Unraveling the molecular pathogenesis of acute myeloid leukemia with a normal karyotype

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A B S T R A C T

Acute myeloid leukemia (AML) is the most common myeloid malignancy and the most common acute leukemia in adults with an incidence of 3-4 cases per 100,000 people. Clinical presentation is relatively uniform and principally secondary to hematopoietic failure; however, the disease is highly heterogeneous at the molecular level. Steady progress in deciphering this heterogeneity has been made over the last few decades and this has accelerated dramatically as a consequence of recent advances in genomics. Our understanding of all subtypes of AML has benefited from these advances, but the particular beneficiary has been AML with a normal karyotype (AML-NK), the largest subgroup, representing 45% of all cases. Initially defined by the absence of recurrent chromosomal abnormalities, AML-NK as a group has an intermediate prognosis. However, it is now clear that AML-NK hides within it an extensive degree of diversity. In fact, at least 23 genes are significantly mutated in AML-NK and individual cases arise as a result of different mutational combinations, creating an almost limitless number of permutations. Nevertheless, as well as presenting direct drug targets, individual mutations can impart their own distinct molecular signatures, which in turn influence the clinical features, therapeutic vulnerabilities and prognosis of their cognate leukemias.

Learning goals

At the conclusion of this activity, participants should:

- recognize the spectrum and molecular groups of mutations driving AML-NK and related myeloid neoplasms;
- appreciate the pre-clinical clonal evolution and subclonal structure of AML-NK;
- understand the known molecular effects of the most common mutations and how these promote leukemogenesis alone and in collaboration;
- identify the prognostic impact and therapeutic implications of particular mutations.

Introduction

With an incidence of 3-4 cases per 100,000 people, acute myeloid leukemia (AML) is the commonest myeloid malignancy and the commonest acute leukemia in adults.[1] The clinical presentation of AML is relatively uniform and principally secondary to hematopoietic failure; however, the disease is highly heterogeneous at the molecular level. Steady progress in deciphering this heterogeneity has been made over the last few decades[2-5] and this accelerated dramatically as a consequence of recent advances in genomics.[6-8] Our understanding of all subtypes of AML has benefited from these advances, but the particular beneficiary has been AML with a normal karyotype (AML-NK), the largest subgroup representing 45% of all cases. Initially defined by the absence of recurrent chromosomal abnormalities, AML-NK as a group has an intermediate prognosis.[9] However, it is now clear that AML-NK hides within it an extensive degree of diversity. In fact, at least 23 genes are significantly mutated in AML-NK and individual cases arise as a result of different mutational combinations, creating an almost limitless number of permutations.[8] Nevertheless, as well as presenting direct drug targets, individual mutations can impart their own distinct molecular signatures,[8,10-12] which in turn influence the clinical features,[13-15] therapeutic vulnerabilities[16-19] and prognosis[9,20-23] of their cognate leukemias.

Genetic mutations in AML-NK and related myeloid neoplasms

The cellularity, morphology and surface phenotype of AML reveal the two important processes commandeered by leukemogenesis, namely *block of differentiation* and *uncontrolled proliferation*. In AML with recurrent cytogenetic abnormalities, the former can be primarily attributed to mutations such as the fusion genes RUNX1-RUNX1T1 or CBFB-MYH11, which disrupt hematopoietic transcription factors (Class II mutations), and the latter to proliferative mutations such as those affecting FLT3 and RAS genes (Class I).[24] However, whilst its phenotype may not be noticeably different, the mutations found in AML-NK are less easy to categorize into one or other class, alluding to the fact that the leukemic phenotype relies on the synthesis of complimentary molecular effects. Nonetheless, AML-NK mutations can be grouped into classes according to their gene

family or their anticipated molecular consequences (Figure 1), bearing in mind that their particular roles in leukemogenesis are understood to very different degrees. When examining the patterns of these mutations, it becomes clear that certain mutations co-occur regularly in the same AML (e.g. *NPM1* and *FLT3*), whilst others almost never co-occur (e.g. *NPM1* and *ASXL1*). When these observations are combined with what we know about the function of individual mutations, it becomes apparent that mutations that co-occur more often than expected by chance (co-occurrence) collaborate with each other in leukemogenesis. By contrast, mutations with similar function do not co-occur or do so less often than expected by chance (mutual exclusivity).

Another attribute of AML-associated mutations, which has important therapeutic implications, is their hierarchical position in the clonal evolution of the disease. Once again, there is good evidence that leukemia-associated translocations are likely to be initiating or "founder" events[25,26] and that proliferative mutations are usually acquired late in disease evolution.[27] However, until recently, the clonal hierarchy of the many mutations identified in AML-NK was poorly understood. The finding that *DNMT3A* mutations were consistently stable through the course of AML[28] and that they can be present in pre-leukemic hematopoietic stem cells (HSCs)[29] indicated that these mutations were founder events. Similarly, *TET2* mutations had been identified in elderly individuals with clonal hematopoiesis but without any hematologic abnormalities.[30] Subsequent studies have now shown that AML and related myeloid neoplasms are often preceded, probably by many years or even decades, by clonal hemopoiesis driven most commonly by mutations in *DNMT3A*, *TET2*, *JAK2* and *ASXL1*, a phenomenon that becomes more frequent with age[31-33] [age-related clonal hemopoiesis (ARCH)] and is probably present in the majority of persons aged over 90 years.[34]

These and other relevant findings propose that whilst Darwinian selection underlies the clonal evolution of AML, the speed with which the sufficient set of leukemogenic mutations is acquired (and by extension, the likelihood of AML developing in a person's lifetime) is influenced by the ability of individual mutations to generate large numbers of clonal cells susceptible to the acquisition of subsequent mutations[35] (Figure 2). This model also provides an explanation for the frequently subclonal nature of AML, as the large number of susceptible cells required to make onward progression likely, invites the acquisition of different mutations in different cells, each with a distinct impact on growth kinetics. Viewed from a different perspective, the model also explains the extensive overlap in the mutational spectra of AML-NK with those of myeloid neoplasms such as the myelodysplastic syndromes (MDS)[36] and the myeloproliferative disorders (MPD),[37] with these disorders representing alternate or overlapping evolutionary routes to neoplasia, as reflected, for example, by the increased proportion of elderly patients with *de novo* AML-NK carrying MDS-associated mutations.[38] The

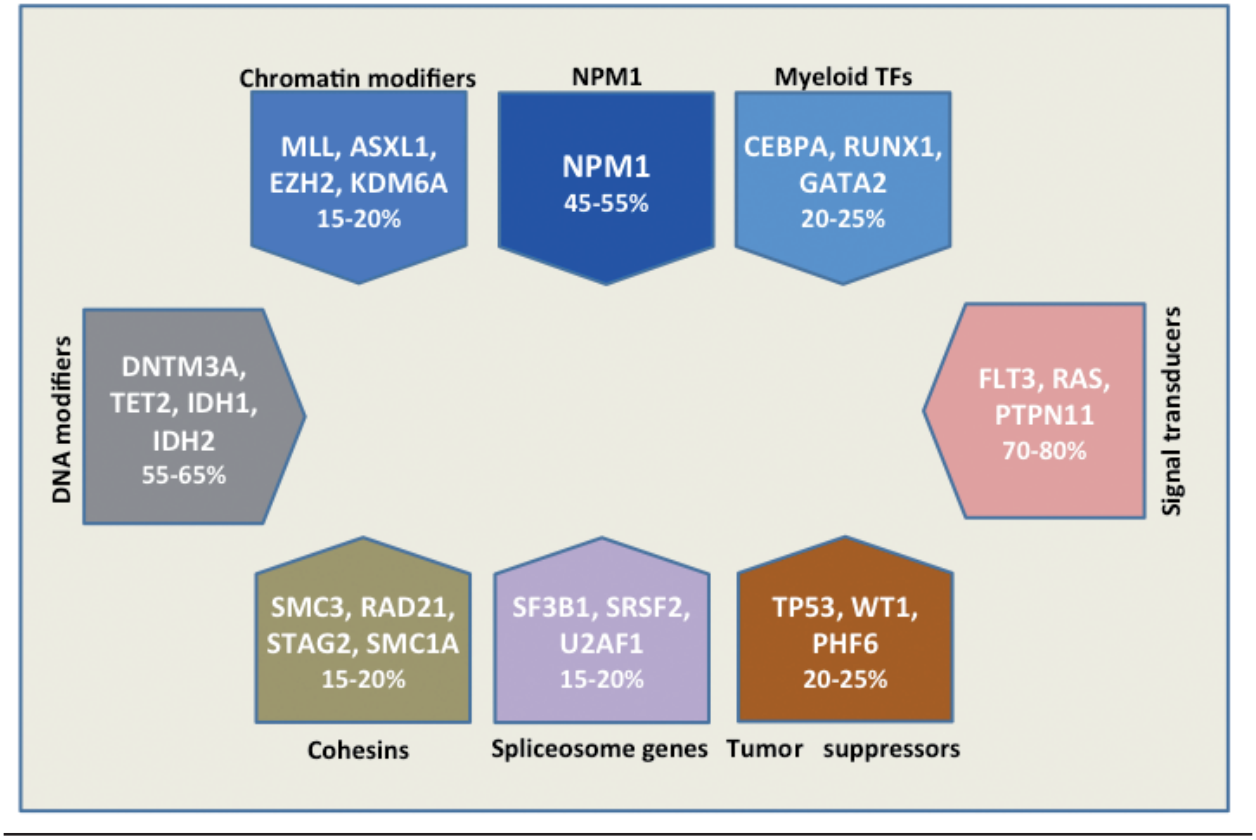

Figure 1. Mutation classes in acute myeloid leukemia with a normal karyotype and their frequencies.

alternative model, that somatic mutations increase the rate of mutagenesis, is not supported by evidence that AML-NK carries only a small total number of coding mutations (average n=13, of which 5 are "drivers"), which is not significantly different to that of individual normal HSCs from a person of a similar age.[8]

The above observations are intriguing and reveal many interesting facets of AML-NK pathogenesis. A deeper understanding of their basis, and in turn of the biology and therapy of this disease, can be gained by examining what is known about the effects of individual genes and genetic pathways corrupted in AML-NK and related malignancies. The most important mutations are discussed below, with greater emphasis given to those that are more common or clinically relevant.

Genes involved in DNA modifications

Genomic DNA is packaged into a macromolecular structure known as chromatin, which is composed of DNA, proteins and RNA. Eukaryotic chromatin carries chemical modifications that are added to either DNA or chromatin proteins. These epigenetic modifications vary across the genome and their presence or absence regulates local gene transcription. The addition and removal of these chemical marks by proteins referred to collectively as epigenetic modifiers, is a closely regulated process whose disruption plays a role in the pathogenesis of many cancers, including AML.[39] Cytosine methylation is the most important direct modification of DNA and can be found throughout mammalian genomes, with the exception of short regions rich in CpG dinucleotides and known as CpG islands (CGIs).[40] Most CGIs are located within gene promoters and their methylation provides an important means for controlling transcription, with increased methylation associated with reduced gene expression and *vice versa*.[41] Several genes are involved in the process of CpG methylation (Figure 3) and, strikingly, the majority of cases of AML-NK harbor mutations in one or more of these.

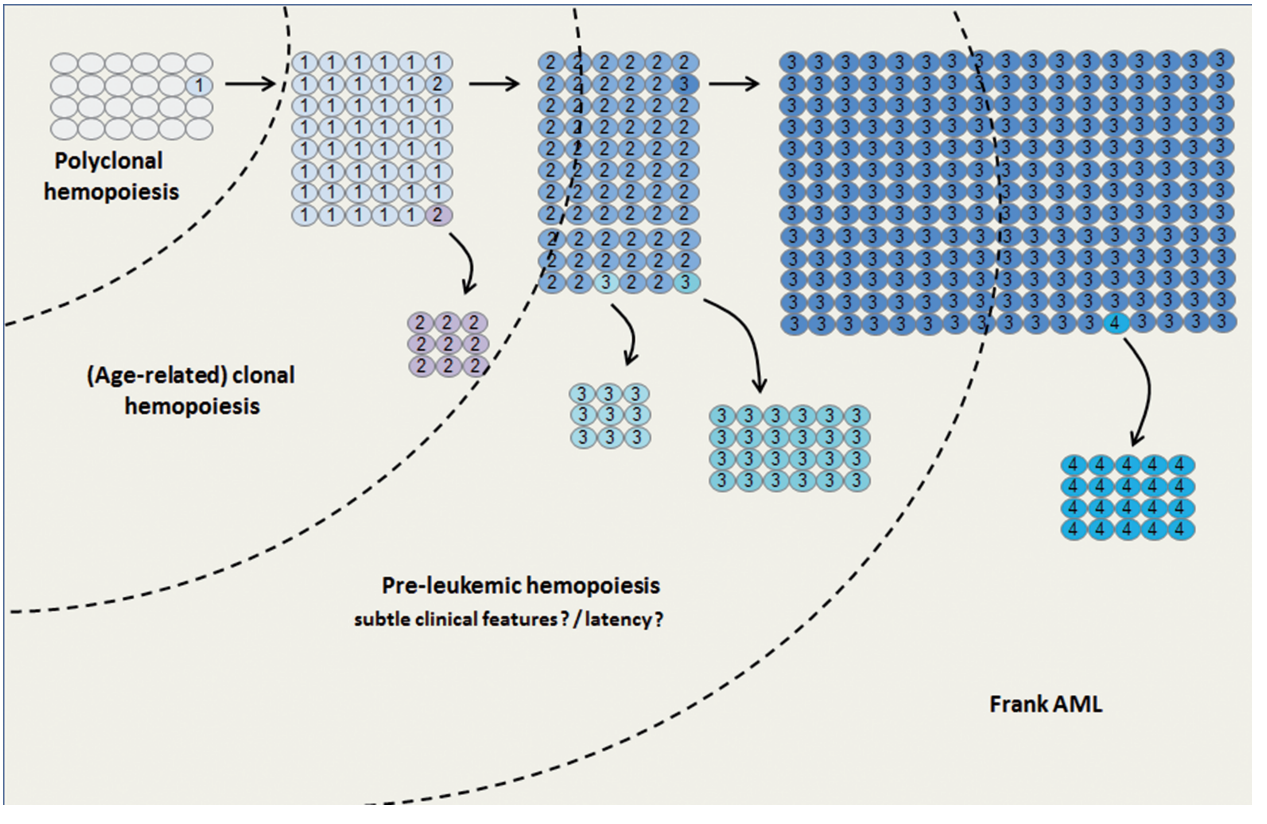

Figure 2. Proposed kinetics for the clonal evolution of acute myeloid leukemia. Hematopoietic stem/progenitor cells harboring the same set of driver mutations are depicted in the same color and the number of such mutations indicated. The acquisition of one of a limited number of founder mutations such as those affecting *DNMT3A, TET2 or ASXL1* leads to the establishment of a founder clone of stem cells that drive clonal hemopoiesis in the absence of detectable clinical abnormalities. As this clone of cells expands, the acquisition of a second mutation in a collaborating gene becomes more likely, and when it does occur, a new clone is founded harboring both mutations. This process continues, selecting cell clones along Darwinian principles until a set of mutations occurs that imparts on its host cells an acute myeloid leukemia phenotype. The total number of mutations required for this will vary depending on their potency and the extent to which they collaborate with each other. Importantly, whilst mutation acquisition is stochastic, the time to leukemic progression is likely to be shorter for mutations that expand cell clones the fastest as this increases the likelihood of acquiring subsequent mutations ("opportunity" hypothesis[35]). Along the path of leukemic evolution, one or more independent subclones of different sizes can develop and these will form part of the leukemic bulk at the time of diagnosis.

DNMT3A mutations

The DNA methyltransferase family includes DNMT1, DNMT3A, DNMT3B, and DNMT3L. DNMT3A and DNMT3B are *de novo* DNA methylases responsible for the establishment of genome-wide DNA methylation patterns during development and play important roles in HSC differentiation.[42,43] *DNMT3A* mutations, identified through whole genome and exome sequencing of primary AML samples,[44-46] have been shown to occur in more than 30% of cases of AML-NK.[18,47] Two types of DNMT3A mutations are seen: heterozygous missense mutations affecting codon R882 (60%) and mutations dispersed through the length of the gene which are usually biallelic and often lead to premature chain termination (40%).[8] The mutations are associated with changes in gene expression and DNA methylation patterns, whilst DNMT3A mutants displayed a reduced affinity to histone H3 *in vitro*.[46] The effect of these mutations on DNMT3A protein function has not been fully defined, but there is evidence that they are associated with loss of methylase activity,[48] and in the case of R882 mutations, that they do this is a dominant negative manner (i.e. they inhibit wild-type DNMT3A)[49] offering an explanation for why R882 mutations are almost always heterozygous. Methylation changes associated with loss of DNMT3A function preferentially affect non-CGI regions[50] and occur throughout the genome,[50,51] although there is evidence that certain genes involved in leukemogenesis, such as the *HOX* genes, are hypomethylated in AML-NK with mutant *DNMT3A*.[50] However, it is most probable that rather than any individual effect of disrupted DNMT3A function, it is the sum total of all its effects that drives clonal outgrowth and promotes the development of AML and related myeloid neoplasms which also frequently carry *DNMT3A* mutations.[37,52] With *DNMT3A* as the founding mutation, the phenotype of the eventual malignancy is to a significant extent determined by the nature of secondary collaborating mutations,[35] and this can also be said for mutations affecting *TET2*. In AML, *DNMT3A* mutations probably have an adverse overall impact,[47,53] although this effect is more significant for older patients,[18] and can be at least partially mitigated by the choice or dose of anthracycline used.[19, 22]

TET2 mutations

The Ten-eleven Translocation dioxygenases (TET1, TET2 and TET3) are responsible for the modification of methyl marks in methylcytosine nucleotides of DNA. Their enzymatic activity is dependent on iron and 2-ketoglutarate (also known as 2-oxoglutarate) and acts to convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC)[54] (Figure 3). The precise role of 5hmC is not fully understood, but its introduction may change chromatin structure by recruiting selective 5hmC-binding proteins and displacing chromatin-modifying complexes recruited by 5mC, thus altering gene transcription.[55] Also conversion of 5mC to 5hmC may provide a means for removal of the methyl mark and restoration of the unmodified cytosine in DNA.[56]

Mutations affecting *TET2* disrupt its enzymatic function, are found in up to 27% of patients with AML-NK, can affect one or both alleles of the gene[57] and are associated with a worse prognosis.[58,59] Myeloid malignancies with mutant *TET2* have decreased global levels of 5hmC, whilst disruption of *Tet2* in mouse hematopoietic progenitors promotes myeloid differentiation[60] and enhances HSC self-renewal.[61] The changes in 5hmC associated with mutant *TET2*, are likely to vary throughout the genome in a similar way to those of mutant *DNMT3A*, but have not yet been fully deciphered. The recent development of technologies for the quantitative analysis of 5hmC at single base resolution promises to provide new insights into the molecular effects of these mutations.[62]

IDH1 and IDH2 mutations

IDH1 and IDH2 are the cytosolic and mitochondrial isoenzymes of isocitrate dehydrogenase, a key enzyme in the citric acid (Krebs) cycle. Mutations in *IDH1* were initially isolated in colonic adenocarcinoma and then glioblastomas, before they were identified in AML, where mutations in *IDH2* were also identified.[6,63] Overall, mutations in *IDH1* (*IDH1* R132H) are found in 10%-16% and those in *IDH2* (*IDH2* R140Q and R172H) in 10%-19% of AML-NK.[22,64-66] The role of IDH enzymes in the citric acid cycle is to convert isocitrate to alpha ketoglutarate (α-KG), while reducing NADP+ to NADPH (Figure 3). The mutant forms of IDH1/2 change their enzymatic activity leading to the conversion of α-KG to 2-hydroxyglutarate (2-HG),[67] an oncometabolite that inhibits enzymes which use α-KG as a substrate, including TET2 and the Jumonji-C domain-containing (JMJC) family of histone lysine demethylases leading to impaired hematopoietic differentiation.[68,69] Mice expressing *Idh1* R132H conditionally in the hematopoietic compartment developed splenomegaly due to extramedullary hematopoiesis and expansion of the multipotent progenitor compartment.[70] However, as with *Dnmt3a*[43] and *Tet2*[61] knock-out mice, *Idh1* R132H mice did not go on to develop AML.

Reports on the prognostic impact of *IDH1* and *IDH2* mutations are conflicting[71] and may be compounded by their significant association with *NPM1*[22] and mutual exclusivity with *TET2*[68] mutations. Nevertheless, it is probable that *IDH2*^{R172H} mutations impart a poorer than average prognosis.[72,73] Interestingly, a neighboring single nucleotide polymorphism appears to have an adverse impact on the prognosis of IDH1 mutant AML. With regards to treatment, early data describing the effects of IDH1/2 inhibitors[74,75] promise to open new therapeutic avenues for patients with these mutations.[76] Also, human AML cells carrying *IDH1* or *IDH2* mutations were recently shown to be dependent on BCL2 for their survival and were sensitive to treatment with a BCL2 inhibitor.[77]

NPM1 mutations

AML-associated mutations in *NPM1*, the gene for nucleophosmin, were identified through the fact that they lead to mislocalization of this nucleolar/nuclear protein to the cytoplasm,[78] hence their annotation as "*NPM1c*". *NPM1c* mutations occur in 40%-55% of AML-NK and confer a favorable prognosis, although this is annulled when they co-exist with *FLT3-ITD*.[21,79-82] Unlike many of the other mutations discussed here, *NPM1c* mutations rarely occur in neoplasms other than AML-NK[83] and, despite being the most common mutations in this disease, were not found to drive ARCH, placing them in the role of "gatekeeper" for the development of AML-NK.[34] In fact,

the three most common mutations in AML-NK, namely those affecting *DNMT3A*, *NPM1* and *FLT3*, are acquired in this order and co-exist in up to 10% of AML-NK, often in the absence of any other known driver mutations.[8]

Cases of AML-NK carrying *NPM1c* mutations exhibit overexpression of anterior homeobox genes with an established pedigree in leukemogenesis, including *HOXA5*, *HOXA9 and HOXA10*.[10] In fact, mice expressing the human mutation from the endogenous locus also overexpress these genes and prime HSCs to transformation by additional proliferative mutations.[12] Also, mice carrying both *NPM1c* and *FLT3-ITD* develop aggressive leukemia within 4-6 weeks from birth, emphasizing the extraordinary complementarity of these two types of mutation.[84,85] However, whilst the mutant nucleophosmin was shown to displace a number of other proteins to the cytoplasm, it is not known which of its many interactions drive its molecular and cellular effects.[86]

Mutations in chromatin modifiers

Post-translational modifications of histone tails play a central role in the regulation of gene expression. Mutations in genes responsible for these modifications and others influencing their function are common in hematopoietic and other cancers. The longest known of these affect the *MLL* (*KMT2A*) gene, which codes for a

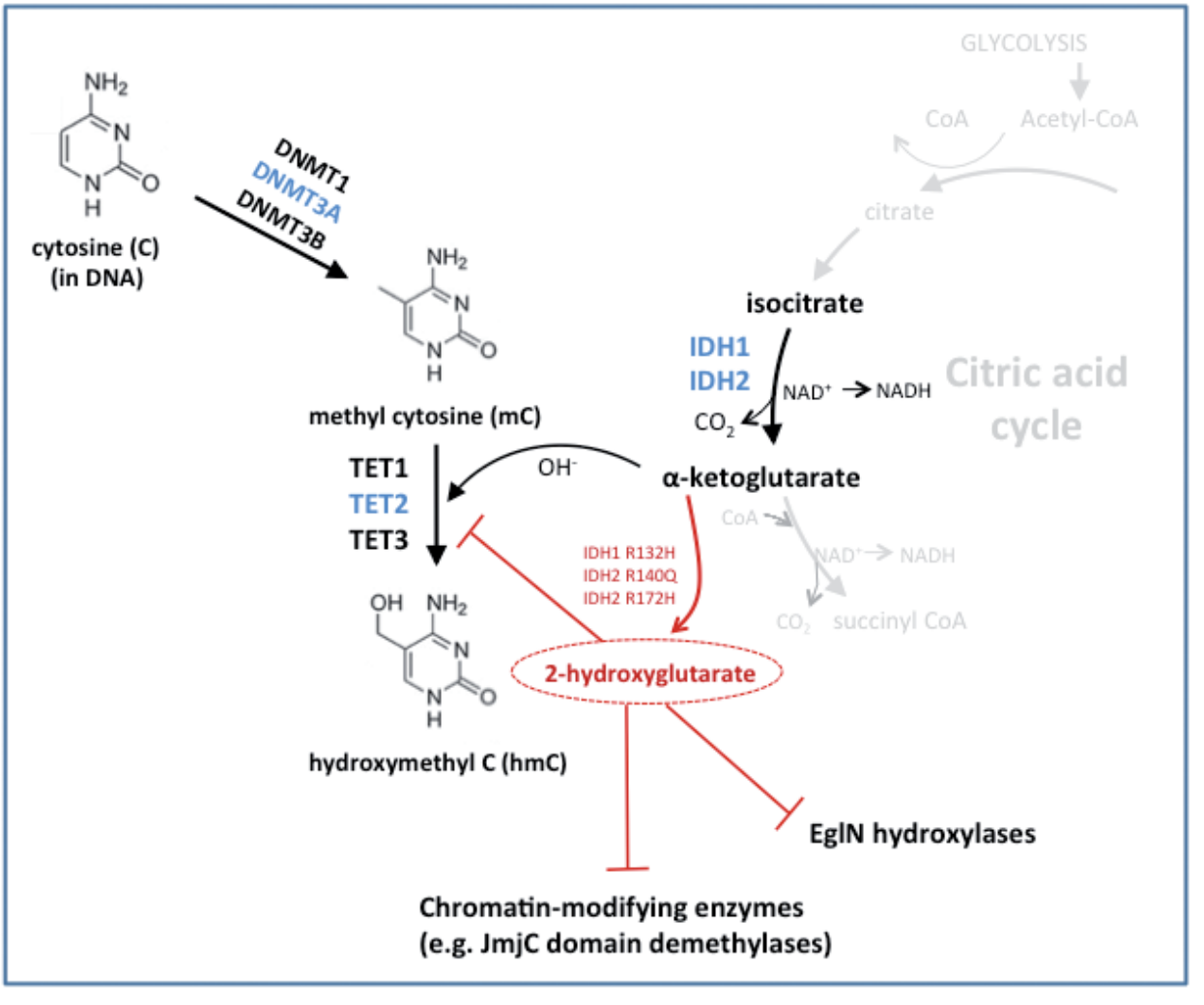

Figure 3. Enzymes involved in cytosine modification and acute myeloid leukemia with a normal karyotype. Methylation of cytosines in DNA is an important node for the control of gene expression. Cytosine (C) is methylated by DNA methylases to methylcytosine (mC) and can then be further modified to hydroxymethylcytosine (hmC) by TET deoxygenases. The latter reaction uses α-ketoglutarate as substrate and this is generated from isocitrate by the action of isocitrate dehydrogenases (IDH1 in the cytosol and IDH2 in the mitochondria) within the citric acid cycle. These biochemical reactions are frequently corrupted in acute myeloid leukemia (AML) with normal karyotype as a result of loss-of-function mutations in the *DNMT3A* and *TET2* genes or gain of function mutations in *IDH1* and *IDH2*. Particular *IDH1* and *IDH2* mutations (red text) lead to the generation of the oncometabolite 2-hydroxyglutarate, which exerts its oncogenic effects through the inhibition of TET dioxygenases (including TET2), chromatin modifying enzymes (such as JmjC domain containing demethylases) and EGLN prolyl hydroxylases, a family of α-ketoglutarate-dependent dioxygenases that regulate the activity of hypoxia-inducible factor (HIF). Genes/proteins subject to recurrent somatic mutations in AML are indicated in blue text.

histone methyltransferase and is involved in chromosomal translocations and partial tandem duplications (PTDs) in AML.[87] *MLL*-PTDs mediate overexpression of *HOX* genes including *HOXA7* and *HOXA9*,[11] occur in 8%-10% of AML-NK,[88] and are associated with worse clinical outcomes.[22,88] More recently, mutations in another gene in this category, *ASXL1*, were identified in diverse myeloid malignancies.[89,90] These mutations occur in up to 6%-25% of AML, their incidence increases with age, and they are associated with a poor prognosis.[90-92] ASXL1 interacts with several proteins including LSD1 and RARa,[93] and leukemia-associated mutations result in loss of protein expression and an associated reduction in histone H3 lysine 27 methylation, a repressive mark normally introduced by the chromatin modifier PRC2.[94] Mouse models with reduced *Asxl1* expression developed hematopoietic defects including MDS/MPD that accelerated in the presence of mutant *NrasG12D* mutations, which co-occur with *ASXL1* in human myeloid malignancies.[94]

Mutations in myeloid transcription factors

Somatic mutations affecting hematopoietic transcription factors occur in up to 20% of AML-NK and most commonly affect the genes *CEBPA*, *RUNX1* and *GATA2*. Also germ-line mutations in each of these genes are associated with familial MDS/AML syndromes.[95]

C/EBPα is an important hematopoietic transcription factor that controls differentiation of myeloid cells to mature granulocytes.[96] Mutations in *CEBPA* are found in 10%-15% of patients with AML-NK[4,97] and can affect one or both alleles of the gene.[97] The mutant (truncated) proteins acted in a dominant negative fashion to inhibit wild-type C/EBPα from binding DNA resulting in a failure of myeloid differentiation.[4] Single *CEBPA* mutant cases had a better than average prognosis in some series;[98] however, only double mutations were found to be independent favorable prognostic predictors and these are associated with long-term survivals beyond 60%.[98-100] Also, double *CEBPA* mutations were found to frequently co-occur with mutations in *GATA2*,[101] a hematopoietic transcription factor known to interact directly with C/EBPα,[102] whilst single mutations can co-occur with mutant *NPM1* and *FLT3*.[98,100]

RUNX1 is a master hematopoietic regulator[103] which, as well as participating in the *RUNX1-RUNX1T1* (AML1-ETO) fusion gene in AML associated with t(8;21),[104] is also a target of substitutions and indels in approximately 5% of *de novo* AML-NK.[22,105] These mutations usually lead to protein truncation, frequently co-occur with trisomy of chromosome 13 and *MLL*-PTD mutations, and are associated with distinct gene mRNA and microRNA signatures[106,107] and a worse overall prognosis.[106-108]

Mutations in tumor suppressor genes

WT1 (Wilm Tumor 1) loss-of-function mutations occur in 10% of AML-NK and are associated with a worse than average prognosis.[109-111] Unlike other tumor suppressor gene mutations, *WT1* mutations usually affect only one allele in AML-NK, are more common in AML with biallelic *CEBPA* mutations (where they do not have an adverse prognostic impact),[112] and rarely co-occur with *TET2* or *IDH1/2* mutations,[22,112] suggesting they may have similar molecular effects to these. In fact, the WT1 protein physically interacts with TET2, and reduction of WT1 levels was recently shown to reduce 5hmC levels, as is seen with *TET2* mutations.[113] Interestingly *WT1* mutations are lost in relapsed disease in approximately 1 in 3 cases and occur more commonly in female patients.[112]

The X-linked genes *BCOR* (BCL6 co-repressor), *BCORL1* (BCOR-like 1) and *PHF6* [plant homeodomain (PHD) Finger Protein 6] are affected by what appear to be loss-of-function mutations in AML-NK. *BCOR* mutations in AML are similar to germline BCOR *BCOR* mutations found in the X-linked dominant oculo-facio-cardio-dental syndrome and are associated with reduced BCOR mRNA and absence of the full-length protein. AML *BCOR* mutations were present in 3.8% of AML-NK and frequently associated with mutant *DNMT3A*, whilst they were virtually mutually exclusive of NPM1 mutations and associated with inferior patient outcomes.[114] Mutations in the related gene *BCORL1* were also identified in around 6% of *de novo* AML patients[115] and in 7%-9% of other myeloid malignancies, including CMML and post-MDS AML.[116] *BCORL1* mutations were associated with *RUNX1* and *DNMT3A* mutations.[116] *PHF6* mutations are present in 20% of T-cell acute lymphoblastic leukemia (T-ALL)[117] and in approximately 3% of adult AML.[118] In both T-ALL and AML mutations are inactivating (frameshift or nonsense) or affect the second PHD-like domain of the protein,[117,118] and are almost exclusively found in male patients (unlike *BCOR* and *BCORL1* that are found in males and females). *PHF6* mutations in AML were associated with a worse prognosis in univariate but not multivariate analysis.[22]

Mutations affecting *TP53* are found in up to 15% of *de novo* AML, are frequently biallelic and strongly associated with a complex karyotype, but are only seen in 1%-2% of AML-NK.[8,119] They behave as a distinct subgroup of AML that displays primary resistance to therapy and a very poor prognosis.[120] Recently, *TP53* mutations associated with therapy-related AML were shown to have been present prior to the administration of chemotherapy, suggesting that a pre-leukemic subclone harboring a TP53 mutation gained a survival advantage after chemotherapy, rather than the chemotherapy causing the mutation.[121] This suggests that therapy-related and *de novo* AML associated with *TP53* mutations may have more in common than was previously thought.

Mutations in cohesin genes

Cohesin is a molecular complex composed of four major subunits, SMC1A, SMC3, RAD21 and STAG1/2, with a key role in sister chromatid exchange[122] and important functions in gene expression and DNA damage repair.[123] Mutations in cohesin genes were found in 5%-13% of AML, are almost always mutually exclusive of each other, and are significantly associated with *NPM1c* and *RUNX1* mutations.[8,23,124,125] The incidence of aneuploidy is not increased in AMLs with cohesin gene mutations, suggesting that their role in sister chromatid exchange is not central to their leukemogenic properties and turning the focus to their effects on gene expression. One of the probable ways through which cohesin regulates

gene expression is by mediating long-range communication between gene promoters and distant regulatory elements,[126] the disruption of which forms a well-established paradigm in the pathogenesis of hematologic cancers, including AML.[127]

Mutations in spliceosome genes

RNA splicing, the removal of intronic sequences from newly synthesized pre-mRNA, is performed by a large molecular complex composed of multiple proteins and small nuclear RNAs (snRNA) known as the spliceosome. Spliceosome gene mutations in myeloid malignancies can affect many different genes, including *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*. They were first identified in patients with MDS,[128] but are also present in other myeloid neoplasms, including 6%-15% of *de novo* AML.[8,129] Of the two most commonly mutated spliceosome genes in MDS, *SF3B1* is strongly associated with refractory anemia with ring sideroblasts and a favorable prognosis,[128,130] and *SRSF2* with a worse prognosis.[18] Patients with *SF3B1* and *SRSF2* mutations are significantly older than other MDS patients, and so are patients with hematologically silent clonal hematopoiesis driven by these mutations, suggesting that they may only give a clonal advantage in the context of an aging hematopoietic compartment.[34] It is not known how spliceosome gene mutations drive leukemogenesis, as their effects on RNA splicing, and therefore protein expression, appear to be genome-wide.[129] However, recent studies have identified effects on particular genes that include known players in leukemogenesis, such as *RUNX1* and *ASXL1* (reviewed by Boultwood *et al*.[131]). From a therapeutic standpoint, the role of spliceosome inhibitors is being investigated as a way to exploit the fact that cells with spliceosome mutations may be particularly sensitive to these compounds, as they already have impaired splicing.[131]

Mutations in signal transduction genes

Mutations affecting the tyrosine kinase gene *FLT3* and *RAS* pathway genes are widespread in myeloid malignancies and present in more than 70% of AML-NK.[8] *FLT3* is targeted by ITD and tyrosine kinase domain (TKD) mutations (Figure 4), both of which are associated with constitutive activation of the kinase and of downstream signaling pathways that drive cellular proliferation, such as RAS (ITD and TKD) and STAT (ITD). *FLT3*-ITD mutations are found in 35%-40% of AML-NK,[8,132] confer a poor prognosis,[79,82,133] and significantly co-occur with *NPM1* and *DNMT3A* mutations.[8] *FLT3*-TKD mutations are less common, but may also impart a less favorable prognosis amongst younger AML patients.[134] Non-synonymous *NRAS* and *KRAS* mutations are widespread in human can-

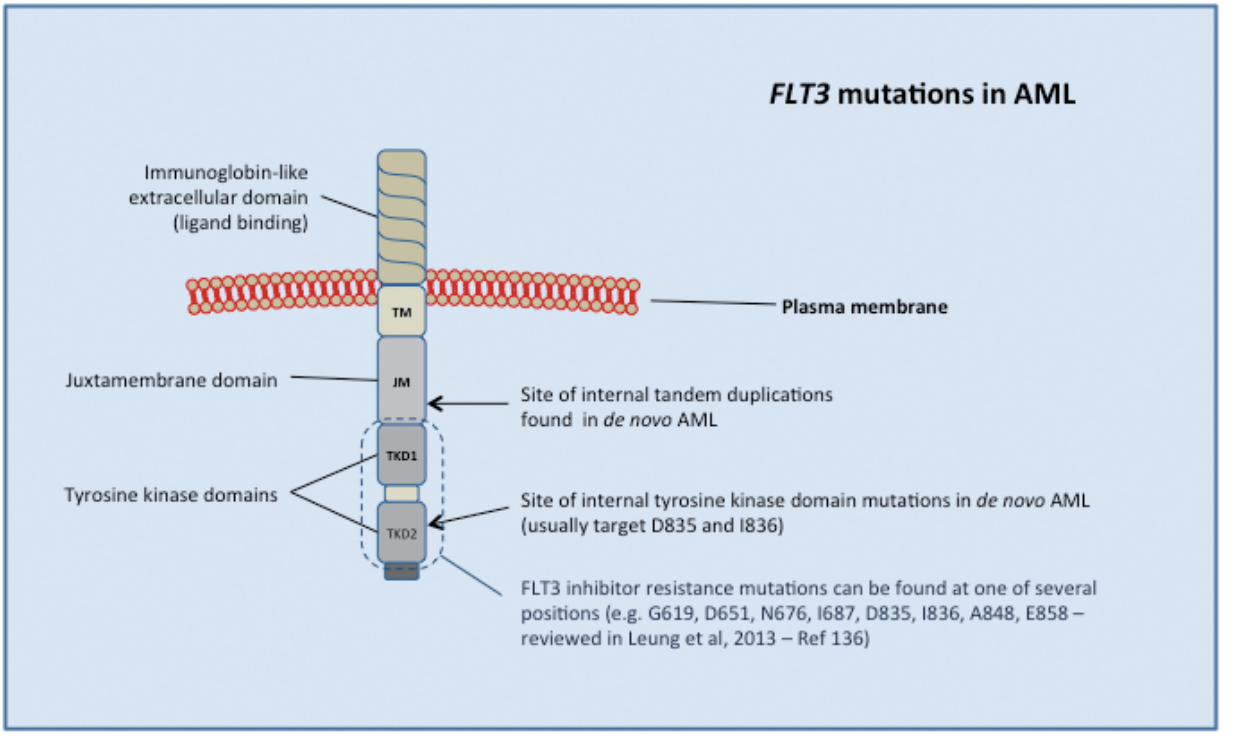

Figure 4. Mutations affecting the gene for the FLT3 tyrosine kinase in acute myeloid leukemia. The protein domains of the FLT3 tyrosine kinase are shown and the sites of somatic mutations found in *de novo* acute myeloid leukemia (AML) and those that mediate resistance to FLT3 inhibitors indicated. Of note, mutations affecting D835, I836 and N676 can be found in both *de novo* AML and during/after therapy with FLT3 inhibitors as they mediate resistance to these drugs.

cer and are also found in 10%-15% of AML-NK,[8,82] whilst mutations in the *RAS* regulator *PTPN11* are mutated in just under 10%.[8] *FLT3*-ITD mutations present an attractive therapeutic target, and FLT3 kinase inhibitors can indeed achieve initial disease control in AML. However, their efficacy is limited by acquired resistance associated with mutations in the tyrosine kinase domain of the gene.[135,136] Strategies to overcome acquired resistance, such as the use of FLT3-specific inhibitors (most of the currently available agents inhibit other tyrosine kinases such as KIT[137] causing dose-limiting hematopoietic toxicity), and the development of new molecules active against TKD mutant FLT3[136,138] are being pursued. However, these approaches need to take into account the fact that signal transduction mutations including those affecting *FLT3* are usually acquired late in AML evolution and their targeting is unlikely to eliminate ancestral clones or co-existing subclones of the disease carrying different driver mutations.[28,139]

Conclusion

More than 40 years after they were first introduced, cytotoxic chemotherapies continue to form the mainstay of AML therapy. Scientific advances made over this period, and accelerated by recent developments in genomic technologies, have brought our understanding of the molecular pathogenesis of the least well understood AML subgroup, AML-NK, to a level which permits optimism that significant rational therapeutic advances are impending. However, although some of the leukemogenic mutations discussed above offer themselves as drug targets, others do not, are less well understood and pose much more difficult challenges. Nevertheless, progress in the last few years has continued at an unprecedented rate and there is a pervasive sense that therapeutic breakthroughs are forthcoming.

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