

## New Strategies in Acute Myeloid Leukemia: Redefining Prognostic Markers to Guide Therapy

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### Abstract

Although standard therapy for AML has been relatively constant over the past 2 decades, this may be changing with enhanced technologies allowing for the classification of acute myeloid leukemia (AML) into molecularly distinct subsets. Some specific subsets of AML have an excellent prognosis in response to standard therapy, whereas the poor prognosis of AML associated with specific sets of mutations or chromosomal anomalies requires the development of new therapies. Elucidation of the molecular pathogenesis of AML has led to the development of therapies that affect signaling, apoptosis, protein and intermediate metabolism, the surface of the leukemia cell, leukemia cell/stromal interaction, and epigenetic regulation of gene expression. *Clin Cancer Res*; 18(19); 5163–71. ©2012 AACR.

### Background

Despite decades of clinical research, induction therapy for acute myeloid leukemia (AML) has remained virtually unchanged for 30 years. Between 20% and 40% of patients fail to achieve remission with induction chemotherapy, and 50% to 70% of patients who achieve a complete remission relapse within 3 years. Over the past decades our insight into the pathogenesis and prognosis of AML has evolved substantially. In the 1980s markers of poor prognosis included age, elevated leukocyte count, and an antecedent hematologic disorder. In the late 1980s and 1990s recurrent chromosomal anomalies were formally incorporated into the World Health Criteria for the diagnosis of AML (1). Nearly 15% of patients have favorable karyotypic abnormalities composed of t(15;17)—acute promyelocytic leukemia and the core binding factor (CBF) leukemias, which include t(8;21) or inversion 16, with 5-year survival rates of 65%. Another 15% have poor karyotypic features, including deletion of chromosome 7, 5q, or a complex karyotype comprising 3 or more chromosomal abnormalities with 5-year survival rates of 10% to 15%. However, cytogenetic prognostication is limited by heterogeneity in the intermediate-risk group, comprising 50% of patients with AML. This group encompasses patients characterized by +8, -Y, +6, del(12p), or cytogenetically normal AML (CN-AML). This cytogenetic prognostication was validated in large clinical trials (2, 3). A more comprehensive classification

system proposed by the European Leukemia Network incorporates cytogenetic and molecular abnormalities resulting in 4 prognostic subgroups—favorable; intermediate I; intermediate II; and adverse (4).

### Advances in the prognostic classification of AML

Advances in molecular technologies have led to ever-finer classification of AML. Cytogenetic studies of the 1970s and 1980s were followed by the identification of fusion genes in the 1990s and elucidation of their mode of action in blocking myeloid differentiation and stimulating proliferation. In the 1990s and 2000s, point mutations in key signaling molecules and transcription factors were identified. More recently, microarray technologies allowed for the classification of AML by virtue of gene expression patterns, patterns of gene segment gain or loss, DNA methylation patterns, and microRNA (miRNA) expression. Over the past 3 to 4 years, high-throughput sequencing of leukemia genomes has led to yet further subclassification of AML (5). Hence, morphologically identical diseases may encompass dozens of different molecular genetic subsets. This represents a challenge in designing therapy for the individual patient.

### Gene mutations

To date, assays for *NPM1*, *CEBPA*, *FLT3*, and *cKIT* mutations have entered clinical practice, affecting risk stratification and guiding therapy. Whole-genome sequencing of AML identified new recurrent mutations in AML, including *DNMT3A* (DNA methyl transferase 3A) and *IDH1*, and *IDH2* (isocitrate dehydrogenase 1 and 2). Subsequently, mutations in *DNMT3A*, *TET2*, and *ASXL1* have emerged as important adverse prognosticators in subsets of AML patients independent of *FLT3* (6). Molecular profiling will guide dosing in induction chemotherapy as cases harboring certain mutations [(mixed lineage leukemia (*MLL*) and Nucleophosmin 1 (*NPM1*)] respond better to higher doses of daunorubicin although others, such as *FLT3*, do not

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benefit. Furthermore, genotyping of AML patients will be used to guide postinduction strategies by avoiding high-risk procedures such as stem cell transplantation in the favorable prognostic subgroup. (See Table 1 for a compendium of recurrent AML mutations).

### Single gene expression

Misexpression of specific genes has been associated with AML prognosis. For example, brain and acute leukemia cytoplasmic (BAALC) gene expression is frequently associated with other adverse molecular prognostic features, including *fms*-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD), lack of *NPM1* expression, and high *ERG* (*ETS*-related gene) transcription factor expression (7). Among the favorable prognosis FLT3-ITD-negative/*NPM1*-positive patient subset, high *ERG* expressers had an approximately 4-fold higher risk of adverse outcome (8). High expression of the meningioma-1 gene (*MN1*) also confers an unfavorable outcome in CN-AML (9).

### Methylation profiles

Methylation profiling of newly diagnosed AML revealed distinct subgroups. Some methylation profiles segregated according to known cytogenetic and molecular abnormalities, and additional clusters showed unique epigenetic signatures. Moreover, a 15-gene methylation classifier was defined and validated as an independent risk factor for survival in a multivariate analysis (10). The presence of a strong hypermethylation signature in some gene clusters is associated with a poor prognosis, and this subgroup may benefit from hypomethylating agents.

### Gene expression profiling

Early gene expression profiling (GEP) of AML identified molecular subgroups with distinct gene expression signatures (11). The clinical utility of GEP was established with the Microarray Innovations in Leukemia multiple-laboratory study to assess the clinical accuracy of gene expression profiles of 16 acute and chronic leukemia subclasses in 3,334 patients (12). Profiling of *CD34*<sup>+</sup> leukemic stem cells revealed that elevated expression of *ANKRD28*, *GNA15*, and *UGP2* was correlated with poorer overall survival in CN-AML (13). Moreover, GEP of AML cells exposed to cytarabine allowed identification of critical mediators of AML cell survival such as the cell-cycle checkpoint protein *WEE1* (14).

### MicroRNA analysis

Expression profiles of miRNA in AML correlate with cytogenetic and molecular profile. Although genome-wide microarray profiling is relatively cumbersome for risk assessment in individual patients, changes in a single miRNA (i.e., miR-181a) were shown to independently predict for remission, disease-free survival (DFS), and overall survival in CN-AML (15). Subsequently, the functional relevance of miRNAs has been shown in leukemogenesis, with some miRNAs acting as oncogenes, and others as tumor suppressors. For example, *miR-29b* modulates DNA methylation by targeting *DNMT3a* and *DNMT3b*, and elevated levels can

be used as a predictor of response to decitabine, a DNMT inhibitor (16).

### Alterations in metabolic pathways

Neomorphic mutations in the metabolic enzymes IDH1 and IDH2 in AML result in the conversion of  $\alpha$ -ketoglutarate to 2-hydroxyglutarate (2HG), an oncogenic metabolite that inhibits the action of the methylcytosine oxidase TET2 and results in DNA hypermethylation. 2HG-producing IDH mutants can also prevent the histone demethylation that is required for lineage-specific progenitor cells to differentiate (17). These mutations were identified in 30% of patients with CN-AML and confer an adverse prognosis in younger (<60 years), molecular low-risk patients (18). Loss of function mutations in the  $\alpha$ -ketoglutarate-dependent enzyme TET2 are mutually exclusive with IDH mutations and are similarly associated with a worse prognosis among favorable-risk patients (19). The growth of myeloid cells harboring IDH1/2 mutations can be blocked by a specific inhibitor of the mutant enzyme, representing a future clinical strategy (20).

### On the Horizon

#### First-generation FLT3 inhibitors—lestaurtinib (CEP-701), midostaurin (PKC412), and sunitinib

The FLT3-ITD mutation leads to constitutive activation of the FMS-like tyrosine kinase 3 (FLT3) and proliferation of leukemic blasts. The first generation of FLT3 inhibitors had limited specificity and potency. A multicenter phase III trial that randomized patients with relapsed AML to induction chemotherapy alone or followed by lestaurtinib showed no survival benefit (21), attributed to the small proportion of patients that achieved FLT3 inhibition *in vivo*. Based on encouraging phase I data (22), a phase III study of daunorubicin/cytarabine with or without midostaurin for newly diagnosed FLT3<sup>+</sup> AML was completed with results forthcoming.

#### Second-generation FLT3 inhibitors

AC220 shows greater potency and selectivity in biochemical and cellular assays compared with first-generation inhibitors (23). A phase I study of AC220 in relapsed/refractory AML reported 24% transient clinical responses, and 4 of 45 patients achieved a complete remission. The majority of the responders harbored FLT3 mutations (24). A phase II trial of AC220 in relapsed/refractory patients with mutant FLT3 AML showed reductions in marrow blasts in 45% of patients, and one third of these patients were successfully bridged to hematopoietic stem cell transplantation (HSCT; 25).

#### Future of FLT3 inhibition

A retrospective study showed that the FLT3-ITD mutation resulted in a higher relapse rate after HSCT (26). As a result, a phase I trial is under way to find a safe dose of sorafenib, a Raf kinase inhibitor that also affects FLT3, for maintenance therapy after HSCT (NCT01398501). A phase I trial is also studying the combination of AC220 plus

**Table 1.** Common gene mutations in AML and their clinical importance

Gene mutation	Prevalence in CN-AML	Function	Prognostic impact	Management strategy
<b>NPM-1 (Nucleophosmin-1)</b>	50%	Nuclear shuttle protein that is aberrantly located in cytoplasm in leukemic blasts	Favorable in absence of Flt3-ITD mutation, benefit in older population	Standard induction chemotherapy followed by 3 to 4 cycles of high-dose cytarabine. Possible benefit for all- <i>trans</i> retinoic acid + chemotherapy in patients with NPM1 mutation
<b>Flt-3 ITD (Fms-like tyrosine kinase internal tandem domain mutation)</b>	20%	Class III receptor tyrosine kinase; constitutively activated, causing proliferation, survival, and differentiation	Significantly worse than unmutated FLT3; a higher ratio of mutant wild-type alleles predicts worse outcome	Overall survival with allogeneic stem cell transplant equivalent to patients with wild-type FLT3; new FLT3 inhibitors currently in clinical trials
<b>CEBP-<math>\alpha</math> (CCAAT/enhancer binding protein <math>\alpha</math>)</b>	15%–19%	Transcription factor critical to granulocyte maturation	With biallelic mutations, benefit in overall survival and recurrence-free survival (RFS) equivalent to favorable subgroup of AML	Standard induction therapy followed by repeated cycles of high-dose cytarabine is first-line treatment option; patients may not benefit from allogeneic HSCT in first complete remission
<b>c-Kit</b>	Only relevant in CBF leukemias, mutated or overexpressed in 15%–20% of CBF-AML	The SCF receptor tyrosine kinase; mutation results in constitutive activation	In patients with CBF-AML, greater probability of relapse following complete remission	Low-dose cytarabine (LDAC) with imatinib in elderly with c-kit overexpression noninferior to standard chemotherapy; NCT00850382 is currently looking at dasatinib in combination with 7+3 in CBF-AML.
<b>MLL</b>	7%–10%	Partial tandem duplication associated with silencing of wild-type allele; MLL-PTD yields aberrant expression of HOX genes	Shorter remission duration	Improved outcomes with stem cell transplant; preclinical data suggest a therapeutic role of HDAC inhibitors and hypomethylating agents.
<b>WT1</b>	10%	Transcriptional regulator	Inferior prognosis Frequent coincident FLT3 mutations	No impact on treatment at this time
<b>RUNX</b>	10%	Transcription factor	Chemotherapy-resistant disease; worse, RFS and overall survival	Patients do better with allogeneic stem cell transplants
<b>RAS</b>	12%–27%	Constitutively activating mutations in N-Ras and K-Ras, members of GTPase family	No prognostic significance	Patients benefit from high-dose cytarabine consolidation; consider MEK inhibitors on clinical trials in patients with Ras mutations
<b>TET2</b>	23%	Reduced levels of 5-OH methylcytosine, which is needed for DNA demethylation	Lower complete remission rate and shorter DFS among favorable-risk CN-AML in CALGB study; in AMLSG study, no prognostic impact	None known

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**Table 1.** Common gene mutations in AML and their clinical importance (Cont'd)

Gene mutation	Prevalence in CN-AML	Function	Prognostic impact	Management strategy
<b>DNMT3A</b>	20%	Reduced enzymatic activity of DNA methyltransferase may result in decreased DNA methylation	Independently predict shorter overall survival; often concomitant with FLT3 mutation	Benefit from high-dose anthracycline induction chemotherapy (90 mg/m <sup>2</sup> daunorubicin)
<b>IDH1, IDH2</b>	33%	Gain-of-function mutations allowing enzymes to convert $\alpha$ -ketoglutarate to 2-hydroxyglutarate, a competitive inhibitor of TET2 and histone demethylases	Prognostic impact depends on molecular context; IDH mutations coexistent with NPM-1 are associated with a good prognosis	None known

Abbreviations: AMLSG, German AML study group; CALGB, Cancer and Leukemia Group B; HDAC, histone diacetylase.

daunorubicin/cytarabine induction followed by consolidation high-dose cytarabine plus AC220 (NCT01390337).

#### MEK inhibitors

Aberrant signaling through growth factor and cytokine receptors, *RAS* mutations, and *RAF* overexpression all converge on the MEK/ERK cascade. The oral mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitor AZD6244 (27) showed only minor responses in AML, perhaps because of concomitant activation of the phosphoinositol 3-kinase (PI3K)/AKT/mTOR pathway. Leukemia cells can be more effectively targeted by combination using combined MEK and PI3K/mTOR inhibitors (28), and clinical trials of these combinations should be encouraged.

#### PI3K/mTORC inhibitors

Constitutive activation of the PI3K/AKT/mTOR pathway occurs in 60% to 80% of cases of AML and is associated with shorter disease-free and overall survival (29). *In vitro*, the PI3K inhibitor LY294002 induced apoptosis of AML cells in a dose-dependent manner (30). mTOR, a key kinase that activates metabolic pathways and cell growth, exists in functionally distinct TORC1 and TORC2 complexes. Rapamycin analogues targeting mTORC1 were used in AML (31) with no evidence of synergy when combined with chemotherapy. This may be because of the formation of mTORC2 and rapamycin-insensitive mTORC1 complexes. Dual TORC1/2 inhibitors such as OSI-027 elicited far more potent suppression of leukemic cells (32). Dual inhibition of PI3K and mTORC1/2 with BEZ-235 also suppresses growth of AML cells (33).

#### Epigenetic Modulation

Histone deacetylase (HDAC) inhibitors can potentially activate genes abnormally suppressed in cancer cells, hence reversing the malignant phenotype. These agents also alter chromatin structure and may lead to increased genomic fragility and DNA damage. Single-agent HDAC inhibitor therapy only yields 10% to 20% response rates. Vorinostat,

the first approved HDAC inhibitors, had modest single-agent activity, but combined with 5-azacitidine yielded a response rate of 30% (34). The combination of vorinostat, with idarubicin and cytarabine (AI) has synergistic activity with optimal effect when vorinostat precedes cytarabine. In a phase II trial, the response rate of 85% of the combination was superior to that of AI alone (35); notably, there were responses in all patients with FLT3-ITD mutations. Median survival was 82 weeks, with a trend toward better survival in the FLT3-ITD patients. A phase I trial (NCT00875745) is examining the combination of vorinostat and sorafenib in AML and high-risk myelodysplastic syndrome (MDS). Encouraging results have also been reported for HDAC inhibitors MGCD0103 (36) with 5-azacytidine. Entinostat, another HDAC inhibitor, synergizes with granulocyte macrophage colony-stimulating factor to promote growth of mature myeloid cells and improves marrow function, minimizing the need for platelet transfusions (37). This strategy may be applied to patients with low-disease burden.

#### Histone methyltransferase activity

The MLL translocated leukemias result in recruitment of DOT1L, a histone 3 lysine 79 (H3K79) methyltransferase activity, to activate critical target genes (38). EPZ01, a small-molecule DOT1L inhibitor, blocked tumor growth in a mouse model of MLL-fusion gene-mediated leukemia (39). Because of its short half-life, further medicinal chemistry may be required to develop a clinical reagent, or the agent will need to be given by continuous infusion for a prolonged period to block histone methylation.

#### Targeting histone-protein interactions

*BRD4*, a protein that binds to specific acetylated histone residues to "read" the histone code is critical for the growth of AML cells (40). JQ1 is a recently developed small molecule that blocks BRD4 binding to chromatin (41). Treatment of AML cells with JQ1 suppressed the expression of the *c-myc* proto-oncogene and resulted in marked cell death (42). Another bromodomain inhibitor, GSK1210151A,

was highly effective against human MLL-fusion cell lines and mouse models of MLL-fusion leukemia (43).

### Aberrant DNA methyltransferase activity

Through empirical clinical research, the DNA demethylating agents 5-azacitidine and decitabine have come into clinical use. The use of 5-azacitidine predated knowledge of the *DNMT3A* mutations or documentation of aberrant patterns of gene methylation in AML. These agents yield a response rates of 20% to 30% in MDS and AML (44). Clinical factors associated with 5-azacitidine response included untreated disease and leukocyte count  $<10 \times 10^6/\text{dL}$  (45). Although the administration of these agents leads to large-scale loss of DNA methylation *in vivo*, it is uncertain whether this leads to normalization of aberrant patterns of gene regulation or leads to responses through induction of DNA damage. In AML, aberrant DNA methyltransferase activity may play a role in epigenetic silencing of genes involved in hematopoiesis. *DNMT3A* mutation status was evaluated in 46 older patients with untreated AML who received decitabine, and patients with low *DNMT3A* activity appeared to benefit (46). Further genome-wide studies of DNA methylation patterns in AML will be required to determine if the response to DNMT inhibitors can be linked to the presence of *DNMT3A* or other mutations that affect DNA methylation, such as *TET2*, *IDH1*, or *IDH2*.

### Targeting Protein Metabolism

#### Hsp90 inhibitors

Heat-shock protein 90 (Hsp90) is a molecular chaperone for many oncogenic client proteins, such as receptor tyrosine kinases. In a phase I trial, the Hsp90 inhibitor alvespimycin increased apoptosis of marrow blasts and induced complete responses in 3 of 17 patients (47). Hsp90 inhibition can be potentiated through its increased acetylation in response to HDAC inhibitors (48). Mutated forms of FLT3 are more dependent on chaperone molecules than the wild-type molecules. 17-AAG, an Hsp90 inhibitor shows additive efficacy with the FLT3 inhibitor PKC412 in pre-clinical models (49).

#### Thalidomide/lenalidomide

Patients with del(5q) MDS display a unique sensitivity to lenalidomide where the drug exerts karyotype-specific clonal suppression. Lenalidomide may upregulate tumor suppressor genes activated by azacitidine (50). In addition, lenalidomide can upregulate the p21 gene through activation of lysine demethylases (51). A phase I trial used sequential azacitidine and lenalidomide in elderly, untreated AML patients, resulting in a 44% complete remission rate with a median response of 6.2 months. In a phase II study, elderly patients received high-dose lenalidomide at 50 mg daily for up to 2 cycles followed by maintenance. The complete remission rate was 30%, with a median duration of 10 months (52). The elusive target of this class of drugs was recently shown to be cereblon (53). When bound by thalidomide, cereblon inhibits the oncogenic Cul4A E3

ligase. Whether this is how lenalidomide affects AML remains to be determined.

### Targeting Apoptosis

Elevated expression of antiapoptotic molecules is associated with chemotherapy resistance in AML. Oblimersen sodium, a BCL-2 antisense oligonucleotide, was evaluated in combination with daunorubicin/cytarabine in a phase I trial, but a phase III trial was halted as this combination did not result in improved overall survival. ABT-737, another small-molecule BCL-2 inhibitor, slowed tumor growth in xenograft models of AML and potentiated a number of chemotherapeutic agents (54). AEG35156, an X-linked inhibitor of apoptosis (XIAP) antisense oligonucleotide, when combined with idarubicin/cytarabine reinduction, resulted in a complete remission/pathologic complete remission rate of 91% (10 of 11 patients) in refractory patients, and 9 of these 11 patients could then undergo transplantation (55).

### Leukemia/Stromal Interactions

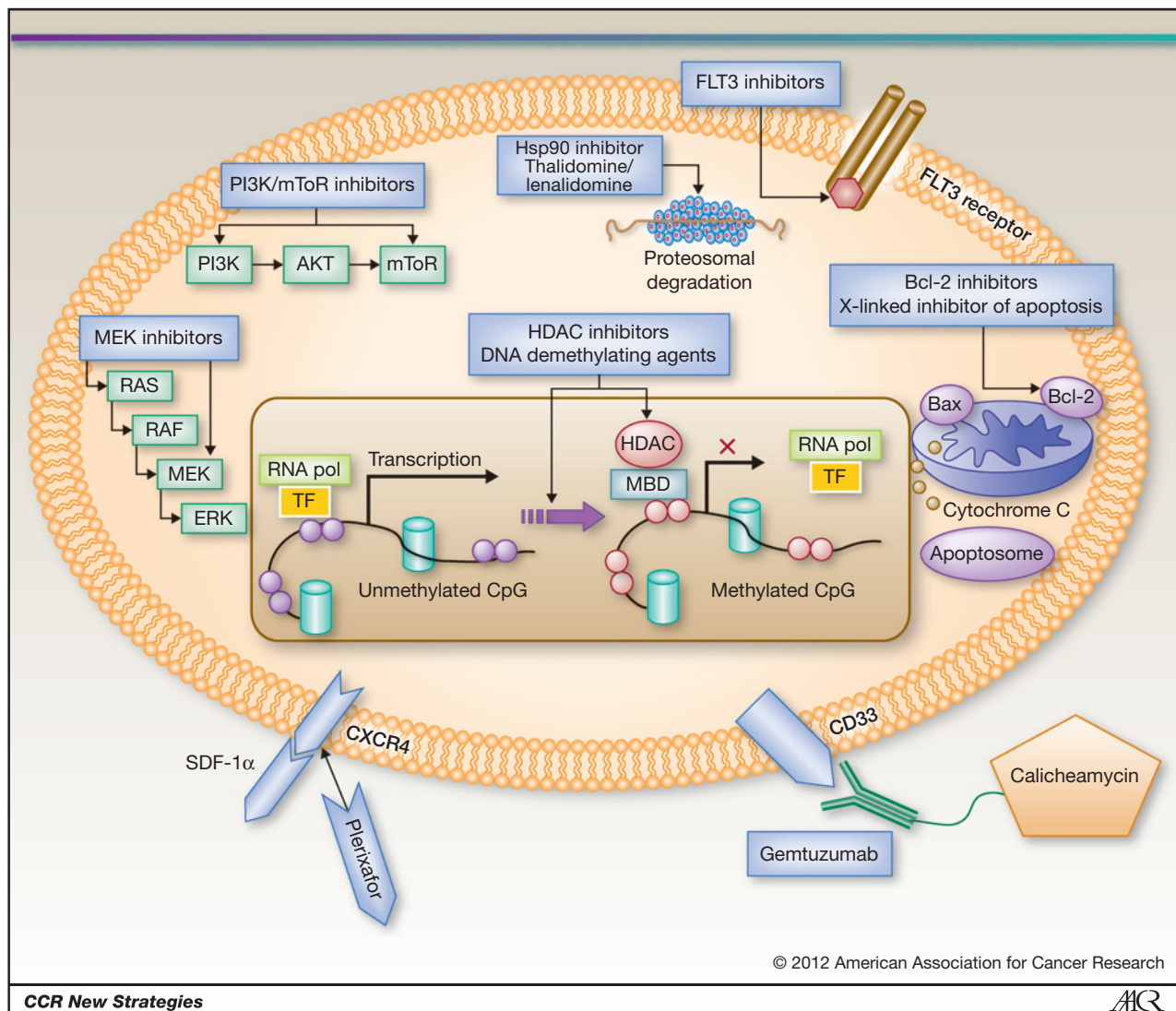
The interaction of AML blasts with the marrow microenvironment through the CXCR4/CXCL1 axis appears to be an important mediator of resistance to chemotherapy. In a murine model, the CXCR4 antagonist plerixafor released leukemic cells from protective marrow niches, enhancing the efficacy of chemotherapy (56). In a phase I/II study, 52 patients with relapsed/refractory AML were treated with plerixafor plus mitoxantrone, etoposide, and cytarabine, leading to a 46% complete remission rate (57) in association with a 2-fold mobilization in leukemic blasts into the peripheral circulation. The utility of this strategy requires confirmation in a randomized trial.

### Targeting the Cell Surface—Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (GO), an anti-CD33 immunconjugate, showed remission rates of about 25% in relapsed/refractory AML, resulting in U.S. Food and Drug Administration approval of GO nearly a decade ago. GO was subsequently removed from the market after a large Southwest Oncology Group trial showed that GO plus chemotherapy yielded no survival benefit and a high early death rate (58). GO may yet have a role in specific subsets of AML. In young patients with favorable cytogenetics, GO increased survival when it was combined with induction chemotherapy (59). Two phase III trials using a fractionated dose of GO in combination with daunorubicin/cytarabine in elderly AML patients showed significant improvement in relapse-free and overall survival (60, 61). Whether this drug will be reintroduced into clinical practice remains to be determined.

### Conclusions

Molecular profiling of AML is now affecting treatment decisions in AML. Genetic analysis of samples from E1900 showed that the more intensive 90-mg/m<sup>2</sup> dose of daunorubicin in induction chemotherapy was associated with



**Figure 1.** Therapeutic targets in AML. Therapy may be directed against signals generated by growth factor receptors (interleukin-3 receptor, FLT3) by direct kinase inhibition, by inhibition of downstream signaling (MEK), (PI3K/AKT/mTOR), or by interference with the Hsp90 chaperone protein. Additional targets on the horizon include migration/stromal interactions (SDF-1/CXCR4), antiapoptotic molecules (BCL2), or aberrant DNA methylation and histone methylation. The leukemia cell surface may be targeted by anti-CD33 antibodies, other monoclonal antibodies, or tumor vaccines. RNA pol, RNA polymerase; TF, transcription factor.

improved survival in patients with *DNMT3A*, *NPM1* mutations, or *MLL* translocations. The favorable impact of *IDH1/NPM1* mutations in CN-AML, if confirmed, might obviate aggressive consolidation and stem cell transplantation in these patients. Conversely, patients with *FLT3* mutations have a poor prognosis and may need antikinase therapy added to typical regimens. Other agents, such as MEK/ERK and AKT axis inhibitors, are well tolerated and display modest efficacy but may yet find a place in AML treatment in combination with chemotherapy. Hypomethylating agents have been empirically used in hypoproliferative AML with moderate success, in some cases prolonging survival in the absence of achieving a complete remission. Whether specific anomalies in DNA methylation patterns of mutations in the DNA methylation machinery

predicts success of these agents remains to be determined. HDAC inhibitors may have a role in combination with either chemotherapy or hypomethylating agents.

It is important to emphasize the need for biologic insight-directed use of novel agents. The high expression of CD33 on the leukemic blasts of patients with *NPM1* mutations suggests that trials using GO in this population may have yielded better results. Interest remains in developing targeting monoclonal antibodies against antigens unique to the leukemia stem cells, and vaccine trials against antigens such as WT1 continue.

Advances in next-generation sequencing technologies should soon lead to implementation of comprehensive genetic profiling in the clinical care of AML patients. As the list of genes mutated and pathways deregulated in AML grows, more

targets for therapy will be investigated (Fig. 1). AML will be subclassified into ever-smaller subsets, some of which may not be amenable to specific targeted therapy. Challenges ahead include dissecting the hierarchical significance of multiple mutations identified in these patients and finding therapies robust enough to cross over many of the genetic subsets.

#### Disclosure of Potential Conflicts of Interest

J.D. Licht has research support from Epizyme, Inc. J.K. Altman is a consultant advisory board member for Cellgene, Astellas, and Teva. No potential conflicts of interests were disclosed by the other author.

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