The Changing Mutational Landscape of Acute Myeloid Leukemia and Myelodysplastic Syndrome

Connie A. Larsson, Gilbert Cote, and Alfonso Quintás-Cardama

Abstract

Over the past few years, large-scale genomic studies of patients with myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) have unveiled recurrent somatic mutations in genes involved in epigenetic regulation (DNMT3A, IDH1/2, TET2, ASXL1, EZH2 and MLL) and the spliceosomal machinery (SF3B1, U2AF1, SRSF2, ZRSR2, SF3A1, PRPF40B, U2AF2, and SF1). The identification of these mutations and their impact on prognostication has led to improvements in risk-stratification strategies and has also provided new potential targets for the treatment of these myeloid malignancies. In this review, we discuss the most recently identified genetic abnormalities described in MDS and AML and appraise the current status quo of the dynamics of acquisition of mutant alleles in the pathogenesis of AML, during the transformation from MDS to AML, and in the context of relapse after conventional chemotherapy.

Implications: Identification of somatic mutations in AML and MDS suggests new targets for therapeutic development.

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DNA hypomethylating agents and histone deacetylase (HDAC) inhibitors are widely used for the treatment of patients with AML or MDS. In addition, recent studies have reported the presence of mutations in genes that encode components of the splicing machinery in patients with MDS and AML (Table 1). Here, we discuss the most exciting recent developments about recurrent mutations in AML and MDS, the prognostic impact of such mutations (Table 2), and their potential as therapeutic targets as well as the dynamics of mutant alleles during the progression from MDS to AML and between genomes at diagnosis and after failure to standard therapy.

### Epigenetic Deregulation in the Pathogenesis of MDS and AML

Regulation of gene expression through epigenetic reprogramming is fundamental to development and cellular differentiation of higher eukaryotes. DNA methylation and histone modifications that change the conformation of chromatin stably alter the gene expression potential through mechanisms that do not change the sequence of the genome. Notably, many malignancies, including AML and MDS, exhibit aberrant DNA methylation and altered histone modifications that result in the alteration of gene expression, such as silencing of tumor suppressors and/or activation of oncogenes. The main epigenetic modification in humans is the methylation of DNA, in which a methyl group is covalently attached to the 5' carbon of cytosine [5'-methylcytosine (5mC)] within a CpG dinucleotide. There are regions of the genome that are rich in CpG dinucleotides (i.e., CpG islands), which generally span the 5' regulatory region of genes and are present in approximately half of all human genes. The methylation status of promoter CpG islands is modulated by DNA methyltransferases (DNMT) and dictates the outcome of gene expression (Fig. 1). Under normal conditions, CpG islands are unmethylated, which is characteristic of active gene expression. Conversely, promoter CpG methylation is a hallmark of gene repression and, under normal conditions, is generally restricted to X-chromosome inactivation in women and imprinted germ-line-specific and tissue-specific gene (13). Using mouse models, the specific roles of different DNMTs have been elucidated and well characterized. Dnmt3a−/− mice displayed normal levels of DNA methylation and were born normal but eventually succumbed to developmental defects and died by 4 weeks of age. Dnmt3b−/− mice exhibited slightly lower levels of DNA methylation and were not viable. However, de novo DNA methylation was severely impaired in Dnmt3a−/−; Dnmt3b−/− double mutants, showing that DNMT3a and DNMT3b have overlapping functions in de novo DNA methylation and embryonic development (14). Dnmt1−/− mice are embryonic lethal and displayed a global reduction in DNA methylation levels, which is consistent

### Table 1. Frequency of gene mutations in patients with MDS or AML

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene function</th>
<th>Mutation description</th>
<th>Mutation frequency in MDS (%)</th>
<th>Mutation frequency in CN-AML (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>De novo DNA methylation</td>
<td>Recurrent heterozygous missense mutation at R882</td>
<td>8</td>
<td>18–25</td>
</tr>
<tr>
<td>IDH1/2</td>
<td>Catalyzes interconversion of isocitrate and α-KG</td>
<td>HεR1-heterozygous R132 missense IDH2-heterozygous R172 or R140 missense mutation</td>
<td>4–11</td>
<td>6–19</td>
</tr>
<tr>
<td>TET2</td>
<td>Oxidizes 5-mC to 5-hmC, a demethylation intermediate</td>
<td>Primarily biallelic mutations resulting in loss of function</td>
<td>19–26</td>
<td>24–27</td>
</tr>
<tr>
<td>EZH2*</td>
<td>Progressive trimethylation of H3K27</td>
<td>Inactivating mutations and overexpression observed in myeloid malignancies</td>
<td>6.4</td>
<td>Rare</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Transcription factor that maintains HOX expression</td>
<td>Primarily heterozygote, monoallelic truncating mutations that deletes the PHD domain</td>
<td>14</td>
<td>5.2</td>
</tr>
<tr>
<td>MLL</td>
<td>H3K4 methyltransferase</td>
<td>Translocations involving MLL that result in a fusion protein or partial tandem duplication mutations of MLL</td>
<td>Rare</td>
<td>10 (de novo), 30 (secondary)</td>
</tr>
<tr>
<td>SF3B1</td>
<td>Spliceosomal gene important for HOX gene repression</td>
<td>Predominantly heterozygous missense mutation at R625, H662, and K700</td>
<td>80% in MDS-RARS and 6% in MDS without RS</td>
<td>3–5</td>
</tr>
<tr>
<td>SRSF2</td>
<td>Spliceosomal gene implicated in genomic stability</td>
<td>Heterozygous missense mutation at P95</td>
<td>~2% in MDS-RARS and ~12% in MDS w/o RS</td>
<td>7–8</td>
</tr>
<tr>
<td>U2AF35</td>
<td>Spliceosomal gene implicated in proper splicing of genes</td>
<td>Heterozygous missense mutation at S34 and Q157</td>
<td>12% (only in MDS without RS)</td>
<td>11</td>
</tr>
</tbody>
</table>

Abbreviations: PHD, plant homeodomain; RS, ringed sideroblasts. *EZH2 mutations are generally activating mutations in other malignancies.
DNA methylation patterns being a common feature of MDS. Not surprisingly, these mutations are consistent with aberrant DNA methylation such as

\[ \text{DNMT3A}^a \]

... and the notion that DNMT1 is essential for maintaining hemi-methylated DNA during replication (13). 5-mC, a mark of DNA methylation, can be further modified by the α-ketoglutarate (α-KG)–dependent oxygenases TET1, TET2, and TET3, which catalyze the conversion of 5-mC to 5′-hydroxymethylcytosine (5-hmC; ref. 15). α-KG is obtained from isocitrate in a process catalyzed by isocitrate dehydrogenase 1 (IDH1) in the cytoplasm and by IDH2 in the mitochondria. Sequencing analysis of MDS and AML patient samples revealed mutations in genes important for DNA methylation such as TET2, IDH1/2, and DNMT3A. Not surprisingly, these mutations are consistent with aberrant DNA methylation patterns being a common feature of MDS and AML (7, 16).

### Mutations in genes involved in DNA methylation

**DNMT3A**. DNMT3A, which encodes a DNMT and is related to the DNMT1 and DNMT3B genes, has been found mutated in 18% to 25% of patients with AML and approximately 8% of those with MDS. The most common mutation observed is a missense mutation that results in a substitution of arginine-882 to histidine, cysteine, proline, or serine (7, 17). Patients with DNMT3A R882 missense mutations have reduced DNA methylation compared with patients with wild-type DNMT3a matched AML patients wild-type for DNMT3a.

**IDH1/2**. Mutations in IDH1/2 have been reported in 15% to 20% of MDS and 20% to 30% of AML. Specifically, a substitution of arginine-857 to histidine, methionine, aspartate, or glutamic acid is observed in approximately 8% of those with MDS. The most common mutation observed is a missense mutation that results in a substitution of arginine-857 to histidine, methionine, aspartate, or glutamic acid (18). This mutation reduces the methyltransferase activity of the enzyme in a dominant-negative manner, which is further supported by the fact that all R882 mutations are

#### Table 2. Prognostic implications of gene mutations in patients with MDS or AML.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>Patients, n</th>
<th>% with mutation</th>
<th>Prognosis of patients carrying mutation vs. wild type</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNMT3A</strong></td>
<td>AML</td>
<td>415</td>
<td>23</td>
<td>Worse OS [HR, 1.38 (95% CI, 1.13–2.05); P = 0.022]</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>605</td>
<td>12</td>
<td>Worse OS (median, 15.0 ± 1.9 mo; P &lt; 0.001); worse EFS (median, 8.0 ± 1.2 mo; P = 0.01)</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>398</td>
<td>23</td>
<td>Improved survival to high-dose daunorubicin in patients enrolled in ECOG E1900 carrying mutant DNMT3a compared with patients with wild-type DNMT3a</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>150</td>
<td>8</td>
<td>Worse OS (log-rank P = 0.005); worse EFS (P = 0.009); higher rate of transformation to AML (P = 0.007)</td>
<td>(24)</td>
</tr>
<tr>
<td><strong>TET2</strong></td>
<td>AML</td>
<td>783</td>
<td>7</td>
<td>No impact on response or OS</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>318</td>
<td>27</td>
<td>Inferior OS (median, 20.4 vs. 62.2 mo; P = 0.173), shorter EFS (median, 6.7 vs. 18.7 mo; P = 0.009), shorter time to relapse (median, 10.3 vs. 41.3 mo; P = 0.005)</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>439</td>
<td>20</td>
<td>No impact on survival</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>96</td>
<td>23</td>
<td>Independent prognostic factor for OS [HR, 5.2 (95% CI, 1.6–16.5); P = 0.005]</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>MDS or AML</td>
<td>86</td>
<td>13</td>
<td>No impact on OS, but favorable response to azacitidine (82% mutant vs. 45% wild type; P = 0.007)</td>
<td>(29)</td>
</tr>
<tr>
<td><strong>IDH1/2</strong></td>
<td>AML</td>
<td>358</td>
<td>14 in IDH1, 19 in IDH2</td>
<td>Worse DFS in patients with NPM1 mutations without FLT3 mutations</td>
<td>(30)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>893</td>
<td>6 in IDH1, 11 in IDH2</td>
<td>Worse OS in patients carrying wild-type FLT3 and mutant NPM1 alleles</td>
<td>(31)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>520 and 805</td>
<td>9-16 in IDH1 3-7 in IDH2</td>
<td>Lower complete remission rate and shorter OS in patients with CN-AML carrying NPM1 mutations</td>
<td>(32)</td>
</tr>
<tr>
<td><strong>ASXL1</strong></td>
<td>MDS</td>
<td>439</td>
<td>14</td>
<td>Worse OS [HR, 1.38 (95% CI, 1.00–1.89); P = 0.006]</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>882</td>
<td>5</td>
<td>Independent prognostic factor for shorter OS [HR, 1.52 (95% CI, 1.12–2.4); P = 0.010]</td>
<td>(34)</td>
</tr>
<tr>
<td><strong>EZH2</strong></td>
<td>MDS</td>
<td>439</td>
<td>6</td>
<td>Worse OS [HR, 2.13 (95% CI, 1.36–3.33); P = 0.001]</td>
<td>(35)</td>
</tr>
<tr>
<td><strong>MLL</strong></td>
<td>AML</td>
<td>605</td>
<td>14</td>
<td>Translocations involving the MLL gene portend very poor OS (median, 15.0 ± 1.9 mo; P &lt; 0.001) and EFS (median, 8.0 ± 1.2 mo; P &lt; 0.01)</td>
<td>(36)</td>
</tr>
<tr>
<td><strong>SF3B1</strong></td>
<td>MDS</td>
<td>354</td>
<td>20</td>
<td>Favorable prognosis, longer EFS [HR, 0.1 (95% CI, 0.0–0.7); P = 0.02]</td>
<td>(37)</td>
</tr>
<tr>
<td><strong>SRSF2</strong></td>
<td>MDS</td>
<td>193</td>
<td>12</td>
<td>Shorter OS (median, 17 vs. 39% at 5 y; HR, 1.76; 95% CI, 1.0–3.1); P = 0.049; shorter 5-y DFS (median, 39 vs. 69% [HR, 2.5; 95% CI, 1.2–5.1]; P = 0.012)</td>
<td>(38)</td>
</tr>
<tr>
<td><strong>U2AF1</strong></td>
<td>MDS</td>
<td>193</td>
<td>7</td>
<td>More rapid transformation to AML [HR, 2.53 (95% CI, 0.9–7.13); P = 0.079]; no impact on OS [HR, 1.49 (95% CI, 0.54–4.1); P = 0.44]</td>
<td>(39)</td>
</tr>
</tbody>
</table>

**Abbreviations**: CI, confidence interval; DFS, disease-free survival; EFS, event-free survival; OS, overall survival.

*aPrognostic value remains controversial and is not currently used in clinical practice.*
heterozygous (7). Surprisingly, however, differences in gene expression, methylation patterns, or altered total 5-mC content could not be robustly linked to DNMT3A mutational status (7, 19). Serial transplantsations of Dnmt3a-null HSCs, which were derived and purified from a conditional Dnmt3a knockout mouse, into wild-type recipient mice resulted in a competitive advantage of mutant cells over the wild-type ones, suggesting that loss of Dnmt3a may contribute to clonal dominance. Reduction in DNA methylation at sites that correlated with increased expression of multipotency genes and downregulation of differentiation factors was also observed in the differentiated progeny of Dnmt3a-null HSCs (19). This suggests that loss of de novo DNA methylation impairs the differentiation potential of HSCs, providing a possible explanation of how DNMT3A mutations contribute to the pathogenesis of MDS and AML. DNMT3A mutations also tend to cluster in patients carrying NPM1, FLT3, and/or IDH1 mutations (20). In a study including AML patients under the age of 60 years, DNMT3A mutations were observed in 23% of patients and were associated with worse overall survival (OS) and relapse-free survival than that observed in patients with wild-type DNMT3A alleles (21). Similar results were observed in a cohort of 1185 Chinese patients with AML (22). DNMT3A mutations, typically R822H, were also noted in patients with all subtypes of MDS. Similar to AML, DNMT3A mutations correlated with shorter survival and increased risk of transformation to AML (23).

TET2. Uniparental disomy and deletions spanning chromosome 4q24 were first described in patients with myeloid malignancies (16). Sequencing of the commonly deleted region unveiled acquired somatic mutations to TET2 (the ten-eleven-translocation gene 2) in patients with myeloid cancers, including patients with MDS (19%–26%) or AML (24%–27%; refs. 16, 24, 25). When they occur, TET2 mutations are present in the vast majority of bone marrow precursor hematopoietic cells (including CD34+ progenitors; ref. 25). TET2 mutations can be observed concomitantly with NPM1, FLT3, JAK2, RUNX1, CEBPA, CBL, and KRAS mutations but are mutually exclusive with IDH1/2 mutations (24, 26). Frequently, patients will carry more than one TET2 mutation (16). Missense mutations frequently occur in regions that are evolutionary conserved and are typically C-terminus truncation mutations that result in loss of TET2 protein function (25). TET2 catalyzes the oxidation of 5-mC to 5-hmC, which has been shown to be a demethylation intermediary (27, 28). The role of TET2 mutations in the pathogenesis of MDS and AML, however, is not well understood, and published reports are often conflicting. In general, loss of TET2 function leads to a global decrease in 5-hmC levels and accumulation of 5-mC, which is a feature of DNA hypermethylation that results in gene repression (28). Surprisingly, an analysis of 88 patients with different myeloid malignancies harboring TET2 mutations revealed that a reduction in 5-hmC levels was associated with CpG hypomethylation, contradicting the paradigm that loss of TET2 function results in hypermethylation of differentially methylated CpG sites (27). Furthermore, the dysplastic phenotype of MDS cannot be attributed to TET2 mutations alone. Experiments using mouse models have shown that loss of Tet2 in the hematopoietic compartment results in an increase in HSC self-renewal with enhanced HSC function and expression of myeloid-specific and self-renewal gene programs, leading to progressive myeloproliferative and extramedullary hematopoiesis (Table 3; ref. 29). In addition, TET2 mutations are relatively frequent in patients with myeloproliferative neoplasms, in whom dysplastic features are very infrequently present. The impact of TET2 mutations on the outcomes of patients with AML or MDS remains controversial. In one study, among patients with AML who had achieved complete remission after induction chemotherapy, the incidence of TET2 mutations was 17% but had no impact on survival. This finding is further supported by another study in 783 younger adult
Table 3. Mouse models genetically engineered to express gene mutations encountered in patients with MDS or AML

<table>
<thead>
<tr>
<th>Gene (reference)</th>
<th>Mouse model</th>
<th>Phenotype</th>
<th>Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A &amp; DNMT3B (14)</td>
<td>Dnmt3a&lt;sup&gt;−/−&lt;/sup&gt;, Dnmt3b&lt;sup&gt;−/−&lt;/sup&gt;, and [Dnmt3a&lt;sup&gt;−/−&lt;/sup&gt;, Dnmt3b&lt;sup&gt;−/−&lt;/sup&gt;] straight KO</td>
<td>Dnmt3a&lt;sup&gt;−/−&lt;/sup&gt; died by 4 wk of age. Dnmt3b&lt;sup&gt;−/−&lt;/sup&gt; are embryonic lethal. Dnmt3 double mutants severely impaired in de novo DNA methylation and died by E11.5</td>
<td>Intercrossed Dnmt3a and Dnmt3b heterozygotes, which are grossly normal and fertile, to generate nulls</td>
</tr>
<tr>
<td>DNMT3A (19)</td>
<td>Dnmt3a&lt;sup&gt;fl/fl&lt;/sup&gt; conditional KO</td>
<td>Dnmt3a loss in HSCs leads to expansion of morphologically normal HSC with improper differentiation</td>
<td>Transplantation of purified Mx1-Cre; Dnmt3a&lt;sup&gt;fl/fl&lt;/sup&gt; HSCs and deletion of Dnmt3a induced 4 weeks after transplanting</td>
</tr>
<tr>
<td>IDH1 (34)</td>
<td>Idh1&lt;sup&gt;lsl/wt&lt;/sup&gt; R132H conditional KI</td>
<td>Increase in hematopoietic progenitors, splenomegaly, and anemia with extramedullary hematopoiesis</td>
<td>Expression of R132H mutation in all hematopoietic cells or specifically myeloid cells by crossing Idh1&lt;sup&gt;lsl/wt&lt;/sup&gt; with VavCre or LysM-Cre, respectively</td>
</tr>
<tr>
<td>TET2 (29)</td>
<td>Tet2&lt;sup&gt;fl/fl&lt;/sup&gt; conditional KO</td>
<td>Biallelic deletion of Tet2 results in progressive myeloproliferation, increase HSC self-renewal, CMML phenotype</td>
<td>Deletion of Tet2 in the hematopoietic compartments by crossing Tet2&lt;sup&gt;fl/fl&lt;/sup&gt; mice with different tissue-specific Cre mice</td>
</tr>
<tr>
<td>EZH2 (50)</td>
<td>Ezh2&lt;sup&gt;fl/fl&lt;/sup&gt; conditional KO</td>
<td>Loss of Ezh2 results in development of T-cell leukemia with almost complete penetrance in less than 1 year; implicates Ezh2 as a tumor suppressor</td>
<td>Ezh2&lt;sup&gt;fl/fl&lt;/sup&gt; mice crossed with Mx-Cre Tg. Deletion of the set domain induced by poly(I) poly(c) injections</td>
</tr>
<tr>
<td>EZH2 (54)</td>
<td>Ezh2fl/fl conditional KO in a myeloid leukemia mouse model</td>
<td>Increased numbers of differentiated leukemic cells and perturbed leukemic progression after Ezh2 deletion suggesting Ezh2 is oncogenic in AML</td>
<td>Transplanted MLL-AF9-transformed GMPs from Cre-ERT;Ezh2&lt;sup&gt;fl/fl&lt;/sup&gt; mice into lethally irradiated mice; deletion of Ezh2 by tamoxifen injections</td>
</tr>
<tr>
<td>ASXL1 (42)</td>
<td>Asxl&lt;sup&gt;tm12bc/tm12bc&lt;/sup&gt; KO</td>
<td>Asxl1 loss displays mild phenotype; differentiation defects of hematopoietic precursors</td>
<td>Insertion of a PGKneo cassette upstream from the PHD domain to disrupt Asxl1 expression globally.</td>
</tr>
<tr>
<td>ASXL1 (43)</td>
<td>NrasG12D/Asxl1 shRNA</td>
<td>Asxl1 loss causes myeloproliferation with myeloid infiltration in spleen and liver resulting in splenomegaly and hepatomegaly and impaired survival</td>
<td>Transplantation of bone marrow cells expressing NrasG12D in combination with Asxl1 short hairpin RNA construct or empty vector with GFP into lethally irradiated mice</td>
</tr>
<tr>
<td>SF3B1 (64)</td>
<td>Sf3B1&lt;sup&gt;−/−&lt;/sup&gt; mutant</td>
<td>Sf3B1 haplosufficiency leads to various skeletal defects; homozygous null are embryonic lethal</td>
<td>Mutant allele generated by replacing 4 exons with neo gene to disrupt gene expression globally</td>
</tr>
</tbody>
</table>

Abbreviations: KO, knockout; KI, knockin.
catalytic activity of the TET proteins. Mutations mainly map to codons R132 of IDH1 and R140 or R172 of IDH2. All IDH mutations reported thus far occur in the active catalytic site and participate in isocitrate binding (8, 15). An analysis of 385 patients with de novo AML revealed that those harboring an IDH1 or IDH2 mutation displayed global DNA hypermethylation and impaired hematopoietic differentiation, correlating with an increase in stem and progenitor cell markers (15). Mutations to IDH1 are always heterozygous and exclusively occur at residue 132, with an arginine to histidine being the most common amino acid substitution. It is important to note that IDH mutations are mutually exclusive with TET2 mutations, suggesting a common pathogenetic nexus leading to TET2 inactivation through alterations in either enzyme (24). Although the mechanism for the hypermethylation and leukemogenesis in IDH-mutant AML is not entirely known, it has been linked to downregulation of α-KG levels, which are needed by a variety of enzymes for their function, such as TET proteins, thus affecting differentiation of myeloid cells, or the Jumonji family of histone lysine demethylases (which demethylate H3K9 and H3K36), thus resulting in increased methylation of lysine residues (33). Recently, a mouse model that specifically expresses the Idh1R132H missense mutation in the hematopoietic or in the myeloid compartment was generated to gain a better understanding of how IDH1/2 mutations promote leukemogenesis. These mice showed an increase in hematopoietic progenitors and developed splenomegaly and anemia with extramedullary hematopoiesis, which occurs in 10% to 15% of patients with MDS harboring an IDH1/2 mutation who progress to AML. Furthermore, expression of the mutant in the myeloid compartment led to hypermethylated histones and patterns of aberrant DNA methylation that are consistent with those observed in patients with AML with IDH1/2 mutations (34).

The impact of IDH mutations in the prognosis of AML was investigated in patients with CN-AML, with frequencies of 14% and 19% for IDH1 and IDH2, respectively (35). IDH1 mutations tend to cluster in cases with mutant NPM1 but wild-type FLT3. In that setting, IDH1 mutations were associated with worse disease-free survival (DFS; ref. 35). Large studies from the United Kingdom, France, and Germany reported IDH mutations in 8% to 16% of patients, which were associated with worse complete remission rates and shorter survival among patients with CN-AML carrying NPM1 mutations (36–38). An analysis of 398 patients younger than 60 years randomly assigned to receive standard-dose or high-dose daunorubicin in the Eastern Cooperative Oncology Group (ECOG) E1900 trial revealed that the favorable effect of NPM1 mutations was limited to patients with simultaneous expression of NPM1 and IDH1 or IDH2 mutations (20). IDH1/IDH2 mutations are less frequent in MDS (4%–11%), compared with AML, but have been linked to shorter OS compared with unmutated patients (39). However, as with TET2 mutations, the prognostic value of IDH mutations remains controversial and needs to be further evaluated both experimentally and in additional cohorts. Importantly, given that mutant IDH proteins acquire neomorphic enzymatic activity, they might lend themselves to inhibition by molecules designed to inhibit the synthesis of 2-HG.

Mutations in chromatin remodeling genes

Although DNA methylation is the best characterized epigenetic modification, a combination of different high-throughput sequencing applications has recently revealed recurrent mutations to chromatin remodelers in MDS and AML, suggesting a role in neoplastic transformation (Table 1). This led to the identification of recurrent mutations to genes that encode histone modifiers, such as ASXL1 (additional sex-comb like-1), and EZH2. ASXL1, and EZH2 encode proteins that are members of the polycomb-group (PCG) family. PCG family members form protein complexes that function to maintain the transcriptionally repressive state of genes, such as the clustered (class I) homeobox genes that encode HOX proteins, which are frequently overexpressed in AML and confer a poor prognosis. For instance, the H2.0-like homeobox (Hlx) gene is overexpressed by 2- to 6.8-fold in 87% of patients with AML compared with CD34+ cells of healthy donors, and increased Hlx expression correlated with inferior survival (40).

ASXL1. ASXL1 has opposing functions in maintaining both the activation and suppression of HOX genes, depending on the histone-modifying protein that interacts with ASXL1 (41). Mutations in ASXL1 that map to the C-terminus of the protein have been identified in 3% to 22% of patients with MDS or AML, and correlate with poor OS (Table 2; ref. 20, 26). In vivo studies using Asxl1 knockout mice showed that loss of ASXL1 resulted in a mild phenotype characterized by differentiation defects in the myeloid and lymphoid progenitors without ever progressing to a more severe disease state that is typically observed in patients with MDS and AML harboring an ASXL1 mutation (42). It is possible that loss of ASXL1 is compensated for by homologous proteins with redundant functions, such as ASXL2 and ASXL3, thus resulting in a milder phenotype. However, mutations conferring loss of both ASXL1 loci are rare events. In fact, 90% of ASXL1 mutations are truncating mutations that result in the loss of the plant homeodomain (PHD) of the protein, yet the function of this mutant protein remains unclear (26). It is possible that a truncating mutation in ASXL1 results in gain-of-function or dominant-negative activity of the mutated protein. Recently, using a panel of human myeloid leukemia cell lines, it was shown that leukemia cells with homozygous ASXL1 frameshift/nonsense mutations had undetectable ASXL1 protein levels, whereas leukemia cells with heterozygous ASXL1 mutations showed a reduction in ASXL1 protein levels. Furthermore, knockdown of ASXL1 in these cell lines is associated with global loss of the trimethylation mark of H3K27 and upregulation of HOX4 gene expression (43). Interestingly, ASXL1 is a cofactor essential for the enzymatic activity of BAP1, and coimmunoprecipitation assays showed that...
ASXL1 forms a complex with BAP1 in human myeloid leukemia cells that are wild-type for ASXL1. Selective BAP1 haploinsufficiency in hematopoietic cells in mice induces a myelodysplastic phenotype reminiscent of chronic myelomonocytic leukemia (CMML), suggesting that the ASXL1–BAP1 axis may play an important role in MDS (44). Contrary to this, loss of BAP1 in human leukemia cell lines with wild-type ASXL1 did not lead to an increase in HOXA gene expression, suggesting that leukemic transformation as a result of ASXL1 loss occurs through a mechanism that is independent of BAP1 (43). In a study involving 439 patients with MDS, ASXL1 mutations were found in 14% of patients and were independent prognostic factors for survival [HR for death from any cause, 1.38; 95% confidence interval (CI), 1.00–1.89; ref. 26]. ASXL1 mutations were found in 5% of patients with AML and were inversely associated withFLT3-ITD and mutually exclusive with NPM1 mutations, and represented an unfavorable prognostic factor for survival (45).

EZH2. EZH2 makes up the catalytic component of the polycomb repressive complex 2 (PRC2), where it interacts with EED and SUZ12. Mutations to any of these genes have been described in early T-cell precursor acute lymphoblastic leukemia (T-ALL; ref. 46). The SET domain of EZH2 catalyzes the progressive trimethylation of H3K27 (H3K27me3) to induce the repression of target genes (47). EZH2 is overexpressed in many different epithelial cancers and lymphomas, whereas inactivating mutations are observed in myeloid malignancies, suggesting that H3K27me3 alterations have biologic consequences that are tissue specific (48). In patients with MDS, mutations to EZH2 are generally missense or truncating mutations that disrupt or delete the SET domain, leading to a reduction of H3K27me3 levels (48, 49). Another study reported that biallelic deletion of Ezh2 in the HSCs of mice was sufficient to cause T-ALL, which occurred with almost complete penetrance in less than 1 year (50). PRC proteins play a significant role in regulating HOX gene expression, providing a possible causal link between inactivating mutations to EZH2 and its implication in myeloid malignancies. Certain HOX proteins, such as HOXAX1-11, are frequently overexpressed in AML, and in vivo studies showed that overexpression of HOXAX10 in HSCs was sufficient to cause AML in mice (51, 52). One study showed that repression of certain HOX genes involves both promoter CpG methylation and NSPc1-mediated H2A ubiquitination in a process that requires the cooperation of EZH2, DNMT3A, and NSPc1, a component of PRC1. Trimethylation of H3K27 is required for the recognition and binding of DNMT3A and NSPc1, and loss of H3K27 trimethylation, as a result of EZH2 depletion, led to impairments in NSPc1-mediated H2A ubiquitination and CpG methylation in HOXA gene clusters (53). EZH2 mutations are observed in 6% of patients with MDS and are associated with worse OS compared with patients with wild-type EZH2 (26, 48, 49). This is not surprising, as EZH2 maps to chromosome 7, which is a genomic area frequently lost in MDS and associated with very poor prognosis. Interestingly, EZH2 mutations are infrequently found in patients with AML (20). In fact, loss of Ezh2 perturbs the progression of AML by promoting the differentiation of leukemic cells in mice transplanted with MLL–AF9–transformed granulocyte-macrophage progenitors (GMP), suggesting that EZH2 is oncogenic in AML (54).

MLL. MLL (mixed lineage leukemia) is another SET domain-containing protein that represents one of the first identified epigenetic modifiers that is frequently altered in leukemia. MLL is translocated in 10% of AML cases and in 30% of secondary AML. MLL is recruited to many promoters where it mediates H3K4 methyltransferase activity, resulting in activated gene expression (55). It has been speculated that mislocalized activity of the H3K79 histone methyltransferase, DOT1L, is the main leukemogenic driver in cases of AML carrying MLL rearrangements. Importantly, the novel DOT1L inhibitor EPZ004777 has been shown to selectively inhibit H3K79 methylation, resulting in blocked expression of leukemogenic genes with negligible impact on non-MLL–translocated cells (56). These data suggest that pharmacologic inhibition of DOT1L may represent a potential therapeutic option for patients with MLL rearrangements.

BCOR. The genome of a female patient with AML and devoid of mutations to FLT3, NPM1, and CEBPA was sequenced as a means to gain a better understanding of the molecular characterization of the subgroup of patients with CN-AML who lack NPM1 and CEBPA mutations. Further mutational screenings and additional sequencing of CN-AML cases identified recurrent mutations to BCOR, which encode a corepressor of BCL6. Mutations to BCOR occur at a frequency of 3.8% in unselected CN-AML cases. However, in the subgroup of CN-AML that is least characterized, BCOR mutations are observed in 17% of cases. This subgroup includes patients with no MLL-PTD and lacking mutations to CEBPA, NPM1, FLT3, or IHD1/2 (4). In addition, a single BCOR mutation has been observed in a small MDS examination that included 26 patient samples (57). BCOR has been shown to interact with HDACs, and mutations to BCOR are associated with DNMT3A mutations, implicating a cooperative epigenetic mechanism for AML pathogenesis. Furthermore, this study showed that BCOR mutations portend inferior survival (4).

Mutations in spliceosomal genes

The spliceosome plays a fundamental role in the processing of nascent RNA transcripts. Spliceosomes are complexes of small nuclear RNAs and proteins that remove (splice) introns and unconventional exons from primary transcripts (pre-mRNA) to assemble recognized exons (splicing) into the mature mRNA (Fig. 2). Disease-associated aberrant RNA splicing is a well-described paradigm that is primarily gene specific and due to mutations in cis-regulatory sequences that guide spliceosomal recognitions of exons (58). However, a growing number of mutations are known to target components of the spliceosome, and thus potentially to impart global rather than gene-specific RNA splicing changes. In patients with myelodysplasias, somatic
mutations have been described in eight core splicingosomal genes—SF3B1, SRSF2, U2AF35 (U2AF1), ZRSR2, SF3A1, PRPF40B, U2AF65 (U2AF2), and SF1. Mutations were highly recurrent in SF3B1, SRSF2, ZRSR2, and U2AF1, whereas mutations in the other splicingosomal genes (U2AF2, SF1, PRPF40B, and SF3A1) occurred with a frequency of 1% or less (57, 59–61). Mutations to splicingosomal genes are highly specific for patients with refractory anemia with ring sideroblasts (RARS; 84.9%), or MDS without ringed sideroblasts (43.9%), and CMML (54.5%) compared with patients with de novo AML (6.6%) or myeloproliferative neoplasms (9.6%). The spectrum of splicingosomal mutations also seems to be specific to disease type (57). As mutations to U2AF2, SF1, PRPF40B, and SF3A1 are rare events in MDS and AML, there will be no further discussion in regards to these genes within this review. This review focuses on the four most frequently mutated splicing genes, SF3B1, SRSF2, ZRSR2, and U2AF1, occurring in all subtypes of MDS. It is important to note that with the exception of ZRSR2, the majority of the mutations described to date are targeted missense rather than nonsense or frameshift mutations, suggesting a gain-of-function rather than a reduction in splicing factor levels. Because these genes play a fundamental role in the recognition of the 3′ splice site during early spliceosome formation, functional changes in these proteins, such as in their ability to stabilize U2 interactions, have the potential to differentially affect both alternative and constitutive splicing decisions.

**SF3B1.** The SF3B1 gene encodes the 155-kDa subunit of the SF3B complex, which serves to target the U2snRNP complex to the branch point during complex A formation (Fig. 2). Mutations are found in more than 80% of patients with RARS, whereas it is mutated in only 6% of patients with MDS without ringed sideroblasts, specifically implicating SF3B1 mutation in the pathogenesis of this subtype of MDS (57, 62). Mutations to SF3B1 confer a more favorable prognosis in MDS, MDS/MPN, and RARS cases and are associated with lower risk of progression to AML compared with patients with wild-type SF3B1 (59, 62). SF3B1 mutations are predominantly heterozygous missense mutations at residues K700, K666, R625, and H662, but the specific effect of these mutations on protein function remains unknown. It is hypothesized that mutations in SF3B1 alter branch point and 3′ splice site recognition, which contributes to changes in the mature mRNA pool. Indeed, a recent examination of splicing of 81,564 exons in 9,069 genes revealed that 423 exons in 350 genes were differentially used in mutants compared with a representative healthy donor. Notable genes with aberrant RNA splicing included ASXL1, CBL, EZH2, and RUNX1. Some consequences of the aberrant splicing pathways were structural differences in iron distribution contributing to the pathogenesis of RARS (63). Importantly, SF3B1 is involved in repressing HOX genes by physically interacting with PcG proteins, suggesting a potential role for SF3B1 in epigenetic regulation. Loss of one Sf3b1 allele leads to developmental defects characterized by various skeletal alterations as a result of impaired Hox gene silencing in murine models. Furthermore, Mll mutations rescue the Sf3b1+/− phenotype (64). It is important to emphasize that translocations or insertions involving MLL, resulting in an MLL gene fusion, occur in about 10% of adult AML and confer a poor prognosis.

**SRSF2.** SRSF2 is a member of the SR family of RNA-binding proteins that predominantly function to enhance exon recognition by binding regulatory splicing sequences. At the 3′ splice site region, SRSF2 facilitates the recruitment of both U2AF1 and SF3A2, which is consistent with the model whereby gene mutation alters regulation of exon recognition (Fig. 2). For the myeloid diseases, SRSF2 is currently the second most mutated splicing gene. Mutations...
to SRSF2 are almost always heterozygous missense mutations that specifically occur at P95 and are more prevalent in CMML cases (28%) when compared with patients with RARS (1.5%), MDS without ringed sideroblasts (1.6%), and AML (~7%; ref. 57). MDS harboring an SRSF2 mutation is associated with a higher rate of transformation to AML with shorter progression time and lower OS compared with MDS with wild-type SRSF2 (65). Depletion of SRSF2 leads to an accumulation of DNA damage and genomic instability and to induction of G_{1}–M cell-cycle arrest, uncovering pathways that may be targeted as a result of aberrant RNA splicing (66). Thus, gain-of-function mutations could provide a compelling reason as to why mutations to SRSF2 confer a poor prognosis. Furthermore, SRSF2 mutations are associated with concomitant mutations in IDH1 and RUNX1, a transcription factor that is frequently mutated and/or misspliced in MDS (61). Both mutations and missplicing of RUNX1 are associated with inferior event-free survival (EFS) and OS. The fact that SRSF2 mutations frequently co-occur with mutations in RUNX1, which also happens to be abnormally spliced when SRSF2 is mutated, may explain why deregulations in these two genes lead to a similar phenotype and prognosis.

**ZRSR2.** ZRSR2 is a member of the U2AF1-related protein family, which functions in early spliceosome assembly through direct interactions with the U2AF 65-kDa subunit (U2AF2). Unlike the other members of this group, ZRSR2 does not seem capable of functionally replacing U2AF1, and while required its precise role in U2-dependent splicing is unclear (67). An important consideration, however, is that ZRSR2 plays a different role in assembly of the minor U12-dependent spliceosome, where it is capable of completely replacing the U2AF complex (68). Mutations of the ZRSR2 gene occur at similar frequency in MDS (~8%) and CMML (~8), and unlike other splicingosomal gene mutations they do not occur in select “hot spots” (60). Instead, nearly all reported mutations are nonsense or frameshift mutations, clearly establishing a loss-of-function role. In one study, patients with ZRSR2 mutations seemed to cluster with RAEB-1 and RAEB-2, MDS subtypes with further associated pronounced thrombocytopenia (60). Furthermore, these mutations were associated with a higher AML transformation rate and poor OS. A specific link to U12-dependent gene splicing remains to be established.

**U2AF1.** The U2AF1 gene encodes the 35-kDa subunit of U2AF and functions to recognize the AG dinucleotide marking the end of the intron. For introns with a weak U2AF2-binding sequence, U2AF1 is critical for splice site recognition (69). U2AF1-related proteins, ZRSR1, ZRSR2, and U2AF1L4, substitute for U2AF1 in some spliceosomal reactions, clearly defining the potential to affect a subset of genes (68). Mutations to U2AF1 were not observed in patients with RARS but occurred with relatively equal frequencies in MDS without ringed sideroblasts (11.6%), CMML (2.9%), and AML (11.0%) cases (57, 59, 62, 70). Mutations to U2AF1 occur exclusively at codons S34 or Q157 and correlate with a more rapid transformation from MDS to AML, although their impact on OS remains unclear (65). Expression of mutant U2AF1 in HeLa cells leads to a reduction in cell proliferation and enhanced apoptosis, which are common features of MDS (57). Much like SRSF2 mutations, mutations to U2AF1 are implicated in abnormal splicing of certain genes, namely RUNX1 and TET2, and are also associated with mutations in ASXL1 and TET2. Furthermore, expression of mutant U2AF1 resulted in upregulation of nonsense-mediated RNA decay (NMD) pathway genes, indicating that U2AF1 mutations lead to increases in RNA splicing defects that require targeting for degradation (61).

Overall, mutations to SF3B1, SRSF2, ZRSR2, and U2AF1 are the most frequent gene aberrations occurring in all subtypes of MDS and are mutually exclusive (57, 59–62).

**TP53 mutations in the pathogenesis of MDS/AML**

TP53 is the tumor suppressor more frequently mutated in human cancer. Mutations to TP53 occur in approximately 10% of de novo MDS and AML cases, whereas it is mutated in more than 25% of therapy-related MDS (t-MDS) and AML (t-AML) cases. Although the presence of TP53 mutations has long been recognized as a poor risk factor for survival, novel aspects of TP53 biology have been linked to the pathogenesis of myeloid malignancies. It is now widely recognized that patients with TP53 mutations typically have a complex karyotype (CK; ref. 71). It has been recently shown that the events leading to complex genomic rearrangements in CK-AML are orchestrated by a one-step catastrophic event termed chromothripsis (Fig. 3; ref. 72). Genomic rearrangements caused by chromothripsis are characterized by complex somatic rearrangements with alternating copy-number states affecting one or a few chromosomes. Two scenarios describing the events leading to p53-mediated chromothripsis have been discussed. One scenario describes critical telomere shortening, followed by chromosomal end-to-end fusion, and subsequent breakage as the events leading to complex interchromosomal rearrangements. The fact that progressive telomere shortening occurs as a function of patient age may also explain why chromothripsis is more frequent in advanced aged TP53-mutated AML and why the frequency of CK-AML increases with age. The other scenario postulates that chromothripsis occurs when double-stranded DNA breaks, caused by ionizing radiation or DNA-damaging agents, are subjected to repair by the error-prone nonhomologous end joining (NHEJ) pathway, the dominant repair pathway in p53-deficient (e.g., mutated) cells (72). TP53 function is also implicated in the pathogenesis of 5q– syndrome. In vitro studies showed that downregulation of RPS14, a 40S ribosomal subunit, in human bone marrow cells phenocopied the erythroid failure observed in patients with 5q– syndrome (73). Deletion of the Cdh7-Nid67 interval (syntenic to the 5q– syndrome commonly deleted region that contains RPS14) in the bone marrow of mice resulted in elevated levels of p53 and increased bone marrow apoptosis. Crossing Cdh7-Nid67+/− mice with p53-null mice (74) or pharmacologic inhibition of p53 rescues the...
erythroid defect (75), thereby definitely implicating p53 in the pathogenesis of this syndrome.

Dynamics of mutated alleles in MDS and AML

Although the use of next-generation sequencing platforms has shed invaluable new light into the mutational landscape in MDS and AML, several questions remain to be answered. First, available studies only provide a snapshot of the mutational make-up of MDS or AML but do not address the mutational clonal hierarchy within the bulk of leukemic cells or aid in establishing which mutations bear real weight in the pathogenesis of the disease (i.e., “driver” vs. “passenger” mutations). Second, serial mutational screenings will be necessary to establish the genetic lesions involved in the progression from MDS to AML. Third, extensive work is warranted to exploit the available wealth of information for diagnostic, prognostic, and therapeutic purposes.

A recent study has shown that hematopoietic stem/progenitor cells (HSPC) of normal individuals contain hundreds of random mutations, that the number of mutations is positively correlated with age, and that the mutational spectrum between normal healthy HSPCs and that of AML genomes is very similar. This suggests that normal HSPCs accumulate random mutations over time that are irrelevant for AML pathogenesis but will be transmitted (i.e., passengers) to all AML clones if and when a driver mutation is acquired and provides a growth advantage.
advantage for an HSPC. Furthermore, a comparative analysis of 12 FAB-M1 CN-AML and 12 FAB-M3 AML genomes identified 13 recurring mutations in M1-AML, including NPM1, DNMT3a, and IDH1, which only coexisted in M1-AML, suggesting that the latter are likely M1-AML-initiating mutations rather than cooperating mutations (12). Not surprisingly, an analysis of the dynamics of mutated alleles during the progression from MDS to AML in 7 patients by high-throughput sequencing, DNA copy-number analysis, and microarray-based gene-expression studies found that the MDS clones also contained hundreds of acquired mutations. More importantly, the AML arising from them were derived from at least one subclone acquiring new driver mutations or genomic rearrangements and that 85% to 90% of MDS and secondary AML unfractonated bone marrow cells were clonal, even with a myeloblast count of less than 5% (76). In this group of patients, 11 genes were found mutated recurrently: CDH23, NPM1, PTPN11, RUNX1, SMC3, STAG2, TP53, U2AF1, UMODL1, WT1, and ZWIM4 (76). It is also important to consider that mutation of spliceosomal genes may serve as complete genetic drivers of tumorigenesis, a concept originating from the observation that SRSF1 is capable of inducing tumor formation (77). It is clear that mutations involving spliceosomal genes specifically affect genes known to be sequentially targeted by individual mutations. Although most patients with AML respond to initial induction chemotherapy, a large number of them will relapse and exhibit refractoriness to subsequent therapy that almost invariably leads to the patient's death. It is therefore important to understand the genetics underlying primary tumorigenesis as well as disease recurrence. The genome sequences of 8 patients with AML at diagnosis have been compared from the same patients at the time of relapse by deep sequencing (44). Such analysis unveiled novel mutations in AML such as those in WAC, SMC3, DIS3, DDX41, and DAXX. More importantly, two patterns of clonal evolution at relapse were identified. These include one in which the founding clone at diagnosis acquired new mutations that were later identified in clones at relapse, and another one in which a subclone of the founding clone without initial therapy and acquired additional mutations that led to clonal expansion and relapse (Fig. 3). In both instances, initial therapy failed to eradicate all or specific subclones from the founding clone. Interestingly, some of the mutations observed at relapse were due to DNA damage secondary to cytotoxic chemotherapy, highlighting potential deleterious effects of current AML therapeutic regimens (44).

**Perspective and Conclusions**

Available mutational data challenge the conceptual "two-hit" dictum in AML and MDS, whereby the pathogenesis of these diseases is driven by a mutation in a gene-regulating cell growth or proliferation that is complemented by a second mutation in a transcription factor regulation myeloid maturation and depicts a much more complex picture about the pathogenesis of these malignancies. It is now becoming more recognized that mutations driving neoplastic transformation and progression are not restricted to tumor suppressors, oncogenes, or pathway-specific genes involved in growth signaling, proliferation, migration, and survival. Regulatory proteins that are not defined to a specific pathway but have functional roles in the regulation of global processes (i.e., transcription and translation) are frequently targeted to functionally deregulating specific mutations in many different blood disorders, including AML and MDS. Cross-examinations of the epigenetic profile in patients with myeloid cancers harboring mutations to epigenetic genes suggest that aberrations to the epigenome play a significant role in the pathogenesis of these malignancies. This is not surprising considering that many of these epigenetic regulators are important for maintaining the repressive state of genes involved in growth, stem cell renewal, and survival. Importantly, epigenetic-modifying agents such as HDAC inhibitors and hypomethylating agents have already shown clinical activity in patients with AML and MDS. More importantly, the number of different genetic lesions in epigenetic regulators provides researchers with more potential therapeutic targets and currently remains an area of focus in cancer drug discovery. Furthermore, a high proportion of patients with MDS carry somatic mutations in several different spliceosome genes, namely SF3B1, SRSF2, and U2AF1. Although the roles of these genes in RNA splicing are well described, little is known about their roles in the context of hematopoietic function or how splicing mutations contribute to the pathogenesis of MDS and in some cases, the transformation to AML. With the accumulating data correlating spliceosome mutations with mutations in epigenetic regulators, it is arguable that abnormal RNA splicing parallels aberrant epigenetic reprogramming in the pathogenesis of MDS or that a direct link may exist between RNA splicing and epigenetic regulation. The overlap and frequency of mutations to these genes and the data correlating splicing mutations with the missplicing of epigenetic regulators recurrently mutated in myeloid malignancies further supports this notion. Studying the interplay between concomitantly mutated genes will undoubtedly shed new light into the mechanisms whereby normal myeloid cells become malignant and will aid in developing more biologically informed therapeutic approaches.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: C.A. Larson, A. Quintás-Cardama

Development of methodology: A. Quintás-Cardama

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Quintás-Cardama

Writing, review, and/or revision of the manuscript: C.A. Larson, G. Cote, A. Quintás-Cardama

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Quintás-Cardama

Study supervision: A. Quintás-Cardama

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