

Advances in the Treatment of Acute Myeloid Leukemia: New Drugs and New Challenges



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ABSTRACT

The therapeutic armamentarium of acute myeloid leukemia (AML) has rapidly expanded in the past few years, driven largely by translational research into its genomic landscape and an improved understanding of mechanisms of resistance to conventional therapies. However, primary and secondary drug resistance remains a substantial problem for most patients. Research into the mechanisms of resistance to these new agents is informing the development of the next class of AML drugs and the design of combination regimens aimed at optimally exploiting therapeutic vulnerabilities, with the ultimate goal of eradicating all subclones of the disease and increasing cure rates in AML.

Significance: AML is a heterogeneous disease, characterized by a broad spectrum of molecular alterations that influence clinical outcomes and also provide potential targets for drug development. This review discusses the current and emerging therapeutic landscape of AML, highlighting novel classes of drugs and how our expanding knowledge of mechanisms of resistance are informing future therapies and providing new opportunities for effective combination strategies.

INTRODUCTION

Driven by intense basic and translational research, the past 10 to 15 years have greatly improved our understanding of the pathobiology and genetic diversity of acute myeloid leukemia (AML). This effort has led to the discovery of several new, promising therapies for AML as well as the FDA approval of eight agents for the treatment of AML between 2017 and 2019 (1). In particular, large-scale genomic analyses have led to significant improvements in understanding the molecular landscape of AML, including the impact of multiple recurrent mutations and clusters of co-occurring mutations that frequently hold prognostic and, in some cases, therapeutic importance (2–5). The successful development of effective targeted therapies for some of the common genetic lesions in AML has led to the regulatory approval of inhibitors of mutant *fms*-like tyrosine kinase 3 (FLT3) and isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2), improving response rates and outcomes for patients whose leukemia harbors these mutations. An increasing knowledge of the

importance of the apoptotic machinery in chemotherapy resistance and AML propagation has also led to the development of apoptosis-inducing therapies that appear to be efficacious irrespective of the presence or absence of targetable genetic mutations (6, 7). Despite the advances that these new therapies represent, primary and secondary resistance remains an issue, and investigational agents to further target these resistance mechanisms are being studied in both early- and late-phase clinical trials.

This review will discuss the evolving therapeutic armamentarium for AML, with a focus on some of the most promising and active areas of research in the field, particularly the development of mutation-specific targeted therapies, combined and sequential approaches to targeting apoptotic pathways, and the broad range of immunotherapeutics in different stages of clinical development. We also discuss the next frontier in AML therapy that will focus on identifying and abrogating mechanisms of resistance to these novel agents by developing effective, rationally designed combination therapies.

CYTOGENETIC AND MUTATIONAL LANDSCAPE OF AML

AML is characterized by a number of recurrent cytogenetic abnormalities and mutations that influence disease phenotype, response to conventional therapies, risk of relapse, and survival (8). For example, *t(8;21)* and *inv(16)/t(16;16)*, which lead to the balanced translocations *RUNX1–RUNX1*

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and *CBFB-MYH11*, respectively, constitute a cytogenetically favorable risk group that is highly curable with cytotoxic combination chemotherapy, whereas the presence of a complex karyotype (defined as ≥ 3 cytogenetic abnormalities) or specific chromosomal aneuploidies (e.g., -5/-5q, -7, and -17/-17p) is associated with a relatively chemoresistant phenotype and poor prognosis (9). Although cytogenetics have historically been one of the primary determinants of prognosis in AML, up to 60% of patients have cytogenetically normal AML at the time of diagnosis, limiting the utility of karyotypic analysis to provide prognostic information in a large proportion of patients. For such patients with AML without a well-established prognostic or predictive karyotypic abnormality, identification of recurrent gene mutations is particularly important for risk stratification, decision to proceed to allogeneic hematopoietic stem cell transplantation (HSCT), and, increasingly, selection of targeted therapeutics.

The number of mutations in the AML genome is significantly lower than most solid-tumor malignancies, with an average of only 5 recurrent mutations per genome. However, at least one driver mutation is identified in 96% of patients with *de novo* AML, with 86% harboring ≥ 2 driver mutations (3, 4). In recent years, great advances have been made in understanding the genomic landscape of AML and how some of these recurrent alterations cooperate to influence disease phenotype and prognosis (3, 10). The prognostic and therapeutic implications of frequently mutated genes in AML are summarized in Table 1. In a comprehensive analysis of 1,540 patients with AML that incorporated cytogenetic analysis with genomic profiling, 11 mutually exclusive subtypes of AML were identified (3). In addition to 8 previously established AML subsets defined by the presence of an *NPM1* mutation, biallelic *CEBPA* mutations, or recurrent gene fusions [i.e., *inv(16)/t(16;16)*, *t(8;21)*, *t(15;17)*, *inv(3)/t(3;3)*, *t(6;9)*, and *KMT2A* translocations], 3 new heterogeneous subtypes of AML were defined, including AML with mutations of genes regulating RNA splicing (e.g., *SRSF2* and *SF3B1*), and/or chromatin modification (e.g., *ASXL1*), AML with chromosomal aneuploidy and/or mutation of *TP53*, and AML with *IDH2*^{R172} mutation. The presence of comutations also significantly influenced prognosis within these individual subgroups. This important study was one of the first to highlight the remarkable heterogeneity of AML, a principle that is increasingly appreciated as more knowledge continues to accumulate about the complex cytogenetic and molecular landscape of AML.

CONVENTIONAL AML THERAPY

In the 1970s, an intensive induction regimen of cytarabine and an anthracycline (commonly called the “7+3 regimen”) was developed for the treatment of AML. After receiving induction chemotherapy, patients with favorable-risk disease features [e.g., core-binding factor (CBF) AML or *NPM1* mutation without high allelic burden *FLT3*-internal tandem duplication (ITD) mutation] who achieve remission are generally recommended to continue with consolidative chemotherapy with a high-dose cytarabine-based regimen, whereas patients with adverse-risk disease features (e.g., poor-risk cytogenetics or genetic mutations) and frequently patients

with intermediate-risk features (i.e., those not falling into either favorable or adverse risk categories) should be referred for allogeneic HSCT, as the risk of relapse for these patients when treated with chemotherapy alone is unacceptably high. This risk-stratified treatment approach is curative in approximately 35% to 45% of patients <60 years of age. However, the cure rates with this approach are <15% in patients 60 years of age and older, a group that often has poor tolerance of intensive chemotherapy and increased risk of treatment-related mortality, as well as a higher rate of adverse-risk cytogenetics and mutations (8). Because AML is a disease primarily of older adults (median age at diagnosis: 68 years), a substantial proportion of patients are not suitable for intensive chemotherapy or allogeneic HSCT due to prohibitive rates of treatment-related mortality, which further contributes to the poor outcomes in this population (2). Historically, effective options have been limited for this frailer population and consisted primarily of low-dose cytarabine (LDAC) or inhibitors of DNA methyltransferases (e.g., azacitidine or decitabine), also commonly referred to as “hypomethylating agents” (HMA). Although these less-intensive regimens are associated with lower rates of treatment-related mortality than combination chemotherapy, median survival with LDAC or HMAs is only 6 to 10 months, highlighting the need for more effective, low-intensity regimens for older patients with AML.

Although it is largely true that there were few substantial therapeutic advances in the treatment of AML until the past few years, a notable exception was the treatment of acute promyelocytic leukemia (APL), which has been transformed from one of the most fatal subtypes of AML to now the most curable. APL is a biologically and clinically distinct subtype of AML characterized by the balanced translocation *t(15;17)(q24.1;q21.2)* (11). The resultant *PML-RARA* gene fusion transcribes an oncoprotein that binds to DNA, blocking transcription and differentiation of granulocytes. The use of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) have significantly improved the outcomes of patients with APL. Both ATRA and ATO bind to the *PML-RAR α* oncoprotein, resulting in its degradation and promoting differentiation, inducing apoptosis of the malignant APL cells, and restoring normal hematopoiesis (12, 13). These chemotherapy-free regimens can achieve a complete remission (CR) rate of nearly 100% and long-term survival rates of >98%, serving as a paradigm for targeted therapy and drug development in AML (14–16).

MUTATION-SPECIFIC TARGETED THERAPIES

FLT3 Inhibitors

Mutations in the *FLT3* gene occur in approximately one third of all patients with newly diagnosed AML; 20% to 25% of these mutations are ITD and 5% to 10% are point mutations of the tyrosine kinase domain (TKD; ref. 17). Both types of mutations lead to constitutive activation of the *FLT3* receptor tyrosine kinase, promoting cellular proliferation and survival and inhibiting differentiation (18, 19). In particular, *FLT3*-ITD mutations are associated with more proliferative disease (e.g., increased white blood cell count and increased peripheral and bone marrow blasts), increased risk of relapse, and inferior survival, which is influenced by both

Table 1. Recurrent genomic alterations in newly diagnosed AML in adults

Mutation	Functional mutation class	Frequency	Impact on prognosis	Comments	Targeted agents in advanced clinical development ^a
<i>FLT3</i>	Signaling and kinase pathway	20%–25% (ITD) 5%–10% (TKD)	<ul style="list-style-type: none"> Inferior survival for ITD mutations No impact on survival for TKD mutations 	<ul style="list-style-type: none"> More common in NK-AML (up to 35% for ITD mutations) Prognosis influenced by concomitant <i>NPM1</i> mutation status 	FLT3 inhibitors: midostaurin , gilteritinib , sorafenib, quizartinib, crenolanib, ponatinib, FF-10101
<i>NPM1</i>	Nucleophosmin	~30%	<ul style="list-style-type: none"> Superior survival in the absence of high allelic burden <i>FLT3</i>-ITD mutation 	<ul style="list-style-type: none"> More common in NK-AML (up to 60%) Associated with concomitant <i>FLT3</i>, <i>IDH1/2</i>, and <i>DNMT3A</i> mutations May be used to monitor for MRD 	BCL2 inhibitors: venetoclax ? All- <i>trans</i> -retinoic acid + arsenic trioxide
<i>DNMT3A</i>	Epigenetic modifier	~20%	<ul style="list-style-type: none"> Conflicting reports on impact on survival 	<ul style="list-style-type: none"> Increased incidence in older adults CHIP mutation 	? Epigenetic therapies
<i>IDH1</i> and <i>IDH2</i>	Epigenetic modifier	5%–15% (<i>IDH1</i>) 10–15% (<i>IDH2</i>)	<ul style="list-style-type: none"> Conflicting reports on impact on survival 	<ul style="list-style-type: none"> More common in NK-AML (up to 30%) <i>IDH2</i>^{R172} may represent distinct AML disease subtype 	IDH1 inhibitors: ivosidenib , FT-2102 IDH2 inhibitors: enasidenib ? Dual <i>IDH1/IDH2</i> inhibitors (in early development)
<i>NRAS</i> and <i>KRAS</i>	Signaling and kinase pathway	10%–25%	<ul style="list-style-type: none"> Conflicting reports on impact on survival 	<ul style="list-style-type: none"> Associated with <i>NPM1</i> and biallelic <i>CEPBA</i> mutations, and with <i>inv(16)/t(16;16)</i> and <i>inv(3)/t(3;3)</i> 	MEK 1/2 or AKT inhibitors: trametinib
<i>TET2</i>	Epigenetic modifier	5%–20%	<ul style="list-style-type: none"> Conflicting reports on impact on survival 	<ul style="list-style-type: none"> Increased incidence in older adults CHIP mutation 	? Epigenetic therapies
<i>RUNX1</i>	Transcription factor	5%–20%	<ul style="list-style-type: none"> Inferior survival 	<ul style="list-style-type: none"> Increased incidence in older adults Associated with secondary AML progressing from antecedent hematologic malignancy 	None currently available
<i>TP53</i>	Tumor suppressor	5%–20%	<ul style="list-style-type: none"> Inferior survival 	<ul style="list-style-type: none"> Increased incidence in older adults Associated with complex karyotype, monosomal karyotype, and secondary AML 	Agents that reactivate p53: APR-246 ? Immunotherapies (e.g., T-cell or macrophage checkpoint inhibitors)
<i>ASXL1</i>	Epigenetic modifier	5%–15%	<ul style="list-style-type: none"> Inferior survival 	<ul style="list-style-type: none"> Increased incidence in older adults CHIP mutation Associated with secondary AML progressing from antecedent hematologic malignancy 	? Epigenetic therapies
<i>CEBPA</i>	Transcription factor	~10%	<ul style="list-style-type: none"> Superior survival (only if biallelic) 	<ul style="list-style-type: none"> More common in NK-AML (up to 20%) 	None currently available
<i>KIT</i>	Signaling and kinase pathway	~10%	<ul style="list-style-type: none"> Inferior survival in CBF AML 	<ul style="list-style-type: none"> More common in CBF AML (present in 25%–35%) Poor prognosis more notable in AML with <i>t(8;21)</i> 	c-KIT inhibitors: dasatinib, midostaurin, avapritinib

Abbreviations: CHIP, clonal hematopoiesis of indeterminate potential; MRD, measurable residual disease; NK, normal karyotype.

^aAgents in bold are FDA-approved for use in AML.

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comutations (particularly *NPM1* and *DNMT3A* mutations) and the ratio of *FLT3*-ITD to wild-type *FLT3* alleles (3, 5, 20).

Over the past 15 years, several *FLT3* inhibitors have entered clinical trials (17). These agents act through competitive inhibition of the ATP-binding sites in the *FLT3* receptor; however, they vary substantially in their pharmacodynamic properties, including their potency in inhibiting *FLT3*, their activity on *FLT3*-ITD versus TKD mutations, and their activity on non-*FLT3* targets (i.e., kinome specificity), the latter of which may influence their off-target toxicities (17). First-generation *FLT3* inhibitors (e.g., midostaurin and sorafenib) have a broad kinome profile, whereas second-generation *FLT3* inhibitors (e.g., quizartinib and crenolanib) generally have more *FLT3*-specific kinome profiles. Some *FLT3* inhibitors bind to the *FLT3* receptor only in the inactive conformation (“type II inhibitors,” e.g., sorafenib and quizartinib) and thus lack significant activity against TKD mutations, which favor the active conformation (21). In contrast, type I inhibitors (e.g., midostaurin, gilteritinib, and crenolanib) bind regardless of the receptor conformation and thus should have activity against both ITD and TKD mutations.

Three *FLT3* inhibitors (midostaurin, quizartinib, and gilteritinib) have demonstrated improvement in overall survival (OS) in randomized phase III studies compared with conventional therapies. Midostaurin is a multitargeted protein kinase inhibitor that targets c-KIT, PKC, PDGFR, and VEGFR, in addition to ITD and TKD mutations of the *FLT3* protein (22). In the multinational, randomized phase III RATIFY (CALBG 10603) study, 717 adults <60 years of age with newly diagnosed *FLT3*-mutated AML (either ITD or TKD) were randomized to receive standard 7+3 induction followed by consolidation with high-dose cytarabine (or HSCT, if appropriate) in combination with either midostaurin or placebo (23). Midostaurin or placebo were given during induction and consolidation, and could be given for up to one year as post-consolidation maintenance. Treatment with midostaurin was associated with a significant improvement in OS (4-year OS rate: 51.4% versus 44.3%; median OS: 74.7 months versus 25.6 months; $P = 0.009$); an incremental improvement in survival was observed regardless of the type of *FLT3* mutation (e.g., ITD or D835 TKD) or the ITD allele burden, suggesting that midostaurin could be used in all *FLT3*-mutated patients. On the basis of these results, midostaurin was approved by the FDA in April 2017 for the treatment of adults with newly diagnosed *FLT3*-mutated AML in combination with induction and consolidation chemotherapy.

In patients with relapsed or refractory *FLT3*-mutated AML, two second-generation *FLT3* inhibitors, quizartinib and gilteritinib, have shown improvement in response rates and OS compared with standard salvage chemotherapies in randomized studies (24, 25). Quizartinib is a potent type II *FLT3* inhibitor with activity against *FLT3*, c-KIT, PDGFR, and RET. In addition to high single-agent activity (marrow remission rates of 45% to 50%) in relapsed/refractory *FLT3*-mutated AML, quizartinib was associated with a composite complete remission (CRc) rate of 36% in patients with *FLT3*-wild-type disease in a phase II study, suggesting it may play a broader role in the treatment of AML (26). It must, however, be noted that most responses in *FLT3*-wild-type patients occurred at dose levels >120 mg daily, doses that are no longer being

evaluated due to the higher incidence of corrected QT (QTc) prolongation (grade 3 QTc prolongation rates of 20% to 25%) at these dose levels. Despite meeting the primary objective of OS improvement with quizartinib over investigator choice salvage chemotherapy in a phase III randomized study of 367 patients with relapsed or refractory *FLT3*-ITD mutated AML (CRc rate 48% vs. 27%; median OS 6.2 months vs. 4.7 months, $P = 0.0177$), quizartinib was not granted FDA approval for this indication, due in part to concerns over treatment equipoise and robustness of OS improvement. However, quizartinib secured approval in Japan in June 2019 and is being considered for approval in other countries. Gilteritinib is another potent second-generation type I inhibitor with activity against AXL, a receptor tyrosine kinase that may play a role in mediating resistance to earlier generation *FLT3* inhibitors (27). Gilteritinib was found to be well tolerated with marrow remission rates of 45% to 50% as a single agent in relapsed or refractory *FLT3*-mutated AML in a phase I-II study (28). In a randomized phase III study with single-agent gilteritinib versus investigator choice salvage chemotherapy (both high- and low-dose chemotherapy), gilteritinib was associated with higher CRc rates (54% vs. 22%), higher CR/CR with partial hematologic recovery (CRh) rates (34% vs. 15%), and longer median OS (9.3 months vs. 5.6 months; $P = 0.007$). More patients (26% vs. 15%) were able to proceed to HSCT with gilteritinib compared with salvage chemotherapy. Gilteritinib was approved by the FDA for the treatment of relapsed/refractory *FLT3*-mutated AML (both ITD and TKD) in November 2018.

Given the expanding spectrum of *FLT3* inhibitors that are FDA-approved or in advanced development, the optimal use of *FLT3* inhibitors in clinical practice is becoming increasingly complex. One important area of uncertainty is whether more selective *FLT3* inhibitors might be superior to multitargeted protein kinase inhibitors such as midostaurin. Randomized phase III studies of conventional chemotherapy in combination with midostaurin versus gilteritinib (NCT03836209) and with midostaurin versus crenolanib (NCT03258931) are ongoing to address this question. Initial data are promising with the combination of azacitidine and quizartinib for older adults (i.e., age ≥ 60 years) with previously untreated *FLT3*-ITD-mutated AML in which a CR/CR with incomplete hematologic recovery (CRi) rate of 83% and median OS of 18.6 months have been reported (29). For patients who require successive lines of *FLT3* inhibitor treatment, response rates decline substantially with each subsequent salvage attempt (in one analysis, from 49% to 27% to 17% for first-line, second-line, and third-line *FLT3* inhibitor-based therapies, respectively; ref. 30). The optimal sequence of these *FLT3* inhibitors is largely unknown, although initial data suggests that combination strategies for patients with relapsed/refractory *FLT3*-mutated AML may be substantially more effective than single-agent *FLT3* inhibitors (29, 30).

Despite the efficacy of *FLT3* inhibitors, therapeutic failure is still common, particularly in the relapsed/refractory setting in which 2-year OS rates even with quizartinib and gilteritinib remain <20%, with only patients who receive HSCT experiencing long-term survival. Several mechanisms of resistance to *FLT3* inhibitors have been described (Fig. 1). Secondary mutations of the *FLT3* gene frequently lead to resistance,

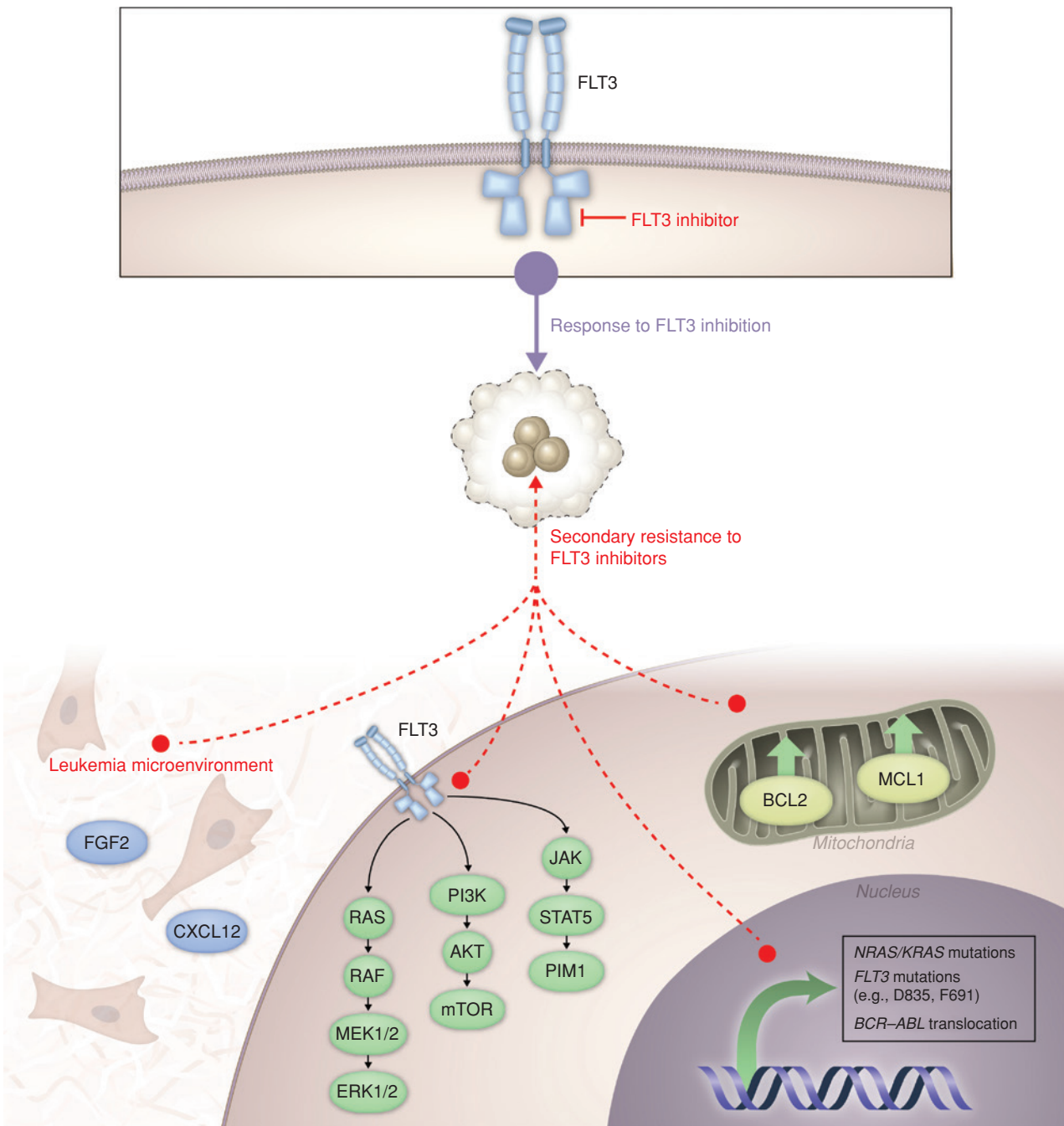


Figure 1. Mechanisms of resistance to FLT3 inhibitors. Several pathways of resistance have been described in patients treated with FLT3 inhibitors and serve as targets to develop rational combinations. Alterations of the leukemia microenvironment, including increased FGF2 and CXCL12/CXCR4 signaling, may protect *FLT3*-mutated progenitors. Increased signaling through parallel prosurvival pathways, including RAS-RAF-MEK-ERK, PI3K-AKT-mTOR, and JAK-STAT5-PIM1 pathways may also contribute to FLT3 inhibitor resistance. Agents targeting these pathways are in clinical trial development. Resistance mutations are commonly observed (30%-40%) in FLT3 inhibitor-resistant cases and may be treatment-emergent or due to expansion of a preexisting resistant subclone. These include *NRAS/KRAS* mutations, alternative *FLT3* mutations (e.g., D835, F691, and others), and *BCR-ABL* translocation. Although these mutations occur on the DNA level within the nucleus (as shown in the figure), their encoded proteins exert oncogenic effects within the cytoplasm. Upregulation of antiapoptotic proteins, including BCL2 and MCL1, has been observed in cases of FLT3 inhibitor resistance, and a number of FLT3 inhibitors also inhibit MCL1, providing the rationale for combining FLT3 inhibitors with BCL2 inhibitors. This graphic reprinted with permission, The University of Texas MD Anderson Cancer Center © 2019.

particularly for patients treated with type II inhibitors, which do not have activity against TKD mutations (31, 32). Frequently recurrent locations for such secondary mutations are in the activating loop residues (e.g., D835, I836, D839, and Y842) or in the gatekeeper residues (e.g., F691) of FLT3 (32). Recent studies using single-cell sequencing have shed further light on the nature of this mutation-driven resistance. For example, in patients treated with quizartinib, FLT3-D835 mutations commonly occur in both *FLT3*-ITD and *FLT3*-wild-type subclones, leading to complex, polyclonal architecture at the time of treatment failure (33). In patients treated with gilteritinib, mutations in *NRAS/KRAS* were the most common secondary resistance mutations identified; less common mutations were F691L gatekeeper mutations or *BCR-ABL1* gene fusions (34). The novel FLT3 inhibitor FF-10101 that has preclinical activity against the F691L gatekeeper mutation may help to overcome this mutation and is being evaluated in phase I trials (35). In this longitudinal single-cell sequencing study of gilteritinib-treated patients, 19 of 41 cases (46%) with secondary clinical resistance to gilteritinib could not be explained by treatment-emergent mutations, suggesting alternative pathways contribute to FLT3 inhibitor resistance.

These alternate pathways may include microenvironmental factors such as increased FGF2 and CXCL12/CXCR4 signaling, which have been reported to protect *FLT3*-mutated progenitors (36–40). Signaling through several parallel, pro-survival pathways may also be upregulated in cases of FLT3 inhibitor resistance. These include both the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR pathways, which may be potentially targetable using available therapies, such as MEK, AKT, or mTOR inhibitors (18, 41–44). Activation of STAT5 and its effector oncogenic serine/threonine kinase PIM1 has also been reported as a mechanism of FLT3 inhibitor resistance (45–47). Notably, the transcription of both FLT3 and PIM1 is regulated by CDK6 (48), and therefore the CDK4/6 inhibitor palbociclib in combination with a FLT3 inhibitor may be a promising therapeutic option for these patients (NCT03132454). PIM1 upregulates antiapoptotic proteins (e.g., BCL2, BCL-XL, MCL1), which also contributes to resistance to FLT3 inhibition (49–55). Therapeutic inhibition of several of these pathways (specifically inhibition of BCL2 with venetoclax) demonstrated synthetic lethality with FLT3 inhibitors in preclinical models, in some cases restoring sensitivity to FLT3 inhibition, and has led to ongoing clinical trials combining gilteritinib or quizartinib with venetoclax (NCT03625505; NCT03735875). Building on the improved understanding of mechanisms of resistance, many clinical trials evaluating rationally designed combination therapies with FLT3 inhibitors have been initiated.

IDH1 and IDH2 Inhibitors

IDH1 and IDH2 are enzymes critical for the oxidative carboxylation of isocitrate to α -ketoglutarate (α -KG). Mutations in *IDH1* or *IDH2* are present in 5% to 15% and 10% to 15% of patients with newly diagnosed AML, respectively (2). These mutations alter the function of IDH dimers, resulting in increased concentrations of 2-hydroxyglutarate (2-HG), which, in turn, interferes with the epigenetic regulation of hematopoietic differentiation via competitive inhibition of α -KG-dependent enzymatic activities. Oral, small-molecule

inhibitors of both mutant IDH1 (e.g., ivosidenib) and IDH2 (e.g., enasidenib) have shown efficacy in patients with the corresponding mutations. In a phase I study in 258 patients with relapsed/refractory *IDH1*-mutated AML, ivosidenib produced an overall response rate (ORR) of 41.6% and a CR rate of 21.6%, with a median OS of 8.8 months (56). Similarly, enasidenib, a covalent inhibitor of R140Q- and R172K-mutated *IDH2*, produced an ORR of 40.3%, a CR rate of 20.6%, and a median OS of 9.3 months in patients with relapsed/refractory *IDH2*-mutated AML (57). Notably, IDH inhibitors can induce differentiation of malignant cells, leading to a clinical IDH differentiation syndrome in 10% to 20% of patients (58, 59). This is analogous to the differentiation syndrome seen in patients with APL treated with ATRA-based regimens, except that it may occur at any time during therapy, may occur recurrently in the same patient, and may not be heralded by leukocytosis, making the IDH differentiation more difficult to diagnose than the APL differentiation. On the basis of the clinical activity of these agents, the FDA approved ivosidenib and enasidenib for patients with relapsed or refractory *IDH1*- and *IDH2*-mutated AML, respectively, in 2018. Single-agent ivosidenib was also approved in May 2019 for patients with newly diagnosed *IDH1*-mutated AML who are ≥ 75 years of age or unfit for intensive chemotherapy, based on a CR/CRh rate of 42% and a CR rate of 30% with median OS of 12.6 months with single-agent IDH1 inhibitor in older patients not eligible for intensive therapy (60, 61).

Interestingly, 2-HG is reduced in nearly all patients treated with enasidenib and does not predict clinical response. This suggests that there is a nearly universal on-target therapeutic effect, but alternative mechanisms of primary resistance may dictate the degree of clinical benefit (59, 60). Mechanisms of secondary resistance have also been described with enasidenib. Some of these include mutations in the *IDH* genes, including isoform switching from *IDH2* to *IDH1* mutation (or vice versa; ref. 62) or development of second-site *IDH2* missense mutations on the nonmutant allele (63). These mutations, which in one study were detected in 2 of 9 cases of acquired resistance to enasidenib, occurred in areas where enasidenib binds to the IDH2 dimer (e.g., Q316E and I319M mutations; ref. 63). They were not found in any of the 14 cases of primary resistance and could not be detected by digital droplet PCR at baseline, suggesting that these *trans* mutations were treatment-emergent under the selective therapeutic pressure of enasidenib therapy.

Mutations in *NRAS/KRAS* or in other MAPK pathway effectors (e.g., *PTPN11*, *NF1*, *FLT3*) are enriched at baseline in patients with primary resistance to enasidenib or ivosidenib and are likely mechanisms of resistance (64). Such resistance may be mediated through the expansion of preexisting resistant clones or the acquisition of treatment-emergent mutations. In both cases, 2-HG remains suppressed at the time of relapse. However, in rare cases, 2-HG levels may rise at the time of relapse, which suggests alterations of one of the *IDH* genes as the driver of resistance (e.g., the acquisition of an *IDH1* mutation in a patient treated with enasidenib).

To increase response rates and prolong the duration of remission with IDH inhibitors, combination therapies that can address clones or subclones driven by IDH-independent

pathways are needed. To this end, studies of IDH inhibitors in combination with chemotherapy and/or HMAs are ongoing. *IDH* mutations lead to a hypermethylated signature, providing additional scientific basis for the use of HMAs in this setting (65). There is also a strong rationale for the combination of IDH inhibitors with BCL2 inhibitors (e.g., venetoclax), as the accumulation of 2-HG caused by *IDH* mutations leads to the inhibition of cytochrome *c* oxidase activity, mimicking an oxygen-deprived state and decreasing the mitochondrial threshold for induction of apoptosis (66). BCL2 inhibition is therefore synthetically lethal to *IDH*-mutated AML, opening up the exciting possibility of a chemotherapy-free, oral combination therapy for these patients. Initial clinical results were striking, with 9 of 12 (75%) patients with relapsed or refractory *IDH1*-mutated AML achieving a CR/CRi/CRh with ivosidenib plus venetoclax in an ongoing phase Ib/II clinical trial (NCT03471260; ref. 67).

RAS Pathway Inhibitors

Alterations of the MAPK signaling pathway are common in AML, and mutations in *KRAS* or *NRAS* are present in 10% to 25% of patients at the time of diagnosis (2). *RAS* mutations are also common mechanisms of resistance to FLT3 and IDH inhibitors, and to BCL2 inhibitor-based therapies. Across cancers, therapeutic targeting of mutated RAS and other downstream proteins has been an area of active investigation, with mixed clinical success (68, 69). In AML, the MEK1/MEK2 inhibitors selumetinib and trametinib have led to modest response rates of 17% to 20% in relapsed or refractory *RAS*-mutated AML (70, 71). An established mechanism of resistance to MEK inhibition is the compensatory activation of parallel signaling through the PI3K–AKT–mTOR pathway. In a study of 23 patients with *RAS*-mutated AML treated with trametinib plus an AKT inhibitor (GSK2141795), expected targeted downmodulation of pERK and pS6 was shown, but disappointingly no responses were observed (71). *RAS* pathway–targeting agents may be of particular clinical importance either concomitantly with FLT3 or IDH inhibitors to avoid primary resistance in patients with *RAS* mutations at baseline, or sequentially in patients who have a newly detectable *RAS* mutation while on therapy with a FLT3, IDH, or BCL2 inhibitor.

KIT Inhibitors

Among patients with CBF AML (i.e., those with *CBFB–MYH11* or *RUNX1–RUNX1T1* translocations), *KIT* mutations (particularly the D816V missense variant) can be detected in up to 25% of cases and may be associated with an inferior prognosis compared with *KIT*–wild-type disease when treated with 7+3 induction (72). Midostaurin and dasatinib are both multikinase inhibitors with activity against c-KIT, among other targets. In a phase II study of 89 patients with newly diagnosed CBF AML, dasatinib was added to 7+3 induction and high-dose cytarabine consolidation and then continued for 1 year as maintenance (73). The 4-year cumulative incidence of relapse and event-free survival were 33% and 58%, respectively, which compared favorably with historical outcomes with chemotherapy alone. A phase III study is further evaluating this approach (NCT02013648). Interestingly,

among patients with CBF AML treated with FLAG (fludarabine, cytarabine, granulocyte colony-stimulating factor) in combination with either idarubicin and/or gemtuzumab ozogamicin, the presence of a *KIT* mutation was not associated with inferior outcomes, suggesting that more intensive regimens may overcome the negative prognostic impact of mutant *KIT* (74).

Targeted Therapies for *TP53*-Mutated AML

TP53 mutations are detected in 5% to 20% of patients with newly diagnosed AML, with higher incidence in older patients and those with AML arising from an antecedent hematologic disorder [e.g., myelodysplastic syndrome (MDS)] or with prior exposure to cytotoxic agents or radiation (2). Presence of one or more mutations in *TP53* is associated with a poor prognosis in AML (75). Although long thought “undruggable,” there has been intense research into agents that could target or overcome the negative impact of the mutant p53 protein. One such approach that generated significant interest was the use of a prolonged course of decitabine given at 10 days per cycle (rather than the standard 5-day schedule). In one study of a 10-day schedule of decitabine, the marrow remission rate was 100% in *TP53*-mutated patients with AML or MDS (76). Unfortunately, this benefit was not confirmed in a subsequent randomized study of 5-day versus 10-day schedules of decitabine as first-line therapy for older patients with AML, neither in the entire cohort nor in the *TP53*-mutated patients (77).

APR-246 is a novel agent that can restore transcriptional activity of unfolded wild-type or mutant p53, leading to induction of apoptosis in cancer cells with mutant p53 (78). In early results from an ongoing phase Ib/II study in patients with high-risk *TP53*-mutated MDS or oligoblastic AML (20% to 30% blasts), the combination of APR-246 and azacitidine resulted in a composite CR, CRi, and morphologic leukemia-free state (MLFS) rate of 100% (11 of 11 evaluable patients), with 82% of patients achieving CR and 72% of responders having undetectable *TP53* mutation by next-generation sequencing (79). Transcriptomic analysis after run-in of single-agent APR-246 confirmed on-target effects, including transcriptional activation of p53 targets. A phase III randomized study of azacitidine with or without APR-246 in MDS and AML with 20% to 30% blasts has been initiated (NCT03745716).

TARGETING THE APOPTOTIC PATHWAY

Control of apoptosis is tightly regulated in normal human cells, and evasion of this process is one of the hallmarks of cancer (80). Apoptosis is controlled by two parallel pathways, intrinsic and extrinsic, that converge with activation of intracellular caspases, ultimately leading to cell death. The intrinsic pathway is under the control of the BCL2 family of proteins, which includes antiapoptotic proteins (e.g., BCL2, BCL-XL, and MCL1), proapoptotic BH3-only proteins (e.g., BIM, BAD, PUMA, and NOXA), and proapoptotic effector proteins (e.g., BAK and BAX). In AML and other malignancies, functional loss of p53 or an altered balance of antiapoptotic and proapoptotic protein expression impairs downstream apoptotic signaling, leading to

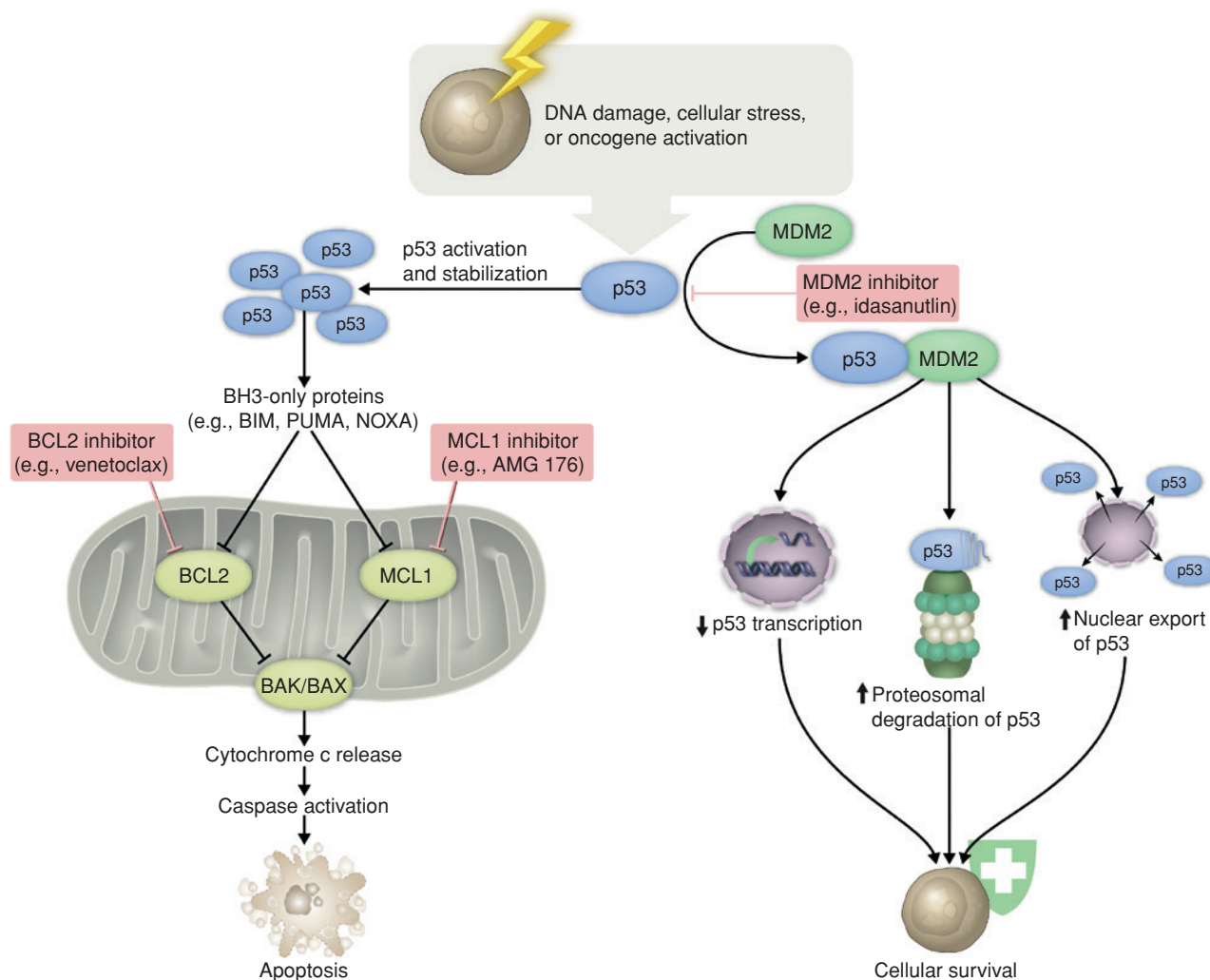


Figure 2. Targeting the intrinsic apoptotic pathway in AML. Functional p53 is integral to the activation of the intrinsic apoptotic pathway. MDM2 complexes with p53, promoting cellular survival through decreased p53 transcription, increased proteasomal degradation, and increased nuclear export of p53. Oral inhibitors of MDM2 such as idasanutlin or milademetan inhibit this p53-MDM2 interaction, thereby promoting activation and stabilization of p53 in response to cellular stress. Activated p53 can then trigger the intrinsic apoptotic pathway through upregulation of proapoptotic proteins (e.g., BIM, PUMA, NOXA) and inhibition of the antiapoptotic proteins BCL2 and MCL1. BH3 mimetics that inhibit BCL2 (e.g., venetoclax) or MCL1 (e.g., AMG176, S64315) may concurrently release inhibition on proapoptotic effector proteins such BAK and BAX, ultimately causing apoptotic cell death. This graphic reprinted with permission, The University of Texas MD Anderson Cancer Center © 2019.

unrestricted leukemic growth and survival (81). Targeting of the apoptotic pathway in an effort to restore balance to a proapoptotic phenotype has emerged as a major component of AML therapeutics (Fig. 2).

BCL2 and MCL1 Inhibitors

Antiapoptotic proteins, including BCL2, BCL-XL, and MCL1, are frequently overexpressed in AML and are associated with resistance to chemotherapy (82). This observation led to the development of BH3 mimetics that structurally mimic BH3-only proteins and are capable of binding to antiapoptotic proteins, effectively inhibiting their functional activity and inducing apoptosis. Although an initial study of the pan-BCL2 inhibitor obatoclax in AML was disappointing due to excessive toxicity and lack of meaningful clinical activity (83), the second-generation, oral, selective BCL2 inhibitor

venetoclax has shown exciting activity in AML in multiple combination regimens.

In a phase II study of single-agent venetoclax in 32 patients with relapsed/refractory AML, the CR/CRi rate was 19%; another 19% of patients had a bone marrow blast reduction not meeting formal response criteria (84). Notably, 4 of 12 patients (33%) with an *IDH* mutation achieved CR/CRi with venetoclax monotherapy, further supporting the preclinical rationale for BCL2 inhibition in these patients (66). Baseline BCL2 dependence, as assessed by either the ratio of BCL2 to BCL-XL or functional assessment of BCL2 dependency by BH3 profiling, correlated with time on study, suggesting that these may serve as meaningful biomarkers for BCL2 inhibitor-based therapies in AML. In addition to the relative proportions of apoptotic proteins influencing the clinical efficacy of venetoclax, preexisting and treatment-emergent mutations

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in *FLT3*-ITD and *PTPN11* were identified as genomic mechanisms of primary and secondary resistance, respectively, on longitudinal whole-exome sequencing performed in patients treated with venetoclax monotherapy (85).

Subsequent studies have evaluated venetoclax in combination with low-intensity therapy in older adults with newly diagnosed AML deemed unfit for intensive chemotherapy. This is a population of patients in whom standard therapy over the last 10 to 15 years has consisted of either an HMA (e.g., azacitidine or decitabine) or LDAC, with published CR/CRi rates of 18% to 28% and median OS of 6 to 10 months with HMA, and CR/CRi rates of 10% to 15% and median OS of 5 to 7 months with LDAC (86, 87). In a phase Ib study, 145 patients ≥ 65 years of age with newly diagnosed AML received azacitidine or decitabine in combination with venetoclax (6). Overall, the CR/CRi rate was 67%, with similar response rates seen in adverse risk subsets, including patients with poor-risk cytogenetics (60%) and secondary AML (67%). The median duration of response was 11.3 months, and the median OS was 17.5 months, with a 2-year OS of 46%. In another study of 82 older adults ≥ 60 years of age treated with LDAC in combination with venetoclax, the CR/CRi rate with the combination was 54%, with a median remission duration of 8.1 months, and median OS of 10.1 months (7). Notably, 29% of these patients had prior HMA exposure, which may at least partially explain the difference in outcomes compared with the study of azacitidine or decitabine plus venetoclax, in which patients with prior HMA exposure were excluded. These response rates, remission durations, and median OS were dramatically better than seen with historical comparisons of single-agent HMA or LDAC, and also appear superior to outcomes of older patients treated standard 7+3 chemotherapy where 2-year OS rates are 25% to 30% (88). Based on these exciting results in this historically difficult-to-treat patient population, in November 2018 the FDA approved venetoclax in combination with either LDAC, azacitidine, or decitabine for patients with newly diagnosed AML who are ≥ 75 years of age or have comorbidities that preclude the use of standard intensive chemotherapy; confirmatory phase III trials are ongoing to confirm the OS benefit compared with LDAC or HMA alone. Although these combinations represent an important advance in the treatment of older and unfit patients with AML, the long-term outcomes of patients with poor-risk disease features remain poor. For example, among patients harboring a *TP53* mutation, although the CR/CRi rates of 45% to 55% attained with venetoclax doublet regimens are only slightly lower than in the non-*TP53*-mutated population, the median duration of response for patients with *TP53*-mutated AML is only 5 to 7 months with median OS < 8 months (6, 89). This suggests that even though outcomes in *TP53*-mutated patients with AML are improved with venetoclax-based doublets compared with HMA or LDAC alone, they remain suboptimal, and new therapies and combinations are needed in this population.

Prolonged courses of decitabine (e.g., 10 days rather than 5 days per cycle) have also been evaluated in AML, with the rationale that this longer schedule may capture a larger proportion of leukemic cells as they asynchronously enter the S phase (90). Early results with a 10-day regimen of decitabine in combination with venetoclax are encouraging, especially in

patients with newly diagnosed AML with CR/CRi rates $> 90\%$, 4-week mortality of 0%, and median OS not yet reached. Results in relapsed or refractory AML are more modest, with CR/CRi rates of 30% to 35% and median OS of 6 to 8 months (91). Recently, a phase II trial studying the new backbone of cladribine plus LDAC alternating with decitabine in 118 older patients with newly diagnosed AML demonstrated an ORR of 68% with a median OS of 13.8 months (92). On the basis of these data, the addition of venetoclax to this regimen is currently undergoing evaluation (NCT03586609). Confirmatory phase III trials of azacitidine with or without venetoclax (VIALE A) and of LDAC with or without venetoclax (VIALE C) have completed enrollment, and results are eagerly anticipated. For patients who are younger and fit for intensive chemotherapy, studies are evaluating the combination of venetoclax with conventional chemotherapies such as FLAG plus idarubicin, 3+7, and CPX-351 (NCT03214562, NCT03709758, and NCT03629171), and results are awaited (93, 94).

Despite the encouraging results observed with venetoclax-based regimens in AML, relapses are still common, and it is becoming clear that these regimens are unlikely to be curative in the vast majority of patients. The best defined mechanism of resistance to venetoclax is the upregulation of MCL1 (95). Preclinical data with MCL1 inhibitors are promising across tumor types, including in AML (96). Preclinically, inhibition of MCL1 appears to be synergistic with venetoclax and may also reverse venetoclax resistance (94, 97, 98). MCL1 inhibitors are therefore now being explored in early clinical trials, both as single agents and in combination with venetoclax (99). In AML, the balance between BCL2 and MCL1 antiapoptotic dependence varies from patient to patient, with some patients exhibiting mixed co-dependence (98). This relative dependence on BCL2 versus MCL1 is also dynamic over the course of therapy. These considerations further support the development of clinically validated functional assays such as BH3 profiling to determine baseline dependency on apoptotic proteins to predict sensitivity to specific BH3 agents and to decide which patients may preferentially benefit more from BCL2 inhibition or MCL1 inhibition (or a combination of both; refs. 84, 95). The goal of such an approach is to deliver an individualized therapeutic approach to maximize efficacy while minimizing toxicity when possible.

In addition to combination with MCL1 inhibitors, several other strategies that indirectly suppress MCL1 expression or function are being evaluated. Pevonedistat is a first-in-class inhibitor of NEDD8-activating enzyme (NAE) that catalyzes the rate-limiting step in the process of protein neddylation, a critical step in the degradation of a wide variety of cellular proteins that takes place upstream of the proteasome (100, 101). In preclinical studies, pevonedistat synergizes with venetoclax by upregulation of NOXA, which, in turn, neutralizes MCL1 (102). Pevonedistat may therefore function as an indirect MCL1 inhibitor, and a first-line study of azacitidine in combination with venetoclax and pevonedistat is ongoing (NCT03862157). MEK1/2 and CDK9 inhibitors also modulate MCL1 expression and may hence prevent the development of venetoclax resistance (99, 103). Mitochondrial oxidative phosphorylation promotes the persistence of leukemia stem cells, and is

regulated by both BCL2 and glutamine levels (104). In pre-clinical models, the inhibition of glutaminase was shown to induce arrest of proliferation and activate apoptotic pathways, which may sensitize AML cells to venetoclax (105). These data support the development of glutaminase inhibitors in AML and their evaluation in combination with BCL2 inhibition.

MDM2 Inhibitors

In the absence of *TP53* mutation or loss, cell-cycle arrest and apoptosis are dysregulated through functional inactivation of the p53 protein or its downstream pathways, including overexpression of MDM2 or MDMX. MDM2 forms a complex with p53, leading to decreased p53 transcription, increased nuclear export, and increased degradation of p53 through the proteasome (106). Various compounds have been developed that disrupt this MDM2-p53 interaction. These MDM2 inhibitors appear to be synergistic with genotoxic chemotherapeutics in AML and other malignancies because both drug classes activate the p53 pathway (107). Because the antitumor activity of MDM2 inhibitors is dependent on the presence of functional p53 proteins, it is believed that this class of drugs will be largely ineffective in *TP53*-mutated disease. A number of MDM2 inhibitors (e.g., idasanutlin, milademetan, and others) are being evaluated in patients with AML. An ongoing phase III study is evaluating intermediate-dose cytarabine with or without the oral MDM2 inhibitor idasanutlin in first relapsed AML, and results are expected in the near future (NCT02545283).

Dual inhibition of MDM2 and BCL2 synergistically increases apoptosis in AML models, as MDM2 inhibition negatively regulates the RAS-RAF-MEK-ERK pathway and promotes degradation of MCL1 (108, 109). In an ongoing international phase Ib study of venetoclax and idasanutlin, in patients >60 years of age with relapsed or refractory AML, the marrow remission rate was 37% (11 of 30 evaluable patients) in the entire population, and 50% (9 of 18 evaluable patients) at the recommended phase II dose of venetoclax (110). This is substantially higher than the response rates reported with venetoclax monotherapy or venetoclax with HMA-based combinations in a similar population, suggesting that targeting dual apoptotic pathways may have additive or synergistic clinical benefit and may be effective even in the absence of chemotherapy (84). As with other venetoclax-based regimens, higher ratios of BCL2/BCL-XL and BCL2/MCL1 predicted for an increased likelihood of response. Emergence of new detectable *TP53* mutations was a mechanism of secondary resistance to the combination, as has been shown in other studies of MDM2 pathway inhibitors in other malignancies (111). Ongoing efforts are under way to determine whether MDM2 expression and more comprehensive gene-expression profiling may help to predict antitumor effects with MDM2 inhibitors and combinations (112, 113).

IMMUNE-BASED THERAPIES

In AML, the value of harnessing the immune system has long been appreciated, given the established benefit of allogeneic HSCT or donor lymphocyte infusions, both of which are effective through the induction of a graft-versus-leukemia

effect (114). A number of immune-based therapies have recently emerged and are now being evaluated in the therapy of AML. Development of effective and safe immune therapies will likely complement and further enhance the efficacy of cytotoxic, targeted, and apoptosis-inducing agents.

mAbs Targeting Leukemia Surface Antigens

Naked antibodies, which rely primarily on antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity, have historically been largely ineffective in AML, due in part to the qualitative defects of natural killer cells in these patients (115). Therefore, the development of mAbs in AML has largely focused on antibody constructs capable of delivering a toxic payload (e.g., toxin, chemotherapy, or radioisotope) or antibodies that can increase host T-cell engagement with AML cells (e.g., bispecific T-cell engagers or dual-affinity retargeting antibodies). A wide variety of antibody constructs targeting different target antigens are currently in clinical trials in AML (Fig. 3).

Gemtuzumab ozogamicin (GO) is an antibody-drug conjugate (ADC) of a recombinant IgG4 humanized mAb against CD33 that is conjugated to calicheamicin, a potent DNA-damaging toxin (116). Upon engagement with CD33, GO undergoes receptor-mediated endocytosis and delivers the calicheamicin into leukemic cells. GO was initially approved by the FDA in 2000 but was voluntarily withdrawn from the market in 2010 due to concerns from a confirmatory phase III study of GO with induction therapy in newly diagnosed AML (S0106), suggesting a lack of efficacy and increased toxicity (117). However, subsequent studies, including a meta-analysis of 5 randomized first-line trials of GO added to different induction regimens in newly diagnosed AML, showed a significant OS benefit (6-year OS 34.3% with GO vs. 30.6% without GO; $P = 0.01$), with no increased early mortality and low rates of veno-occlusive disease (1% to 2%). The benefits were most pronounced in patients with favorable-risk cytogenetics, with a more modest benefit observed in patients with intermediate-risk cytogenetics (118). After reanalysis of these data, GO was reapproved by the FDA in September 2017 for use in the first-line setting in combination with standard induction therapy based on the ALFA-0701 regimen (119), as a single agent for older patients who are unfit for intensive chemotherapy, or in patients with relapsed/refractory AML (120, 121). Recent work has suggested that in addition to karyotype, SNPs of *CD33* and *ABCB1*, encoding proteins that mediate GO resistance in preclinical studies, may predict clinical responses in pediatric AML (122, 123). The role of these polymorphisms is less clear in adults, with subsequent similar analyses in adults showing contradictory findings (124, 125).

On the basis of the established clinical efficacy of GO in AML, various other CD33 antibody constructs have been developed in AML. The anti-CD33 ADCs vadastuximab (conjugated to pyrrolobenzodiazepine dimer) and IMGN779 (conjugated to DGN462, a DNA-alkylating agent) showed promising clinical activity in phase I studies (126, 127). Excessive hematologic toxicity, especially neutropenia, appeared to be an issue when vadastuximab was combined with HMAs, resulting in increased early mortality and termination of the frontline phase III CASCADE study of azacitidine with or without vadastuximab in older patients with AML

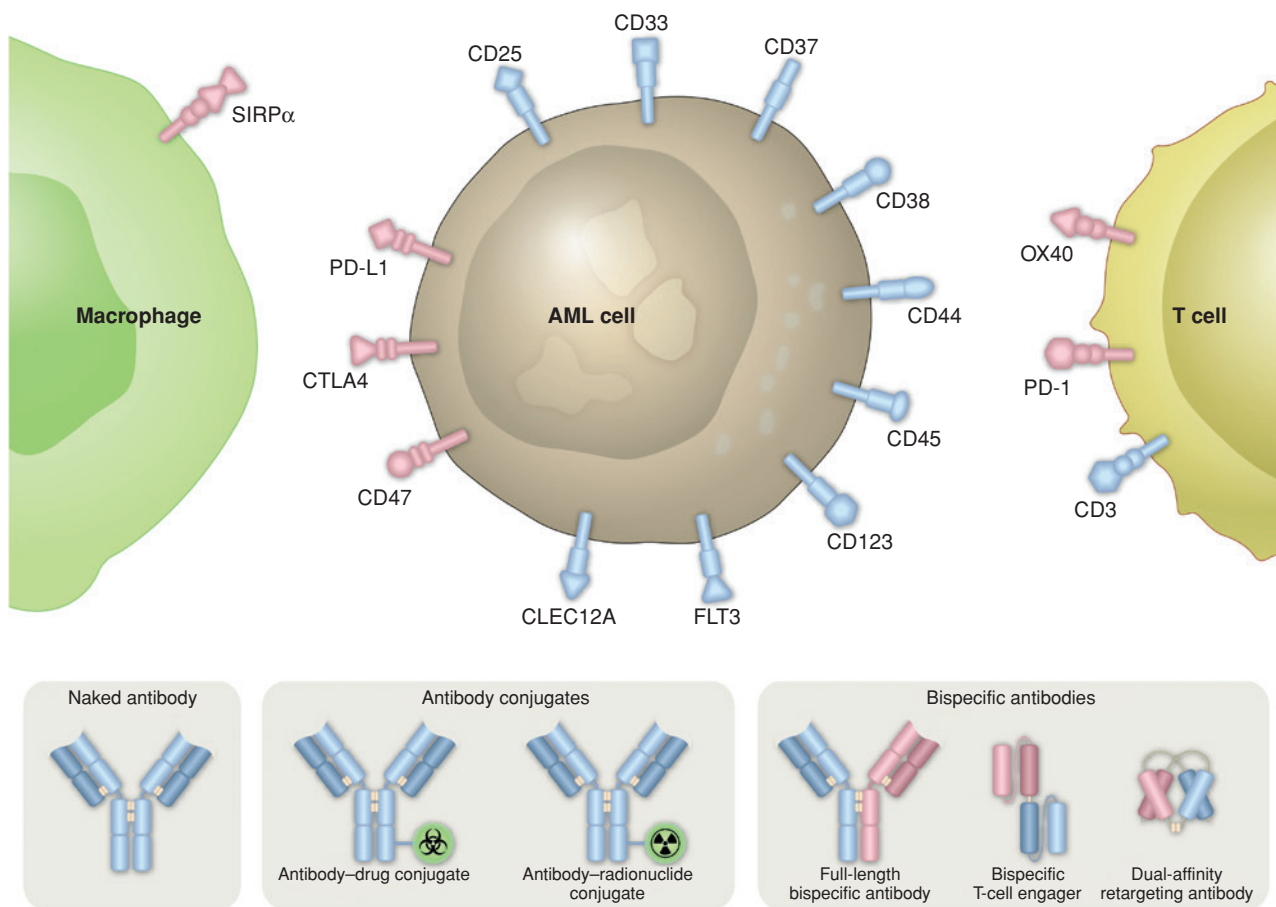


Figure 3. Surface antigen targets of monoclonal/bispecific antibody constructs and T-cell/macrophage checkpoint pathways in AML. Various mAbs are currently in clinical trials for the treatment of AML, including naked antibodies, antibody–drug (e.g., gemtuzumab ozogamicin, IMGN632) or antibody–radionuclide (e.g., lomab-B) conjugates, and various bispecific antibody constructs (e.g., MGD006, AMG330, XmAb123). The surface antigens shown in blue represent targets of antibody–drug or antibody–radionuclide conjugates or bispecific antibodies. The surface antigens in pink represent costimulatory ligands or coreceptor targets of T-cell or macrophage immune checkpoint inhibitors. This graphic reprinted with permission, The University of Texas MD Anderson Cancer Center ©2019.

(128). Bispecific antibodies targeting CD33 and CD123 are currently being evaluated in AML, with initial responses observed, although response rates have generally been lower (CR/CRi rates 15% to 25%) and less robust than were achieved with the CD3–CD19 bispecific T-cell engager antibody blinatumomab in relapsed B-cell acute lymphoblastic leukemia (ALL; refs. 129–131). Combining the bispecific antibodies with complementary immune-enhancing strategies such as checkpoint inhibitors may further enhance their efficacy, and such approaches are being evaluated in the clinic. As observed with other bispecific antibodies, such as blinatumomab in ALL, cytokine release syndrome can occur with these therapies but is usually grade 1 to 2 and responds rapidly to corticosteroids or the anti-IL6 receptor mAb tocilizumab (132).

Ongoing studies are also evaluating the delivery of radioisotopes using surface antigen–targeting mAbs. In a phase II study of older patients unfit for intensive chemotherapy, the anti-CD33 antibody–radioisotope conjugate ²²⁵Ac-lintuzumab led to a response rate of 69% at a dose of 2 μCi/kg, albeit with significant myelosuppression, supporting the

future development of this construct in AML (133). Such strategies are also being explored using CD45-targeted antibodies (e.g., Iomab-B or ⁹⁰Y-BC8-DOTA; refs. 134, 135). As CD45 is more ubiquitously expressed in the hematopoietic system than CD33, CD45-targeting agents can lead to significant myeloablation and are therefore being studied as part of pre-HSCT conditioning in transplant-eligible patients. A randomized phase III registrational study using Iomab-B versus investigator choice salvage therapy prior to HSCT in patients with relapsed/refractory AML is ongoing (NCT02665065).

Other promising antibody targets are being evaluated in AML, some of which offer theoretical advantages over targeting CD33, including expression profiles that are more restricted to leukemic targets, thereby reducing off-target toxicity to the hematopoietic system or other organs. The IL3 receptor alpha chain, CD123, is notably expressed on leukemic stem cells (LSC) and is expressed at lower levels on normal hematopoietic stem cells (HSC) than CD33 (136). ADCs and bispecific antibodies targeting CD123 have shown promising clinical activity in phase I studies and are rapidly moving to

multicenter studies as single-agent expansions and in combination approaches (130, 131, 137). Targeting CD123 may also be an effective strategy to target measurable residual disease (MRD) or as a maintenance therapy in high-risk AML. Similarly, C-type lectin domain family 12 member A (CLEC12A) is a transmembrane glycoprotein present on LSCs but not expressed on HSCs or nonhematopoietic tissues, making it a promising potential target for AML antibody therapies (138).

Checkpoint Inhibitors

The emerging understanding of how both immune evasion by malignant cells as well as exhaustion of the host's own immune system contribute to cancer growth and resistance to therapy has revolutionized the treatment of a number of solid-tumor malignancies. However, despite the rationale for development of T-cell checkpoint inhibitors (e.g., anti-PD-1, PD-L1, and CTLA4 antibodies) in AML and other hematologic malignancies, their development has lagged behind that of many solid tumors. Compared with healthy controls, patients with AML have increased T-regulatory cell infiltration and increased inhibitory coreceptor expression on CD8⁺ T cells, including PD-1, TIM3, and LAG3 (139). Increased expression of these immune checkpoint proteins has been associated with immune exhaustion and early relapse in AML in murine and human studies (140–145). In murine models of AML, blockade of these inhibitory checkpoints, including CTLA4, PD-1, PD-L1, and TIM3, demonstrated promising immune-mediated antileukemic effects (144–148).

Emerging clinical data also suggest that targeting these checkpoints may be effective in AML. The anti-CTLA4 antibody ipilimumab was evaluated as a single agent in patients with hematologic malignancies who relapsed after allogeneic HSCT (149). Among 12 patients with AML, there were 4 CRs; all of these patients had extramedullary disease at the time of relapse. These responses were durable with most lasting 1 year or more. In contrast, activity with single-agent PD-1 inhibition in AML and relapsed MDS has been more modest (150). The development of checkpoint inhibitors in first-line and relapsed AML over the last 4 to 5 years has largely focused on rationally designed combinations, with the exception of ongoing studies evaluating these agents as single-agent maintenance for patients who are in remission after standard therapy but at high risk for relapse, or as single-agent maintenance after HSCT.

Checkpoint proteins are significantly increased after AML-directed treatment (to some extent with cytotoxic chemotherapy but even more so with HMAs; refs. 139, 151), suggesting that this may be a mechanism of resistance to these conventional therapies. Furthermore, these therapies may prime leukemic cells for immune-based destruction. Exposure to chemotherapy and HMAs releases neoantigens, which leads to increased antigen presentation by macrophages and dendritic cells (152, 153). Similarly, HMAs can upregulate previously silenced leukemic neoantigens or endogenous retroviruses, promoting cytotoxic T-cell expansion (154). In a study of azacitidine plus the anti-PD-1 inhibitor nivolumab in 70 patients with relapsed or refractory AML, the ORR with the combination was 33% (CR/CRi rate: 22%), with an ORR of 58% in patients with no prior HMA exposure (155). Among patients receiving this combination as first salvage therapy, the median OS was 10.6 months compared with 5.3 months

with a historical cohort of patients treated with HMA-based regimens on contemporary clinical trials ($P = 0.01$) at the same institution. An ongoing phase III trial is evaluating the combination of azacitidine with or without nivolumab as frontline therapy in older patients with AML (SWOG 1612, NCT03092674). A phase II study is evaluating single-agent nivolumab as maintenance therapy for high-risk patients in remission after induction and consolidation. The treatment was well tolerated with manageable immune toxicities that did not require discontinuation. The 1-year CR duration and OS estimates were 71% and 86%, respectively, which compare favorably with the historical expectations of these high-risk patients (156). A randomized phase II trial is currently evaluating maintenance nivolumab versus observation in patients with AML who are in remission and have completed their planned induction and consolidation (NCT02275533).

Across studies of patients with AML treated with PD-1 or PD-L1 inhibitors, increased pretherapy CD3⁺ or CD8⁺ T-cell infiltrate in the bone marrow and increased diversity of T-cell receptors were associated with an increased likelihood of clinical benefit. These may be potential biomarkers to prospectively select patients most likely to respond. Conversely, increased CD4⁺ effector T cells with dual PD-1/TIM3 or PD-1/LAG3 expression and increased T-regulatory cell infiltration in bone marrow appeared to be associated with lack of response (155, 157, 158). Notably, in the study of azacitidine plus nivolumab in relapsed or refractory AML, a dynamic increase in CTLA4 expression was observed with treatment, suggesting that upregulation of CTLA4 or other inhibitory checkpoint proteins may be a mechanism of resistance to PD-1 blockade, as has been frequently shown with solid tumors (155). This observation has led to a trial combining azacitidine, nivolumab, and ipilimumab in patients with relapsed or refractory AML (159). Among 20 patients treated with this regimen, CR/CRi was achieved in 43% and the projected 1-year OS rate was 58%; 26% of the patients experienced grade 3 to 4 immune-related adverse events, particularly pneumonitis that was potentially reversible with rapid steroid initiation. The 60-day mortality was 8%. Thus, although such combination strategies appear to be effective in AML, awareness and monitoring for immune-related toxicity is critical if such approaches are to succeed.

In addition to T-cell checkpoint inhibitors, targeting “macrophage checkpoints” may also prove to be an effective therapeutic strategy in AML. CD47 is a leukemic antigen that is highly expressed on LSCs and is associated with poor clinical outcomes (160). Upregulation of CD47 on AML cells allows for immune evasion from phagocytosis by binding to the signal-regulatory protein α (SIRP α) receptor on macrophages, providing a “don't eat me” signal (161). Hu5F9-G4 is an anti-CD47 antibody that blocks this interaction with SIRP α , promoting macrophage-mediated phagocytosis (162). The toxicity profile of this agent in other hematologic malignancies appears favorable compared to T-cell checkpoint inhibitors (163). In a study of Hu5F9-G4 in combination with azacitidine in adults with newly diagnosed AML unsuitable for intensive therapy, CR/CRi/MLFS was achieved in 9 of 14 patients (64%); the ORR in patients with newly diagnosed MDS was 100% (11 of 11 patients; ref. 164). With limited follow-up, the responses have been durable, and the drug

demonstrated a favorable safety profile in this high-risk older population. Elimination of putative LSCs was also observed. The study is ongoing at multiple centers (NCT03248479).

Vaccines and Cellular Therapies

Vaccine-based approaches in AML have primarily been explored as maintenance therapy after chemotherapy or allogeneic HSCT. Wilms tumor antigen 1 (WT1) is highly expressed on leukemic blasts, and vaccines against WT1 have been evaluated in high-risk MDS and AML in phase I studies (165, 166). Multivalent peptide vaccines that use antigens containing both MHC-I and MHC-II peptides to induce CD4⁺ and CD8⁺ T-cell responses may be particularly effective, given the low-avidity T-cell response seen with HLA-class-restricted peptides. In one study of a multivalent WT1 peptide vaccine in patients with AML in first CR, the median estimated OS after vaccination was ≥ 67.6 months, which compares favorably to historical expected outcomes in a similar population, although a phase III trial will be needed to confirm its benefit (167). Similar vaccine therapies have been tested by loading dendritic cells *in vitro* with known tumor antigens. However, both antigen-specific peptides and dendritic cell vaccines are limited by a small number of known suitable targets in AML [e.g., WT1, preferentially expressed antigen of melanoma (PRAME), receptor for hyaluronan-mediated motility (RHAMM), etc.]. More individualized vaccines composed of patient-derived AML cells fused with autologous dendritic cells may stimulate a potent immune response against both known and unknown tumor antigens (168). By targeting a broader spectrum of tumor antigens, T-cell response may be less susceptible to immune escape through target downregulation.

On the basis of the success of chimeric antigen receptor (CAR) T-cell therapies in other hematologic malignancies (namely ALL, diffuse large B-cell lymphoma, and multiple myeloma), early-phase trials are ongoing in AML (169). Unlike other hematologic malignancies, a challenge in the development of safe and effective CAR T-cell therapies in AML is the lack of truly AML-specific surface antigens. Many putative targets, including CD33, CD123, CLEC12A, and others, are not restricted to malignant cells but are rather overexpressed on malignant cells relative to the normal hematopoietic cells or organ tissues, resulting in the potential for “on-target, off-tumor” toxicity. Several strategies have been developed to optimize the efficacy and safety profile of CAR T-cell therapies in AML, including temporary expression of the CARs using mRNA electroporation, “suicide switch” control of the CARs using inducible caspase-9, and modification of the affinity of the CARs to target only cells with high target expression (170). However, as of today, no CAR T-cell construct has shown a clear efficacy and safety signal in AML.

FUTURE DIRECTIONS: MOVING TOWARD “TOTAL THERAPY” IN AML

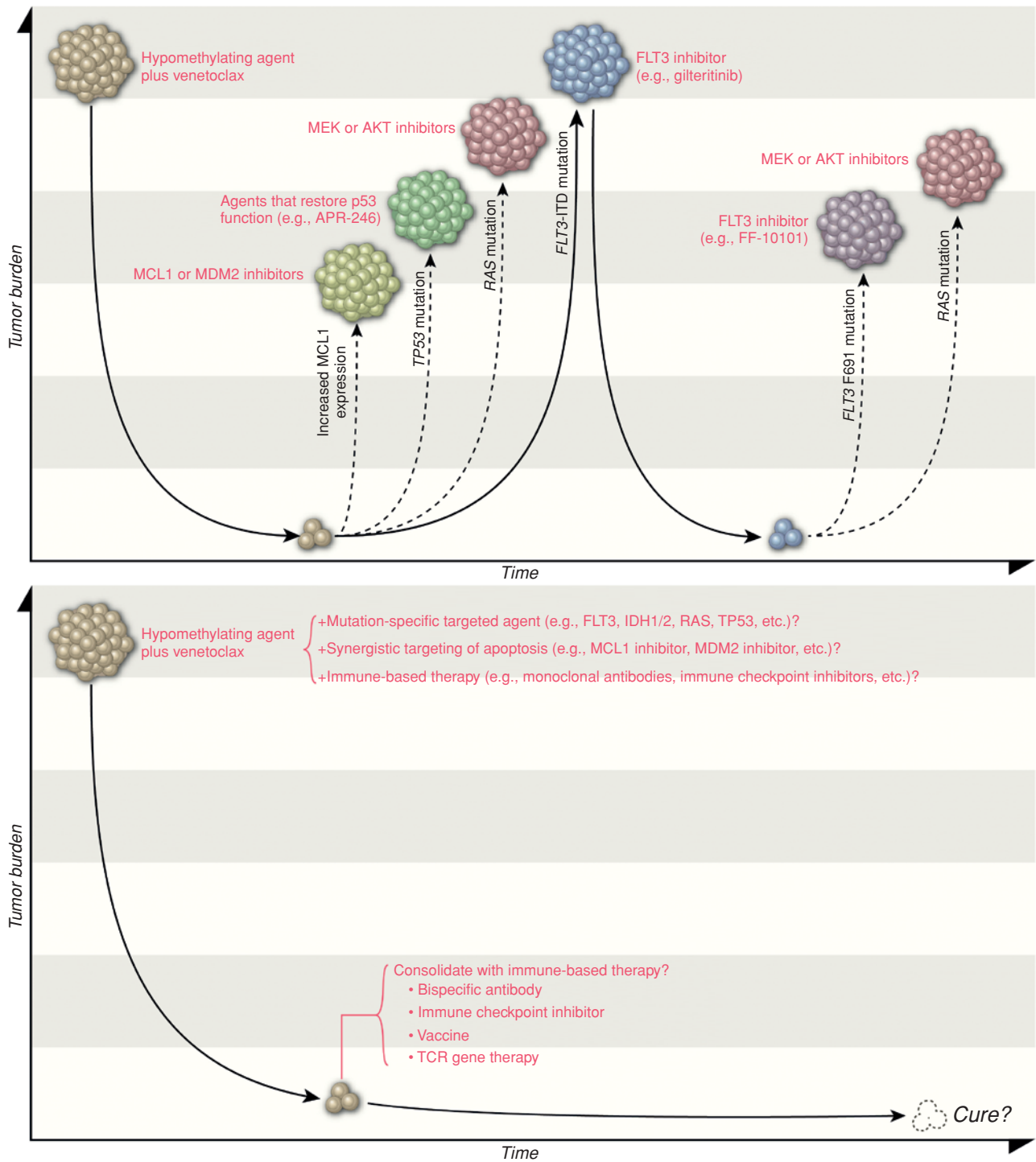
Along with the development and implementation of new, effective drugs and combinations of these drugs for patients with AML has come a myriad of new challenges and questions. Primary and secondary resistance to these novel therapies remains a pervasive issue, and the mechanisms by which resistance to many of these new drugs occurs are

only beginning to be elucidated. The complex and dynamic clonal architecture of AML remains a key driver of variability in response and the eventual development of secondary resistance in many patients (171–173). Single-cell sequencing studies have provided important information on subclonality and clonal evolution, mutual exclusivity of mutations, and order of mutation accrual to specific targeted and apoptosis-inducing therapies in a way that was previously not possible (33, 174–178). It is also being increasingly recognized that the interpatient and inpatient heterogeneity of AML extends beyond genomic mutations and encompasses variations in the epigenome and RNA and protein expression (179, 180). Furthermore, this clonal heterogeneity is dynamic and affected by both competition from the microenvironment and selective pressure from administered therapeutics (e.g., emergence of an *NRAS*-mutated clone as a mechanism of resistance to FLT3 inhibition). Thus, with our improving knowledge of clonal diversity and clonal evolution in AML as well as the expanding therapeutic options at our disposal, the major question is: How best can we sequence or combine these available agents?

Because of the polyclonal nature of AML, single-agent therapies are not curative, even in the presence of a clear drug and corresponding target (e.g., FLT3 or IDH2 inhibitors in relapsed or refractory AML), as treatment-emergent mutations or expansion of preexisting resistant subclones eventually leads to relapse. In contrast, combinations of active agents have traditionally been shown to improve response rates and long-term outcomes in most hematologic malignancies, as long as safety can be maintained (6, 7, 23). Rationally combining or sequencing therapies to preemptively target known mechanisms of resistance will likely further improve outcomes (Fig. 4). Enrollment of patients into carefully selected, molecularly triaged clinical trials is needed to evaluate these novel drugs and combinations and their utility in the general population. One such initiative in this direction is the multicenter, multiarm Beat AML umbrella trial that uses next-generation sequencing–based genetic analysis to assign older patients with newly diagnosed AML to personalized therapies based on the genomic features of their disease.

LSCs are rare and quiescent cells that are generally chemoresistant and serve as an important reservoir of disease that can lead to relapse after initial response to therapy (2, 181). To increase the potential for durable remissions and cure in AML, strategies that are capable of eradicating self-renewing LSCs are imperative, and several such compounds are actively being tested in clinical trials. For example, glasdegib is an inhibitor of Smoothened (SMO), an integral component of the Hedgehog pathway that plays a critical role in the maintenance of LSCs (182). In a randomized trial, the addition of glasdegib to LDAC in older adults with AML who were unsuitable for intensive chemotherapy resulted in improved OS compared with LDAC alone (CR rates 17% vs. 2.3%, $P < 0.05$; median OS 8.8 months vs. 4.9 months; $P = 0.0004$) (183). Although these results are more modest than observed with venetoclax-based combinations, they nevertheless highlight that targeting of LSCs, including through inhibition of Hedgehog pathway signaling, may be a fruitful therapeutic approach in AML.

Even when clinical remission is achieved with combination therapies, low-volume residual disease often persists and predisposes to relapse (184). In ALL, the CD3–CD19 bispecific



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Figure 4. Mechanisms of resistance to conventional AML therapy and paradigms of future treatment. Top, established mechanisms of primary or secondary resistance to the combination of a hypomethylating agent plus the BCL2 inhibitor venetoclax, including alterations of protein expression (e.g., upregulation of MCL1) or genetic mutations (e.g., *FLT3*, *NRAS/KRAS*, or *TP53*). Drugs targeting each of these mechanisms of resistance are currently in clinical trials or already FDA-approved; for example, for patients who relapse with a *FLT3* mutation, gilteritinib is FDA-approved for this indication. Established genomic mechanisms of resistance with gilteritinib include the F691 *FLT3* gatekeeper mutation and mutations in *NRAS* or *KRAS* and *BCR-ABL* translocation. Identifying the resistance mechanism in a particular patient may lead to selection of a subsequent therapeutic option with increased chance of efficacy. Bottom panel shows a potential future treatment paradigm in AML where combination regimens that preemptively target common mechanisms of resistance are concomitantly or sequentially incorporated into first-line regimens, which may lead to an increased likelihood of deep remission and potential cure. The incorporation of immune-based therapies either into induction or consolidation regimens or as a post-consolidation therapy to eradicate residual leukemia and leukemia stem cells may further deepen the depth and duration of response. TCR, T-cell receptor. This graphic reprinted with permission, The University of Texas MD Anderson Cancer Center ©2019.

T-cell engager antibody blinatumomab achieves high rates of MRD eradication with improved survival and low rates of cytokine release syndrome when used in patients with low disease burden (i.e., in morphologic remission but with detectable MRD; ref. 185). Better outcomes are also achieved with allogeneic HSCT when pre-HSCT disease burden is low or undetectable (186). Similarly, the optimal role of non-HSCT immune therapies in AML, including checkpoint inhibitors, vaccines, and adoptive T-cell therapies, may be in the setting of low disease burden after chemotherapy or HSCT. Several studies of consolidative or maintenance immune modulation in this context are ongoing (184). These approaches may be particularly important for the large proportion of patients with AML who are unfit for allogeneic HSCT due to advanced age or comorbidities. A total therapy approach involving initial chemotherapy or HMA with targeted or apoptosis-inducing therapy sequentially adjusted on the basis of emerging early clones with immune-based therapies to eradicate reservoirs of residual disease has always been envisioned, but was previously not feasible given the limited efficacy and high toxicity of antileukemic therapy. However, with recent expansion of the repertoire of effective AML therapeutics, this approach will likely become a reality in the near future.

Disclosure of Potential Conflicts of Interest

N.J. Short is a consultant at Takeda Oncology and AstraZeneca, reports receiving commercial research grants from Takeda Oncology and Astellas Pharma Inc., and has received speakers bureau honoraria from Amgen. T.M. Kadia is a consultant at Agios, Genentech, Jazz, Novartis, Pfizer, and AbbVie and reports receiving commercial research support from Bristol-Myers Squibb, Pfizer, Amgen, Jazz, Genentech, Celgene, and AbbVie. F. Ravandi is a consultant at Astellas, Amgen, Celgene, Novartis, and AstraZeneca and reports receiving commercial research grants from Amgen, Bristol-Myers Squibb, AbbVie, Xencor, and Orsenix. C.D. DiNardo has received speakers bureau honoraria from Agios, AbbVie, Celgene, Daiichi-Sankyo, Jazz, Medimmune, and Syros. N. Daver is a consultant/advisor for BMS, Pfizer, AbbVie, Genentech, Daiichi-Sankyo, Astellas, Celgene, Immunogen, Servier, Roche, and Forty-Seven; and has received commercial research grants from BMS, Incyte, Pfizer, AbbVie, Genentech, Immunogen, Servier, Roche, Daiichi-Sankyo, and Forty-Seven. No potential conflicts of interest were disclosed by the other authors.

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