Driver mutations in acute myeloid leukemia

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Purpose of review
The mutational landscape of acute myeloid leukemia (AML) has revised diagnostic, prognostic, and therapeutic schemata over the past decade. Recurrently mutated AML genes have functional consequences beyond typical oncogene-driven growth and loss of tumor suppresser function.

Recent findings
Large-scale genomic sequencing efforts have mapped the complexity of AML and trials of mutation-based targeted therapy has led to several FDA-approved drugs for mutant-specific AML. However, many recurrent mutations have been identified across a spectrum from clonal hematopoiesis to myelodysplasia to overt AML, such as effectors of DNA methylation, chromatin modifiers, and spliceosomal machinery. The functional effects of these mutations are the basis for substantial discovery.

Summary
Understanding the molecular and pathophysiologic functions of key genes that exert leukemogenic potential is essential towards translating these findings into better treatment for AML.

Keywords
acute myeloid leukemia driver mutations, epigenetics, methylation, splicing, transcription factors

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogenous and complex disease characterized by differentiation blockade and clonal proliferation of hematopoietic stem and progenitor cells (HSPCs) at the expense of normal hematopoiesis. The application of next generation of sequencing technologies has led to the identification of 40–50 genes harbor recurrent somatic mutations in various AML subtypes [1–4]. These discovery studies have led to a greater understanding of AML biology, especially the striking frequency of epigenetic dysregulation in AML pathogenesis. In this review, we will provide an overview of genomic and epigenomic alterations in AML by addressing the role of underlying molecular events, which contribute to AML (Fig. 1). Consequently, our evolving understanding of the molecular basis of AML is refining prognostic schema and leading to new therapeutic approaches.

DRIVER MUTATIONS IN ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia-licensing mutations

NPM1

Mutations in nucleophosmin 1 (*NPM1*) represents the largest of these genetically defined AML groups, constituting ~30% of AMLs. NPM1 is a multifunctional protein involved in histone chaperoning, ribosome biogenesis, centrosome duplication, and the DNA damage response [5]. *NPM1*-mutant AML requires nuclear export of NPM1 to the cytoplasm, and the resultant overexpression of stem cell gene signature including *HOXA*, *HOXB* genes, and *MEIS1* [6▪,7]. A potential explanation of this unique gene regulatory network in *NPM1* mutation AML is that *NPM1*c chaperones PU.1 (SPI1) into the cytoplasm, which releases PU.1-mediated repression of *HOX/MEIS1* [7]. AML with *NPM1* mutations is a distinct entity in the classification and prognostication of myeloid neoplasms and are associated with high remission rates with intensive chemotherapy [8,9▪▪]. AML patients with *NPM1* mutations generally carry a favorable prognosis in the absence of *FLT3-ITD*,

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Curr Opin Hematol 2020, 27:49–57
DOI:10.1097/MOH.0000000000000567

KEY POINTS

- Recurrent mutations in acute myeloid leukemia have functional consequences beyond gain of oncogene/loss of tumor suppressor.
- Mutations, such as NPM1c often are sufficient for transformation.
- Other alleles require cooperating mutations and are frequently found in preleukemic entities, such as myelodysplastic syndrome and clonal hematopoiesis.

or when the *FLT3-ITD* allelic ratio is low [9▪▪] (Table 1). The molecular underpinnings of the favorable prognostic effect of *NPM1c* is unclear, though the observations that *NPM1c* does not occur in clonal hematopoiesis and the infrequent occurrence of *NPM1c* in myelodysplastic syndrome, both suggest that the unique licensing effect of *Npm1c* for transformation carries a shorter lead time to acquire additional mutational hits and may result in less complex clonal architecture.

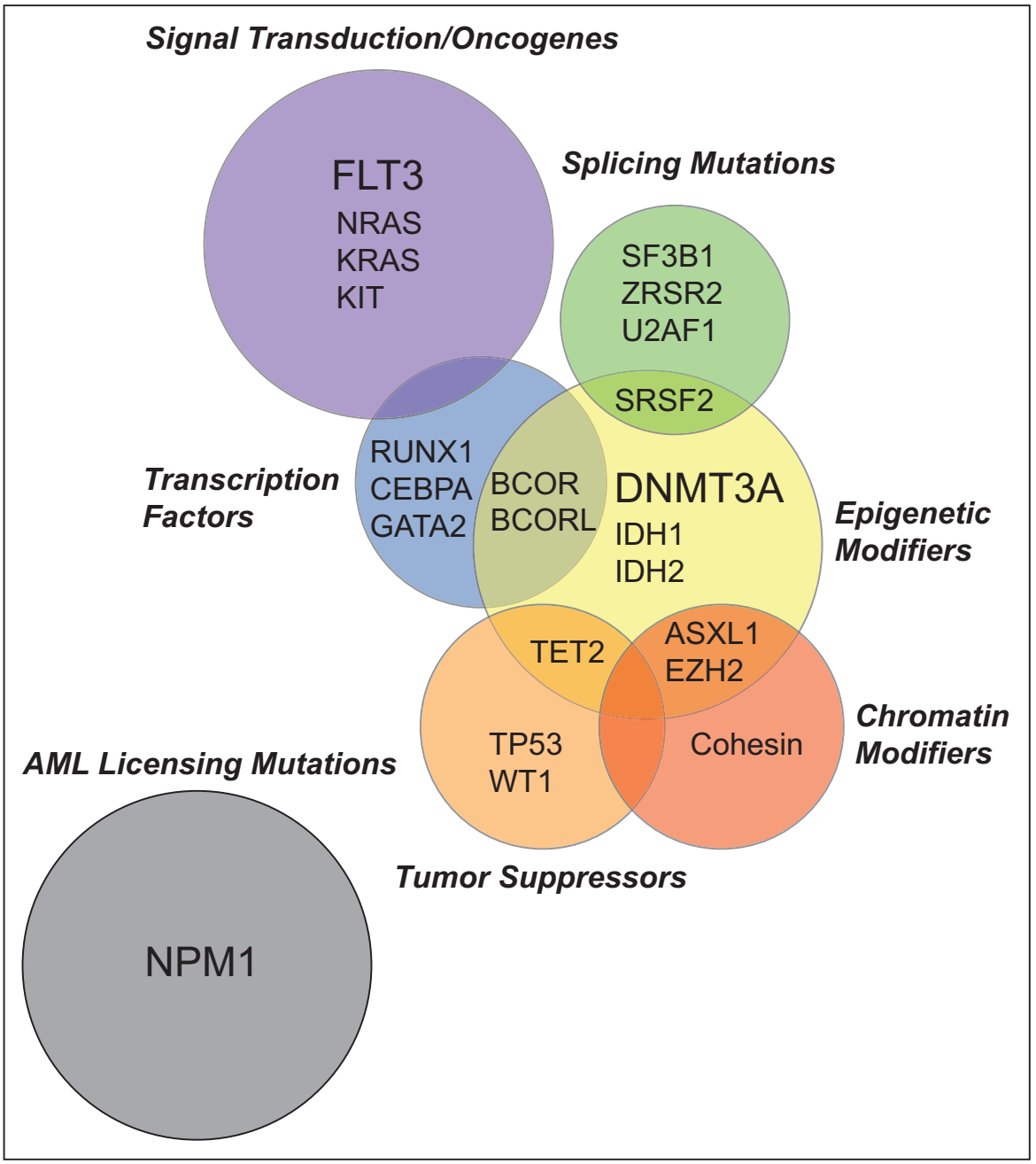

FIGURE 1. Functional overlap of acute myeloid leukemia driver mutations. Driver mutations in AML stratified by mechanistic consequence. Size of circles reflect mutational frequency with three most common mutations in bold. AML, acute myeloid leukemia.

Table 1. The European LeukemiaNET 2017 risk stratification of acute myeloid leukemia

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low} = allelic ratio < 0.5 Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3-ITD*^{high} = allelic ratio > 0.5 Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low} (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* −5 or del(5q); −7; −17/abn(17p) Complex karyotype monosomal karyotype Wild-type *NPM1* and *FLT3-ITD*^{high} Mutated *RUNX1* Mutated *ASXL1* Mutated *TP53*

Data from [9▪▪].

Mutations altering signal transduction

FLT3-ITD and *FLT3-TKD*

Mutations in the transmembrane growth factor receptor fms-related tyrosine kinase 3 (*FLT3*) can occur either as an in-frame internal tandem duplication within the juxtamembrane domain of the receptor (*FLT3-ITD*), seen in ~20 to 25% of AML, or as point mutations most commonly in the activation loop of the tyrosine kinase domain (*FLT3-TKD*), seen in ~7 to 8% of AML patients. These mutations lead to autoactivation of kinase activity and constitutive activation of downstream signaling pathways, including PI3K/AKT/mTOR, RAS/MAPK, and STAT5 [10]. *FLT3-ITD* mutations are frequently accompanied with leukocytosis, high percentage of blasts in the bone marrow and presages a poor prognosis, with most of these patients relapse after chemotherapy and allogeneic-HSCT [11,12,13▪▪]. The poor prognosis is particularly significant in the presence of *DNMT3A*, the absence of *NPM1*, or when a high ITD allele burden is present, and shows inferior overall survival (OS) compared with *FLT3-TKD* or wild-type *FLT3* [2,4] (Table 1). The development of the FLT3 inhibitors represents a paradigm of targeted therapy in AML and have changed clinical practice. There are currently two Food and Drug Administration (FDA)-approved small-molecule-targeted inhibitors (midostaurin and gilteritinib) of FLT3 kinase activity [14▪▪,15▪▪]. Although improved outcomes have been observed with the use of these agents, the magnitude of the effects on survival have only been modest, suggesting either the immense strength of subclonal resistance or the need for agents with better pharmacokinetic properties. There is recent evidence suggesting the measurement of fms-related tyrosine kinase 3 ligand (Flt3L) during induction chemotherapy and follow-up provides prognostic information and can serve as a biomarker with the potential to inform management of these patients [16].

KIT

KIT, also known as CD117, is a transmembrane glycoprotein type III receptor tyrosine kinase. Upon binding of stem cell factor (KIT ligand), the monomeric KIT receptor dimerizes and becomes autophosphorylated at key tyrosine sites and activates downstream signaling pathways (Ras/ERK, PI3K, and JAK/STAT pathways) important for cell proliferation, differentiation, and survival [17]. Gain-of-function mutations in the *KIT* proto-oncogene results in ligand-independent constitutive activation. *KIT* mutations occur in less than 10% of patients, but they are enriched in patients with AML with core-binding factor [t(8;21)/*RUNX1/RUNX1T1*, inv(16)/*CBFB-MYH11*] rearrangements and portend a poorer outcome in this otherwise favorable disease group [18].

NRAS and *KRAS*

The Ras family of small GTPases, *NRAS* and *KRAS*, activate downstream signaling effectors, such as Raf and PI3K, thereby transducing signals from activated growth factor receptors. *NRAS* and *KRAS* encode proteins that accumulate in the active GTP-bound conformation, leading to constitutive activation [19]. These mutations have been reported in ~12% (*NRAS*) and ~5% (*KRAS*) of AML patients [20]. Mutations in epigenetic modifiers (*TET2/IDH/WT1*) often co-occur and cooperate with *NRAS* mutations in AML [4] and show preferential sensitivity to MAPK kinase (MEK) inhibition in mouse models and patient samples [21▪]. Although RAS mutations tend to be mutually exclusive with FLT3 mutations, *N/KRAS* mutations are a common and clinically important mechanism of resistance to *FLT3* inhibitors including gilteritinib and crenolinib [22▪].

Mutations disrupting transcription factor function in acute myeloid leukemia

RUNX1

The master hematopoietic transcription factor Runt-related transcription factor 1 (*RUNX1*) is an

essential regulator of cell lineage specification, proliferation, and differentiation [23]. RUNX1 contains a runt-homology domain (RHD), a protein motif responsible for both DNA-binding and heterodimerization with CBFβ. Somatic mutations and chromosomal rearrangements involving *RUNX1* are relatively common in AML. *RUNX1* mutations occur in approximately 5–15% of all patients with AML and are enriched in intermediate risk (including normal karyotype AML) disease [2,4]. *RUNX1* mutations are mutually exclusive with *NPM1* and *CEBPA* mutations, and are associated with lower CR rates and inferior OS in both younger and older adults with AML [24]. Germline mutations in *RUNX1* are associated with familial platelet disorder with propensity to myeloid malignancy (FPDMM), with a 20–60% estimated rate of transformation to myeloid neoplasm [25].

CEBPA

The lineage master hematopoietic transcription factor CCAAT/enhancer-binding protein α (*CEBPA*) is involved in cell fate decisions including a key role in governing myeloid differentiation [26]. Loss-of-function mutations in *CEBPA* are reported in ∼10% of AML patients and are enriched in younger patients and normal karyotype. *CEBPA* mutations can occur in either the N-terminus, leading to expression of a truncated dominant negative protein, which retains the DNA-binding domain, or in the C-terminus, leading to impaired DNA binding and disrupted protein–protein interactions [27]. Biallelic mutations in *CEBPA* constitute a defined patient subgroup and are associated with a specific gene expression signature with a markedly favorable prognosis in normal karyotype AML owing to disease chemosensitivity [28] (Table 1). Germline mutations in *CEBPA* are associated with autosomal dominant familial AML with near complete penetrance [29].

GATA2

The GATA transcription factor family members, GATA1 and GATA2, play critical roles in hematopoiesis. The *GATA2* gene encodes a zinc-finger transcription factor involved in transcriptional regulation of hematopoietic stem/progenitor cell differentiation [30]. Somatic mutations in *GATA2* are relatively rare in AML, occurring in less than 5% of cases overall, and are described in normal karyotype AML arising in the context of bi-allelic mutations in *CEBPA* [31]. These mutations are most often missense mutations that target the zinc finger domains impairing DNA binding and affecting transcriptional activity. Heterozygous germline mutations in *GATA2* cause a spectrum of disorders with overlapping features and predisposition to MDS and AML [32,33].

Mutations in epigenetic modifiers

DNMT3A

DNA methylation is a key epigenetic modification involved in normal hematopoiesis, which is altered during leukemogenesis. DNA methyltransferase 3A (*DNMT3A*) is a highly conserved member of the DNA methyltransferase family. *DNMT3A* catalyzes *de novo* methylation of cytosine residues in DNA. Mutations in *DNMT3A* are present in ∼30% of AML cases, mostly in AML patients who present with a normal karyotype [3,34]. Mutations include nonsense, frameshift, and missense alterations throughout the open-reading frame, with a significant enrichment (∼40 to 60% of *DNMT3A*) for mutations at codon R882 [3]. This mutation has been shown to exert a dominant-negative effect on the wild-type *DNMT3A* and *DNMT3B* that reduces DNA methylation activity by ∼80% *in vitro* [35,36]. *DNMT3A*-mutant AMLs frequently have co-occurring mutations *NPM1* and *FLT3-ITD* and confer adverse-risk [37], and have been shown to promote anthracycline-resistance through impaired DNA damaging sensing [38]. Clonal hierarchy studies show that mutations in *DNMT3A* are one of the earliest events in leukemogenesis [39], making this an attractive therapeutic target for novel therapeutic approaches. This is underscored by studies showing that the most common mutations seen in preleukemic clonal hematopoiesis are in *DNMT3A* and that these mutations are often initiating events in myeloid malignancies [40–43]. Though important insights into the role of *DNMT3A* mutations in AML have emerged from human/preclinical studies, the fundamental mechanism by which these mutations lead to enhanced AML and increased self-renewal has not been delineated.

TET2

Ten-eleven-translocation (TET) proteins are α-ketoglutarate dependent DNA dioxygenases (TET1–3), which in the presence of oxygen, Fe^{2+} and ascorbic acid, catalyze the successive oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and other oxidation products down to 5-carboxylcytosine (5caC) and promote passive and active DNA demethylation [44–47]. *TET2* inactivation (but not *TET1* or *TET3*) through loss-of-function mutations is a common clonal event in myeloid neoplasms [48,49], indicating that *TET2* functions as a tumor suppressor. Mutations in *TET2* have been identified in ∼20% of patients with AML, and are enriched in

patients with prior MDS or MPN, and has been found to be a variable prognostic indicator [50]. *TET2* mutations co-occur with *NPM1*, *FLT3-ITD*, and *DNMT3A,* and largely mutually exclusive with *IDH1/2* mutations [4]. The majority of the evidence indicates that *TET2* is an early event ('first hit') in the multihit model of leukemogenesis, although additional hits are necessary for further progression. Consistent with this observation, *TET2* mutations are also common in clonal hematopoiesis [51].

IDH1/2 mutations

Neomorphic mutations in the genes encoding isocitrate dehydrogenase 1 and 2 (*IDH1* at R132 or in *IDH2* at R140 or R172) in AML have been shown to lead to the production of the oncometabolite R enantiomer of 2-hydroxyglutratrate (R-2-HG), which inhibits dioxygenases-including TET family of enzymes-by competing with α-ketoglutarate [1,52,53]. The oncometabolite R-2-HG leads to DNA and histone hypermethylation, leading to a repressive chromatin landscape that disrupts cellular differentiation and contributes to leukemogenesis [54,55]. *IDH1/2* mutations are respectively found in ∼5 to 10% and ∼15 to 20% of patients with AML, and are enriched in normal karyotype [56]. The prognostic significance of these mutations is controversial but appears to be influenced by co-mutational status (*NPM1*) and the specific location of the mutation. Ivosidenib and enasidenib are first-in-class, oral, selective inhibitors of the *IDH1* and *IDH2*, respectively, and are FDA-approved for the management of adults with refractory or relapsed AML [57▪,58▪].

ASXL1

Additional sex combs-like 1 (*ASXL1*) is 1 of 3 mammalian homologs of the *Drosophila* additional sex combs (*Asx*), a protein that was originally identified as an enhancer of *trithorax* and *polycomb* genes and plays a critical role in regulating *Hox* gene expression [59]. *ASXL1* forms the polycomb repressive deubiquitinase complex with BRCA1-associated protein 1 (BAP1), which deubiquitinates H2AK119Ub [60], a repressive mark. *ASXL1* mutations promote myeloid transformation, at least in part, through attenuated PRC2-mediated histone H3K27 trimethylation [61], a repressive mark. It is not clear whether mutant-*ASXL1* in myeloid malignancies are loss-of-function, dominant-negative, or gain-of-function mutations, which promote BAP1 deubiquitinase activity. ASXL1 mutations occur in ∼10 to 20% of patients with AML and are enriched in those with underlying myelodysplasia and confer a poor prognosis [62]. These mutations are more common in older patients and coexist with *RUNX1* mutations [63].

EZH2

The enhancer of zeste homolog 2 (*EZH2*) is a histone methyltransferase and functional core subunit of the PRC2, a key epigenetic regulator, which catalyzes the methylation of histone H3 at lysine 27 (H3K27me2/3) [64]. *EZH2* play a critical role in epigenetic regulation during hematopoiesis. *EZH2* mutation exert context-specific and sometimes opposing effects to the development of hematologic malignancies. Oncogenic gain-of-function *EZH2* mutations are reported in lymphoid malignancies. In contrast, loss-of-function *EZH2* mutations resulting in abrogation of histone methyltransferase activity occur in MDS/AML, suggesting that *EZH2* functions as a tumor suppressor for myeloid malignancies [65,66]. These mutations are associated with shorter OS and event-free-survival and are enriched in AML patients with a previous history of MDS/MPN. The effects of loss-of-function *EZH2* mutations on epigenetic and metabolic reprogramming (branch chain amino acid metabolism) may be exploited as potential therapeutic targets [67▪].

BCOR and *BCORL1*

BCL6 corepressor (*BCOR*) and BCL6 corepressor like 1 (*BCORL1*), are key transcription factors and function as components of PRC1.1, a noncanonical PRC1 complex, which monoubiquitinates histone 2A at lysine 119 (H2AK119ub) and mediates transcriptional repression [68▪]. Loss of *Bcor* function results in expansion of myeloid progenitor cells and cooperates with $Kras^{G12D}$ to drive leukemogenesis [69]. Mutations in *BCOR* and *BCORL1* are reported in ∼4% of AML patients with normal karyotype [70] and carriers an unfavorable prognostic significance.

Cohesin complex

The cohesin core complex constitutes a tripartite ring composed of SMC1A, SMC3, and RAD21 that are bound to a HEAT-repeat protein (STAG1 or STAG2). The cohesin complex functions to stabilize sister chromatids during metaphase, stabilize the replication fork, and structure the chromatin of the interphase genome [71]. Recurrent loss-of-function cohesin mutations are heterozygous and mutually exclusive from each other with a reported frequency of 6–12% of AML and are more prevalent in Downs syndrome-associated acute megakaryoblastic leukemia [72] and secondary AML [73]. These mutations are not associated with aneuploidy and are not independently prognostic [73]. Functional

studies in hematopoietic cells have demonstrated that cohesin mutations occur in preleukemic clones [74] and affect transcriptional regulation and lineage priming. This leads to enhanced self-renewal and defective differentiation of HSPCs [75–77]. *STAG2* is commonly mutated across many hematopoietic and solid tumors. A recent study describing the consequences of *Stag2* ablation in the HSPCs demonstrated a specific role for *STAG2* in balancing self-renewal and differentiation in HSPCs, a critical feature of leukemogenesis [78▪].

Mutations in splicing factors

Alternative premRNA splicing is a primary source of diversity in messenger RNA species orchestrated by the macromolecular spliceosome complex [79]. Somatic mutations in the genes encoding splicing factors have been discovered at high frequency in patients with hematologic malignancies, including MDS and AML [80]. The most common mutations occur in *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2* and they tend to be mutually exclusive to one another, suggests synthetic lethal interactions when coexpressed and/or convergent biological effects of these mutations to hyperactivate innate immune signaling [81,82]. These mutations are most common in secondary AML [83]. A more recent study including 1540 patients with AML performed targeted sequencing of 111 genes and cytogenetic analysis and classified 11 subgroups including ~18% of AML patients with mutations in chromatin modifiers and spliceosome genes. The chromatin–spliceosome group was the second largest subgroup and was composed of older patients with lower white blood cell counts, a lower percentage of blasts, decreased responsiveness to chemotherapy, and overall poor survival [4]. A recent study analyzed the transcriptomes of 982 AML patients and found an overlap of mutations between *SRSF2* and *IDH2*. This study demonstrated the synergy between RNA splicing and epigenetic regulation, which is partially because of co-regulation of the gene *INTS3*, a member of the integrator complex [84▪▪]. The deeper understanding of underlying mechanisms through which these mutations promote leukemogenesis are still being investigated, whereas several groups have demonstrated therapeutic targeting using genetic or pharmacologic perturbation of splicing [85–87].

Mutations in tumor-suppressor genes

TP53

The tumor suppressor gene *TP53* encodes for the transcription factor p53 and is the most frequently mutated gene in human cancer. It plays a central role in multiple pathways in response to cellular stress, including cell cycle arrest, senescence and apoptosis [88]. Mutations in *TP53* occur in less than 10% of patients with AML but are enriched in AML cases with genomic instability, including therapy-related, complex karyotype, and relapsed disease. *TP53* mutations are mutually exclusive with mutations in *NPM1*, *RUNX1*, *FLT3-ITD*, and *CEBPA*. The majority of *TP53* mutations are missense mutations and occur in the DNA-binding domain. A recent study has shown that missense *TP53* mutation in myeloid malignancies do not lead to neomorphic gain-of-function activities but instead drive leukemogenesis through a dominant negative effect [89▪]. The resulting loss of activity of p53 favors genomic instability and resistance to chemotherapy. AML patients with *TP53* mutations have been associated with a poor response to chemotherapy and dismal overall survival rates (median of 5–9 months) [90].

WT1

Wilm's tumor 1 (*WT1*) is a zinger finger transcription factor that is mutated in less than 10% of patients with AML and the wild-type WT1 protein is often overexpressed in AML [91]. Loss-of-function mutations in *WT1* led to marked reduction in 5hmC levels and a defect in hematopoietic differentiation, similar to that observed in *TET2* mutation [92]. WT1 physically interacts and recruits TET2 to WT1-target genes to activate their expression [93]. Mutations in *WT1* are mutually exclusive with *TET2* and *IDH1/2* mutations suggesting that these genes function in the same epigenetic pathway. These mutations are associated with younger age and adverse prognostic significance likely secondary to chemoresistance [94].

CONCLUSION

The heterogeneity of the genetic landscape of AML hallmarks the 40-year old notion of clonal evolution in cancer proposed by Peter Nowell. These genetic events have variance in their strength of leukemic drive and disease constitution is a reflection of either few strong drivers (i.e. *NPM1*) or serial acquisition of mutations of additive lower tumorigenic potential. Co-mutational interaction also has a major influence that is challenging to decode. For example, while *DNMT3A* and *NPM1* mutations often co-occur, the presence of an *NRAS* mutation as compared with a *FLT3-ITD* have dramatically different effects on the prognosis and chemosensitivity of the resultant disease.

The toolbox of clinical leukemia physicians has recently been augmented by targeted agents with

inhibitors against *FLT3* and *IDH1/2*. Although both have improved outcomes, the response has not been akin to inhibitors of onco-fusion proteins, such as *BCR-ABL* with Imatinib. One challenge ahead is how to handle the complexity and multigenic nature of AML. Better still, as the detection of many of the disease alleles are found in clonal hematopoiesis, can we identify indications for earlier intervention, most notably in clonal cytopenia of undetermined significance. These targeted agents may find use in, as Peter Nowell once urged, 'controlling the evolutionary process in tumors before it reaches the late stage'.

Acknowledgements

This work was supported by a National Cancer Institute career development grant K08 CA215317 (A.D.V), the William Raveis Charitable Fund Fellowship of the Damon Runyon Cancer Research Foundation (DRG 117–15) (A.D.V), and an Evans MDS Young Investigator grant from the Edward P. Evans 35 Foundation (A.D.V). A.K. is supported by the VeloSano Catalyst Award. R.L.L. was supported by National Cancer Institute awards R35 CA197594-01A1 (R.L.L.), R01 CA216421 (R.L.L.), and PS-OC U54 CA143869-05 (R.L.L.) and a grant from Leukemia & Lymphoma Society Translational Research Foundation 6499-17 (R.L.L.).

Financial support and sponsorship

None.

Conflicts of interest

A.D.V. received travel support from Mission Bio and is on the Editorial Advisory Board of Hematology News. R.L.L. is on the supervisory board of QIAGEN and is a scientific advisor to Loxo, Imago, C4 Therapeutics, and Isoplexis. He receives research support from and consulted for Celgene and Roche and has consulted for Janssen, Astellas, Morphosys, and Novartis. He has received honoraria from Roche, Lilly, and Amgen for invited lectures and from Gilead for grant reviews. A.K. has no competing interests to disclose.

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