**Introduction**

Acute myeloid leukemia (AML) is an aggressive malignancy characterized by clonal proliferation of neoplastic immature precursor cells. AML impacts older adults, and has a poor prognosis. Despite recent advances in treatment, AML is complex, with both genetic and epigenetic aberrations in the malignant clone and elaborate interactions with its microenvironment. We are now able to stratify patients on the basis of specific clinical and molecular features in order to optimize individual treatment strategies. However, our understanding of the complex nature of these molecular abnormalities continues to expand the defining characteristics of high-risk mutations. In this review, we focus on genetic and microenvironmental factors in adverse risk AML that play critical roles in leukemogenesis, including those not described in an European LeukemiaNet adverse risk group, and describe therapies that are currently in the clinical arena, either approved or under development.

As we have continued to dissect the molecular pathogenesis and pathophysiology of AML, we are able to stratify patients on the basis of specific clinical and molecular features in order to optimize individual treatment strategies. Herein, we describe selected factors associated with adverse...
risk AML, focusing on high risk mutations, including those not described in an ELN adverse risk group (Table 2). We discuss selected newer treatment options available for high-risk patients that target specific genetic and microenvironmental factors as well as selected treatments currently under development for future early phase clinical trials.
The clinical and molecular factors associated with a drug-resistant phenotype and overall poor prognoses are delineated in Table 1.

### Cytogenetics

Cytogenetic findings are classified according to favorable, intermediate, and unfavorable risk categories. Unfavorable cytogenetics define adverse ELN risk and thus provide critical prognostic information that can inform treatment options.

Nonetheless, −7, −5/del(5q), monosomal karyotypes, and complex cytogenetics with at least three abnormalities carry an adverse prognosis independent of treatment type. Adverse risk cytogenetics often accompany secondary AMLs, including myelodysplasia-related (MDS/AML) and therapy-related (t-AML) variants, older age, high risk molecular pathways implicated in leukemogenesis (e.g., TP53), and multidrug resistance.

Of the high-risk cytogenetic translocations, we have chosen to highlight mixed lineage leukemia (MLL)-rearranged AML. AMLs with MLL-based

### Table 2. High-risk mutations, their functional class and mechanisms of leukemogenesis.

| High-risk mutations | Functional class | Mechanisms of leukemogenesis | Incidence in AML |
|---------------------|------------------|------------------------------|------------------|
| FLT3, KRAS, NRAS, KIT | Signaling and kinase pathway | These mutations lead to the aberrant activation and proliferation of cellular signaling pathways. | ~2/3 of AML cases |
| DNMT3A, ASXL1 | Epigenetic modifiers (DNA methylation and chromatin modification) | Felt to be inciting mutations in leukemogenesis and are often found in age-related clonal hematopoiiesis. These mutations likely promote clonal outgrowth, but require additional mutations to initiate leukemic transformation. Of note: DNMT3A mutations in conjunction with mutation NPM1 confers particularly poor prognosis. NPM1 participates in a variety of cellular functions, which include protein formation, ribosome biogenesis, DNA replication, and the cell cycle. | ~1/2 of AML cases |
| SRSF2, SF3B1, U2AF1, and ZRSR2 | Spliceosome complex | Spliceosome complex is important for RNA splicing of mRNA precursors. Mutations in RNA spliceosomes causes mis-splicing of mRNA precursors leading to abnormal epigenetic regulation, transcription, and genome integrity, ultimately leading to cancer. These are often seen in older individuals with less proliferative disease. | ~1/10 of AML cases |
| RUNX1 | Transcription factors | This is an important core-binding factor family of transcription factors involved in embryogenesis of HSC generation and regulation of HSC differentiation and homeostasis. When mutated, may lead to a stem cell phenotype characterized by early HSC exhaustion. | ~1/10 of AML cases |
| TP53 | Tumor suppressors | Tumor suppression occurs via apoptosis, DNA repair and cell cycle arrest/senescence, and when disrupted, will lead to survival of cancerous cells. | ~1/6 of AML cases |

AML, acute myeloid leukemia; HSC, hematopoietic stem cells.
Translocations are associated with poor outcomes in adults and frequent relapses, despite initial response to standard induction chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT). The MLL gene, located on chromosome 11q23, encodes the histone-lysine-N-methyltransferase 2A (KMT2A), which plays a critical role in differentiation and homeostasis through chromatin remodeling and regulation of homeobox (HOX) genes. MLL-rearranged leukemia is characterized by balanced translocation and fusion with over 80 different partner genes including AF4, Afadin, AF9, ELL, and ENL, with the resultant translocations t(4;11)(q21;q23), t(6;11) (q27;q23), t(9;11)(q22;q23), t(11;19)(q23;p13.1), and t(11;19)(q23p13.3). For leukemia to develop, MLL fusion proteins must interact with the protein menin, a tumor suppressor protein responsible for regulating cell growth for endocrine organs (encoded on the MEN1 gene). When cofactor menin and MLL fusion proteins interact, there is an upregulation of HOX9 and MEIS1 genes, which ultimately promotes leukogenesis and proliferation. In fact, when menin is blocked in MLL transformed leukemic blasts, HOX gene upregulation and cell differentiation arrest ceases, supporting menin’s crucial role for oncogenesis. MLL-rearrangement is found more frequently in t-AML (9.4%) than in de novo AML (2.6%, p < 0.0001), with particular occurrence in the setting of agents that freeze the topoisomerase II-DNA complex, such as anthracyclines and epipodophyllins.

The histone H3K79 methyltransferase DOT1L is recruited in MLL-rearranged AML, leading to methylation of oncogenic downstream targets HOX9 and Meis1. In a murine model, inhibition of DOT1L led to the suppression of downstream MLL target genes with significant tumor regression. The DOT1L inhibitor Pinometostat – a potent and selective small molecule inhibitor of DOT1L methyltransferase activity – has the ability to abrogate HOX cluster gene expression in AML cells, which leads to leukemia cell apoptosis. A phase I study of Pinometostat in MLL-rearranged relapsed/refractory (R/R) myeloid malignancy patients demonstrated tolerability and modest including morphologic changes in the bone marrow consistent with myeloid differentiation. An ongoing phase Ib/II open-label, single-arm trial enrolling R/R MLL-rearranged AML patients will evaluate the tolerability and early efficacy of pinometostat in combination with azacitidine.

New therapeutic agents targeting the menin-KMT2A protein–protein interaction are being investigated in early phase clinical trials. Preliminary results of KO-539 in a phase I/II trial of adults with R/R disease, show that the drug is generally well tolerated with no dose interruptions or discontinuations due to drug-related adverse events. There is also some suggestion of good anti-leukemic activity.

**Genetic mutations**

**FLT3-ITD high + and wild type NPM1.** FLT3 is a transmembrane ligand-activated receptor tyrosine kinase and is expressed on hematopoietic progenitor cells. Mutations in FLT3 occur in 25–30% of all AMLs and result in aberrant activation of RAS/RAF/MEK/mammalian target of rapamycin (mTOR) pathways, as well as through phosphatidylinositol 3 kinase (PI3K)/AKT pathways, all of which lead to cell growth and survival. Higher allele frequencies/ratios, have been associated with poorer outcomes, especially with wild type NPM1.

Prior to ELN 2017, all FLT3 mutations irrespective of allelic ratio were considered to be high risk. A low ITD allelic ratio is considered <0.5, whereas a high allelic ratio is over ≥0.5. ELN now lists patients with wild-type NPM1 without FLT3-ITD or with FLT3-ITDlow (without adverse-risk genetic lesions) and mutated NPM1 and FLT3-ITDhigh as intermediate risk. Patients with mutated NPM1 with FLT3-ITDlow are listed as having a favorable risk. We do not have prospective data that suggests FLT3 mutated patients in favorable and intermediate risk groups have better outcomes without allogeneic HSCT. Several tyrosine kinase inhibitors (TKIs) have been studied either as monotherapy or in combination with various chemotherapies. Midostaurin is now approved in combination with 7+3 as front-line treatment for newly diagnosed adults, who can tolerate induction treatment. This phase III trial found significantly improved event-free survival (EFS) and OS in the combination midostaurin and daunorubicin/cytarabine (7+3) arm compared with the 7+3 arm alone (Table 3). Interestingly, the CR rates were similar in both arms, suggesting that the improvement in OS could reflect a
### Table 3. Efficacy of drugs used in AML patients with high-risk mutations.

| High risk mutations/ cytogenetics and frequency in newly diagnosed AML | Trial | Drug | Population | Efficacy (95% CI) for high risk mutations |
|---|---|---|---|---|
| FLT3 | RATIFY phase III trial<sup>19</sup> | Midostaurin in combination with standard induction chemotherapy [7 + 3] versus 7 + 3, followed by HiDAC +/- transplant? | Adults with newly diagnosed AML, with FLT3-ITD high + and low allele frequency | Midostaurin group (n = 360) versus placebo (n = 357)  
CR: 58.9% (53.6–64.0) versus 53.5% (48.2–58.8)  
EFS: 8.2 months [5.4–10.7] versus 3.0 months [1.9–5.9]  
mOS: 74.7 months [31.5 to not reached] versus 25.6 months [18.6–42.9] |
| FLT3-ITD: 20–25%  
FLT3-TKD: 5–8% | SORAML phase II trial<sup>20</sup> | 7 + 3 induction with HiDAC consolidation with sorafenib versus placebo [continued into maintenance for 12 months] | Adults, ages of 18–60 years, with newly diagnosed AML | Explorative analysis (n = 44) with FLT3-ITD, in the sorafenib group (n = 23) compared with the placebo group (n = 21)  
EFS: 18 months [0–36] versus 6 months [0–16]  
OS: not reached versus 19 months [0–39] |
| Phase I/II gilteritinib and azacitidine trial<sup>21</sup> | Gilteritinib and azacitidine | Adults with newly diagnosed AML, FLT3 positive (FLT3-ITD or FLT3-TKD), unfit to receive standard induction chemotherapy | Exploratory analyses from safety phase I, cohort ORR: 80%. Composite CR rate of 67% (n = 10 out of 15 patients), CR of 27%, (4 out of 15 patients) and CRi of 40% (6 out of 15 patients) |
| Phase II trial, Goldberg et al.<sup>22</sup> | 7 + 3 induction with HiDAC consolidation with Crenolanib [continued as maintenance for 12 months after HSCT] | Newly diagnosed AML patients with FLT3 mutations, aged 18–60 years old | Exploratory analyses of the survival phase I, cohort ORR: 80%. Composite CR rate of 67% (n = 10 out of 15 patients), CR of 27%, (4 out of 15 patients) and CRi of 40% (6 out of 15 patients) |
| VLALE-A phase III trial<sup>2</sup> | Azacitidine plus either venetoclax or placebo. | 75 years and older, or with significant co-morbidities, ineligible for standard induction | Subgroup analysis from FLT3 mutation in azacitidine and venetoclax (n = 29 of 163 patients) versus azacitidine alone (n = 22 of 86 patients)  
Composite remission: 72.4% (52.8–87.3%) and 36.4% (17.2–59.3%), respectively (p = 0.02)  
HR for OS: 0.66 (0.35–1.26) [death in 19 out of 29 patients in azacitidine and venetoclax group versus 19 out of 22 patients in azacitidine alone group] |
| BRIGHT AML1003 phase II<sup>23</sup> | Glasdegib and LDAC or LDAC | >55 years and older and not suitable for intensive chemotherapy. | Subgroup analysis from Glazdegib and LDAC or LDAC; FLT3 ITD n = 5  
ORR (n = 51) 33% versus 0% (n = 2) |
| M14-387 phase IIb/II<sup>24</sup> | Venetoclax and low-dose cytarabine | 60 years or older and ineligible for intensive chemotherapy | Subgroup analysis from poor cytogenetic risk versus intermediate cytogenetic risk [FLT3 n = 16]  
CR/CRi rates poor risk = 42% in combination group [27%, CRi, and 15% CR] versus CR/CRi intermediate risk = 63% in combination group [29%, CR, and 35% CR] |
| ALFA 0701 phase III trial<sup>25</sup> | 7 + 3 with or without GO | Patients between 50–70 years with previously untreated de novo CD33+ AML | Subgroup analysis from FLT3 ITD+ (n = 22 in GO group and n = 27 patients in control CR + CRp: 95.5% [21/22 patients] versus 85.2% [23/27 patients]; p value 0.36  
EFS at 2 years: 53.5% [30.1–71.5%] versus 12.3% [2.8–29.5%]; p value 0.002  
OS at 2 years: 64.2% [38.3–81.4%] versus 33.9% [15.8–53.1%]; p value 0.005  
RFS at 2 years: 55.9% [32.2–74.3%] versus 14.5% [3.2–33.8%]; p value 0.004 |
| Lancet et al., JCO phase III clinical trial<sup>26</sup> | CPX-351 versus 7 + 3 standard induction | Patients were aged 60–75 years with newly diagnosed therapy-related AML, AML with antecedent MDS or CMML, or de novo AML with MDS-related cytogenetic abnormalities (per 2008 WHO criterion) | Subgroup analysis from concurrent FLT3 mutation  
ORR (n = 22 in CPX-351 group and n = 21 in 7 + 3 group)  
mOS: 10.25 months in CPX-351 group versus 4.60 months in the 7 + 3 group;  
HR 0.76 (0.34–1.66); trend but no statistical significance |
| RUNX1 | BRIGHT AML1003 phase II<sup>23</sup> | Glasdegib versus LDAC or LDAC | >55 years and older and not suitable for intensive chemotherapy. | Subgroup analysis from concurrent RUNX1 (n = 35): glasdegib versus LDAC  
ORR 36% [10 out of 28] versus 0% [0 out of 7] |

(Continued)
| High risk mutations/ cytogenetics and frequency in newly diagnosed AML | Trial | Drug | Population | Efficacy (95% CI) for high risk mutations |
|---|---|---|---|---|
| ASX1 10–20% | No human trials as of yet have investigated this mutation in the context of new, targeted agents. |
| TP53 uncommon in de novo AML, ~15% of therapy-related AML or AML with MDS-related changes | VIALE: A phase II trial<sup>2</sup> | Azacitidine plus either venetoclax or placebo. | 75 years and older, or with significant co-morbidities, ineligible for standard induction | Subgroup analysis for TP53 mutation in azacitidine and venetoclax (n = 38) versus azacitidine alone (n = 14). Composite remission: 55.3% [38.3–71.4] and 0% (p < 0.001) HR for death: 0.76 [0.40–1.45] (death in 34 out of 38 patients in azacitidine and venetoclax group versus 13 out of 14 patients for azacitidine alone group). |
| M14-387 phase Ib/II trial | Venetoclax and low-dose cytarabine | 60 years or older and ineligible for intensive chemotherapy | Subgroup analysis TP53 mutation n = 10 CR/CRi rate 30% in combination group |
| Welch et al., NEJM 2016, prospective uncontrolled trial<sup>27</sup> | Decitabine, 10day course | Over the age of 60, newly diagnosed AML (47%), and included R/R AML (31%) and transfusion dependent MDS (22%) | Bone marrow blast clearance (<5% blasts) [CR, CRi, or morphologic complete remission]: 21 of 21 patients with TP53 mutations (100%) versus 32 of 78 patients with wild-type TP53 (41%) (p < 0.001) |
| AZA-AML-001 study<sup>28</sup> | Azacitidine versus conventional care (CC) | Newly diagnosed AML ≥age of 65years, ECOG scores ≤2, white blood cell counts ≤15 × 10<sup>9</sup>/L, and intermediate- or poor-risk cytogenetics with >30% bone marrow blasts. | CCR group: TP53 mutation n = 17, WT p53 = 56. Azacitidine group: TP53 mutation n = 15, WT p53 = 68. In CC arm, median OS 2.4 months in TP53 mutation group versus 12.5 months in wild type TP3 group; p = 0.026. In azacitidine group, median OS 7.2 months in TP53 mutation group versus 12.0 months in WT-TP3 mutation group (not statistically different with p = 0.40). |
| GFM phase II study<sup>29</sup> | APR-246 combined with azacitidine | Adults, with treatment naive intermediate, high, or very high Revised International Prognostic Scoring System (IPSS-R) TP53-mutants (N = 55) | Evaluable group (n = 43): The ORR 87%. CR/CRi 53%. Marrow CR: 9%. Marrow CR and hematologic improvement: 18%. Hematological improvement alone: 7%. Median time to response was 2.1 months [0.1–5.4] and median duration of response of 6.5 months. CR rate AML (4/8 patients). An isolated mTP53 was predictive for a higher CR rate (69% versus 25%; p = .006). |
| Sallman et al. ASH 2020, phase Ib trial<sup>30</sup> | Magrolimab combined with azacitidine | Adults, with treatment-naive AML, unfit for intensive chemotherapy (N = 52) | Evaluable group (n = 21 patients with TP53 mutations): ORR 71% [15 out of 21 patients] CR rate of 48% [10 out of of 21 patients] CRi rate of 5% [1 out of 21 patients] Median duration of response of 9.9 months mOS for the TP3 mutated patients 12.9 months [95% CI: 6.2–14.4 months – not reached] versus 18.0 months for TP3 wild type patients [95% CI: 4.3–24.4 months – not reached]. |
| Swords et al. Blood. Phase Ib trial<sup>31</sup> | Pevonedistat combined with azacitidine | Adults ≥60years old with treatment-naive AML, who were considered unlikely to benefit from standard induction with at ≥1 of the following: age ≥75years, presence of antecedent MDS, adverse cytogenetic risk, and EOGD of ≥2 (N = 64) | Subgroup analysis (n = 5 patients with TP53 mutations): CR or PR in 80% [four of five patients]. Subgroup analysis including all sites after further molecular review (n = 8 with TP53 mutations): CR/CRi or PR in 75% [six out of eight patients]. |

Table 3. (Continued)
Table 3. [Continued]

| High risk mutations/ cytogenetics and frequency in newly diagnosed AML | Trial | Drug | Population | Efficacy (95% CI) for high risk mutations |
|---|---|---|---|---|
| **KIT** 20% in core binding factor [CBF]-AML | Phase II pilot study\(^{22}\) | Imatinib | Adult patients, c-kit–positive AML refractory to or not eligible for standard chemotherapy | ORR 5/21 patients [two with complete hematologic CR; one with no evidence of leukemia and two with partial responses] |
| **CALGB 10801\(^{23}\)** | Standard induction with 7 + 3 and oral dasatinib | Adult patients, newly diagnosed AML with CBF fusion transcripts [RUNX1/RUNX1T1 or CBFB/MYH11] | Subgroup, post hoc analysis; Concurrent KIT mutation in 19% of patients KITmut+ patients had comparable outcome with those with wild-type KIT. 3 year DFS: 67% versus 75% respectively [83% versus 77% for younger patients]; 3 year OS: 73% versus 76% respectively [88% versus 84% for younger patients]; 3 year DFS and OS for patients with higher versus lower levels of KITwt expression were 82% versus 72% and 75% versus 80% respectively |
| **AMLSG 11–08** phase Ib/II trial\(^{14}\) | Intensive induction treatment [7 + 3] and consolidation chemotherapy [HIDAC] with dasatinib, and 1-year dasatinib maintenance | Adults, with newly diagnosed AML patients positive for core-binding factor [factor] | Subgroup exploratory analysis; concurrent KIT mutation in n=191; 23% of patients [I18e211, n=7 [20%]; inv16, n=12 [25%]]. Inferior outcomes in patients with KIT mutations as opposed to wild type with regards to EFS, p=0.01 and CIR, p=0.09; OS, p=0.003 |
| **RAS [NRAS / KRAS]** \(\sim 10–15\%\) | BRIGHT AML1003 phase II\(^{27}\) | Glasdegib +LDAC or LDAC | >55 years and older and not suitable for intensive chemotherapy. | Subgroup post hoc analysis for Glasdegib + LDAC versus LDAC; Concurrent KRAS mutation n=4; NRAS mutation n=8 ORR % in KRAS: 0% [zero out of two patients] versus 0% [zero out of two patients]; ORR % in NRAS: 20% [one out of five patients] versus 0% [zero out of three patients] |
| **aza-AML--001 study\(^{18}\)** | Azacitidine versus conventional care [CC] with either intensive chemotherapy, low-dose cytarabine, or best supportive care only | Newly diagnosed AML >age of 65 years, ECOG scores ≤2, white blood cell counts ≤15 x 109/L, and intermediate- or poor-risk cytogenetics with >30% bone marrow blasts. | Subgroup, post-hoc analysis; concurrent NRAS mutation in CCR arm n=8; versus WT-NRAS n=65. Concurrent NRAS mutation azacitidine arm n=10, versus WT-NRAS n=73. CCR arm: Median OS 4.3 months in NRAS mutated subgroup treated versus 10.3 months in wild type group, p=0.020. Azacitidine arm: Median OS 11.8 months in NRAS mutated subgroup versus 8.9 months in wild type group, p=0.95 |
| **RNA Spliceosomes [SRSF2, SFP3B, U2A/F1, and ZRS2]\(\sim 10\%\)** | First in human, phase I trial, Steensma et al. Blood\(^{29}\) | H3B-8800 | Adult patients with patients with MDS, AML or CMML 88% of the cohort had spliceosome mutations of interest | No objective CR or PR were seen in 84 patients enrolled. One patient with CMLM had a durable platelet response that began in Cycle 1 and persisted through Cycle 13. Nine RBC transfusion-dependent patients with MDS or CMML and two patients with AML did not require RBC transfusions for >8 weeks and up to 28 weeks. One platelet transfusion-dependent patient with low risk-MDS did not require platelet transfusions for >8 weeks. |
| **DNMT3A** 20% in de novo and secondary AML\(^{24}\)** | No human trials as of yet have investigated this mutation in the context of new, targeted agents. | | |
| **Poor or unfavorable cytogenetics 10–12%\(^{27}\)** | VIALE-A phase III trial\(^{2}\) | Azacitidine plus either venetoclax or placebo. | 75 years and older or with significant co-morbidities, ineligible for standard induction | Subgroup analysis with unfavorable risk: Azacitidine plus venetoclax versus azacitidine with placebo n=56 patients in control and n=104 in GO group. Median OS 7.6 months [5.3–9.9] and 6.0 months [3.6–10.1] [HR0.78, 95% CI 0.54–1.1]. CR/CRI 53.7% versus 23.2% with risk difference of 30.6 months (95% CI 15.03–44.31) |
| **BRIGHT AML1003 phase II\(^{23}\)** | Glasdegib & LDAC versus LDAC | >55 years and older and not suitable for intensive chemotherapy. | Subgroup with poor cytogenetic risk n=55 [LDAC & Glasdegib versus LDAC alone CR, n%=5/36 [13.9%]; exact CI 6.9–24.21 versus 1/19 [5.3%], 80% exact CI 0.6–19.0] |
| High risk mutations/ cytogenetics and frequency in newly diagnosed AML | Trial | Drug | Population | Efficacy (95% CI) for high risk mutations |
|---|---|---|---|---|
| Welch et al., NEJM, prospective uncontrolled trial | Decitabine, 10 day course | Over the age of 60, newly diagnosed AML (47%), and included R/R AML (31%) and a transfusion dependent MDS (22%) | Subgroup with unfavorable cytogenetic risk (n=43) Bone marrow blast clearance ≤5% blasts, CR, CRi, or morphologic complete remission 29 of 43 or 67% patients with karyotypes associated with unfavorable risk versus 24 of 71 or 34% patients with karyotypes associated with intermediate or favorable risk |
| ALFA 0701 phase III trial | 7 + 3 with or without GO | Patients between 50–70 years of age with previously untreated de novo CD33+ AML | Subgroup with unfavorable risk (n=28 in the GO group versus 30 patients in control) CR + CRp: 50% versus 50%; p value 1.00 EFS at 2 years: NA versus 5.0% [0.5–18.8]; p value 0.91 OS at 2 years: NA versus 20.1% [6.5–38.9]; p value 0.23 RFS at 2 years: NA versus 10.0% [0.8–33.5]; p value 0.84 |
| EORTC-GIMEMA AML-19 phase III trial | G0 versus BSC | Patients over the age of 75 years, and patients 61–75 years of age with a WHO performance score greater than two or who were unwilling to receive standard chemotherapy | Subgroup with unfavorable risk (n=32 patients in control and n=33 in GO group) OS: 33/33 deaths for GO in unfavorable cytogenetic group versus 29/33 for BSC in unfavorable cytogenetic group; HR 1.11 [0.67–1.83]. |
| MyloFrance-1, phase II study | G0 monotherapy | Patients over 18 years of age, with R/R AML excluding secondary AML and patients with prior HSCT | Subgroup with unfavorable risk (n=12), intermediate risk (n=43) ORR poor risk 35% and intermediate risk 25% (p=0.25) |
| AZA-AML-001 study | Azacitidine versus conventional care (ICR) with either intensive chemotherapy, low-dose cytarabine, or best supportive care only | Newly diagnosed AML >age of 65 years, ECOG scores ≤2, white blood cell counts <15 × 10⁹/L, and intermediate- or poor-risk cytogenetics with >30% bone marrow blasts. | Median OS for adverse risk karyotypes in azacitidine group was 5.3 versus 2.9 months with CCR; HR 0.71 [95%CI 0.51, 0.99]; p=0.046 One-year survival rates of 29.1% versus 14.7% for patients treated with azacitidine and CCR, respectively. |
| Lancet et al. JCO, phase III clinical trial | CPX-351 versus 7 + 3 standard induction | Patients were aged 60–75 years with newly diagnosed therapy-related AML, AML with antecedent MDS or CMML, or de novo AML with MDS-related cytogenetic abnormalities (per 2008 WHO criteria) | Subgroup with concurrent unfavorable cytogenetics n=72 in CPX-351 group and n=83 in 7 + 3 group Median OS: 6.60 months in the CPX-351 group versus 5.16 in the control arm, HR of 0.73 [0.51–1.06]; trend towards CPX-351 but not statistically significant |
| Garcia-Manero et al. Blood Adv, phase II trial | Pracinostat and azacitidine | Patients were >age of 65 years, with newly diagnosed AML, ineligible for induction chemotherapy | Subgroup analysis (n=27) had intermediate-risk and 21 (42%) had high-risk cytogenetic abnormalities CRi/CRi/MLFS rates: 38.5% [95% CI, 38.8–77.6%] versus 47.6% [95% CI, 24.7–70.2%] in patients with intermediate versus unfavorable-risk cytogenetics, median OS: 24.1 months for patients with intermediate-risk cytogenetics (95% CI, 10.7 months—not estimable) versus 13.5 months in unfavorable-risk cytogenetics (95% CI, 2.4–26.5 months) |

*Composite remission includes CR or CRi. Investigator-reported ORR = CR + CRi + MLFS. 7 + 3, induction treatment with daunorubicin and cytarabine; AML, acute myeloid leukemia; BSC, best supportive care; CI, confidence interval; CIR, cumulative incidence of relapse; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; DFS, disease-free survival; EFS, event-free survival; GO, Gemtuzumab ozogamicin; HIDAC, high dose Ara-C; LDAC, low dose Ara-C; MDS, Myelodysplastic syndrome; MPN, myeloproliferative neoplasms; ORR, overall response rate; OS, overall survival; RFS, Relapse free survival; R/R, relapsed/refractory.
greater depth of CR and/or an increased number of patients able to go to HSCT.

Sorafenib has been most successful in the post-transplant setting for patients in CR after allogeneic HSCT. Sorafenib was instituted between 30 days after induction/consolidation and 120 days post-transplant once patients achieved count recovery. The OS at 24, 36, and 48 months was 76% [95% confidence interval (CI), 63–91%), 76% (95% CI, 63–91%), and 57% (95% CI, 31–91%), respectively. The EFS at 24, 36, and 48 months was 74% (95% CI, 62–90%), 64% (95% CI, 48–85%), and 64% (95% CI, 48–85%), respectively. Sorafenib in this study population was well tolerated and did not impair engraftment, with a non-relapse mortality rate at 3 years post-transplant of 10% (95% CI, 1–20%). The phase II SORMAIN trial (N=83) evaluated the use of sorafenib for 24 months in adults with FLT3-ITD positive AML after obtaining complete hematologic remission with HSCT. At a median follow up of 41.8 months, they found a 24-month relapse free survival of 53.3% (95% CI, 0.36–0.68) in the placebo group versus 85.0% (95% CI 0.70–0.93) with hazard ratio (HR) of 0.26 (95% CI 0.10–0.65, p = 0.002).

Next-generation TKIs (crenolanib, quizartinib, and gilteritinib) have more specific activity with fewer off-target effects. Of these TKIs, gilteritinib demonstrates the greatest clinical benefit to date, as seen in the R/R AML ADMIRAL trial, and is approved for R/R AML. Compared with salvage chemotherapy, gilteritinib had a higher CR rate (34% versus 15.3%) and OS of 9.3 months versus 5.6 months (p < 0.001). Post hoc analyses of phase II and III combination trials suggest improved efficacy in the subgroup of patients with FLT3 mutations relative to standard approaches. Combinations of TKIs with diverse chemotherapies (CPX-351, azacitidine, 7+3, cladribine) and targeted agents such as venetoclax are in clinical trials, with over 20 open and recruiting trials within the United States for FLT3-positive AML. Other studies with subgroup analysis of FLT3 positive patients are listed in Table 3.

RUNX1

RUNX1, located on chromosome 21q22, is an important transcription factor involved in hematopoietic stem cell (HSC) growth, differentiation, and homeostasis. Different types of RUNX1 mutations are found in AML, including missense mutations, deletions, truncation mutations, and frameshift mutations in the “Runt” homology domain. These diverse mutations lead to loss-of-function mutations with attendant chemotherapy resistance and poor prognosis. RUNX1 mutations are typically associated with older age, male gender, more immature morphology, and MDS/AMLs.

RUNX1 mutations are seen in approximately 8–16% of AML patients and are typically associated with ASXL1 mutations and other epigenetic modifiers (IDH2, KMT2A, EZH2) as well as spliceosome mutations. They are typically inversely associated with NPM1 and CEBPA mutations, and are associated with lower CR rates, shorter disease-free survival (DFS), EFS, and OS. Co-mutation with ASXL1, SRSF2, or PHF6 confers a significantly worse prognosis relative to pairing with other mutations such as IDH2.

To date, there are no approved targeted agents for mutated RUNX1 (mtRUNX1), but preclinical studies demonstrate that the depletion of RUNX1 by short-hairpin RNA as well as editing-out mtRUNX1 eR1 (CRISPR/Cas9-mediated) leads to AML cell death. In vitro treatment with bromo-domain and extraterminal (BET) protein antagonists has also proven efficacious in causing mtRUNX1 AML cell death. BET proteins recognize acetylated lysine moieties on histones and act as a scaffold to recruit promoters and enhancers to co-activate expression of genes involved in cell growth and survival. AML mtRUNX1 cell engrafted in mice treated with a BET protein inhibitor exhibited increased apoptosis and subsequently improved survival. A phase I trial of the BET inhibitor OTX015 in 41 patients yielded CR or CR with incomplete recovery (CRi) in 3, but did not detect a correlation between response and mtRUNX1. Other early trials with other BET inhibitors have shown similar findings. The first generation BET protein inhibitor, ABBV-075, in combination with venetoclax, was found to significantly reduce AML cell-burden and prolong survival in AML engrafted immune depleted mice, which may provide a springboard for BET inhibitor/venetoclax combination trials.

ASXL1

Additional sex comb-like 1 (ASXL1) is a chromatin-binding polycomb protein required for normal
embryogenesis through epigenetic activation and repression of gene transcription, and is located on chromosome band 20q11.34 ASXL1 mutations are detected in 10–20% of AMLs and consist predominantly of heterogenous nonsense/frameshift mutations that appear to result in loss of function.55,56 Nonetheless, gain-of-function mutations have also been suspected with homozygous mutations. Similar to RUNX1, they are found in older patients, those with secondary AML, and are often co-mutated with RUNX1 and spliceosome mutations.54 They are inversely associated with FLT3 ITD mutations, mutually exclusive with NPM1 mutations, and associated with lower rates of CR and overall poorer prognosis when compared with wild type ASXL1.55,57 There are currently no targeted agents that have shown improved outcomes in this subgroup of patients. Nonetheless, BET inhibitors may afford complementary effects on chromatin remodelling.51,52

**Tumor protein 53**

The tumor protein 53 (TP53) gene, located on chromosome 17p13.1, is a prototypical tumor suppressor gene, and is mutated in up to 50% of cancers.58 The encoded TP53 protein is involved in transcriptional regulation of downstream pathways crucial in tumor suppression through apoptosis, DNA repair, and cell cycle arrest/senescence. Multiple forms of TP53 mutations are detected in AML, including missense mutations, deletions, insertions, and nonsense mutations.59 TP53 gain-of-function mutations can also lead to proliferation and survival of tumor cells, as well as angiogenesis and metastasis.60 The most common TP53 mutations are missense alterations occurring in the DNA-binding domain.61 Though mutations in other domains also impact TP53 protein function, the implication of these mutations remains uncertain.

While germline TP53 mutations define the archetypal familial cancer syndrome known as Li-Fraumeni, only 1.1% of AML cases have a germline TP53 mutation. On the other hand, abnormalities in TP53 arise as somatic mutations in 10% of de novo AMLs, 20% in t-AMLs, and up to 90% in erythroleukemias.58 Prognosis with TP53 mutations is dismal, with high rates of disease refractory to both chemotherapy and HSCT with CR rates <30% and 3-year OS <15%.58,62 A recent study showed that patients with TP53 mutations with variant allele frequency (VAF) >40% had significantly worse cumulative incidence of relapse (p=0.030), relapse-free survival (RFS [p=0.001] and OS (p=0.003) than patients with a VAF ≤40%.63 In patients treated with a cytarabine-based regimen, the median OS of patients with a VAF >40% was 4.7 months versus 7.3 months for patients with a VAF ≤40% (p=0.006). In patients treated with HMA, the VAF did not affect OS significantly. Interestingly, patients with VAF ≤40% treated with cytarabine based regimen, had improved OS compared with those treated with HMA (1-year OS rates of 44% and 31%, respectively; p=0.04) whereas patients with VAF >40% had poorer OS regardless of treatment choice (1-year OS rate <25% in all groups). Similarly, Sasaki et al. assessed VAF in various common driver mutations in 421 patients with newly diagnosed AML.64 TP53 mutations, found in 20% of their cohort, were the major contributor to decreased OS with each increasing increment in VAF associated with a 1% higher risk of death. The median VAF in their cohort was 45.7% (range 1.15–93.74%).

APR-246 (PRIMA-1MET) is a small molecule that restores wild-type TP53 transcriptional activity of unfolded wild-type or mutant TP53 protein, resulting in apoptosis of TP53 mutated cancer cells. Preliminary results from a phase II trial of APR-246 in combination with azacitidine in 45 treatment naïve patients with intermediate/high-risk of death. The median VAF of TP53 (25% at the start of the trial) was serially assessed during treatment using next generation sequencing (NGS). At a VAF cut off of 5%, 39% achieved NGS negativity with treatment, which was associated with improvement in OS (12.8 versus 9.2 months, p = 0.02). Additionally, the median VAF at maximum mutation clearance was 0.63% (0.0–5%), with 5 (11%) becoming minimal residual disease (MRD) negative. There is an ongoing phase III trial with APR-246/azacitidine [ClinicalTrials.gov identifier: NCT03745716]. In addition, APR-246 is being studied in a phase I trial in combination with azacitidine and venetoclax [ClinicalTrials.gov identifier: NCT04214860].
Magrolimab (Hu5F9-G4) is a blocking antibody directed against CD47, which is also known as the macrophage immune checkpoint or the “don’t eat me” signal on cancer cell surfaces. This antibody induces phagocytosis and AML cell death, particularly in TP53 mutated patients. A phase Ib study combining magrolimab and azacitidine in 34 treatment-naïve AML patients unfit for intensive chemotherapy resulted in transfusion independence in 56%, ORR 65%, CR 44%, and CRi 12%, with a median time to response of 2.04 months. Of the 19 patients achieving CR/CRi, 37% (7/19) became MRD negative by flow cytometry. Importantly, patients with a TP53 mutation had a 71% objective response (15/21) with CR 48% (10), and 1 CRi, with a median duration of response of 9.9 months. The median OS for the TP53 mutated patients was 12.9 months (95% CI: 6.24 months–not reached) compared with 18.9 months for TP53 wild-type patients (95% CI: 4.34 months–not reached).

Pevonedistat (MLN4924) is a first-in-class small molecule inhibitor of Nedd8-activating enzyme (NAE), thereby inactivating E3 ubiquitin ligases known as ring ligases (CRLs), which in turn leads to accumulation of CRL protein substrates such as c-MYC, leading to eventual cell death through activation of pro-apoptosis pathways. CRLs are crucial for cell proliferation and survival. Based on clinical efficacy as a single agent in the R/R setting, a phase Ib study combining pevonedistat with azacitidine in adults aged ≥60 years with newly diagnosed AML showed an ORR of 50% in the intent-to-treat analysis, with a median duration of response of 8.3 months in 44% who were able to receive at least six cycles of the combination. Of the five patients with TP53 mutation identified at baseline, CR or PR was achieved in four. A total of six out of eight (75%) total TP53-mutated patients achieved CR/CRi. There is an ongoing phase Ib trial in adults ≥50 years with de novo or secondary AML including those with adverse cytogenetics and/or TP53 mutations, combining venetoclax and azacitidine with escalating doses of pevonedistat (PAVE) [ClinicalTrials.gov identifier: NCT04172844].

Flotetuzumab, a bispecific DART (dual-affinity retargeting agent) antibody-based molecule to CD3ε and CD123, is being investigated as monotherapy in the R/R setting, and early findings suggest that the 42 patients with TP53 mutations have higher rates of CD+ T cell infiltration, expression of immune checkpoints, and IFN-γ signalling than patients with other risk defining mutations such as ASXL1, TET2, and DNMT3A. CR was achieved in 47% (7/15) of patients with R/R AML and TP53 abnormalities, with 2 remaining in CR >6 months. Responders exhibited significantly higher tumor inflammation signature at baseline defined by FOXP3, CD8, inflammatory chemokine, and PD1 gene expression scores, compared with nonresponders. Immune infiltration in the tumor microenvironment is an important predictor of treatment outcomes with immunotherapy, and is discussed further in the section Microenvironmental Targets: Immunotherapy below. Patients with TP53 mutations who achieved CR had a median OS of 10.3 months (range 3.3–21.3 months), suggesting that flotetuzumab immunotherapy could improve treatment outcomes in this high-risk group compared with standard of care – a hypothesis that needs to be tested. Other studies with TP53 mutations analysis are listed in Table 3.

Mutations not included in the 2017 ELN risk stratification

KIT

Some chromosomal abnormalities seen in AML are t(8;21)(q22;q22) and inv(16)(p13;q22), known as core binding factor-AML (CBF-AML), which produce corresponding abnormal fusion genes RUNX1-RUNX1T1 and CBFβ-MYH11. CBF-AML is seen in approximately 15–20% of newly diagnosed AML cases and generally has a better prognosis except in the presence of a c-kit mutation; seen in approximately 60–80% of AML patients, with activating mutations seen in 20% of CBF-AML adult patients. KIT is located at chromosome band 4q12 and encodes a transmembrane glycoprotein that activates downstream signaling pathways involved in cell proliferation, differentiation, and survival. Discrepancies exist in terms of outcomes in patients with c-kit mutations, with some studies showing worse OS and decreased remission duration, while others show similar outcomes in those with and without the mutation.

Clinical trials have evaluated the effects of adding a TKI to standard treatment options in CBF-AML. Dasatinib in combination with 7 + 3 induction followed by consolidation and maintenance treatment was studied in the CALGB 10801...
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phase II trial. This trial enrolled 61 adult patients with newly diagnosed CBF-AML, of whom 19% harbored a c-kit mutation (Table 3). In a post hoc analysis of this limited cohort, patients with c-kit mutations had similar outcomes to those with wild-type KIT with 3-year rates of DFS (67% versus 75%) and OS (73% versus 76%), and there were no differences related to the magnitude of KIT wildtype expression.

The phase II AMLSG 11-08 trial also examined the effects of dasatinib in combination with intensive induction and consolidation chemotherapy in newly diagnosed CBF-AML. In contrast to CALGB 10801, patients with c-kit mutation (n = 19) had inferior outcomes compared with c-kit wild type (n = 70). There is an ongoing phase III trial of intensive chemotherapy with or without dasatinib in newly diagnosed adult patients with CBF AML with RUNX1-RUNX1T1 (or variant form) or CFB-MYH11 fusion transcripts [ClinicalTrials.gov identifier: NCT02013648]. Additionally, there is a phase II study combining midostaurin with standard induction, consolidation, and maintenance therapy in adult patients ≤65 years of age with de novo AML harboring rearrangements in CBF-AML genes (RUNX1-RUNX1T1 and CFB-MYH11); [ClinicalTrials.gov identifier: NCT03686345] and [ClinicalTrials.gov identifier: NCT01830361].

RAS genes encode a family of proteins that are crucial in cell signaling networks that regulate cell function across multiple tissue types. RAS oncoproteins are the most common somatic mutations in human cancer and occur in 12–27% of patients with AML. In AML, the incidence of NRAS mutations is about 11%, compared with approximately 5% in KRAS mutations. The RAS pathway is activated by mutations in upstream receptor tyrosine kinases (FLT3 or c-KIT) or mutations/overexpression of downstream effector pathways in RAS/RAF/MEK/ERK kinase pathways. Therefore, patients without RAS mutations but with FLT3, KIT or PDGFR mutations have activation of RAS-dependent pathways, thereby increasing proliferation and preventing apoptosis of leukemic cells. The exact prognostic implication of RAS mutations is unclear, particularly in the newly diagnosed setting. Nonetheless, a retrospective study found decreased median EFS in AML patients that harbored NRAS or KRAS mutations (4.9 months) compared with RAS wild-type patients (11.4 months; p < 0.01) at a median follow up of 25 months. RAS was associated independently with increased risk of death with HR of 1.85 (p = 0.016). Interestingly, relapse occurred despite ongoing RAS mutation clearance in 6 out of 10 patients.

Acquisition of RAS or FLT3 ITD mutations at the time of progression from MDS to AML clearly leads to worse outcomes. The median OS after leukemia transformation in those with a detectable RAS and/or FLT3-ITD mutations was 2.4 months compared with 7.5 months in patients with wild type RAS and FLT3 (HR: 3.08, 95% CI 1.9–5.0, p < 0.0001). For RAS mutation alone, the median survival after leukemic transformation was 3.6 months compared with 7 months in patients with wild-type RAS (p = 0.0008).

For patients with IDH mutations, co-mutations with RAS have been associated with higher rates of resistance to monotherapy with targeted treatments. Proposed mechanisms include constitutive activation of RAS pathway, bypassing the differentiation induced by IDH1/2 inhibitors, or RAS mutation being a marker of higher mutational burden, which increases rates of resistance. These associations have not been consistently identified, and, more recently, the AG221-AML-005 trial reported that co-mutations with RAS/FLT3 have not affected response rates to combination treatment with the IDH 2 inhibitor enasidenib plus azacitidine in newly diagnosed patients being treated with this regimen in the front line setting. However, a recent phase Ib/II trial combining the IDH1 inhibitor idancinib, venetoclax +/- azacitidine in advanced myeloid malignancies, found that active signaling co-mutations (including RAS) were seen in 66% of patients who had no response or had relapsed.

The emergence of AML subclones with new RAS-MAPK pathway mutations in patients who relapse after the FLT3 inhibitor gilteritinib has also been detected. At least in theory, combinations of FLT3 inhibitors and inhibitors of MEK inhibitors or other downstream intermediaries might prevent or overcome FLT3 inhibitor resistance. In this context, preclinical data have supported the notion that inhibition of downstream pathways such as the mitogen-activated protein kinase...
(MAPK) and/or PI3K/AKT pathway could abrogate net RAS signalling.77

RNA spliceosomes
Normal cells require functional RNA splicing of mRNA precursors by a multiprotein spliceosome complex for complete gene expression. Mutations in RNA spliceosomes leads to mis-splicing of mRNA precursors leading to abnormal epigenetic regulation, transcription, and genome integrity, ultimately leading to cancer.92,93 RNA splicing mutations (e.g., SRSF2, SF3B1, U2AF1, and ZRSR2) are frequently identified in MDS and chronic myelomonocytic leukemia (CMML), with about 10% of AML patients harboring these mutations.45 These mutations are usually exclusive of one another and are expressed with its wild type allele, suggesting the requirement of the wild-type allele function for survival of the cancer cell. These mutations are typically found in older patients with less proliferative disease, and are associated with refractory disease.

H3B-8800 – a small molecule agent that binds to SF3b complex leading to alternative splicing changes – has been studied in patients with MDS, AML, or CMML.34 Pharmacodynamic correlates detected dose-dependent alterations in mature mRNA transcripts in blood mononuclear cells with good overall patient tolerability (mostly grade 1 or 2 toxicities). While there were no objective responses seen in the 84 patients (24 with AML), there were measurable improvements in red blood cell (RBC) transfusion requirements.

In addition to preclinical studies looking at small molecule inhibitors to target the core spliceosome, RNA-binding proteins (RBPs) have also become targets of interest in myeloid leukemias with RNA splicing mutations.94 Specifically, RBM39 is a suspected RBP molecular target of sulfonamide compounds (such as indisulam, E7820, and chloroquinonxalin). RBM39 is an RBP, which has important functions in transcriptional coactivation and pre-mRNA splicing. Hypothetically, depletion or inhibition of RBM39 should lead to splicing alterations that, in turn, lead to cell death. Indisulam, a sulfonamide compound, has been shown to recruit RBM39 to the CUL4-DCAF15 E3-ubiquitin ligase, leading to its degradation and ultimately aberrant splicing. Finally, anti-sense oligonucleotide based-therapies hybridize with RNA and may thereby affect splicing events of pre-mRNA or promote RNA degradation. Early trials in this field are currently underway.

DNMT3A
DNMT3A is a pivotal regulator of the epigenetic processes of DNA methylation and chromatin modification and is mutated in about 20% of de novo AML cases.95 DNMT3A found on human chromosome 2p23, which encodes a 130-kDa protein that is expressed in two major forms: a long isoform known as DNMT3A1, and a short isoform known as DNMT3A2. Both isoforms catalyze the methylation of cytosine residues in cytosine guanine dinucleotide islands with resultant silencing of diverse genes involved in the processes of differentiation and self-renewal. DNMT3A mutations are detected mainly in older patients, and are associated with higher white blood cell counts, normal cytogenetics, and myelomonocytic or monocytic morphology. DNMT3A is often co-mutated with NPM1, IDH1, and FLT3-ITD and, although not yet incorporated into the ELN risk score, confers a poor prognosis.35,95–97 The DNMT3A-R882 mutation has been associated with poor prognosis in patients over age 60 years old, while other DNMT3A mutations have been associated with adverse prognosis in younger patients.98 A recent Chinese study of 870 adults with AML, found that prognosis of DNMT3A R882 was associated with a mutant-allele ratio, with higher allele ratios predicting a relatively poor prognosis.99

The frequency and adverse prognostic features of DNMT3A mutations suggest that the ability to target these mutations directly or through downstream intermediaries could have significant clinical impact. In this regard, the H3 lysine methyltransferase DOT1L as a critical downstream mediator of growth and survival-promoting HOX genes, is a potential therapeutic target for the treatment of DNMT3A-mutated AML,35,100 with interest in DOTL1 inhibitor Pinometostat (discussed above in regards to MLL-rearranged leukemia).

Variants in the DNMT3A gene have also been associated with clonal hematopoiesis of indeterminate potential (CHIP), and clonal cytopenias...
of undetermined significance (CCUS), which have been identified as pre-malignant conditions, often associated with older age.101–103 Though several variants in DNMT3A have been identified in CCUS and CHIP, not every mutation has been later linked to the development of AML, and the recommendation has been to monitor such patients.

Number of driver mutations: combining cytogenetics and mutations

While complex karyotypes or combined chromosomal abnormalities are present in approximately 10–12% of AML and are associated with adverse prognosis, older age, and treatment resistance in AML, little is known regarding the impact of specific co-mutations on prognosis.3,104,105 The tumor-mutational burden is relatively low in AML relative to solid tumors, and, as such, there may be less neoantigen presentation and T cell mediated immune response compared with other malignancies.106 Nonetheless, data suggest that increasing number of mutations is associated with poorer prognosis.105

Metzeler et al. sequenced the entire coding sequences of 37 genes and recurrently mutated regions in 664 untreated AML patients who received intensive induction chemotherapy between 1999 and 2012.107 The majority (97%) of patients had at least one identified driver mutation, and an additional 15 patients had recurrent balanced chromosomal translocations. The number of mutated genes per patient was 4 (range 0–10). The median number of driver gene mutations increased with age (p<0.001). Patients with intermediate-risk cytogenetics had a higher number of mutated driver genes (median of 4) compared with patients with favorable or adverse cytogenetics (median of 1 and 2, respectively). Additionally, they found that patients with secondary AML or t-AML had fewer mutations in genes covered, compared with those with de novo AML.

In a prospective study looking at 28 frequently mutated genes in 271 patients with de novo AML, patients in the intermediate cytogenetic risk group had an average of 2.76 mutations, with co-mutations associated with shorter OS (p=0.006). Mutations in NPM1 (p<0.0001), DNMT3A (p<0.0001), FLT3-ITD (p<0.0001), TET2 (p=0.0001), and IDH1/2 (p=0.0048) were found in higher rates in complex molecular genetic abnormalities involving three or more genes (CMGAs) in the intermediate cytogenetic risk group. Mutations in WT1 and KMT2A-PTD were also seen at higher rates in CMGAs at a high frequency and were mutually exclusive with prognostically favorable CEBPA double mutations (dm) (p=0.0019). CMGAs were detected in 63.0% of patients within intermediate cytogenetic risk and they carried a shorter 5-year OS in CMGA patients (18.1% versus CMGA-negative, 45.9%; p=0.0006) and higher 5-year cumulative incidence of relapse (CIR) (CMGA-positive, 83.2% versus CMGA-negative, 52.6%; p=0.0052). CMGAs were also associated with significantly worse OS and CIR in patients with normal cytogenetics. These trends persisted in patients aged ≤65 years who were also FLT3-ITD negative (OS: p=0.0010; CIR: p=0.1800). The increasing use of NGS (discussed below) will likely allow CMGA positivity to become a strong prognostic marker, which could be incorporated in the adverse risk ELN group.

Microenvironmental targets: immunotherapy

The multifaceted bone marrow microenvironment exerts a critical influence on leukemia pathogenesis, pathophysiology, and the ability to eradicate the leukemic clone.108 A key determinant in leukemia generation versus eradication is the multicompartmental immune system. Dysfunction of this system can lead to immune escape of AML cells throughout the course of the disease. Additionally, dysfunction within CD8+ T cells, and increased regulatory T cells in the peripheral blood has pointed towards important dysregulation of T cells in AML.

Both phenotype and genotype features of exhaustion and senescence are seen in CD8+ T cells identified in AML patients.109 Furthermore, different phenotypes are also seen in patients who respond and those who do not respond to induction treatment. Patients who do respond to treatment have upregulation of co-stimulatory T cell signaling pathways, and downregulation of apoptotic T cell signaling pathways. Additionally, increased levels of regulatory T cells have been associated with inferior outcomes. Preclinical studies have shown that primary AML cells express inducible T-cell co-stimulator (ICOS),
which is a member of the CD28 family of co-stimulatory molecules, and maintains durable immune reactions when binding to ICOS ligand.\textsuperscript{110} ICOS/ICOS ligand binding plays an important role in Treg cell function and differentiation. High levels of ICOS\textsuperscript{+} regulatory T cells secrete high interleukin (IL)-10, which promotes AML cell proliferation. Pomalidomide in an immunomodulatory imide drug (IMiD) that induces T cell proliferation and enhances IL-2 and interferon $\gamma$ (IFN$\gamma$) production through its interaction with cereblon – a substrate receptor for E3 ubiquitin ligase complex.\textsuperscript{111} This second generation IMiD also inhibits regulatory T cells and enhances both natural killer (NK) cell mediated cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) due to increased IL-2 production. In an attempt to modulate this immune response, investigators have done earlier trials with pomalidomide after timed sequential therapy induction treatment with AcDVP16 (cytarabine, daunorubicin, and etoposide) in newly diagnosed AML patients and high risk MDS, at the beginning of early lymphocyte recovery (days 14–21). In 43 patients (AML $n=39$, remainder MDS), the overall CR (CR + CRi) rate was 75\% with a median OS of 27.1 months, and DFS of 20.6 months. Within the AML group, there was an overall 86\% CR/CRi rate in AML patients with unfavorable-risk cytogenetics.

In addition to T cell dysfunction, AML cells in the relapsed setting have been shown to have high levels of PD-1 expression also contributing to immune evasion.\textsuperscript{112} Interestingly, PD-1 expression in CD4$^+$ and CD8$^+$ T cells in newly diagnosed patients is significantly less, and often not observed. It is, however, seen more commonly during therapy, after alloHSCT, and at disease relapse. Having higher PD-1 expression is also associated with poor prognosis.

Early findings in the R/R AML setting, as seen in a single center azacitidine and nivolumab non-randomized phase II study, found moderate responses with an ORR of 33\% (23/70 patients), of which there were 22\% overall CRs (CR/CRi).\textsuperscript{113} A phase II trial of pembrolizumab given post HIDAC salvage chemotherapy in R/R AML ($n=37$), had an overall response (ORR: CR + CRi + PR + MLFS) of 46\% and composite CR (CR + CRi) rates of 38\%.\textsuperscript{114} In the newly diagnosed setting, a large randomized phase II trial evaluated the efficacy and safety of azacitidine and durvalumab versus azacitidine alone in both high risk MDS and older and unfit AML patients not eligible for intensive chemotherapy.\textsuperscript{115} Of the 129 enrolled AML patients, the median OS and median PFS were not statistically different between the two groups at 13.0 versus 14.4 months and 8.1 versus 7.2 months, respectively. Though treatment was well tolerated, there was no clinically relevant benefit to adding immunotherapy to treatment.

There are several large trials currently underway evaluating pembrolizumab, nivolumab, and atezolizumab in combination with various backbone treatments including venetoclax, HMA, or induction chemotherapy in both R/R and newly diagnosed AML patients, largely in the post-transplant and post remission setting, where PD 1 expression is likely to be higher than at baseline.\textsuperscript{112}

**Measuring the depth of AML cell kill: a critical part of assessing efficacy**

MRD is defined as the presence of residual leukemia that was not detected through traditional histopathologic methods and in the presence of $<$5\% blasts.\textsuperscript{116} MRD positivity correlates with an increased risk of relapse either at the end of treatment or prior to allogeneic BMT.\textsuperscript{117} Nonetheless, MRD has not yet been standardized quantitatively or qualitatively, which will be necessary for widespread clinical application.\textsuperscript{118} MRD can be identified using multiparametric flow cytometry (MPFC), digital PCR, allele-specific oligonucleotide quantitative polymerase chain reaction (PCR), and NGS, depending on resources available and the genomic abnormality being followed (Table 4).\textsuperscript{116,119} MPFC can identify the presence of leukemia cells down to levels of $1 \times 10^4$–$1 \times 10^6$. It is fast and widely available, and provides an absolute quantification; however, MPFC has less sensitivity than other methods, and variable antigen expression may yield false negative results. Quantitative PCR methods detect aberrant mutations in leukemic cells compared with a housekeeper gene down to levels of $1 \times 10^6$–$1 \times 10^7$, with variability depending on the mutation. The problem with this technology is that there is often no PCR target, especially in the elderly.

By comparison, NGS can detect the presence of leukemia cells down to levels of $<$1 $\times 10^6$, has a high degree of sensitivity, and does not need
patient-specific primers or probes as compared with allele-specific oligonucleotide PCR.\textsuperscript{120} The downside to NGS is that it remains expensive, requires a high degree of expertise, and is not yet standardized. Extensive discussion of these techniques and upcoming technology is not the focus of this review and is therefore not included.

All the methods listed above allow for detection of significantly smaller amounts of residual disease compared with standard morphology. Evidence now suggests that identifying patients with MRD-positive disease can lead to early intervention to prevent relapse with post remission therapy.\textsuperscript{118} For patients who are MRD positive prior to transplant, outcomes remain poor. As of yet, however, MRD is not a standardized tool used in clinical setting, despite its crucial role in prognosis and outcome. Current and future prospective trials are crucial to examining the role of specific post-induction strategies, including maintenance therapies in HSCT or patients with MRD positivity.

### Conclusion

The molecular dissection of AML has uncovered the genomic architecture and the interactive pathways that are essential for leukemia cell growth and survival. Indeed, AML pathogenesis and pathophysiology represent a complex symphony with diverse components that harmonize to create and sustain malignant cells that are programmed to survive a multiplicity of existential threats including cytotoxic therapies. These components exist not only in the leukemia cells themselves but also in diverse components of the microenvironment.

In this review, we have highlighted a few selected molecular lesions where the development of

| MRD method       | Description                                                                                                                                                                                                 |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| MPFC             | MPFC is based on immunophenotyping technologies. There are two current techniques: (1) leukemia associated immunophenotype uses individual-specific surface makers identified at diagnosis and follows these markers in subsequent assessments. (2) The “different from normal” method identifies aberrant surface marker profiles at follow-up irrespective of profiles at diagnosis and can identify immunophenotype shifts.\textsuperscript{121} |
| dPCR             | Conventional PCR assays amplify a segment of DNA exponentially creating multiple copies; therefore, these segments of nucleic acid can be quantified by comparing the number of amplification cycles and the amount of PCR copies with a reference sample. For dPCR, the exponential signal of PCR is converted into a linear digital signal. It is designed to provide an absolute nucleic acid quantification, making it superior for detecting MRD.\textsuperscript{122} |
| ASO quantitative PCR | This technology uses an ASO probe for detection of specific mutations. ASO probes are synthetic DNA complementary to the sequence of a variable target DNA. A fluorogenic probe is designed for each individual tumor-specific MRD-PCR target.\textsuperscript{123} |
| NGS              | NGS is also known as high throughput sequencing, and is a technology that allows for massively parallel sequencing of multiple genes, whole exomes and genomes. It can be done in a single day, and is precise. There is a large variability in cost between whole genome sequencing, whole exome sequencing and targeted sequencing, where only chosen regions of interest are sequenced. There are a variety of different technologies and companies that run this testing, each with a unique list of targeted genes.\textsuperscript{120} |

ASO, allele-specific oligonucleotide; dPCR, digital polymerase chain reaction; MPFC, multiparametric flow cytometry; MRD, minimal residual disease; NGS, next generation sequencing.
targeted therapies to modulate those targets is underway. In particular, we have focused on specific high-risk genetic mutations that play critical roles in leukemogenesis and for which there are drugs that are currently in the clinical arena, either approved or under rigorous clinical investigation. There are numerous pivotal genomic lesions and molecular pathways that we have not addressed, for instance the intermediaries that regulate cell cycle progression and the diverse pathways that are activated by DNA damage and orchestrate the repair of such damage. In this regard, optimal anti-AML therapy requires complementary strategies, including diagnostic standards, aimed at interdicting multiple aberrations and at preventing those mechanisms, whether primary or in response to initial therapy, that confer net resistance.

Author contributions
CL developed the idea and format for the review. KD constructed all the tables. CL, KD, and JK all contributed equally to the writing and approval of the final manuscript.

Conflict of interest statement
CL has served on an advisory board for Abbvie, Agios, Daiichi-Sankyo, Macrogenics, and Jazz Pharmaceuticals and as a speaker for Astellas and Jazz Pharmaceuticals. KD and JK declare that they have no competing interests.

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References
1. Dohner H, Weisdorf DJ and Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015; 373: 1136–1152.
2. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med 2020; 383: 617–629.
3. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424–447.
4. DiNardo CD and Wei AH. How I treat acute myeloid leukemia in the era of new drugs. Blood 2020; 135: 85–96.
5. Sabine K, Robert KH, Marlise RL, et al. Allogeneic hematopoietic cell transplantation improves outcome of adults with (t;6;9) acute myeloid leukemia: results from an international collaborative study. Haematologica 2020; 105: 161–169.
6. Diaz-Beyá M, Labopin M, Maertens J, et al. Allogeneic stem cell transplantation in AML with (t;6;9)(p23;q34);DEK-NUP214 shows a favourable outcome when performed in first complete remission. Br J Haematol 2020; 189: 920–925.
7. Perl AE. MLL-menin and FLT3 inhibitors team up for AML. Blood 2020; 136: 2369–2370.
8. Daneshbod Y, Kohan L, Taghadosi V, et al. Prognostic significance of complex karyotypes in acute myeloid leukemia. Curr Treat Options Oncol 2019; 20: 15.
9. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. Blood 1998; 92: 2322–2333.
10. Daigle SR, Olhava EJ, Therikelsen CA, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood 2013; 122: 1017–1025.
11. Britten O, Ragusa D, Tosi S, et al. MLL-rearranged acute leukemia with t(4;11)(q21;q23)-current treatment options. Is there a role for CAR-T cell therapy? Cells 2019; 8: 1341.
12. Grembecka J, He S, Shi A, et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. Nat Chem Biol 2012; 8: 277–284.
13. Yokoyama A, Somervaille TC, Smith KS, et al. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. Cell 2005; 123: 207–218.
14. Schoch C, Schnittger S, Klaus M, et al. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 2003; 102: 2395–2402.
15. Stein EM, Garcia-Manero G, Rizzieri DA, et al. The DOT1L inhibitor pinometostat reduces
H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood 2018; 131: 2661–2669.

16. Menghrajani K, Cai SF, Devlin SM, et al. A phase Ib/II study of the histone methyltransferase inhibitor pinometostat in combination with azacitidine in patients with 11q23-rearranged acute myeloid leukemia. Blood 2019; 134: 2655.

17. Wang ES, Altman JK, Pettit K, et al. Preliminary data on a phase 1/2A first in human study of the menin-KMT2A (MLL) inhibitor KO-539 in patients with relapsed or refractory acute myeloid leukemia. Blood 2020; 136: 7–8.

18. Daver N, Schlenk RF, Russell NH, et al. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia 2019; 33: 299–312.

19. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med 2017; 377: 454–464.

20. Röllig C, Serve H, Hüttmann A, et al. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukemia (SORAML): a multicentre, phase 2, randomised controlled trial. Lancet Oncol 2015; 16: 1691–1699.

21. Estève J, Schots R, Bernal Del Castillo T, et al. Multicenter, open-label, 3-arm study of gilteritinib, gilteritinib plus azacitidine, or azacitidine alone in newly diagnosed FLT3 mutated (FLT3mut+) acute myeloid leukemia (AML) patients ineligible for intensive induction chemotherapy: findings from the safety cohort. Blood 2018; 132: 2736.

22. Goldberg AD, Coombs CC, Wang ES, et al. Younger patients with newly diagnosed FLT3-mutant AML treated with crenolanib plus chemotherapy achieve adequate free crenolanib levels and durable remissions. Blood 2019; 134: 1326.

23. Cortes JE, Heidel FH, Hellmann A, et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. Leukemia 2019; 33: 379–389.

24. Wei AH, Strickland SA Jr, Hou JZ, et al. Venetoclax combined with low-dose cytarabine for previously untreated patients with acute myeloid leukemia: results from a phase Ib/II study. J Clin Oncol 2019; 37: 1277–1284.

25. Castaigne S, Pautas C, Terre C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet 2012; 379: 1508–1516.

26. Welch JS, Petti AA, Miller CA, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med 2016; 375: 2023–2036.

27. Kollig C, Serve H, Hüttmann A, et al. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukemia (SORAML): a multicentre, phase 2, randomised controlled trial. Lancet Oncol 2015; 16: 1691–1699.

28. Dohner H, Bolli A, Tang L, et al. Cytogenetics and gene mutations influence survival in older patients with acute myeloid leukemia treated with azacitidine or conventional care. Leukemia 2018; 32: 2546–2557.

29. Sallman DA, DeZern AE, Garcia-Manero G, et al. Phase 2 results of APR-246 and azacitidine (AZA) in patients with TP53 mutant myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia (AML). Blood 2019; 134: 676.

30. Sallman DA, Asch AS, Kambhampati S, et al. The first-in-class anti-CD47 antibody magrolimab combined with azacitidine is well-tolerated and effective in AML patients: phase 1b results [abstract]. ASH Annual Meeting 2020; Session 613.

31. Swords RT, Coutre S, Maris MB, et al. Pevonedistat, a first-in-class NEDD8-activating enzyme inhibitor, combined with azacitidine in patients with AML. Blood 2018; 103: 3644–3654.

32. Kindler T, Breitenbuecher F, Marx A, et al. Efficacy and safety of imatinib in adult patients with c-kit–positive acute myeloid leukemia. Blood 2004; 103: 3644–3654.

33. Marcucci G, Geyer S, Laumann K, et al. Combination of dasatinib with chemotherapy in previously untreated core binding factor acute myeloid leukemia: CALGB 10801. Blood Adv 2020; 4: 696–705.

34. Paschka P, Schlenk RF, Weber D, et al. Adding dasatinib to intensive treatment in core-binding factor acute myeloid leukemia—results of the AMLSG 11-08 trial. Leukemia 2018; 32: 1621–1630.

35. Steensma DP, Wermke M, Klimmek VM, et al. Results of a clinical trial of H3B-8800, a splicing
modulator, in patients with myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) or chronic myelomonocytic leukemia (CMML). *Blood* 2019; 134: 673.

36. Brunetti L, Gundry MC and Goodell MA. DNMT3A in leukemia. *Cold Spring Harb Perspect Med* 2017; 7: a030320.

37. Hong WJ and Medeiros BC. Unfavorable-risk cytogenetics in acute myeloid leukemia. *Expert Rev Hematol* 2011; 4: 173–184.

38. Amadori S, Suciu S, Selleslag D, et al. Gemtuzumab ozogamicin versus best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19 trial. *J Clin Oncol* 2016; 34: 972–979.

39. Taksin AL, Legrand O, Raffoux E, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. *Leukemia* 2007; 21: 66–71.

40. Garcia-Manero G, Abaza Y, Takahashi K, et al. Pracinostat plus azacitidine in older patients with newly diagnosed acute myeloid leukemia: results of a phase 2 study. *Blood advances*. 2019; 3: 508–18.

41. Pratz KW, Rudek MA, Smith BD, et al. A prospective study of peritranplant sorafenib for patients with FLT3-ITD acute myeloid leukemia undergoing allogeneic transplantation. *Biol Blood Marrow Transplant* 2020; 26: 300–306.

42. Burchert A, Bug G, Fritz LV, et al. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with FLT3–internal tandem duplication mutation (SORMAIN). *J Clin Oncol* 2020; 38: 2993–3002.

43. Fernandez S, Desplat V, Villacreces A, et al. Targeting tyrosine kinases in acute myeloid leukemia: why, who and how? *Int J Mol Sci* 2019; 20: 3429.

44. Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. *N Engl J Med* 2019; 381: 1728–1740.

45. DiNardo CD and Cortes JE. Mutations in AML: prognostic and therapeutic implications. *Hematology Am Soc Hematol Educ Program* 2016; 2016: 348–355.

46. Mill CP, Fiskus W, DiNardo CD, et al. RUNX1-targeted therapy for AML expressing somatic or germline mutation in RUNX1. *Blood* 2019; 134: 59–73.

47. Jalili M, Yaghmaie M, Ahmadvand M, et al. Prognostic value of RUNX1 mutations in AML: a meta-analysis. *Asian Pac J Cancer Prev* 2018; 19: 325–329.

48. Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinicopathologic and genetic features. *Leukemia* 2016; 30: 2160–2168.

49. Mendler JH, Maharry K, Radmacher MD, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. *J Clin Oncol* 2012; 30: 3109–3118.

50. Berthon C, Raffoux E, Thomas X, et al. Bromodomain inhibitor OXt015 in patients with acute leukaemia: a dose-escalation, phase 1 study. *Lancet Haematol* 2016; 3: e186–e195.

51. Patel MR, Garcia-Manero G, Paquette R, et al. Phase 1 dose escalation and expansion study to determine safety, tolerability, pharmacokinetics, and pharmacodynamics of the BET inhibitor FT-1101 as a single agent in patients with relapsed or refractory hematologic malignancies. *Blood* 2019; 134: 3907.

52. Dawson M, Stein EM, Hunty BJ, et al. A phase I study of GSK525762, a selective bromodomain (BRD) and extra terminal protein (BET) inhibitor: results from part 1 of phase I/II open label single agent study in patients with acute myeloid leukemia (AML). *Blood* 2017; 130: 1377.

53. Fiskus W, Cai T, DiNardo CD, et al. Superior efficacy of cotreatment with BET protein inhibitor and BCL2 or MCL1 inhibitor against AML blast progenitor cells. *Blood Cancer J* 2019; 9: 4.

54. Metzeler KH, Becker H, Maharry K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. *Blood* 2011; 118: 6920–6929.

55. Pratcorona M, Abbas S, Sanders MA, et al. Acquired mutations in ASXL1 in acute myeloid leukemia: prevalence and prognostic value. *Haematologica* 2012; 97: 388–392.

56. Hsu Y-C, Chiu Y-C, Lin C-C, et al. The distinct biological implications of Asxl1 mutation and its roles in leukemogenesis revealed by a knock-in mouse model. *J Hematol Oncol* 2017; 10: 139.
57. Paschka P, Schlenk RF, Gaidzik VI, et al. ASXL1 mutations in younger adult patients with acute myeloid leukemia: a study by the German-Austrian Acute Myeloid Leukemia Study Group. Haematologica 2015; 100: 324–330.

58. Dutta S, Pregartner G, Rücker FG, et al. Functional classification of TP53 mutations in acute myeloid leukemia. Cancers 2020; 12: 637.

59. Kuykendall A, Duployez N, Boissel N, et al. Acute myeloid leukemia: the good, the bad, and the ugly. Am Soc Clin Oncol Educ Book 2018: 555–573.

60. Liu J, Zhang C and Feng Z. Tumor suppressor p53 and its gain-of-function mutants in cancer. Acta Biochim Biophys Sin 2014; 46: 170–179.

61. Hunter AM and Sallman DA. Current status and new treatment approaches in TP53 mutated AML. Best Pract Res Clin Obstet Gynaecol 2019; 32: 134–144.

62. Bowen D, Groves MJ, Burnett AK, et al. TP53 gene mutation is frequent in patients with acute myeloid leukemia and complex karyotype, and is associated with very poor prognosis. Leukemia 2009; 23: 203–206.

63. Short NJ, Montalban-Bravo G, Hwang H, et al. Prognostic and therapeutic impacts of mutant TP53 variant allelic frequency in newly diagnosed acute myeloid leukemia. Blood Adv 2020; 4: 5681–5689.

64. Sasaki K, Kanagal-Shamanna R, Montalban-Bravo G, et al. Impact of the variant allele frequency of ASXL1, DNMT3A, JAK2, TET2, TP53, and NPM1 on the outcomes of patients with newly diagnosed acute myeloid leukemia. Cancer 2020; 126: 765–774.

65. Feng D, Gip P, McKenna KM, et al. Combination treatment with 5F9 and azacitidine enhances phagocytic elimination of acute myeloid leukemia. Blood 2018; 132: 2729.

66. Swords RT, Coutre S, Maris MB, et al. Pevonedistat, a first-in-class NEDD8-activating enzyme inhibitor, combined with azacitidine in patients with AML. Blood 2018; 131: 1415–1424.

67. Vadakekolathu J, Lai C, Reeder S, et al. TP53 abnormalities correlate with immune infiltration and associate with response to flotetuzumab immunotherapy in AML. Blood Adv 2020; 4: 5011–5024.

68. Aytollahi H, Shajiei A, Sadeghian MH, et al. Prognostic importance of C-KIT mutations in core binding factor acute myeloid leukemia: a systematic review. Int J Hematol Oncol Stem Cell Res 2017; 10: 1–7.

69. Sinha C, Cunningham LC and Liu PP. Core binding factor acute myeloid leukemia: new prognostic categories and therapeutic opportunities. Semin Hematol 2015; 52: 215–222.

70. Goyama S and Mulloy JC. Molecular pathogenesis of core binding factor leukemia: current knowledge and future prospects. Int J Hematol 2011; 94: 126–133.

71. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. Blood 2006; 107: 3463–3468.

72. Kim HJ, Ahn HK, Jung CW, et al. KIT D816 mutation associates with adverse outcomes in core binding factor acute myeloid leukemia, especially in the subgroup with RUNX1/RUNX1T1 rearrangement. Ann Hematol 2013; 92: 163–171.

73. Pollard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. Blood 2010; 115: 2372–2379.

74. Qin YZ, Zhu HH, Jiang Q, et al. Prevalence and prognostic significance of c-KIT mutations in core binding factor acute myeloid leukemia: a comprehensive large-scale study from a single Chinese center. Leuk Res 2014; 38: 1435–1440.

75. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. J Clin Oncol 2006; 24: 3904–3911.

76. Ward AF, Braun BS and Shannon KM. Targeting oncogenic Ras signaling in hematologic malignancies. Blood 2012; 120: 3397–3406.

77. Pomeroy EJ and Eckfeldt CE. Targeting Ras signaling in AML: RALB is a small GTPase with big potential. Small GTPases 2020; 11: 39–44.

78. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 1999; 93: 3074–3080.

79. Ritter M, Kim TD, Lisske P, et al. Prognostic significance of N-RAS and K-RAS mutations in 232 patients with acute myeloid leukemia. Haematologica 2004; 89: 1397–1399.
80. Neubauer A, Dodge RK, George SL, et al. Prognostic importance of mutations in the ras proto-oncogenes in de novo acute myeloid leukemia. Blood 1994; 83: 1603–1611.

81. Radič J, Kopecký KJ, Willman CL, et al. N-ras mutations in adult de novo acute myelogenous leukemia: prevalence and clinical significance. Blood 1990; 76: 801–807.

82. Bos JL, Verlaan-de Vries M, van der Eb AJ, et al. Mutations in N-ras predominate in acute myeloid leukemia. Blood 1987; 69: 1237–1241.

83. Bacher U, Haferlach T, Schoch C, et al. Implications of NRAS mutations in AML: a study of 2502 patients. Blood 2006; 107: 3847–3853.

84. Awada H, Kishitagari A, Kuzmanovic T, et al. Impact and outcomes of RAS gene mutations in core binding factor acute myeloid leukemia. Blood 2019; 134: 2720–.

85. Ball BJ, Hsu M, Devlin SM, et al. RAS mutations are independently associated with decreased overall survival and event-free survival in patients with AML receiving induction chemotherapy. Blood 2019; 134: 18.

86. Badar T, Patel KP, Thompson PA, et al. Detectable FLT3-ITD or RAS mutation at the time of transformation from MDS to AML predicts for very poor outcomes. Leuk Res 2015; 39: 1367–1374.

87. Amatangelo MD, Quer L, Shih A, et al. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. Blood 2017; 130: 732–741.

88. DiNardo CD, Schuh AC, Stein EM, et al. Enasidenib plus azacitidine significantly improves complete remission and overall response compared with azacitidine alone in patients with newly diagnosed acute myeloid leukemia (AML) with isocitrate dehydrogenase 2 (IDH2) mutations: interim phase II results from an ongoing, randomized study. Blood 2019; 134: 643.

89. Lachowiez CA, Borthakur G, Loghavi S, et al. Phase Ib/II study of the IDH1-mutant inhibitor ivosidenib with the BCL2 inhibitor venetoclax +/- azacitidine in IDH1-mutated hematologic malignancies. J Clin Oncol 2020; 38: 7500.

90. Hayashi M, Kikushige Y, Harada T, et al. Somatic mutations potentiating RAS-MAPK signaling confer resistant potential against FLT3-inhibitors to acute myelogenous leukemia. Blood 2019; 134: 910.

91. Smith CC, Levis MJ, Perl AE, et al. Emerging mutations at relapse in patients with FLT3-mutated relapsed/refractory acute myeloid leukemia who received gilteritinib therapy in the phase 3 admiral trial. Blood 2019; 134: 14.

92. Bejar R. Splicing factor mutations in cancer. Adv Exp Med Biol 2016; 907: 215–228.

93. Dvinge H, Kim E, Abdel-Wahab O, et al. RNA splicing factors as oncoproteins and tumour suppressors. Nat Rev Cancer 2016; 16: 413–430.

94. Taylor J and Lee SC. Mutations in spliceosome genes and therapeutic opportunities in myeloid malignancies. Genes Chromosomes Cancer 2019; 58: 889–902.

95. Park DJ, Kwon A, Cho B-S, et al. Characteristics of DNMT3A mutations in acute myeloid leukemia. Blood Res 2020; 55: 17–26.

96. Renneville A, Boissel N, Nibourel O, et al. Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. Leukemia 2012; 26: 1247–1254.

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

98. Marcucci G, Metzeler KH, Schwind S, et al. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol 2012; 30: 742–750.

99. Yuan X-Q, Chen P, Du Y-X, et al. Influence of DNMT3A R882 mutations on AML prognosis determined by the allele ratio in Chinese patients. J Transl Med 2019; 17: 220.

100. Rau RE, Rodríguez BA, Luo M, et al. DOT1L as a therapeutic target for the treatment of DNMT3A-mutant acute myeloid leukemia. Blood 2016; 128: 971–981.

101. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017; 377: 111–121.

102. Malcovati L, Galli A, Travaglini E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood 2017; 129: 3371–3378.

103. DeZern AE, Malcovati L and Ebert BL. CHIP, CCUS, and other acronyms: definition, implications, and impact on practice. Am Soc Clin Oncol Educ Book 2019: 400–410.

104. Mrózek K. Cytogenetic, molecular genetic, and clinical characteristics of acute myeloid leukemia with a complex karyotype. Semin Oncol 2008; 35: 365–377.
105. Wakita S, Yamaguchi H, Ueki T, et al. Complex molecular genetic abnormalities involving three or more genetic mutations are important prognostic factors for acute myeloid leukemia. *Leukemia* 2016; 30: 545–554.

106. Beyar-Katz O and Gill S. Novel approaches to acute myeloid leukemia immunotherapy. *Clin Cancer Res* 2018; 24: 5502–5515.

107. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood* 2016; 128: 686–698.

108. Ladikou EE, Sivaloganathan H, Pepper A, et al. Acute myeloid leukaemia in Its Niche: the bone marrow microenvironment in acute myeloid leukaemia. *Curr Oncol Rep* 2020; 22: 27.

109. Knaus HA, Berglund S, Hackl H, et al. Signatures of CD8+ T cell dysfunction in AML patients and their reversibility with response to chemotherapy. *JCI Insight* 2018; 3: e120974.

110. Han Y, Dong Y, Yang Q, et al. Acute myeloid leukemia cells express ICOS ligand to promote the expansion of regulatory T cells. *Front Immunol* 2019; 9: 2227.

111. Zhu YX, Kortuem KM and Stewart AK. Molecular mechanism of action of immune-modulatory drugs thalidomide, lenalidomide and pomalidomide in multiple myeloma. *Leuk Lymphoma* 2013; 54: 683–687.

112. Giannopoulos K. Targeting immune signaling checkpoints in acute myeloid leukemia. *J Clin Med* 2019; 8: 236.

113. Daver N, Garcia-Manero G, Basu S, et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II study. *Cancer Discov* 2019; 9: 370–383.

114. Zeidner JF, Vincent BG, Esparza S, et al. Final clinical results of a phase II study of high dose cytarabine followed by pembrolizumab in relapsed/refractory AML. *Blood* 2019; 134: 831.

115. Zeidan AM, Cavenagh J, Voso MT, et al. Efficacy and safety of azacitidine (AZA) in combination with the anti-PD-L1 durvalumab (durva) for the front-line treatment of older patients (pts) with acute myeloid leukemia (AML) who are unfit for intensive chemotherapy (IC) and pts with higher-risk myelodysplastic syndromes (HR-MDS): results from a large, international, randomized phase 2 study. *Blood* 2019; 134: 829.

116. Sánchez R, Ayala R and Martínez-López J. Minimal residual disease monitoring with next-generation sequencing methodologies in hematological malignancies. *Int J Mol Sci* 2019; 20: 2832.

117. Buckley SA, Wood BL, Othus M, et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. *Haematologica* 2017; 102: 865–873.

118. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* 2018; 131: 1275–1291.

119. Voso MT, Ottone T, Lavorgna S, et al. MRD in AML: the role of new techniques. *Front Oncol* 2019; 9: 655.

120. Leisch M, Jansko B, Zaborsky N, et al. Next generation sequencing in AML—on the way to becoming a new standard for treatment initiation and/or modulation? *Cancers* 2019; 11: 252.

121. Dix C, Lo T-H, Clark G, et al. Measurable residual disease in acute myeloid leukemia using flow cytometry: a review of where we are and where we are going. *J Clin Med* 2020; 9: 1714.

122. Cilloni D, Petiti J, Rosso V, et al. Digital PCR in myeloid malignancies: ready to replace quantitative PCR? *Int J Mol Sci* 2019; 20: 2249.

123. van der Velden V, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia* 2003; 17: 1013–1034.