

Advances in therapeutic options for newly diagnosed, high-risk AML patients

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Abstract: 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.

Keywords: acute myeloid leukemia, adverse risk, high risk, newly diagnosed, therapy

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Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy characterized by clonal proliferation of neoplastic immature precursor cells. AML impacts older adults, at a median age of 68 years, and has a poor prognosis with a 5-year overall survival (OS) of roughly 25%.¹ Approximately 35–40% of adults under 60 years of age attain complete remission (CR) and are deemed cured with treatment. Unfortunately, the prognosis in older adults remains poor, with those unable to receive intensive chemotherapy traditionally having a median overall survival of 5–10 months. The combination of a hypomethylating agent (HMA) and the small molecule BCL2 inhibitor venetoclax has improved the median OS to 15 months.² Nonetheless, AML remains a complex family of diseases characterized by a panoply of genetic and epigenetic aberrations in both the malignant clone and its microenvironment.

Intensive induction chemotherapy regimens combining cytarabine and anthracycline have been the standard of care for the last 40 years, and

cause significant toxicities, especially in older patients.³ Until the last decade, most clinical trials evaluated cytogenetic and molecular features through secondary or retrospective analysis. More recently, a select few of these aberrations have been targeted pharmacologically with resultant clinical success,⁴ emphasizing the importance of upfront diagnostic testing to optimize treatment options. In 2017, the European LeukemiaNet (ELN) updated their risk classification groups by incorporating more mutations, including those associated with adverse clinical outcomes. The adverse risk group now recognizes FLT3-internal tandem duplication (ITD) with high expression (ITD high) with wild type *NPM1*, mutated *RUNX1*, mutated *ASXL1* and mutated *TP53* (Table 1) as contributing to worse outcomes.

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

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Table 1. High-risk features in newly diagnosed AML.

Clinical factors
Age >60years
Antecedent hematological disorders
Treatment-related
Leukemia with persistence of minimal residual disease after induction chemotherapy ^a
Cytogenetic and molecular features
t(6;9)(p23;q34.1); DEK-NUP214 ^b
t(v;11q23.3);KMT2A rearrangements, multiple fusion partners
t(9;22)(q34.1;q11.2); BCR-ABL1
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2.MECOM(EVI1)
-5 or del(5q); -7; -17/abn(17p)
Complex karyotype or monosomal karyotype
Mutations within ELN adverse risk stratification
FLT3-ITD high+ wild type NPM1
RUNX1 ^c
ASXL1 ^c
TP53
Proposed high risk mutations to be included in ELN
KIT mutations
RNA Spliceomes (NRAS and KRAS)
RNA splicing (e.g., <i>SRSF2</i> , <i>SF3B1</i> , <i>U2AF1</i> , and <i>ZRSR2</i>)
DNMT3A
BCOR
Complex molecular genetic abnormalities involving three or more genes
^a Residual disease defined by multiparameter flow cytometry or positive PCR for disease-specific genes describes a group of patients with significant risk of early recurrence after consolidation therapy, including consolidation in the form of allogeneic transplantation.
^b More recent data suggests that t(6;9)(p23;q34.1) should likely be re-classified within the intermediate risk group if treated intensively with allogeneic HSCT. ^{5,6}
^c Should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.
AML, acute myeloid leukemia; ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; PCR, polymerase chain reaction.

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.

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

High-risk mutations	Functional class	Mechanisms of leukemogenesis	Incidence in AML
<i>FLT3, KRAS, NRAS, KIT</i>	Signaling and kinase pathway	These mutations lead to the aberrant activation and proliferation of cellular signaling pathways.	~2/3 of AML cases
<i>DNMT3A, ASXL1</i>	Epigenetic modifiers (DNA methylation and chromatin modification)	Felt to be inciting mutations in leukemogenesis and are often found in age-related clonal hematopoiesis. 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
<i>SRSF2, SF3B1, U2AF1, and ZRSR2</i>	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
<i>RUNX1</i>	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
<i>TP53</i>	Tumor suppressors	Tumor suppression occurs <i>via</i> 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.

Adverse risk molecular factors in the 2017 ELN risk stratification

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.³ Unfavorable cytogenetics define adverse ELN risk and thus provide critical prognostic information that can inform treatment options.⁷

Nonetheless, -7 , $-5/\text{del}(5q)$, monosomal karyotypes, and complex cytogenetics with at least three abnormalities carry an adverse prognosis independent of treatment type.⁸ 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.^{8,9}

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

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).¹⁰ 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)(q23;p13.3).¹¹ 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 *MEN 1* gene).¹² When co-factor menin and *MLL* fusion proteins interact, there is an upregulation of *HOXA9* 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.¹³ *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.¹⁴

The histone H3K79 methyltransferase *DOT1L* is recruited in *MLL*-rearranged AML, leading to methylation of oncogenic downstream targets *HOXA9* and *Meis1*.¹⁰ 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.¹⁵ 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.¹⁶

New therapeutic agents targeting the menin-*KMT2A* protein-protein interaction are being investigated in early phase clinical trials.⁷ Preliminary results of KO-539 in a phase I/IIA 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.¹⁷

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.¹⁸ 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-ITD*^{low} (without adverse-risk genetic lesions) and mutated *NPM1* and *FLT3-ITD*^{high} as intermediate risk.³ Patients with mutated *NPM1* with *FLT3-ITD*^{low} 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.¹⁹ This phase III trial found significantly improved event-free survival (EFS) and OS in the combination midostaurin and daunorubin/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 ¹⁹	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: 7.4 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 ²⁰	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	Exploratory analysis (n = 46) for patients with FLT3 ITD, in the sorafenib group (n = 23) compared with the placebo group (n = 23) EFS: median 5 months [95% CI 0–12] versus 6 months [1–11] RFS: 18 months [0–36] versus 6 months [0–16] OS: not reached versus 19 months [0–39]
	Phase I/II gilteritinib and azacitidine trial ²¹	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. ²²	7 + 3 induction with HiDAC consolidation with Gremolamib (continued as maintenance for 12 months after HSCT)	Newly diagnosed AML patients with FLT3 mutations, aged 18–60 years old	n = 27 CR: 23 of 27 patients (85%) 19 of 27 patients remain alive and free of disease with a median follow-up of 29.3 months at time of reporting
	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 for 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 for azacitidine alone group]
	BRIGHT AML 1003 phase II ²³	Glasdegib and LDAC or LDAC	>55 years and older and not suitable for intensive chemotherapy.	Subgroup analysis Glasdegib and LDAC or LDAC; FLT3 ITD n = 5 ORR (n = 5) 33% versus 0% (n = 2)
	M14-387 phase Ib/II ²⁴	Venetoclax and low-dose cytarabine	60 years or older and ineligible for intensive chemotherapy	Subgroup analysis 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% CRi, and 35% CR)
	ALFA 0701 phase III trial ²⁵	7 + 3 with or without G0	Patients between 50–70 years with previously untreated de novo CD33+ AML	Subgroup with FLT3 ITD+ (n = 22 in G0 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: 53.0% (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 ²⁶	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 FLT3 mutation: 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 ~10–15%	BRIGHT AML 1003 phase II ²³	Glasdegib versus LDAC or LDAC	>55 years and older and not suitable for intensive chemotherapy.	Subgroup with concurrent RUNX1 n = 35; glasdegib versus LDAC ORR 36% (10 out of 28) versus 0% (0 out of 7)

(Continued)

Table 3. (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 III trial ²	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 (<i>n</i> = 38) versus azacitidine alone (<i>n</i> = 14) Composite remission ^a : 55.3% (38.3–71.4) and 0% (<i>p</i> < 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	Venetoclax and low-dose cytarabine	60 years or older and ineligible for intensive chemotherapy	Subgroup analysis TP53 mutation <i>n</i> = 10 CR/CRi rate 30% in combination group
	Welch <i>et al.</i> , NEJM 2016, prospective uncontrolled trial ²⁷	Decitabine, 10 day course	Over the age of 60, newly diagnosed AML (47%), and included R/R AML (31%) and transfusion dependent MDS (22%)	Subgroup with TP53 mutations <i>n</i> = 21, WT TP53 <i>n</i> = 78 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%) (<i>p</i> < 0.001)
	AZA-AML-001 study ²⁸	Azacitidine versus conventional care (CCR) 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 × 10 ⁹ /L, and intermediate- or poor-risk cytogenetics with > 30% bone marrow blasts.	CCR group: TP53 mutation <i>n</i> = 17, WT TP53 = 56 Azacitidine group: TP53 mutation <i>n</i> = 15, WT TP53 = 68 In CCR arm, median OS 2.4 months in TP53 mutation group versus 12.5 months in wild type TP53 group; <i>p</i> = 0.026 In azacitidine group, median OS was 7.2 months in TP53 mutation group versus 12.0 months in WT-TP53 mutation group (not statistically different with <i>p</i> = 0.40)
	GFM phase II study ²⁹	APR-246 combined with azacitidine	Adults, with treatment naive intermediate, high, or very high Revised International Prognostic Scoring System (IPSS-R) TP53-mutants (<i>N</i> = 55)	Evaluate group (<i>n</i> = 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%; <i>p</i> = .006)
	Sallman <i>et al.</i> , ASH 2020, phase Ib trial ³⁰	Magrolimab combined with azacitidine	Adults, with treatment-naïve AML, unfit for intensive chemotherapy (<i>N</i> = 52)	Evaluate group (<i>n</i> = 21 patients with TP53 mutations) ORR 71% (15 out of 21 patients) CR rate of 48% (10 out of 21 patients) CRi rate of 5% (1 out of 21 patients) Median duration of response of 9.9 months mOS for the TP53 mutated patients 12.9 months (95% CI: 6.24 months – not reached) versus 18.9 months for TP53 wild type patients (95% CI: 4.34 months – not reached)
	Swords <i>et al.</i> , Blood, Phase Ib trial ³¹	Pevonedistat combined with azacitidine	Adults ≥ 60 years old with treatment-naïve AML, who were considered unlikely to benefit from standard induction (with at ≥ 1 of the following: age ≥ 75 years, presence of antecedent MDS, adverse cytogenetic risk, and ECOG of 2) [<i>N</i> = 64]	Subgroup analysis (<i>n</i> = 5 patients with TP53 mutations) CR or PR in 80% (four of five patients). Subgroup analysis including all sites after further molecular review (<i>n</i> = 8 with TP53 mutations) CR/CRi or PR in 75% (six out of eight patients)

(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 ³³	Standard induction with 7 + 3 and oral dasatinib	AML patients, newly diagnosed AML with CBF fusion transcripts (<i>RUNX1/RUNX1T1</i> or <i>CBFB/MYH11</i>)	Subgroup, <i>post hoc</i> analysis; Concurrent <i>KIT</i> mutation in 19% of patients <i>KIT</i> /mut+ patients had comparable outcome with those with wild-type <i>KIT</i> 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) 3yr DFS and OS for patients with higher versus lower levels of <i>KIT</i> wt expression were 82% versus 72% and 75% versus 80% respectively
	AMLSG 11-08 phase Ib/II trial ³⁴	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 <i>KIT</i> mutation in (n = 19) 23% of patients [18/21], n = 7 [20%]; inv(16), n = 12 [25%]] Inferior outcomes in patients with <i>KIT</i> mutations as opposed to wild type with regards to EFS, p = .01 and CIR, p = .09; OS, p = .003
RAS (NRAS / KRAS) ~10-15%	BRIGHT AML1003 phase II ²³	Glasdegib + LDAC or LDAC	>55 years and older and not suitable for intensive chemotherapy.	Subgroup <i>post hoc</i> 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 ²⁸	Azacitidine versus conventional care (CCR) 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 × 10 ⁹ /L, and intermediate- or poor-risk cytogenetics with >30% bone marrow blasts.	Subgroup, <i>post-hoc</i> 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 Spliceomes (<i>SRSF2</i> , <i>SF3B</i> , <i>U2AF1</i> , and <i>ZRSR2</i>) ~10%	First in human, phase I trial. Steensma et al. Blood. ³⁵	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 CMML 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 ³⁶	No human trials as of yet have investigated this mutation in the context of new, targeted agents.			
Poor or unfavorable cytogenetics 10-12% ³⁷	VIALE-A phase III trial ²	Azacitidine plus either venetocicax or placebo.	75 years and older, or with significant co-morbidities, ineligible for standard induction	Subgroup analysis with unfavorable risk: Azacitidine plus venetocicax 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 [3.6-10.7] [HR 0.78; 95% CI, 0.54-1.1]. CR/CRi 53.9% versus 23.2% with risk difference of 29.67 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) in LDAC & Glasdegib versus LDAC alone CR, n(%) : 5/36 (13.9%, exact CI 6.9-24.2) versus 1/19 (5.3%, 80% exact CI 0.6-19.0)

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Table 3. (Continued)

High risk mutations/ cyogenetics and frequency in newly diagnosed AML	Trial	Drug	Population	Efficacy (95% CI) for high risk mutations
Welch <i>et al.</i> , NEJM, prospective uncontrolled trial ²⁷	Decitabine, 10 day course	Over the age of 60, newly diagnosed AML (47%), and included R/R AML (31%) and transfusion dependent MDS (22%)	Subgroup with unfavorable cytogenetic risk (<i>n</i> = 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 ²⁵	7 + 3 with or without GO	Patients between 50–70 years with previously untreated de novo CD33+ AML	Subgroup with unfavorable risk (<i>n</i> = 28 in the GO group versus 30 patients in control) CR + CRp: 50% versus 50%; <i>p</i> value 1.00 EFS at 2 years: NA versus 5.0% (0.5–18.8); <i>p</i> value 0.91 OS at 2 years: NA versus 20.1% (6.5–38.9); <i>p</i> value 0.23 RFS at 2 years: NA versus 10.0% (0.8–33.5); <i>p</i> value 0.84	
EORTC-GIMEMA AML-19 phase III trial ³⁸	GO 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 (<i>n</i> = 32 patients in control and <i>n</i> = 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 ³⁹	GO monotherapy	Patients over 18 years of age, with R/R AML excluding secondary AML and patients with prior HSCT	Subgroup with unfavorable risk (<i>n</i> = 12), intermediate risk (<i>n</i> = 43) ORR poor risk 35% and intermediate risk 25% (<i>p</i> = 0.25)	
AZA-AML-001 study ²⁸	Azacitadine versus conventional care (CCR) 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 × 10 ⁹ /L, and intermediate- or poor-risk cytogenetics with >30% bone marrow blasts.	Subgroup analysis CCR group: <i>n</i> = 81 versus azacitidine group: <i>n</i> = 73 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 <i>p</i> = 0.046) One-year survival rates of 29.1% versus 14.7% for patients treated with azacitidine and CCR, respectively.	
Lancet <i>et al.</i> JCO, phase III clinical trial ²⁶	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 <i>n</i> = 72 in CPX-351 group and <i>n</i> = 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 <i>et al.</i> Blood Adv. phase II trial ⁴⁰	Pracinostat and azacitidine	Patients were ≥ age of 65 years, with newly diagnosed AML, ineligible for induction chemotherapy	Subgroup analysis (<i>n</i> = 27 [54%] had intermediate-risk and 21 [42%] had high-risk cytogenetic abnormalities) CR/CRi/MLFS rates: 59.3% (95% CI, 38.8–77.6%) versus 47.6% (95% CI, 25.7–70.2%) 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)	

^aComposite 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.⁴¹ 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.⁴² 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.⁴³ Of these TKIs, gilteritinib demonstrates the greatest clinical benefit to date, as seen in the R/R AML ADMIRAL trial,⁴⁴ 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.^{45,46} These diverse mutations lead to loss-of-function mutations with attendant chemotherapy resistance and poor prognosis.⁴⁷ RUNX1 mutations are typically associated with older age, male gender, more immature morphology, and MDS/AMLs.⁴⁸

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.⁴⁸ 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.⁴⁹ Co-mutation with *ASXL1*, *SRSF2*, or *PHF6* confers a significantly worse prognosis relative to pairing with other mutations such as IDH2.⁴⁸

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 RUNX1 eR1 (CRISPR/Cas9-mediated) leads to AML cell death.⁴⁶ *In vitro* treatment with bromodomain 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 mtRUNX1.⁵⁰ Other early trials with other BET inhibitors have shown similar findings.^{51,52} 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,⁵³ 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 20q11.⁵⁴ 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 $\leq 40\%$.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 $\leq 40\%$ ($p=0.006$). In patients treated with HMA, the VAF did not affect OS significantly. Interestingly, patients with VAF $\leq 40\%$ 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/very high risk MDS, myeloproliferative neoplasms (MPNs) or oligoblastic AML (blasts $\leq 30\%$) harboring *TP53* mutations yielded an overall response rate (ORR) of 87% and CR rate of 53%.²⁹ The median duration of response was 6.5 months with intent-to-treat OS of 11.6 months (95% CI 9.2–14). 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.³⁰ 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.⁶⁵ 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.⁶⁶ 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 CD3 ϵ 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*.⁶⁷ 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 *CBFB-MYH1*.^{68,69} 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.^{45,68} *KIT* is located at chromosome band 4q12 and encodes a transmembrane glycoprotein that activates downstream signaling pathways involved in cell proliferation, differentiation, and survival.⁷⁰ 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.^{68,71–75}

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

phase II trial.³² 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.³³ 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 CBFB-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 CBFB-MYH11); [ClinicalTrials.gov identifier: NCT03686345] and [ClinicalTrials.gov identifier: NCT01830361].

RAS

RAS genes encode a family of proteins that are crucial in cell signaling networks that regulate cell function across multiple tissue types.⁷⁶ RAS oncogenes 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.⁷⁷ 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.^{78–84} 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.⁸⁵ 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.⁸⁶ 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.⁸⁷ 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.⁸⁸ However, a recent phase Ib/II trial combining the IDH1 inhibitor ivosidenib, 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.⁸⁹

The emergence of AML subclones with new RAS-MAPK pathway mutations in patients who relapse after the FLT3 inhibitor gilteritinib has also been detected.^{90,91} 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 chloroquinoxalin). 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 DOT1L 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.¹¹⁰ ICOS/ICOS ligand binding plays an important role in Treg cell function and differentiation. High levels of ICOS⁺ 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 γ (IFN γ) production through its interaction with cereblon – a substrate receptor for E3 ubiquitin ligase complex.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 early 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.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).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%.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.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.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.¹¹⁶ MRD positivity correlates with an increased risk of relapse either at the end of treatment or prior to allogeneic BMT.¹¹⁷ Nonetheless, MRD has not yet been standardized quantitatively or qualitatively, which will be necessary for widespread clinical application.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).^{116,119} MPFC can identify the presence of leukemia cells down to levels of 1×10^4 – 1×10^6 . 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 1×10^6 – 1×10^7 , 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

Table 4. Technologies for MRD detection.

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. 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. 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. 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. ¹²⁰

ASO, allele-specific oligonucleotide; dPCR, digital polymerase chain reaction; MPFC, multiparametric flow cytometry; MRD, minimal residual disease; NGS, next generation sequencing.

patient-specific primers or probes as compared with allele-specific oligonucleotide PCR.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.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

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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