resulting in inactivation of both *TP53* alleles (one through point mutation, the other through deletion). *TP53* mutations are also closely related to older age, to deletion or loss of 5q, of 7q and complex karyotypes.[200,204] Patients with *TP53* mutation are generally resistant to chemotherapy and have very short survival.[205]

The frequency of *P53* mutation is higher in t-MDS and t-AML, since about 30% of P53-mutated cases occur after previous chemotherapy with alkylating agents, platinum derivatives or topoisomerase II inhibitors.[204,206] In addition, the type of mutations is different between *de novo* and therapy-related mutations, with a higher frequency of A-T transversion in therapy-related cases and G-C mutation in *de novo* cases.[204] A strong correlation between *P53* mutations associated to loss of heterozygosity (LOH) and a typical form of dysgranulopoiesis combining pseudo-Pelger-Huët hypolobulation and small vacuoles in neutrophil granulocytes has been reported in AML and MDS.[207,208] Detection of *P53* mutations is important because, in patients with LOH, response to chemotherapy will be extremely short and, in those cases, allogenic bone marrow transplantation should be considered.[204]

Discussion

Clinicobiological features and prognostic significance of the main gene mutations implicated in AML are summarized in Table 1. The screening of these mutations is of great interest for the understanding of AML pathogenesis and also for the management of AML patients.

Cooperation between gene mutations

The first question is why so many mutational events are found in AML and how they cooperate in the disease pathogenesis? Instability in the genome of blast cells is now clearly suspected in the pathogenesis of AML. Those perturbations probably result from deregulation of genes involved in cell maintenance and DNA repair. Such instability leads to acquisition of additive molecular abnormalities that contribute to disease progression.[209,210] A mechanism of multistep leukemogenesis is in agreement with this hypothesis, particularly in AML, where a combination of events leading to cell proliferation with events leading to block myeloid differentiation (so-called type I mutations and type II mutations, respectively) is strongly suspected.[1,211,212] Due to their prognostic value, the mutational events screening is particularly important in CN-AML patients.

Alcalay *et al*.[210] have shown that these AML fusion proteins, which are especially known to block myeloid differentiation (including AML1-ETO, PML-RARA and PLZF-RARA), deregulate genes involved in stem cell maintenance and DNA repair. Thus, chimeric oncoproteins increase the rate of secondary mutations. Some AML fusion proteins have been shown to induce a mutator phenotype in blast cells. As an example, transfection of PML-

Table 1 Clinicobiological features and prognostic significance of the main gene mutations detected in AML

Mutation	*Occurrence in AML*		*Biological-associated features*			*Association with other molecular abnormalities*	*History of the disease and clinical features*	*Prognostic significance*
	Adult (%)	*Pediatric (%)*	*Cytogenetic*	*FAB subtype*	*High WBC count at diagnosis*			
FLT3-ITD	28–33	15	Normal t(15;17) t(6;9)	NS	Yes	Positive with *NPM1* mutations and *PML-RARA* Negative with *c-KIT* and *RAS* mutations	More often *de novo* AML	In CN-AML: negative impact: increased RR and CIR worse CRD, EFS, and OS
FLT3-D835	5–10	5–10	Normal inv(16)/t(16;16)	NS	Yes	Positive with *CBFβ-MYH11* and *NPM1* mutations	More often *de novo* AML	Still controversial (level of mutant allele may be important)
NPM1	25–35	2–6	Intermediate (normal +++)	All except M3 (M4/M5 ++)	Yes	Positive with FLT3-ITD and *WT1* mutations Negative with *CEBPA* mutations	Nearly exclusively *de novo* AML	In CN-AML: *NPM1* mutated/FLT3-ITD-negative patients have significantly better CR rates, EFS, RFS, DFS and OS *NPM1* mutations have no significant effect on prognosis if associated with FLT3-ITD
CEBPA	4–9	6	Intermediate (normal +++)	M1 and M2 +++, M4 ++	NS	Negative with FLT3-ITD and *NPM1* mutations	Predominantly *de novo* AML	Significantly longer CRD and OS
AML1	6–11	6	Normal acquired trisomy 21	M0 +++ and other subtypes with trisomy 21	Yes	Positive with *FLT3*, *RAS* and *p53* mutations in non-M0 AML	*de novo* AML and t-AML	NS
N-RAS	10–15	15	inv(16)/t(16;16)	NS	NS	Positive with *CBFβ-MYH11* Negative with FLT3-ITD	NS	NS
K-RAS	5	3	inv(3)/t(3;3)					
c-KIT D816	2	ND	inv(16)/t(16;16) t(8;21) trisomy 4	M2 +++ and M4Eo +	Yes	Positive with *AML1-ETO* and *CBFβ-MYH11* Negative with FLT3-ITD	ND	Within CBF AML: higher RR and shorter EFS, RFS and OS, especially for D816 mutation in t(8;21) AML
c-KIT exon 8	6–8							
WT1	10	ND	Normal	ND	ND	Positive with FLT3-ITD	ND	May be associated with induction failure and poor prognosis (needs further investigation)
P53	<10	NS	Unfavorable	ND	NS	Positive with *AML1* mutations	More often t-AML Older age +++	Significantly shorter OS
PTPN11	2.5	4.5	NS	M5	NS	ND	ND	ND
JAK2	1.5 in *de novo* AML	ND	Karyotypic aberrations, CBF AML	ND	ND	Positive with *AML1-ETO*	ND	ND

Abbreviations: AML, acute myeloid leukemia; CBF, core-binding factor; CIR, cumulated incidence of relapse; CN-AML, cytogenetically normal AML; CR, complete remission; CRD, complete remission duration; DFS, disease-free survival; EFS, event-free survival; FAB, French-American-British; ITD, intern tandem duplication; ND, not determined; NPM, nucleophosmin; NS, not significant; OS, overall survival; RFS, relapse-free survival; RR, relapse rate; t-AML, therapy-related AML; WBC, white blood cell.

RARA or AML1-ETO fusion protein in U937 cell line can induce a significant reduction of DNA repair of the damage induced by methanesulfonate, particularly through down regulation of the expression of seven genes (*ADPRTL2*, *FEN1*, *OGG1*, *MPG*, *LIG3*, *POLD2* and *POLD3*) involved in base excision repair.[210] Moreover, a preferential association between one type of class I mutations and some fusion proteins has been observed, for example between *c-KIT* or *RAS* mutations and CBF AML. A cooperation between class I mutations and *AML1* mutations has also been reported in AML, which supports this hypothesis that mutations affecting *AML1* can reproduce those effects.[211,212] The parallel mutational analysis of genes involved in signal transduction pathways (*FLT3*, *RAS*, *PTPN11*) and in myeloid differentiation (*AML1*, *CEBPA*) should help clarify this hypothesis. Based on the two-hit model of AML pathogenesis, the frequent association between *NPM1* and FLT3-ITD mutations argues for a role of NPM1 in hematopoietic differentiation. The absence of preferential association between *CEBPA* mutations and class I mutations does not go along this hypothesis. However, it is likely that other molecular abnormalities affecting proliferation pathways will be discovered, so that the existence of the two complementation groups cannot be excluded.

Other mechanisms of leukemogenesis appear to arise from an underlying mutator phenotype associated to the history of the disease or to a particular genetic background (predisposition to acute leukemia seen in FPD, Down syndrome or familial form of *CEBPA* mutations). In those situations, is the two-hit model relevant? If yes, this suggests that class II mutations preexist to class I mutations. The concept of LSC also supports the hypothesis that class I mutations are secondary to class II mutations.[213,214] In agreement with this hypothesis is that some patients with *FLT3* mutation at diagnosis relapse without mutation.[26,44]

Another event implicated in leukemogenesis is the deregulation, in blast cells, of genes involved in the maintenance of the stem cell phenotype.[215] In AML blast cells, the machinery promoting ability to self-renewing divisions induces an expansion of a compartment of true LSC.[215] The expansion of this compartment increases the likelihood of molecular alterations which contribute to the transformation of those cells. GEP analysis of AML samples clearly shows down regulation of genes involved in differentiation (*CEBP* family, *JUN* family, *CSF3R*, *ELF4*, *STAT5*…) and upregulation of genes involved in stem cell maintenance phenotype including *BCL1*, *LMO1* and *JAG1*.[215–219] It has been reported that AML-specific fusion proteins including AML1-ETO and PML-RARA induce NOTCH signaling pathway through the induction of *JAG1* leading to self autonomous self renewal abilities of blasts. Moreover, GEP unsupervised clustering clearly separated *NPM1* mutated from *NPM1* WT AML, regardless of the presence of FLT3 mutations or non major chromosomal rearrangements, supporting the concept that *NPM1*-mutated AML represent a distinct entity.[188] The GEP signature of *NPM1*-mutated AML shows upregulation of genes involved in the maintenance of a stem-cell phenotype, including the same NOTCH JAG1 pathway. Among the genes upregulated are numerous members of the homeodomain-containing family of transcription factors, including *HOX* (that are highly expressed in hematopoietic stem cells).[219] Acute leukemias carrying *MLL* rearrangements also display induction of *HOX* genes, possibly due to direct binding of MLL fusion proteins to *HOX* promoters.[220] Such LSC can be triggered to proliferate after class I mutations.

Role of gene mutations screening in the practical management of AML patients

Cytogenetic analysis at time of diagnosis constitutes a major prognostic factor in clinical outcome in AML, which identifies subgroups of favorable, intermediate and 'unfavorable' prognosis. Nevertheless, clinical evolution in those three subgroups remains very variable, particularly for CN-AML.

In this last group, identification of FLT3-ITD mutation probably constitutes the most important known pretreatment prognostic factor. There is a general agreement that the presence of FLT3-ITD mutation is associated with a higher risk of relapse, and it constitutes for many groups an indication to proceed to bone marrow transplantation if a donor is available, although this is controversial for other groups.[36] NPM1 mutations are also emerging as a second gene mutation known to have clinical relevance in CN-AML. However, their favorable prognostic value is only observed in patients without FLT3-ITD. Besides, several studies have provided important evidence that *CEBPA* mutation represents also an independent positive prognostic factor in CN-AML. Therefore, *NPM1* and *CEBPA* mutational status are starting to be used for treatment stratification in new clinical trials.

In CBF AML, many studies support that additional FLT3-ITD or *c-KIT* mutations constitute factors associated with an increased risk of relapse. Screening for FLT3-ITD and *c-KIT* mutations is therefore useful to identify patients who may benefit from bone marrow transplantation and/or alternative strategies including new targeted therapies. A classification of AML combining cytogenetic and molecular findings would allow more accurate prediction of the response to therapy and thereby the selection of more appropriate treatments, especially in patients with CN-AML or CBF AML.

Practical guideline for the mutational screening in AML patients

On the basis of those findings, we proposed a schema for molecular screening in AML patients in routine practice represented in Figure 1, according to the frequency of each mutation and to their clinical significance. This guideline aims at showing which gene mutations should be investigated in a particular cytogenetic subgroup. To note that it is valuable only in the state-of-the-art of conventional chemotherapy regimen currently used in AML.

In AML with intermediate karyotype, and particularly in CN-AML, the poor prognosis of FLT3-ITD is only modestly influenced by the concomitant presence of favorable molecular markers identified so far, such as *NPM1* and *CEBPA* mutations. However, given the frequent association and the prognostic interaction between FLT3-ITD and *NPM1* mutations, both alterations should be tested together in all patients with CN-AML. Furthermore, *NPM1* mutations could become relevant in FLT3-ITD-positive patients treated with FLT3 inhibitors, when those drugs are available. If *NPM1* and FLT3-ITD mutations are both negative, *CEBPA* mutations should be tested for further risk stratification. Lastly, screening of other gene mutations with potential clinical relevance, such as FLT3-TKD, MLL-PTD, *RAS* and *WT1* mutations, as well as overexpression of genes like *EVI1*, *ERG*, *MN1* or *BAALC*, may help refine prognosis. However, it has not yet been firmly established if the over-expression of *EVI1*, *ERG*, *MN1* or *BAALC* genes provides an independent poor prognostic factor.

In CBF AML, screening of FLT3-ITD and *c-KIT* mutations should be performed systematically in each case for therapeutic stratification and potential use of TKIs. *RAS* and FLT3-TKD mutations can also be tested in CBF AML, but their clinical impact in those patients needs to be further investigated.

In the remaining subgroups, namely AML with unfavorable karyotype and AML with t(15;17), mutational screening is not

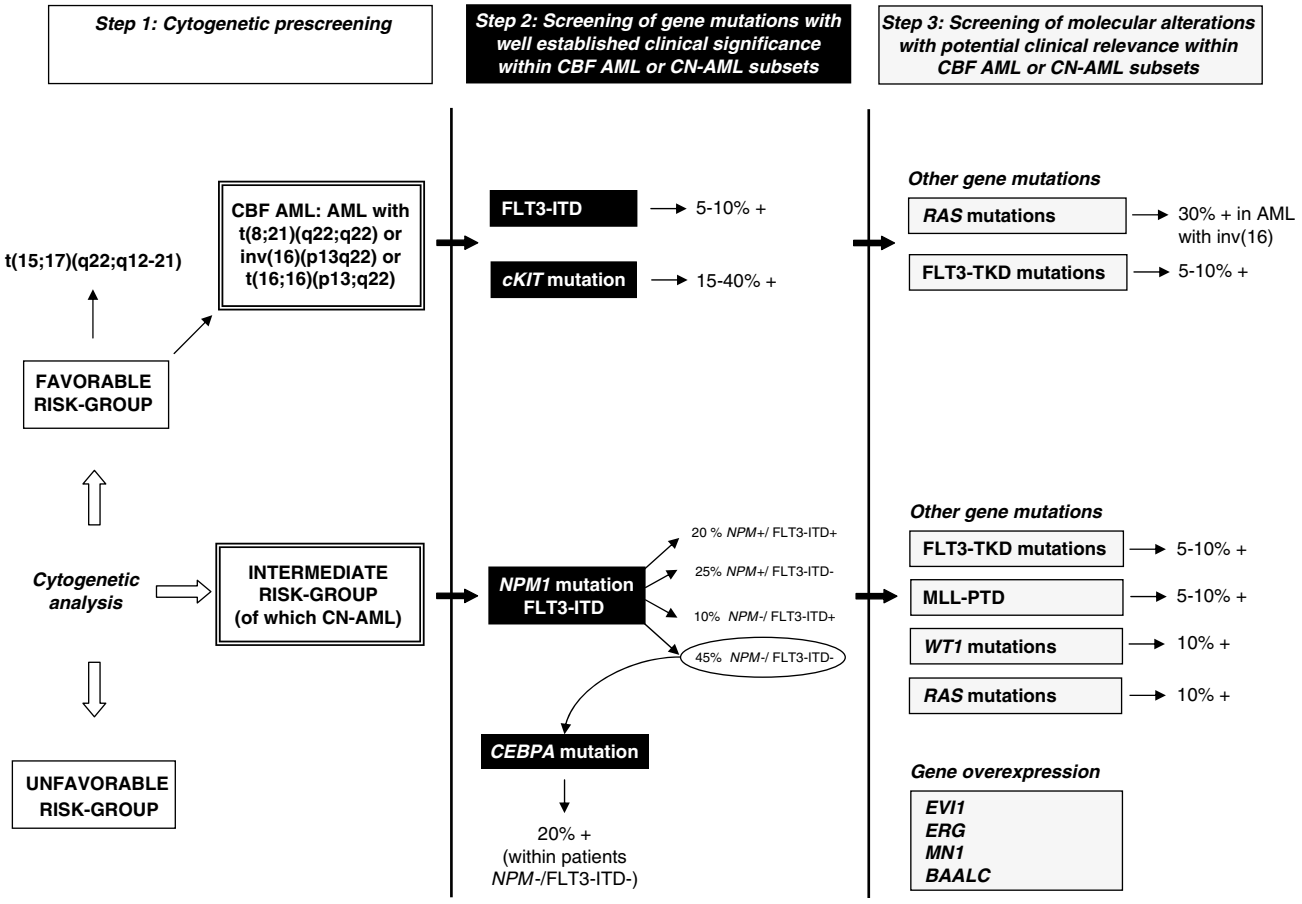

Figure 1 Proposed schema for molecular screening in AML patients in routine practice, depending on a cytogenetic prescreening. Step 1: cytogenetic analysis, including karyotype and FISH analysis for screening of *MLL* rearrangements, is performed. In case of CBF AML or AML with intermediate karyotype, molecular analysis is required to refine prognosis. In the remaining cytogenetic subsets, namely AML with t(15;17) or unfavorable karyotype, molecular analysis is not particularly indicated. Step 2: within CBF AML and AML with intermediate karyotype, mainly CN-AML, screening of gene mutations with well-established prognostic significance is recommended, i.e., FLT3-ITD and *c-KIT* mutations, and FLT3-ITD, *NPM1* and *CEBPA* mutations, respectively. Step 3: screening of other molecular alterations with potential clinical relevance within CBF AML or CN-AML subsets may help to refine prognosis. The percentages indicated correspond to the average proportion of mutated patients in each subgroup. AML, acute myeloid leukemia; CBF, core binding factor; CN-AML, cytogenetically normal AML; ITD, intern tandem duplication; TKD, tyrosine kinase domain; PTD, partial tandem duplication.

particularly recommended since gene mutations have no significant prognostic relevance with current treatment modalities. In acute promyelocytic leukemia, the negative impact of FLT3-ITD on prognosis appears to be redundant to that of increased WBC count, but the analysis of FLT3-ITD status may be helpful for the future use of FLT3 inhibitors.

Gene mutations as potential markers for MRD monitoring

Detection of MRD is of growing importance in the management of AML patients. In AML with balanced chromosomal translocations, evaluation of MRD is possible using the fusion transcripts corresponding to inv(16)/t(16;16), t(8;21), t(15;17) and MLL rearrangements, which are detectable by real-time quantitative polymerase chain reaction (RQ-PCR). In the other cases, representing approximately 70% of AML patients, MRD monitoring is hampered by the lack of reliable molecular markers. For that reason, the use of *WT1* gene expression and gene mutations for PCR-based MRD detection has been proposed. FLT3-ITD and *NPM1* mutations are potentially good candidates for this purpose, since they are applicable to about 30 and 50% of CN-AML, respectively. RQ-PCR techniques using a mutation-specific primer have been developed for both mutations and permit to reach a high sensitivity (about 10^{-4} for FLT3-ITD mutations, and 10^{-4} to 10^{-5} for *NPM1* mutations). However, the suitability of FLT3-ITD mutations as MRD marker remains controversial because of their relative instability during follow-up and their variability from a patient to another and sometimes during disease progression in the same patient.[26,44,46] In addition, RQ-PCR assays based on FLT3-ITD mutations are not without limitations, including the difficulty to sequence the duplicated fragment in cases with low mutated/WT ratio and the requirement for optimal patient-specific primers and probe. FLT3-ITD mutation can still be valuable in case it is the only marker available for MRD follow-up, but should be used with caution as early predictor of relapse. In this respect, the use of several markers in combination, for example FLT3-ITD mutation associated with *WT1* gene expression or *NPM1* mutation, can be helpful to avoid false negative results. Compared to FLT3-ITD mutations, *NPM1* mutations seem to be more stable during disease evolution.[11,186,189] Another

advantage of *NPM1* mutations is that the most frequent variants (A, B and D) account for over 90% of all *NPM1* mutations, thus making easier the routine detection of MRD. Besides, NPM1-MRD levels seem to correlate with response to therapy and clinical status.[189] However, even if those results are encouraging, the use of FLT3-ITD and *NPM1* mutations as MRD markers needs to be validated in large prospective clinical trials. Further studies will determine if these assays will serve to predict outcome and help risk stratification in CN-AML.[189]

Gene mutations as potential therapeutic targets

Many of the identified gene mutations that affect proliferation pathways represent potential targets for the development of new drugs. These targeted therapies could be used as single agent or in combination with conventional chemotherapy in AML. In such a way of evaluation of novel therapeutic approaches, a complete screening of all mutations mentioned above, as well as GEP analysis, should be performed to detect the main predictive factors of response to therapy.

Optimal treatment for CN-AML patients with FLT3-ITD mutations is yet unclear. It has been reported that bone marrow transplantation in first CR may not overcome the chemoresistance/radioresistance inherent in FLT3-ITD-positive blasts.[5,36] Therefore, FLT3-ITD-positive patients could also potentially benefit from treatment with FLT3 inhibitors. Several small molecule TKI with activity against FLT3, such as PKC412 (midostaurin), CEP-701 (lestaurtinib), SU11248 (sunitinib) or MLN518 (tandutinib), are currently in clinical phase I and II trials. In patients with FLT3-ITD or FLT3-TKD mutations treated with TKI alone, clinical responses are usually manifested as a transient reduction in blood and, less frequently, in bone marrow blasts. Few patients achieve a CR rate or prolonged partial response. Several trials combining TKI and conventional chemotherapy are ongoing in patients with newly diagnosed FLT3-mutant AML or in AML in relapse.[221–229] TKI may also target *c-KIT* mutations, although in such cases the exact location and nature of the mutation must be determined individually because specific inhibitors are active against particular *c-KIT* mutations. For example, cells carrying insertion/deletion in exon 8, ITD of exon 11 and 12 or substitutions at codon 822 are sensitive to imatinib, while D816 mutations confer a resistance to imatinib but can be targeted with other TKI such as midostaurin, nilotinib or dasatinib.[54,58,64,230–233] Therefore, testing for *c-KIT* mutations should be performed in all CBF AML patients for risk-adapted treatments.

RAS proteins require post-translational modification by farnesylation to be biologically active. Farnesyl transferase inhibitors have some antitumor activity in the clinic in AML, but they also seem to act through targets other than RAS. Several compounds that block specifically RAS pathway are currently being tested in AML.[234]

Lastly, emerging epigenetic therapies combining hypomethylating agents, including 5-azacytidine and decitabine, to histone deacetylase (HDAC) inhibitors, such as valproic acid (with or without all-*trans* retinoic acid) or more recently designed compounds, appear promising. This new approach has recently shown particular interest in AML patients with MLL-PTD, where this combination was found to reactivate the transcription of *MLL* WT allele, and this was associated with enhanced cell death of MLL-PTD-positive leukemic blasts. These data may provide a rationale to evaluate treatment with DNA methyltransferase and HDAC inhibitors in patients with MLL-PTD.[235]

References

1 Dash A, Gilliland DG. Molecular genetics of acute myeloid leukaemia. *Best Pract Res Clin Haematol* 2001; **14**: 49–64.
2 Agnes F, Shamoon B, Dina C, Rosnet O, Birnbaum D, Galibert F. Genomic structure of the downstream part of the human FLT3 gene: exon/intron structure conservation among genes encoding receptor tyrosine kinases (RTK) of subclass III. *Gene* 1994; **145**: 283–288.
3 Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood* 2002; **100**: 1532–1542.
4 Carow CE, Levenstein M, Kaufmann SH, Chen J, Amin S, Rockwell P *et al.* Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. *Blood* 1996; **87**: 1089–1096.
5 Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD *et al.* Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. *Cancer Res* 2001; **61**: 7233–7239.
6 Schnittger S, Schoch C, Kern W, Mecuci C, Tschulik C, Martelli MF *et al.* Nucleophosmin gene mutations are predictors of favorable prognosis in acute myeloid leukemia with normal karyotype. *Blood* 2005; **106**: 3733–3739.
7 Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K *et al.* Prognostic significance of activating FLT3 mutations in younger adults (16–60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood* 2002; **100**: 4372–4380.
8 Kainz B, Heintel D, Marculescu R, Schwarzinger I, Sperr W, Le T *et al.* Variable prognostic value of FLT3 internal tandem duplications in patients with *de novo* AML and a normal karyotype, t(15;17), t(8;21) or inv(16). *Hematol J* 2002; **3**: 283–289.
9 Beran M, Luthra R, Kantarjian H, Estey E. FLT3 mutation and response to intensive chemotherapy in young adult and elderly patients with normal karyotype. *Leuk Res* 2004; **28**: 547–550.
10 Bienz M, Ludwig M, Leibundgut EO, Mueller BU, Ratschiller D, Solenthaler M *et al.* Risk assessment in patients with acute myeloid leukemia and a normal karyotype [erratum in *Clin Cancer Res* 2005;11: 5659]. *Clin Cancer Res* 2005; **11**: 1416–1424.
11 Boissel N, Renneville A, Biggio V, Philippe N, Thomas X, Cayuela JM *et al.* Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. *Blood* 2005; **106**: 3618–3620.
12 Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M *et al.* Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood* 2006; **107**: 4011–4020.
13 Iwai T, Yokota S, Nakao M, Okamoto T, Taniwaki M, Onodera N *et al.* Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. *Leukemia* 1999; **13**: 38–43.
14 Kondo M, Horibe K, Takahashi Y, Matsumoto K, Fukuda M, Inaba J *et al.* Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. *Med Pediatr Oncol* 1999; **33**: 525–529.
15 Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK *et al.* Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukaemia. *Blood* 2001; **97**: 89–94.
16 Zwaan ChM, Meshinchi S, Radich JP, Veerman AJ, Huismans DR, Munske L *et al.* FLT3 internal tandem duplication in 234 children with acute myeloid leukemia (AML): prognostic significance and relation to cellular drug resistance. *Blood* 2003; **102**: 2387–2394.
17 Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C *et al.* Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* 2002; **100**: 59–66.
18 Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S *et al.* Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* 2001; **97**: 2434–2439.

19 Stirewalt DL, Meshinchi S, Kussick SJ, Sheets KM, Pogosova-Agadjanyan E, Willman CL *et al.* Novel FLT3 point mutations within exon 14 found in patients with acute myeloid leukemia. *Br J Haematol* 2004; **124**: 481–484.
20 Reindl C, Bagrintseva K, Vempati S, Schnittger S, Ellwart JW, Wenig K *et al.* Point mutations in the juxtamembrane domain of FLT3 define a new class of activating mutations in AML. *Blood* 2006; **107**: 3700–3707.
21 Schittenhelm MM, Yee KWH, Tyner JW, McGreevey L, Haley AD, Town A *et al.* FLT3 K663Q is a novel AML-associated oncogenic kinase: determination of biochemical properties and sensitivity to sunitinib (SU11248). *Leukemia* 2006; **20**: 2008–2014.
22 Fröhling S, Scholl C, Levine RL, Loriaux M, Boggon TJ, Bernard OA *et al.* Identification of driver and passenger mutations of FLT3 by high-throughput DNA sequence analysis and functional assessment of candidate alleles. *Cancer Cell* 2007; **12**: 501–513.
23 Grundler R, Miething C, Thiede C, Peschel C, Duyster J. FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. *Blood* 2005; **105**: 4792–4799.
24 Lee BH, Tothova Z, Levine RL, Anderson K, Buza-Vidas N, Cullen DE *et al.* FLT3 mutations confer enhanced proliferation and survival properties to multipotent progenitors in a murine model of chronic myelomonocytic leukemia. *Cancer Cell* 2007; **12**: 367–380.
25 Choudhary C, Schwable J, Brandts C, Tickenbrock L, Sargin B, Kindler T *et al.* AML-associated Flt3 kinase domain mutations show transduction differences compared with Flt3 ITD mutations. *Blood* 2005; **106**: 265–273.
26 Kottaridis PD, Gale RE, Langabeer SE, Frew ME, Bowen DT, Linch DC. Studies of FLT3 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia: implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection, and possible therapy with FLT3 inhibitors. *Blood* 2002; **100**: 2393–2398.
27 Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA *et al.* The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* 2001; **98**: 1752–1759.
28 Kiyoi H, Naoe T, Yokota S, Nakao M, Minami S, Kuriyama K *et al.* Internal tandem duplication of FLT3 associated with leukocytosis in acute promyelocytic leukemia: Leukemia Study Group of the Ministry of Health and Welfare (Kohseisho). *Leukemia* 1997; **11**: 1447–1452.
29 Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U *et al.* Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* 2002; **99**: 4325–4335.
30 Mead AJ, Linch DC, Hills RK, Wheatley K, Burnett AK, Gale RE. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood* 2007; **110**: 1262–1270.
31 Libura M, Asnafi V, Tu A, Delabesse E, Tigaud I, Cymbalista F *et al.* FLT3 and MLL intragenic abnormalities in AML reflect a common category of genotoxic stress. *Blood* 2003; **102**: 2198–2204.
32 Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ *et al.* RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood* 2005; **106**: 2113–2119.
33 Stirewalt DL, Kopecky KJ, Meshinchi S, Appelbaum FR, Slovak ML, Willman CL *et al.* FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood* 2001; **97**: 3589–3595.
34 Yanada M, Matsuo K, Suzuki T, Kiyoi H, Naoe T. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. *Leukemia* 2005; **19**: 1345–1349.
35 Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehninger G. BAALC expression and FLT3 internal tandem duplication mutations in acute myeloid leukemia patients with normal cytogenetics: prognostic implications. *J Clin Oncol* 2006; **24**: 790–797.
36 Gale RE, Hills R, Kottaridis PD, Srirangan S, Wheatley K, Burnett AK *et al.* No evidence that FLT3 status should be considered as an indicator for transplantation in acute myeloid leukemia (AML): an analysis of 1135 patients, excluding acute promyelocytic leukemia, from the UK MRC AML10 and 12 trials. *Blood* 2005; **106**: 3658–3665.
37 Mathews V, Thomas M, Srivastava VM, George B, Srivastava A, Chandy M. Impact of FLT3 mutations and secondary cytogenetic changes on the outcome of patients with newly diagnosed acute promyelocytic leukemia treated with a single agent arsenic trioxide regimen. *Haematologica* 2007; **92**: 994–995.
38 Fitzgibbon J, Smith LL, Raghavan M, Smith ML, Debernardi S, Skoulakis S *et al.* Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. *Cancer Res* 2005; **65**: 9152–9154.
39 Griffiths M, Mason J, Rindl M, Akiki S, McMullan D, Stinton V *et al.* Acquired isodisomy for chromosome 13 is common in AML, and associated with FLT3-itd mutations (letter). *Leukemia* 2005; **19**: 2355–2358.
40 Stirewalt DL, Kopecky KJ, Meshinchi S, Engel JH, Pogosova-Agagjanyan EL, Linsley J *et al.* Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. *Blood* 2006; **107**: 3724–3726.
41 Kusec R, Jaksic O, Ostojic S, Kardum-Skelin I, Vrhovac R, Jaksic B. More on prognostic significance of FLT3/ITD size in acute myeloid leukemia (AML). *Blood* 2006; **108**: 405–406.
42 Ponziani V, Gianfaldoni G, Mannelli F, Leoni F, Ciolli S, Guglielmelli P *et al.* The size of duplication does not add to the prognostic significance of FLT3 internal tandem duplication in acute myeloid leukemia patients. *Leukemia* 2006; **20**: 2074–2076.
43 Radmacher MD, Marcucci G, Ruppert AS, Mrozek K, Whitman SP, Vardiman JW *et al.* 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.
44 Shih LY, Huang CF, Wu JH, Lin TL, Dunn P, Wang PN *et al.* Internal tandem duplication of FLT3 in relapsed acute myeloid leukemia: a comparative analysis of bone marrow samples from 108 adult patients at diagnosis and relapse. *Blood* 2002; **100**: 2387–2392.
45 Noguera NI, Breccia M, Divona M, Diverio D, Costa V, De Santis S *et al.* Alterations of the FLT3 gene in acute promyelocytic leukemia: association with diagnostic characteristics and analysis of clinical outcome in patients treated with the Italian AIDA protocol. *Leukemia* 2002; **16**: 2185–2189.
46 Cloos J, Goemans BF, Hess CJ, van Oostveen JW, Waisfisz Q, Corthals S *et al.* Stability and prognostic influence of FLT3 mutations in paired initial and relapsed AML samples. *Leukemia* 2006; **20**: 1217–1220.
47 Shih LY, Huang CF, Wu JH, Wang PN, Lin TL, Dunn P *et al.* Heterogeneous patterns of FLT3 Asp(835) mutations in relapsed *de novo* acute myeloid leukemia: a comparative analysis of 120 paired diagnostic and relapse bone marrow samples. *Clin Cancer Res* 2004; **10**: 1326–1332.
48 Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001; **411**: 355–365.
49 Reilly JT. Receptor tyrosine kinases in normal and malignant haematopoiesis. *Blood Rev* 2003; **17**: 241–248.
50 Lennartsson J, Jelacic T, Linnekin D, Shivakrupa R. Normal and oncogenic forms of the receptor tyrosine kinase kit. *Stem Cells* 2005; **23**: 16–43.
51 Gari M, Goodeve A, Wilson G, Winship P, Langabeer S, Linch D *et al.* C-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. *Br J Haematol* 1999; **105**: 894–900.
52 Beghini A, Peterlongo P, Ripamonti CB, Larizza L, Cairoli R, Morra E *et al.* C-kit mutations in core binding factor leukemias. *Blood* 2000; **95**: 726–727.
53 Care RS, Valk PJ, Goodeve AC, Abu-Duhier FM, Geertsma-Kleinekoort WM, Wilson GA *et al.* Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. *Br J Haematol* 2003; **121**: 775–777.

54 Kohl TM, Schnittger S, Ellwart JW, Hiddemann W, Spiekermann K. KIT exon 8 mutations associated with core-binding factor (CBF)-acute myeloid leukemia (AML) cause hyperactivation of the receptor in response to stem cell factor. *Blood* 2005; **105**: 3319–3321.
55 Ning ZQ, Li J, McGuiness M, Arceci RJ. STAT3 activation is required for Asp(816) mutant c-KIT induced tumorigenicity. *Oncogene* 2001; **20**: 4528–4536.
56 Chian R, Young S, Danilkovitch-Miagkova A, Ronnstrand L, Leonard E, Ferrao P *et al.* Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. *Blood* 2001; **98**: 1365–1373.
57 Beghini A, Ripamonti CB, Cairoli R, Cazzaniga G, Colapietro P, Elice F *et al.* KIT activating mutations: incidence in adult and pediatric acute myeloid leukemia, and identification of an internal tandem duplication. *Haematologica* 2004; **89**: 920–925.
58 Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K *et al.* KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 2006; **107**: 1791–1799.
59 Goemans BF, Zwaan ChM, Miller M, Zimmermann M, Harlow A, Meshinchi S *et al.* Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia* 2005; **19**: 1536–1542.
60 Boissel N, Leroy H, Brethon B, Philippe N, de Botton S, Auvrignon A *et al.* Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). *Leukemia* 2006; **20**: 965–970.
61 Cairoli R, Beghini A, Grillo G, Nadali G, Elice F, Ripamonti CB *et al.* Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. *Blood* 2006; **107**: 3463–3468.
62 Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M *et al.* KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. *Blood* 2006; **107**: 1806–1809.
63 Paschka P, Marcucci G, Ruppert AS, Mrozek K, Chen H, Kittles RA *et al.* Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol* 2006; **24**: 3904–3911.
64 Corbacioglu S, Kilic M, Westhoff MA, Reinhardt D, Fulda S, Debatin KM. Newly identified c-kit receptor tyrosine kinase ITD in childhood AML induces ligand independent growth and is responsive to a synergistic effect of imatinib and rapamycin. *Blood* 2006; **108**: 3504–3513.
65 Mitin N, Rossman KL, Der CJ. Signaling interplay in Ras superfamily function. *Curr Biol* 2005; **15**: R563–R574.
66 Ayllon V, Rebollo A. Ras-induced cellular events (review). *Mol Membr Biol* 2000; **17**: 65–73.
67 Beaupre DM, Kurzrock R. RAS and leukemia: from basic mechanisms to gene-directed therapy. *J Clin Oncol* 1999; **17**: 1071–1079.
68 Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ *et al.* RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood* 2005; **106**: 2113–2119.
69 Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S. Implications of NRAS mutations in AML: a study of 2502 patients. *Blood* 2006; **107**: 3847–3853.
70 Shih LY, Huang CF, Wang PN, Wu JH, Lin TL, Dunn P *et al.* Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. *Leukemia* 2004; **18**: 466–475.
71 Christiansen DH, Andersen MK, Desta F, Pedersen-Bjergaard J. Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia* 2005; **19**: 2232–2240.
72 Neubauer A, Dodge RK, George SL, Davey FR, Silver RT, Schiffer CA *et al.* Prognostic importance of mutations in the ras proto-oncogenes in *de novo* acute myeloid leukemia. *Blood* 1994; **83**: 1603–1611.
73 Ritter M, Kim TD, Lisske P, Thiede C, Schaich M, Neubauer A. Prognostic significance of N-RAS and K-RAS mutations in 232 patients with acute myeloid leukemia. *Haematologica* 2004; **89**: 1397–1399.
74 Radich JP, Kopecky KJ, Willman CL, Weick J, Head D, Appelbaum F *et al.* N-ras mutations in adult *de novo* acute myelogenous leukemia: prevalence and clinical significance. *Blood* 1990; **76**: 801–807.
75 Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S *et al.* Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood* 1999; **93**: 3074–3080.
76 Tartaglia M, Niemeyer CM, Shannon KM, Loh ML. SHP-2 and myeloid malignancies. *Curr Opin Hematol* 2004; **11**: 44–50.
77 Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H *et al.* Mutations in PTPN11, encoding the protein SHP-2, cause Noonan syndrome. *Nat Genet* 2001; **29**: 465–468.
78 Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH *et al.* Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. *Blood* 2004; **103**: 2325–2331.
79 Tartaglia M, Martinelli S, Iavarone I, Cazzaniga G, Spinelli M, Giarin E *et al.* Somatic PTPN11 mutations in childhood acute myeloid leukaemia. *Br J Haematol* 2005; **129**: 333–339.
80 Goemans BF, Zwaan CM, Martinelli S, Harrell P, de Lange D, Carta C *et al.* Differences in the prevalence of PTPN11 mutations in FAB M5 paediatric acute myeloid leukaemia. *Br J Haematol* 2005; **130**: 795–805.
81 Loh ML, Reynolds MG, Vattikuti S, Gerbing RB, Alonzo TA, Carlson E, *et al.*, Children's Cancer Group. PTPN11 mutations in pediatric patients with acute myeloid leukemia: results from the Children's Cancer Group. *Leukemia* 2004; **18**: 1831–1834.
82 Johan MF, Bowen DT, Frew ME, Goodeve AC, Wilson GA, Peake IR *et al.* Mutations in PTPN11 are uncommon in adult myelodysplastic syndromes and acute myeloid leukaemia. *Br J Haematol* 2004; **124**: 843–844.
83 Nomdedéu J, Carricondo MT, Lasa A, Perea G, Aventin A, Sierra J. Low frequency of exon 3 PTPN11 mutations in adult *de novo* acute myeloid leukemia. Analysis of a consecutive series of 173 patients. *Haematologica* 2005; **90**: 412–413.
84 Watkins F, Fidler C, Boultwood J, Wainscoat JS. Mutations in PTPN11 are rare in adult myelodysplastic syndromes and acute myeloid leukemia. *Am J Hematol* 2004; **76**: 417.
85 Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 2002; **285**: 1–24.
86 Verma A, Kambhampati S, Parmar S, Platanias LC. Jak family of kinases in cancer. *Cancer Metastasis Rev* 2003; **22**: 423–434.
87 James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C *et al.* A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. *Nature* 2005; **434**: 1144–1148.
88 Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779–1790.
89 Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ *et al.* Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005; **7**: 387–397.
90 Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S *et al.* Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; **365**: 1054–1061.
91 Fröhling S, Lipka DB, Kayser S, Scholl C, Schlenk RF, Dohner H *et al.* Rare occurrence of the JAK2 V617F mutation in AML subtypes M5, M6, and M7. *Blood* 2006; **107**: 1242–1243.
92 Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E *et al.* The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood* 2005; **106**: 3377–3379.
93 Steensma DP, McClure RF, Karp JE, Tefferi A, Lasho TL, Powell HL *et al.* JAK2 V617F is a rare finding in *de novo* acute myeloid leukemia, but STAT3 activation is common and remains unexplained. *Leukemia* 2006; **20**: 971–978.
94 Lee JW, Kim YG, Soung YH, Han KJ, Kim SY, Rhim HS *et al.* The JAK2 V617F mutation in *de novo* acute myelogenous leukemias. *Oncogene* 2006; **25**: 1434–1436.

95 Jelinek J, Oki Y, Gharibyan V, Bueso-Ramos C, Prchal JT, Verstovsek S *et al.* JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. *Blood* 2005; **106**: 3370–3373.
96 Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M *et al.* MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 2006; **108**: 3472–3476.
97 Illmer T, Schaich M, Ehninger G, Thiede C, DSIL2003 AML Study Groupss. Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias. *Haematologica* 2007; **92**: 137–138.
98 Schnittger S, Bacher U, Kern W, Haferlach C, Haferlach T. JAK2 seems to be a typical cooperating mutation in therapy-related t(8;21)/ AML1-ETO-positive AML. *Leukemia* 2007; **21**: 183–184.
99 Mercher T, Wernig G, Moore SA, Levine RL, Gu TL, Frohling S *et al.* JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model. *Blood* 2006; **108**: 2770–2779.
100 Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR *et al.* JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med* 2007; **356**: 459–468.
101 Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M *et al.* MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 2006; **3**: e270.
102 Caligiuri MA, Briesewitz R, Yu J, Wang L, Wei M, Arnoczky KJ *et al.* Novel c-CBL and CBL-b ubiquitin ligase mutations in human acute myeloid leukemia. *Blood* 2007; **110**: 1022–1024.
103 Sargin B, Choudhary C, Crosetto N, Schmidt MH, Grundler R, Rensinghoff M *et al.* Flt3-dependent transformation by inactivating c-Cbl mutations in AML. *Blood* 2007; **110**: 1004–1012.
104 Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc Natl Acad Sci USA* 1991; **88**: 10431–10434.
105 Levanon D, Negreanu V, Bernstein Y, Bar-Am I, Avivi L, Groner Y. AML1, AML2, and AML3, the human members of the runt domain gene-family: cDNA structure, expression, and chromosomal localization. *Genomics* 1994; **23**: 425–432.
106 Speck NA, Stacy T, Wang Q, North T, Gu TL, Miller J *et al.* Core-binding factor: a central player in hematopoiesis and leukemia. *Cancer Res* 1999; **59**: 1789s–1793s.
107 Pozner A, Lotem J, Xiao C, Goldenberg D, Brenner O, Negreanu V *et al.* Developmentally regulated promoter-switch transcriptionally controls Runx1 function during embryonic hematopoiesis. *BMC Dev Biol* 2007; **7**: 84.
108 Ogawa E, Maruyama M, Kagoshima H, Inuzuka M, Lu J, Satake M *et al.* PEBP2/PEA2 represents a family of transcription factors homologous to the products of the Drosophila runt gene and the human AML1 gene. *Proc Natl Acad Sci USA* 1993; **90**: 6859–6863.
109 Downing JR. AML1/CBFbeta transcription complex: its role in normal hematopoiesis and leukemia. *Leukemia* 2001; **15**: 664–665.
110 Wang Q, Stacy T, Miller JD, Lewis AF, Gu TL, Huang X *et al.* The CBFbeta subunit is essential for CBFalpha2 (AML1) function *in vivo*. *Cell* 1996; **87**: 697–708.
111 Miyoshi H, Ohira M, Shimizu K, Mitani K, Hirai H, Imai T *et al.* Alternative splicing and genomic structure of the AML1 gene involved in acute myeloid leukemia. *Nucleic Acids Res* 1995; **23**: 2762–2769.
112 Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, Yazaki Y *et al.* An acute myeloid leukemia gene, AML1, regulates transcriptional activation and hemopoietic myeloid cell differentiation antagonistically by two alternative spliced forms. *Leukemia* 1997; **11** (Suppl 3): 299–302.
113 Tsuzuki S, Hong D, Gupta R, Matsuo K, Seto M, Enver T. Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1. *PLoS Med* 2007; **4**: e172.
114 Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, Nishida J *et al.* An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. *EMBO J* 1995; **14**: 341–350.
115 Lutterbach B, Hiebert SW. Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. *Gene* 2000; **245**: 223–235.
116 Rhoades KL, Hetherington CJ, Rowley JD, Hiebert SW, Nucifora G, Tenen DG *et al.* Synergistic up-regulation of the myeloid-specific promoter for the macrophage colony-stimulating factor receptor by AML1 and the t(8;21) fusion protein may contribute to leukemogenesis. *Proc Natl Acad Sci USA* 1996; **93**: 11895–11900.
117 Uchida H, Zhang J, Nimer SD. AML1A and AML1B can transactivate the human IL-3 promoter. *J Immunol* 1997; **158**: 2251–2258.
118 Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hemapoiesis. *Proc Natl Acad Sci USA* 1996; **93**: 3444–3449.
119 Sasaki K, Yagi H, Bronson RT, Tominaga K, Matsunashi T, Deguchi K *et al.* Absence of fetal liver hematopoiesis in mice deficient in transcriptional coactivator core binding factor beta. *Proc Natl Acad Sci USA* 1996; **93**: 12359–12363.
120 Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 1996; **84**: 321–330.
121 Okuda T, Takeda K, Fujita Y, Nishimura M, Yagyu S, Yoshida M *et al.* Biological characteristics of the leukemia-associated transcriptional factor AML1 disclosed by hematopoietic rescue of AML1-deficient embryonic stem cells by using a knock-in strategy. *Mol Cell Biol* 2000; **20**: 319–328.
122 Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. *Blood* 1995; **86**: 1–14.
123 Romana SP, Poirel H, Leconiat M, Flexor MA, Mauchauffe M, Jonveaux P *et al.* A High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood* 1995; **86**: 4263–4269.
124 Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T *et al.* Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. *Blood* 1999; **93**: 1817–1824.
125 Preudhomme C, Warot-Loze D, Roumier C, Grardel-Duflos N, Garand R, Lai JL *et al.* High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. *Blood* 2000; **96**: 2862–2869.
126 Imai Y, Kurokawa M, Izutsu K, Hangaishi A, Takeuchi K, Maki K *et al.* Mutations of the AML1 gene in myelodysplastic syndrome and their functional implications in leukemogenesis. *Blood* 2000; **96**: 3154–3160.
127 Steer EJ, Goldman JM, Cross NC. Mutations of the transcription factor AML1/CBFA2 are uncommon in blastic transformation of chronic myeloid leukaemia. *Leukemia* 2001; **15**: 476–477.
128 Langabeer SE, Gale RE, Rollinson SJ, Morgan GJ, Linch DC. Mutations of the AML1 gene in acute myeloid leukaemia of FAB types M0 and M7. *Genes Chromosomes Cancer* 2002; **34**: 24–32.
129 Yeoh A, Williams K, Behm F, Lenny N, Shih L, Harada Y *et al.* Somatic mutations of the AML1 gene are frequent in acute myeloid leukemia with FAB M0 morphology. *Blood* 2000; **96**: 91a (abstract 389).
130 Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D *et al.* Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* 1999; **23**: 166–175.
131 Cai Z, de Bruijn M, Ma X, Dortland B, Luteijn T, Downing RJ *et al.* Haploinsufficiency of AML1 affects the temporal and spatial generation of hematopoietic stem cells in the mouse embryo. *Immunity* 2000; **13**: 423–431.
132 Harada H, Harada Y, Niimi H, Kyo T, Kimura A, Inaba T. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood* 2004; **103**: 2316–2324.
133 Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. *Leukemia* 2003; **17**: 9–16.

134 Dicker F, Haferlach C, Kern W, Haferlach T, Schnittger S. Trisomy 13 is strongly associated with AML1/RUNX1 mutations and increased FLT3 expression in acute myeloid leukemia. *Blood* 2007; **110**: 1308–1316.
135 Tenen DG, Hromas R, Licht JD, Zhang DE. Transcription factors, normal myeloid development, and leukemia. *Blood* 1997; **90**: 489–519.
136 Tenen DG. Disruption of differentiation in human cancer: AML shows the way. *Nat Rev Cancer* 2003; **3**: 89–101.
137 Nerlov C. C/EBPalpha mutations in acute myeloid leukaemias. *Nat Rev Cancer* 2004; **4**: 394–400.
138 Radomska HS, Huettner CS, Zhang P, Cheng T, Scadden DT, Tenen DG. CCAAT/enhancer binding protein alpha is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors. *Mol Cell Biol* 1998; **18**: 4301–4314.
139 Johansen LM, Iwama A, Lodie TA, Sasaki K, Felsher DW, Golub TR *et al.* c-Myc is a critical target for c/EBPalpha in granulopoiesis. *Mol Cell Biol* 2001; **21**: 3789–3806.
140 Smith LT, Hohaus S, Gonzalez DA, Dziennis SE, Tenen DG. PU.1 (Spi-1) and C/EBP alpha regulate the granulocyte colony-stimulating factor receptor promoter in myeloid cells. *Blood* 1996; **88**: 1234–1247.
141 D'Alo F, Johansen LM, Nelson EA, Radomska HS, Evans EK, Zhang P *et al.* The amino terminal and E2F interaction domains are critical for C/EBP alpha-mediated induction of granulopoietic development of hematopoietic cells. *Blood* 2003; **102**: 3163–3171.
142 Wang QF, Cleaves R, Kummalue T, Nerlov C, Friedman AD. Cell cycle inhibition mediated by the outer surface of the C/EBPalpha basic region is required but not sufficient for granulopoiesis. *Oncogene* 2003; **22**: 2548–2557.
143 Timchenko NA, Wilde M, Nakanishi M, Smith JR, Darlington GJ. CCAAT/enhancer-binding protein alpha (C/EBP alpha) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein. *Genes Dev* 1996; **10**: 804–815.
144 Zhang DE, Zhang P, Wang ND, Hetherington CJ, Darlington GJ, Tenen DG. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. *Proc Natl Acad Sci USA* 1997; **94**: 569–574.
145 Friedman AD. Transcriptional regulation of granulocyte and monocyte development. *Oncogene* 2002; **21**: 3377–3390.
146 Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S *et al.* Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. *Nat Genet* 2001; **27**: 263–270.
147 Radomska HS, Bassères DS, Zheng R, Zhang P, Dayaram T, Yamamoto Y *et al.* Block of C/EBP alpha function by phosphorylation in acute myeloid leukemia with FLT3 activating mutations. *J Exp Med* 2006; **203**: 371–381.
148 Helbling D, Mueller BU, Timchenko NA, Schardt J, Eyer M, Betts DR *et al.* CBFB-SMMHC is correlated with increased calreticulin expression and suppresses the granulocytic differentiation factor CEBPA in AML with inv(16). *Blood* 2005; **106**: 1369–1375.
149 Helbling D, Mueller BU, Timchenko NA, Hagemeijer A, Jotterand M, Meyer-Monard S *et al.* The leukemic fusion gene AML1-MDS1-EVI1 suppresses CEBPA in acute myeloid leukemia by activation of Calreticulin. *Proc Natl Acad Sci USA* 2004; **101**: 13312–13317.
150 Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, Behre G *et al.* AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in t(8;21) myeloid leukemia. *Nat Med* 2001; **7**: 444–451.
151 Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CA, Tielemans D *et al.* Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. *Blood* 2007; **110**: 3706–3714.
152 Perrotti D, Cesi V, Trotta R, Guerzoni C, Santilli G, Campbell K *et al.* BCR-ABL suppresses C/EBPalpha expression through inhibitory action of hnRNP E2. *Nat Genet* 2002; **30**: 48–58.
153 Leroy H, Roumier C, Huyghe P, Biggio V, Fenaux P, Preudhomme C. CEBPA point mutations in hematological malignancies. *Leukemia* 2005; **19**: 329–334.
154 Snaddon J, Smith ML, Neat M, Cambal-Parrales M, Dixon-McIver A, Arch R *et al.* Mutations of CEBPA in acute myeloid leukemia FAB types M1 and M2. *Genes Chromosomes Cancer* 2003; **37**: 72–78.
155 Barjesteh van Waalwijk van Doorn-Khosrovani S, Erpelinck C, Meijer J, van Oosterhoud S, van Putten WL, Valk PJ *et al.* Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. *Hematol J* 2003; **4**: 31–40.
156 Kaeferstein A, Krug U, Tiesmeier J, Aivado M, Faulhaber M, Stadler M *et al.* The emergence of a C/EBPalpha mutation in the clonal evolution of MDS towards secondary AML. *Leukemia* 2003; **17**: 343–349.
157 Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton S, *et al.*, ALFA Group. Favorable prognostic significance of CEBPA mutations in patients with *de novo* acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). *Blood* 2002; **100**: 2717–2723.
158 Gombart AF, Hofmann WK, Kawano S, Takeuchi S, Krug U, Kwok SH *et al.* Mutations in the gene encoding the transcription factor CCAAT/enhancer binding protein alpha in myelodysplastic syndromes and acute myeloid leukemias. *Blood* 2002; **99**: 1332–1340.
159 Frohling S, Schlenk RF, Stolze I, Bihlmayr J, Benner A, Kreitmeier S *et al.* Mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. *J Clin Oncol* 2004; **22**: 624–633.
160 Lin LI, Chen CY, Lin DT, Tsay W, Tang JL, Yeh YC *et al.* Characterization of CEBPA mutations in acute myeloid leukemia: most patients with CEBPA mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. *Clin Cancer Res* 2005; **11**: 1372–1379.
161 Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. *N Engl J Med* 2004; **351**: 2403–2407.
162 Sellick GS, Spendlove HE, Catovsky D, Pritchard-Jones K, Houlston RS. Further evidence that germline CEBPA mutations cause dominant inheritance of acute myeloid leukaemia. *Leukemia* 2005; **19**: 1276–1278.
163 Nanri T, Uike N, Kawakita T, Iwanaga E, Hoshino K, Mitsuya H *et al.* A pedigree harbouring a germ-line N-terminal C/EBP mutation and development of acute myeloblastic leukemia with a somatic C-terminal C/EBP mutation. *Blood* 2006; **108**: 543a abstract 1916.
164 Keilholz U, Menssen HD, Gaiger A, Menke A, Oji Y, Oka Y *et al.* Wilms' tumour gene 1 (WT1) in human neoplasia. *Leukemia* 2005; **19**: 1318–1323.
165 Ellisen LW, Carlesso N, Cheng T, Scadden DT, Haber DA. The Wilms tumor suppressor WT1 directs stage-specific quiescence and differentiation of human hematopoietic progenitor cells. *EMBO J* 2001; **20**: 1897–1909.
166 King-Underwood L, Pritchard-Jones K. Wilms' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance. *Blood* 1998; **91**: 2961–2968.
167 Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F *et al.* Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. *Leukemia* 2007; **21**: 550–551.
168 Mueller BU, Pabst T, Osato M, Asou N, Johansen LM, Minden MD *et al.* Heterozygous PU.1 mutations are associated with acute myeloid leukemia. *Blood* 2002; **100**: 998–1007.
169 Vegesna V, Takeuchi S, Hofmann WK, Ikezoe T, Tavor S, Krug U *et al.* C/EBP-beta, C/EBP-delta, PU.1, AML1 genes: mutational analysis in 381 samples of hematopoietic and solid malignancies. *Leuk Res* 2002; **26**: 451–457.
170 Lamandin C, Sagot C, Roumier C, Lepelley P, De Botton S, Cosson A *et al.* Are PU.1 mutations frequent genetic events in acute myeloid leukemia (AML)? *Blood* 2003; **100**: 4680–4681.
171 Döhner K, Tobis K, Bischof T, Hein S, Schlenk RF, Fröhling S *et al.* Mutation analysis of the transcription factor PU.1 in younger adults (16–60 years) with acute myeloid leukemia: a study of the AML Study Group Ulm (AMLSG ULM). *Blood* 2003; **102**: 3850.

172 Borer RA, Lehner CF, Eppenberger HM, Nigg EA. Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* 1989; **56**: 379–390.
173 Dumbar TS, Gentry GA, Olson MO. Interaction of nucleolar phosphoprotein B23 with nucleic acids. *Biochemistry* 1989; **28**: 9495–9501.
174 Okuda M, Horn HF, Tarapore P, Tokuyama Y, Smulian AG, Chan PK *et al.* Nucleophosmin/B23 is a target of CDK2/cyclinE in centrosome duplication. *Cell* 2000; **103**: 127–140.
175 Bertwistle D, Sugimoto M, Sherr CJ. Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. *Mol Cell Biol* 2004; **24**: 985–996.
176 Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. Nucleophosmin regulates the stability and transcriptional activity of p53. *Nat Cell Biol* 2002; **4**: 529–533.
177 Kurki S, Peltonen K, Latonen L, Kiviharju TM, Ojala PM, Meek D *et al.* Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. *Cancer Cell* 2004; **5**: 465–475.
178 Colombo E, Martinelli P, Zamponi R, Shing DC, Bonetti P, Luzi L *et al.* Delocalization and destabilization of the Arf Tumor suppressor by the Leukemia-associated NPM mutant. *Cancer Res* 2006; **66**: 3044–3050.
179 Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL *et al.* Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 1994; **263**: 1281–1284.
180 Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. *Blood* 1996; **87**: 882–886.
181 Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ *et al.* The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. *Oncogene* 1996; **12**: 265–275.
182 Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L *et al.* Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med* 2005; **352**: 254–266.
183 Dohner K, Schlenk RF, Habdank M, Scholl C, Rucker FG, Corbacioglu A *et al.* Mutant nucleophosmin predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics—interaction with other gene mutations. *Blood* 2005; **106**: 3740–3746.
184 Verhaak RG, Goudswaard CS, van Putten W, Bijl MA, Sanders MA, Hugens 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.
185 Cazzaniga G, Dell'Oro MG, Mecucci C, Giarin E, Masetti R, Rossi V *et al.* Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. *Blood* 2005; **106**: 1419–1422.
186 Chou WC, Tang JL, Lin LI, Yao M, Tsay W, Chen CY *et al.* Nucleophosmin mutations in *de novo* acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. *Cancer Res* 2006; **66**: 3310–3316.
187 Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R *et al.* Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. *Blood* 2005; **106**: 2854–2861.
188 Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP *et al.* Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. *Blood* 2005; **106**: 899–902.
189 Gorello P, Cazzaniga G, Alberti F, Dell'Oro MG, Gottardi E, Specchia G *et al.* Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. *Leukemia* 2006; **20**: 1103–1108.
190 Isobe M, Emanuel BS, Givol D, Oren M, Croce CM. Localization of gene for human p53 tumour antigen to band 17p13. *Nature* 1986; **320**: 84–85.
191 Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell* 1997; **88**: 323–331.
192 Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991; **351**: 453–456.
193 Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 1989; **57**: 1083–1093.
194 Havre PA, Yuan J, Hedrick L, Cho KR, Glazer PM. p53 inactivation by HPV16 E6 results in increased mutagenesis in human cells. *Cancer Res* 1995; **55**: 4420–4424.
195 Bouffler SD, Kemp CJ, Balmain A, Cox R. Spontaneous and ionizing radiation-induced chromosomal abnormalities in p53-deficient mice. *Cancer Res* 1995; **55**: 3883–3889.
196 Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* 1992; **70**: 923–935.
197 Gualberto A, Aldape K, Kozakiewicz K, Tlsty TD. An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control. *Proc Natl Acad Sci USA* 1998; **95**: 5166–5171.
198 Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* 1992; **70**: 937–948.
199 Hsu I, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 1991; **350**: 427–428.
200 Fenaux P, Jonveaux P, Quiquandon I, Lai JL, Pignon JM, Loucheux-Lefebvre MH *et al.* P53 gene mutations in acute myeloid leukemia with 17p monosomy. *Blood* 1991; **78**: 1652–1657.
201 Slingerland JM, Minden MD, Benchimol S. Mutation of the p53 gene in human acute myelogenous leukemia. *Blood* 1991; **77**: 1500–1507.
202 Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994; **54**: 4855–4878.
203 Prokocimer M, Unger R, Rennert HS, Rotter V, Rennert G. Pooled analysis of p53 mutations in hematological malignancies. *Hum Mutat* 1998; **12**: 4–18.
204 Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. *J Clin Oncol* 2001; **19**: 1405–1413.
205 Wattel E, Preudhomme C, Hecquet B, Vanrumbeke M, Quesnel B, Dervite I *et al.* p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. *Blood* 1994; **84**: 3148–3157.
206 Merlat A, Lai JL, Sterkers Y, Demory JL, Bauters F, Preudhomme C *et al.* Therapy-related myelodysplastic syndrome and acute myeloid leukemia with 17p deletion. A report on 25 cases. *Leukemia* 1999; **13**: 250–257.
207 Lai JL, Preudhomme C, Zandecki M, Flactif M, Vanrumbeke M, Lepelley P *et al.* Myelodysplastic syndromes and acute myeloid leukemia with 17p deletion. An entity characterized by specific dysgranulopoiesis and a high incidence of P53 mutations. *Leukemia* 1995; **9**: 370–381.
208 Soenen V, Preudhomme C, Roumier C, Daudignon A, Lai JL, Fenaux P. 17p Deletion in acute myeloid leukemia and myelodysplastic syndrome. Analysis of breakpoints and deleted segments by fluorescence *in situ*. *Blood* 1998; **91**: 1008–1015.
209 Suela J, Alvarez S, Cifuentes F, Largo C, Ferreira BI, Blesa D *et al.* DNA profiling analysis of 100 consecutive *de novo* acute myeloid leukemia cases reveals patterns of genomic instability that affect all cytogenetic risk groups. *Leukemia* 2007; **21**: 1224–1231.
210 Alcalay M, Meani N, Gelmetti V, Fantozzi A, Fagioli M, Orleth A *et al.* Acute myeloid leukemia fusion proteins deregulate genes involved in stem cell maintenance and DNA repair. *J Clin Invest* 2003; **112**: 1751–1761.
211 Matsuno N, Osato M, Yamashita N, Yanagida M, Nanri T, Fukushima T *et al.* Dual mutations in the AML1 and FLT3 genes are associated with leukemogenesis in acute myeloblastic leukemia of the M0 subtype. *Leukemia* 2003; **17**: 2492–2499.
212 Roumier C, Lejeune-Dumoulin S, Renneville A, Goethgeluck AS, Philippe N, Fenaux P *et al.* Cooperation of activating Ras/rtk signal transduction pathway mutations and inactivating myeloid

differentiation gene mutations in M0 AML: a study of 45 patients. *Leukemia* 2006; **20**: 433–436.
213 Huntly BJ, Gilliland DG. Cancer biology: summing up cancer stem cells. *Nature* 2005; **435**: 1169–1170.
214 Li L, Piloto O, Kim KT, Ye Z, Nguyen HB, Yu X *et al.* FLT3/ITD expression increases expansion, survival and entry into cell cycle of human haematopoietic stem/progenitor cells. *Br J Haematol* 2007; **137**: 64–75.
215 Gal H, Amariglio N, Trakhtenbrot L, Jacob-Hirsh J, Margalit O, Avigdor A *et al.* Gene expression profiles of AML derived stem cells; similarity to hematopoietic stem cells. *Leukemia* 2006; **20**: 2147–2154.
216 Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani S, Boer JM *et al.* Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med* 2004; **350**: 1617–1628.
217 Rosenbauer F, Koschmieder S, Steidl U, Tenen DG. Effect of transcription-factor concentrations on leukemic stem cells. *Blood* 2005; **106**: 1519–1524.
218 Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J Exp Med* 2005; **202**: 1599–1611.
219 Scholl C, Bansal D, Dohner K, Eiwen K, Huntly BJ, Lee BH *et al.* The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. *J Clin Invest* 2007; **117**: 1037–1048.
220 Dorrance AM, Liu S, Yuan W, Becknell B, Arnoczky KJ, Guimond M *et al.* MLL partial tandem duplication induces aberrant Hox expression *in vivo* via specific epigenetic alterations. *J Clin Invest* 2006; **116**: 2707–2716.
221 Levis M, Tse KF, Smith BD, Garrett E, Small D. A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. *Blood* 2001; **98**: 885–887.
222 Levis M, Allebach J, Tse KF, Zheng R, Baldwin BR, Smith BD *et al.* A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells *in vitro* and *in vivo*. *Blood* 2002; **99**: 3885–3891.
223 Wadleigh M, DeAngelo DJ, Griffin JD, Stone RM. After chronic myelogenous leukemia: tyrosine kinase inhibitors in other hematologic malignancies. *Blood* 2005; **105**: 22–30.
224 Smith BD, Levis M, Beran M, Giles F, Kantarjian H, Berg K *et al.* Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood* 2004; **103**: 3669–3676.
225 Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E, Nimer SD *et al.* Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood* 2005; **105**: 54–60.
226 O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW *et al.* SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* 2003; **101**: 3597–3605.
227 O'Farrell AM, Foran JM, Fiedler W, Serve H, Paquette RL, Cooper MA *et al.* An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. *Clin Cancer Res* 2003; **9**: 5465–5476.
228 Fiedler W, Mesters R, Tinnefeld H, Loges S, Staib P, Duhrsen U *et al.* A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. *Blood* 2003; **102**: 2763–2767.
229 Fiedler W, Serve H, Dohner H, Schwittay M, Ottmann OG, O'Farrell AM *et al.* A phase I study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. *Blood* 2005; **105**: 986–993.
230 Nanri T, Matsuno N, Kawakita T, Mitsuya H, Asou N. Imatinib mesylate for refractory acute myeloblastic leukemia harbouring inv(16) and a C-KIT exon 8 mutation. *Leukemia* 2005; **19**: 1673–1675.
231 Growney JD, Clark JJ, Adelsperger J, Stone R, Fabbro D, Griffin JD *et al.* Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. *Blood* 2005; **106**: 721–724.
232 Gleixner KV, Mayerhofer M, Aichberger KJ, Derdak S, Sonneck K, Bohm A *et al.* PKC412 inhibits *in vitro* growth of neoplastic human mast cells expressing the D816V-mutated variant of KIT: comparison with AMN107, imatinib, and cladribine (2CdA) and evaluation of cooperative drug effects. *Blood* 2006; **107**: 752–759.
233 Beghini A, Bellini M, Magnani I, Colapietro P, Cairoli R, Morra E *et al.* STI 571 inhibition effect on $KIT^{Asn822Lys}$-mediated signal transduction cascade. *Exp Hematol* 2005; **33**: 682–688.
234 Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2005; **3**: 11–22.
235 Whitman SP, Liu S, Vukosavljevic T, Rush LJ, Yu L, Liu C *et al.* The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. *Blood* 2005; **106**: 345–352.