ANNUAL REVIEWS

Annual Review of Cancer Biology

Mechanisms of Resistance to Targeted Therapies in AML

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Annu. Rev. Cancer Biol. 2024. 8:81–96

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-062822-025055

Keywords

acute myeloid leukemia, targeted therapy, acquired resistance, tumor heterogeneity, tumor evolution

Abstract

The treatment of acute myeloid leukemia (AML) has historically relied on cytotoxic chemotherapy, but modern understanding of AML biology has paved the way for new treatments that target the molecular pathways that drive AML, in particular FLT3, IDH1/IDH2, and BCL2. Many of these targeted therapies are effective, but responses are typically short-lived and resistance remains a ubiquitous clinical problem. Understanding the mechanisms of resistance to targeted therapy is essential to continue improving AML therapy. Recent studies have shed new light on the ways in which AML evades targeted inhibition, including on-target resistance mutations, mutations in parallel molecular pathways, and plasticity in cellular state. In this review, we outline the mechanisms of resistance to commonly used targeted therapies in AML and discuss ideas to overcome the urgent problem of resistance.

INTRODUCTION

Acute myeloid leukemia (AML) is the most common type of leukemia in adults. It is characterized by pathological proliferation and disordered maturation of the myeloid lineage, resulting in accumulation of immature myeloid blasts. Prognosis varies widely between patients depending on clinical and pathological factors. Despite the prevalence and heterogeneity of AML, the treatment landscape has been historically dominated by a one-size-fits-all approach using intensive chemotherapy with a cytarabine and anthracycline regimen called 7+3 (Yates et al. 1973) followed by hematopoietic stem cell transplant for eligible patients. More recently, hypomethylating agents (HMAs) such as azacitidine and decitabine have been introduced as alternatives for patients who are not fit for intensive chemotherapy (Dombret et al. 2015, Kantarjian et al. 2012). Both high-intensity and low-intensity chemotherapy regimens are ultimately unsuccessful in many patients. Therefore, primary treatment of refractory disease and postremission relapse are major clinical problems.

The need for more effective treatments for AML has generated significant interest in therapies targeted at the genetic drivers of disease. The first descriptions of the genomic landscape of AML ushered in a new era for the study of the molecular pathways important in AML biology. This biological understanding has in turn led to the development of therapies that are targeted at key proteins in these pathways with the goal of augmenting or replacing cytotoxic chemotherapy regimens. It has been proposed that the field of AML treatment is in the midst of a shift away from chemotherapy-based treatments and toward a precision medicine paradigm of targeted therapy tailored to the genetic features of an individual patient's leukemia. However, targeted therapy imposes a selective pressure on leukemia that invariably leads to the evolution of resistance.

Targeted therapies in AML currently occupy roles as adjuncts to chemotherapy, salvage treatment for patients who are refractory to first-line chemotherapy, and maintenance therapy after remission. Many targeted therapies have good initial efficacy with high rates of response in selected patients, but primary and acquired resistance are frequent phenomena that prevent any such targeted treatment from being curative. A thorough understanding of resistance is essential to optimize the use of targeted therapies in AML treatment. In this review, we discuss key molecular pathways in AML biology that have been successfully targeted by therapeutic compounds and outline mechanisms of resistance. The field of targeted therapy in AML is rapidly expanding, with dozens of drugs at various stages of development. We confine our scope to those agents that are currently in widespread clinical use with some discussion of promising next-generation targeted therapies.

FLT3

Biology and Clinical Significance

FLT3 encodes the cytokine receptor protein FMS-like tyrosine kinase 3. Wild-type FLT3 is the receptor for the growth factor FLT3-ligand (FLT3L), which causes dimerization and auto- and transphosphorylation of the receptor. This results in activation of the intracellular kinase domain and phosphorylation of downstream signaling pathways, including Ras/mitogen-activated protein kinase (MAPK), PI3 kinase–AKT, and JAK/STAT, which transduce proliferative and prosurvival signals (Hayakawa et al. 2000, Mizuki et al. 2000). In AML, *FLT3* mutations cause constitutive, ligand-independent activation of these downstream signaling pathways.

FLT3 mutations are the most common driver mutations in adult AML, with constitutively activating *FLT3* mutations found in ~30% of patients with AML (Papaemmanuil et al. 2016, The Cancer Genome Atlas Research Network 2013) (**Figure 1**). Of these 30%, ~25% of patients harbor in-frame *FLT3* internal tandem duplications (ITDs) within the juxtamembrane domain

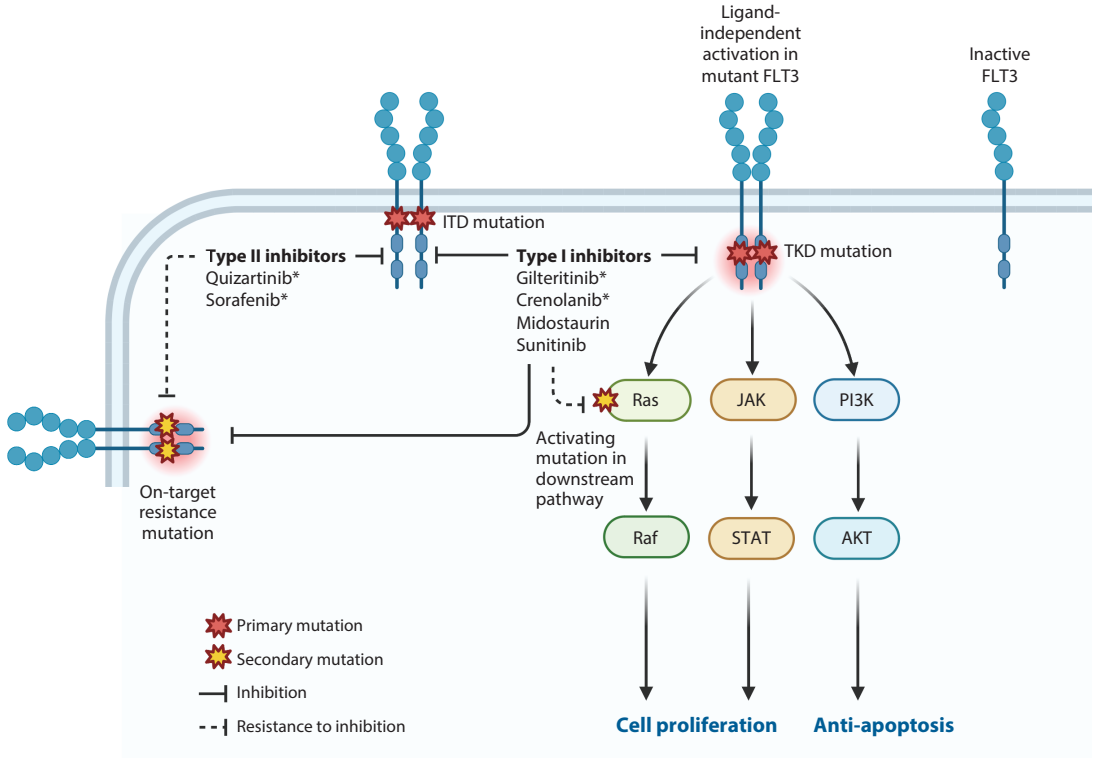

Figure 1

On-target and off-target resistance mutations in FLT3 AML. There are two types of *FLT3* mutations: ITD mutations in the juxtamembrane domain that result in loss of autoinhibition and TKD mutations that favor the active conformation of the TKD. There are also two types of FLT3 inhibitors: Type I inhibits the active conformation of FLT3, and type II inhibits the inactive conformation. Consequently, type I inhibitors are effective against both ITD and TKD mutations while type II inhibitors are generally effective against only ITD mutations. Type II inhibitors are also vulnerable to acquired resistance through on-target secondary mutations that favor the active kinase conformation. Type I inhibitors typically select for off-target mutations that activate pathways downstream of FLT3, most commonly Ras. Asterisks denote second-generation FLT3 inhibitors. Abbreviations: AML, acute myeloid leukemia; FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; TKD, tyrosine kinase domain. Figure adapted from images created with BioRender.com.

that result in loss of autoinhibitory steric hindrance (Nakao et al. 1996). The other 5% harbor missense mutations within the tyrosine kinase domain (TKD) that favor active conformation of the kinase domain (Larrosa-Garcia & Baer 2017). The 2022 edition of the European Leukemia Network guidelines classifies all AML cases with FLT3-ITD to be intermediate risk if they lack other adverse risk features (Döhner et al. 2022).

First-generation FLT3 inhibitors such as midostaurin, sorafenib, and lestaurtinib are multikinase inhibitors without specificity for FLT3. As single agents, they have limited and transient clinical efficacy. However, midostaurin in combination with cytotoxic chemotherapy was superior to chemotherapy alone in the large, randomized phase III RATIFY trial, with a hazard ratio for death from any cause of 0.78 (Stone et al. 2017). These results established midostaurin in combination with 7+3 as the standard-of-care induction regimen for fit patients with *FLT3*-mutated AML. Second-generation FLT3 inhibitors include quizartinib, gilteritinib, and crenolanib, which have enhanced specificity for FLT3 and greater potency compared with first-generation tyrosine kinase

inhibitors. Gilteritinib and crenolanib are type I inhibitors that target the active conformational state of the FLT3 protein, whereas quizartinib is a type II inhibitor that is specific for the inactive state (Daver et al. 2015, Larrosa-Garcia & Baer 2017) (**Figure 1**). The second-generation FLT3 inhibitor gilteritinib is effective in relapsed/refractory (R/R) AML with FLT3-ITD or FLT3-TKD mutations. The phase III ADMIRAL trial randomized patients to either gilteritinib or one of several standard-of-care chemotherapy regimens and showed a significant benefit in the gilteritinib arm versus chemotherapy in outcomes of overall survival (OS) (9.3 versus 5.6 months), complete response (CR) rates (34.0% versus 15.3%), and median duration of response (11 versus 1.8 months) (Perl et al. 2019). These results established single-agent gilteritinib as the standard of care in R/R *FLT3*-mutated AML. Quizartinib also improves OS compared with chemotherapy in patients with R/R FLT3 AML (Cortes et al. 2019). Despite the initial efficacy of single-agent FLT3 inhibitors, patients treated with them invariably relapse with new mutations that confer resistance.

Mechanisms of Resistance to Therapy

The biological dependence or addiction of FLT3-ITD AML to sustained FLT3 signaling, and therefore the conceptual utility of therapeutically targeting mutated FLT3, was first demonstrated by the finding that patients with FLT3-ITD treated with quizartinib relapsed with kinase domain mutations that decrease the binding affinity of FLT3-ITD to quizartinib (Smith et al. 2012). These findings indicate a strong selective pressure to maintain active FLT3 signaling. Subsequent work has shown that this selective pressure leads to outgrowth of AML clones with mutations that bypass FLT3 inhibition, and this acquired resistance remains a common clinical problem for all patients treated with FLT3 inhibitors.

Mechanisms of resistance to FLT3 inhibition are complex and reflect the underlying clonal heterogeneity of AML. In general, FLT3 inhibitor treatment selects for either mutations in *FLT3* that decrease the binding to FLT3 (on-target mutations) or mutations in other genes that activate downstream signaling in an FLT3-independent manner (off-target mutations) (**Figure 1**). The type of on-target mutation seen at relapse is influenced by the type of FLT3 inhibitor used for treatment. Because of their mechanism of action, type II inhibitors, which bind the inactive kinase conformation, are especially vulnerable to on-target resistance mutations that result in constitutive activation of FLT3. In vitro saturation mutagenesis experiments predicted that mutations in the kinase domain residue D835 or F691 would create resistance to quizartinib (Smith et al. 2012). This prediction was confirmed by the observation of D835 and, less often, F691 missense mutations in patients who relapsed after treatment with quizartinib in clinical trials (Smith et al. 2017). On-target mutations at D835 have also been observed in patients with acquired resistance to the type II inhibitor sorafenib (Man et al. 2012). A study using single-cell sequencing to profile the clonal evolution of 11 patients treated with quizartinib showed that 7 of 11 patients developed on-target kinase domain mutations, and none of these mutations was present prior to treatment (Peretz et al. 2021). These findings suggest that on-target mutations typically arise during treatment or are present at very low levels prior to treatment.

Relapse after treatment with type I inhibitors, which bind the active kinase conformation, is infrequently associated with on-target mutations. For example, gilteritinib and crenolanib are type I inhibitors that retain activity against most activating FLT3 kinase domain mutations (Galanis et al. 2014, Lee et al. 2017, Smith et al. 2014). Instead, resistance to type I inhibitors arises through selection for off-target mutations (**Figure 1**). Comparison of sequencing results from patients pre- and posttreatment with gilteritinib and crenolanib shows a variety of mutations at relapse, most often in the Ras pathway (*NRAS*, *KRAS*, and *PTPN11*), but also including mutations in other leukemia-associated genes such as *IDH1/IDH2*, *ASXL1*, *TP53*, and *TET2*. These mutations can be detected

in *FLT3* mutant clones, but they are also selected for in *FLT3* wild-type clones (McMahon et al. 2019, Zhang et al. 2019). Although off-target mutations are the predominant genetic mechanism of resistance seen after treatment with type I inhibitors, they can also be seen after treatment with type II inhibitors (Peretz et al. 2021). Off-target resistance mutations are often present at subclonal levels prior to treatment as part of the underlying clonal heterogeneity of AML and expand under the selective pressure of FLT3 inhibition.

Numerous cell-extrinsic mechanisms of resistance to FLT3 inhibition in vitro have been described. These include increased compensatory secretion of FLT3L and FGF by stroma (Javidi-Sharifi et al. 2019, Sato et al. 2011, Traer et al. 2016, Yang et al. 2014); upregulation of chemokine pathways CXCR4–CXCL12 (Jacobi et al. 2010, Zeng et al. 2009) and CCR5–CCL5 (Waldeck et al. 2020); and upregulation of the AXL pathway (Park et al. 2015). Putative pharmacokinetic mechanisms of resistance include increased expression of P-glycoprotein efflux pump (Hunter et al. 2004), increased expression of CYP3A4 in stroma (Chang et al. 2019), and increased binding to plasma proteins (Young et al. 2021). In general, the clinical relevance of these mechanisms remains uncertain, though they may explain some instances of primary treatment resistance that are poorly accounted for by genetic mechanisms.

Overcoming Resistance

The strong selective pressure to maintain MAPK output after FLT3 inhibition results in clonal outgrowth of Ras/MAPK pathway mutations as a common mechanism of resistance. Novel combinations of therapies targeted at eliminating both FLT3 mutant clones and treatment-emergent resistant clones have provided encouraging data in preclinical studies and early clinical trials. Gilteritinib synergizes with venetoclax and seems to be effective at eliminating FLT3 mutant clones, leading to high rates of clinical response with or without the HMA azacitidine (Chen et al. 2023, Daver et al. 2022, Kennedy et al. 2022, Singh Mali et al. 2020). Such combinations may be effective bridges to transplant for fit patients. In less fit R/R patients who are not transplant candidates, development of new small-molecule inhibitors aimed at the Ras/MAPK pathway combined with prospective monitoring for resistance mutations on treatment with FLT3 inhibitors could allow for precision sequencing of treatments targeted at resistance mutations as they arise.

BCL2

Biology and Clinical Significance

The gene B cell lymphoma 2 (*BCL2*) encodes a protein that is a key regulator of apoptosis in both normal and malignant cells. BCL2 and its family members share BCL2 homology (BH) domains, through which they regulate mitochondrial outer membrane permeability and mitochondria-linked caspase activation. Commitment to the apoptotic pathway is initiated by BH3-only proteins, which in turn trigger heterodimerization of BAX and BAK to form pore complexes in the mitochondrial outer membrane, resulting in cytochrome c release and caspase activation. A counterbalancing prosurvival force is exerted by BCL2, BCL-X_L, and MCL-1 by binding to BH3-only proteins and preventing their association with BAX and BAK (Adams & Cory 2018). The disordered physiology of AML results in stressors that tip this balance toward apoptosis: AML blasts are primed for cell death (Vo et al. 2012). Although circumventing apoptosis is a hallmark of all cancers (Hanahan & Weinberg 2011), the means by which malignant cells evade apoptosis varies considerably between and even within types of cancers. AML depends on high levels of expression of BCL2 and MCL-1 to counterbalance proapoptotic signals. Due to this dependence on BCL2, the binding site between BH3-only domain proteins and BCL2 has emerged as a key target for therapy in AML.

Venetoclax is a BH3 mimetic that binds selectively to BCL2 and prevents its association with proapoptotic BH3-only proteins such as BIM. It has only modest activity as a single agent in AML (Konopleva et al. 2016), but in combination with the HMA azacitidine, venetoclax is now a first-line treatment for AML in patients unfit for intensive chemotherapy. The landmark VIALE-A trial randomized older or medically unfit patients with untreated AML to receive azacitidine plus either venetoclax or placebo and showed significant improvement in the CR rate (36.7% versus 17.9%) and OS (14.7 versus 9.6 months) in the venetoclax arm compared to the placebo arm (DiNardo et al. 2020a).

Mechanisms of Resistance to Therapy

Acquired resistance to BCL2 inhibition with venetoclax is a significant clinical phenomenon. As summarized below, mechanisms of resistance include selection for mutations that confer resistance as well as changes in cell state and cell death pathways without detectable genetic alterations (**Figure 2**).

Selection for RAS/FLT3/TP53 mutant clones. A study of 81 patients who received venetoclax in combination with HMA or low-dose cytarabine identified several genetic correlates of treatment response. Patients with *NPM1* or *IDH2* mutations were likely to respond well, and *NPM1* mutations predicted durable remission. Single-cell DNA sequencing of samples from patients who acquired resistance to venetoclax showed selection for clones with mutations that activate progrowth signaling pathways at relapse time points, most notably *FLT3-ITD*, *FLT3-TKD*, and *RAS* mutations. Biallelic loss of *TP53* was also observed in multiple clones at the time of relapse (DiNardo et al. 2020b). In a review of the large BEAT-AML clinical cohort, *TP53* mutation conferred the lowest odds ratio for CR to venetoclax of any molecular or clinical risk category (Nechiporuk et al. 2019).

Altered apoptotic dependencies. Increased expression of prosurvival BCL2-related proteins is a common mechanism of resistance to venetoclax. A landmark study of the mechanisms of resistance to BCL2 inhibition used patient-derived xenografts (PDXs) to profile the dependency of AML on BCL2 and other BH3 family members during venetoclax treatment. By comparing venetoclax-responsive and venetoclax-resistant PDXs, Bhatt and colleagues (2020) found that loss of sensitivity to venetoclax was accompanied by decreased mitochondrial apoptotic priming. This loss of priming is thought to be due to decreased expression of proapoptotic BAK or increased expression of antiapoptotic MCL-1 and/or BCL-X_L (Bhatt et al. 2020). Similarly, increased expression of the antiapoptotic BH3 protein BCL2A1 correlates strongly with decreased sensitivity to venetoclax, and knockdown of BCL2A1 sensitizes AML cell lines to venetoclax (Zhang et al. 2020). In a cohort of 41 patients treated with venetoclax combinations, inactivating mutations in the apoptosis effector gene *BAX* were seen in 7 patients at relapse (Moujalled et al. 2023), suggesting that *BAX* mutations abrogate the proapoptotic effect of venetoclax.

Cell state plasticity. Mitochondrial apoptotic priming and BH3 family dependencies also vary between differentiation states in AML. The large BEAT-AML study observed that monocytic AML has a unique profile of sensitivity to targeted therapy in ex vivo experiments, including notable resistance to venetoclax (Bottomly et al. 2022). AML with monocytic differentiation shows decreased sensitivity to BCL2 inhibition with venetoclax both in aggregate and when monocytic cells are isolated and compared to immature blasts from the same patient (Kuusanmäki et al. 2020). This decreased venetoclax sensitivity in monocytic AML correlates with increased dependency on MCL-1 as a BH3-only protein sequestering mechanism, whereas AML with a

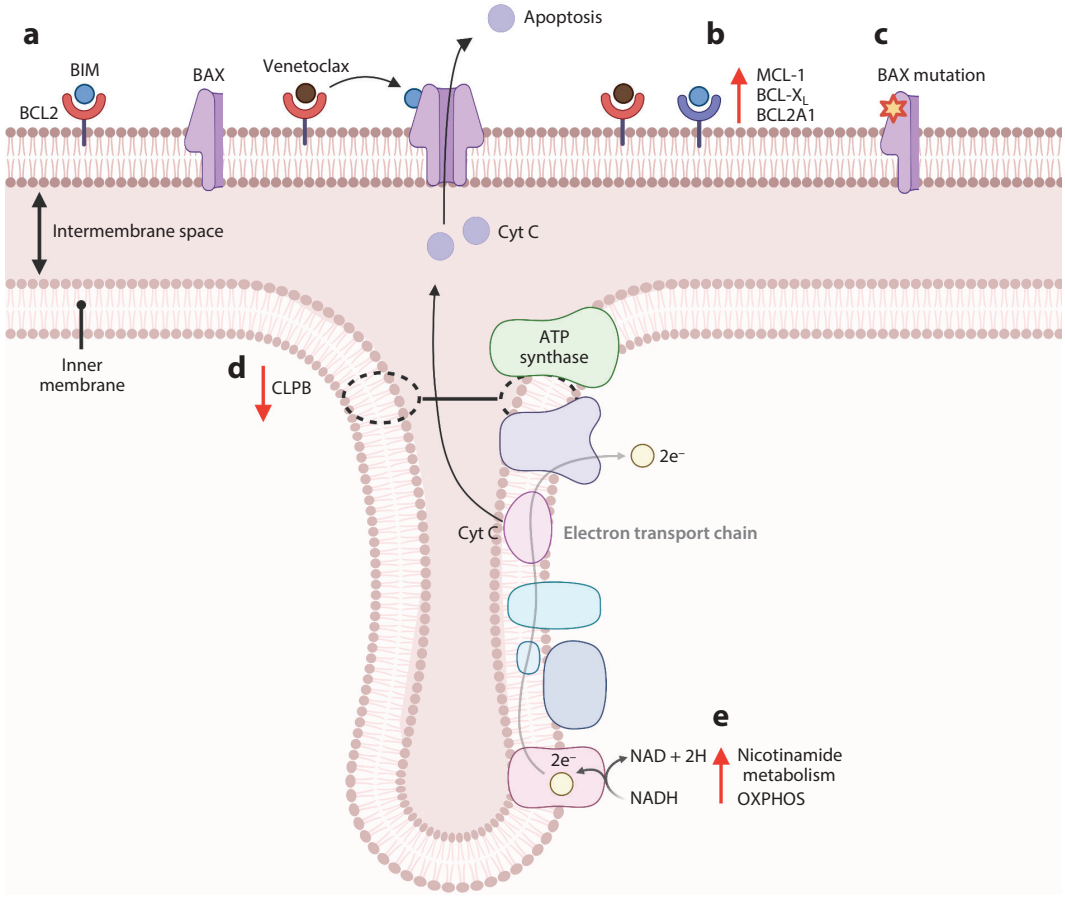

Figure 2

Mitochondrial mechanisms of resistance to BCL2 inhibition. (*a*) Venetoclax potentiates apoptosis by inhibiting the association between BIM and BCL2, allowing BIM to bind to BAX, which results in mitochondrial outer membrane permeabilization and release of cytochrome c (Cyt C) into the cytoplasm, where it triggers apoptosis. Resistance to venetoclax has been reported with (*b*) increased expression of BCL2 relatives MCL1, BCL-X_L, and BCL2A1; (*c*) inactivating mutations in *BAX*; (*d*) remodeling of the cristae due to decreased expression of CLPB; and (*e*) increased OXPHOS and nicotinamide metabolism. Abbreviation: OXPHOS, oxidative phosphorylation. Figure adapted from Mitochondrial Membrane (Phospholipid Bilayers) and Electron Transport Chain by BioRender.com, retrieved from **https://app.biorender.com/biorender-templates**.

primitive differentiation state depends more on BCL2 and has increased venetoclax sensitivity (Pei et al. 2020). Similarly, AML with erythroid and megakaryocytic differentiation shows increased dependency on BCL-X_L, and therefore lower sensitivity to venetoclax but higher sensitivity to the multitargeting BCL2 and BCL-X_L inhibitor navitoclax (Kuusanmäki et al. 2023). Simultaneous single-cell sequencing and immunophenotyping have shown that patients who relapse after treatment with gilteritinib combined with venetoclax have a more monocytic immunophenotype at the relapse time point regardless of which mutations drive relapse (Kennedy et al. 2022).

Metabolic reprogramming. Several studies have shown that venetoclax reprograms cellular metabolism via its effects on the oxidative phosphorylation (OXPHOS) pathway on the inner mitochondrial membrane. The combination of azacitidine and venetoclax decreases the activity

of complex II of the electron transport chain, resulting in impaired OXPHOS in both myeloid and lymphoid malignancies (Guièze et al. 2019, Pollyea et al. 2018). This has been proposed to selectively target the effect of venetoclax to the leukemia stem cell (LSC) population, which has unique metabolic dependence on OXPHOS. Conversely, LSCs that acquire resistance to venetoclax shift their preferred energy source from amino acid catabolism to fatty acid oxidation (Stevens et al. 2020) and upregulate nicotinamide levels to sustain OXPHOS (Jones et al. 2020), which circumvent azacitidine-/venetoclax-mediated inhibition of OXPHOS. The effect of venetoclax on OXPHOS may be related to structural remodeling of the inner mitochondrial membrane, where cytochrome c is enriched at cristae. Permeabilization of the outer mitochondrial membrane requires widening of the cristae to allow adequate release of cytochrome c, and venetoclax-resistant AML cells maintain narrow cristae on inspection with electron microscopy. Maintenance of narrow cristae appears to depend on the chaperonin CLPB, and inhibition of CLPB sensitizes AML to venetoclax in vitro (Chen et al. 2019).

Use of venetoclax in the treatment of chronic lymphocytic lymphoma (CLL) has also revealed several mechanisms of resistance, and comparison of these mechanisms to the AML experience is instructive. In contrast to AML, where mutations in *BCL2* are uncommon, sequencing of samples from patients with CLL who acquire venetoclax resistance has revealed recurrent point mutations that decrease the affinity for binding BH3 mimetics while retaining wild-type binding capacity for BIM (Blombery et al. 2019, Tausch et al. 2019). As for AML, MCL-1 upregulation is also responsible for venetoclax resistance in CLL, with increased NFκB signaling suggested as a causal mechanism for increased MCL-1 and decreased BCL2 dependency (Thijssen et al. 2022). Loss of expression of the proapoptotic p53 target gene *PUMA* via de novo promoter hypermethylation has also been observed in venetoclax-resistant CLL (Thomalla et al. 2022).

There are several possible explanations for the different mechanisms of resistance seen in venetoclax treatment of CLL compared with AML. One major difference is that the time on treatment with venetoclax is shorter for patients with AML, with average treatment durations in AML trials on the order of 6 months compared with 3 years in CLL trials (Blombery et al. 2019, DiNardo et al. 2020b). A longer duration of treatment for CLL may allow for the emergence of on-target *BCL2* mutations, while the more aggressive biology of AML selects for other mechanisms of relapse before *BCL2* mutations are acquired. The selection for *BCL2* mutations in lymphoid malignancies both during leukemogenesis and at relapse suggests a degree of oncogene addiction to BCL2 that is not present in AML, where *BCL2* mutations are rarely observed. In AML biology, BCL2 may be a dependency for survival but not a key oncogenic driver. The more subordinate role of BCL2 in AML may also explain why venetoclax is most effective as a sensitizing agent in combination with other antineoplastics such as HMAs rather than as monotherapy. The distinct selective pressure imposed by venetoclax combination regimens compared with that of monotherapy may also account for differences in resistance mechanisms seen in AML versus those seen in CLL. As novel combinations of venetoclax with other targeted therapies such as gilteritinib or ivosidenib for AML are studied in greater detail, we expect that each combination may be associated with its own patterns of resistance.

IDH1 AND IDH2

Biology and Clinical Significance

Mutations in the genes isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) are AML drivers that operate at the interface of metabolism and epigenetics. The IDH enzymes encoded by these genes catalyze conversion of isocitrate to α-ketoglutarate in the Krebs cycle. α-Ketoglutarate, in turn, is a substrate in several important epigenetic modifications, including histone lysine

demethylation and the conversion of 5-methylcytosine to 5-hydroxymethylcytosine by the TET enzymes, which leads to removal of CpG methylation. Leukemogenesis selects for neomorphic *IDH1/IDH2* mutations, which produce 2-hydroxyglutarate (2HG). 2HG then competitively inhibits downstream reactions governing DNA and histone methylation. The net effect of these mutations on chromatin structure and transcription results in blocked granulocytic differentiation (Figueroa et al. 2010, Lu et al. 2012, Ward et al. 2010).

The first inhibitors of mutant IDH1 and IDH2 to enter clinical use were ivosidenib and enasidenib, respectively. These inhibitors bind to an allosteric site at the homodimer interface and stabilize the inactive conformation of the enzyme (Yen et al. 2017). Inactivation of neomorphic IDH mutations effectively normalizes DNA methylation, relieves the differentiation block, and restores granulocyte maturation. Ivosidenib and enasidenib have single-agent CR rates of approximately 20% in R/R AML (DiNardo et al. 2018, Stein et al. 2017). When studied as first-line therapy for older patients with *IDH1/IDH2* mutations unfit for intensive induction chemotherapy, single-agent ivosidenib or enasidenib showed CR rates of 42% and 21%, respectively (Pollyea et al. 2019, Roboz et al. 2020). Early results from phase I/II trials of first-line ivosidenib or enasidenib in combination with HMA or intensive chemotherapy have been encouraging (DiNardo et al. 2021, Stein et al. 2021). A phase III trial comparing ivosidenib plus azacitidine versus placebo plus azacitidine in older patients showed a CR rate of 38% and median OS of 24 months in the ivosidenib arm compared with 11% and 7.9 months, respectively, in the placebo arm.

Mechanisms of Resistance to Therapy

In contrast to the targeted therapies discussed above, which tend to have high overall response rates with eventual relapse, IDH1/IDH2 inhibitors have the relatively unique problem of primary resistance, in which treatment fails to induce a response in a significant proportion of patients. This may be related to the presence of other driver mutations, most problematically activating mutations in the Ras pathway, for example, with mutations in *PTPN11* and *KRAS* (Amatangelo et al. 2017, Choe et al. 2020, DiNardo et al. 2018, Stein et al. 2019). Primary resistance to IDH1/IDH2 inhibition does not seem to be related to variant allele frequency. Even patients with low mutation burden, which may otherwise suggest that the IDH1/IDH2 mutant clone is not a dominant driver of disease biology, may still respond to ivosidenib and enasidenib. This response indicates possible interactions between mutant clones, perhaps with the metabolic effects of IDH1/IDH2 inhibition resulting in altered expression of cytokines that exert antileukemic effects on other clones that lack IDH mutations. Expression of a stem-like transcriptional program is also associated with primary resistance to IDH inhibitors, perhaps due to inherent resistance to the prodifferentiation clinical effects of IDH inhibition (Wang et al. 2021).

Acquired resistance to IDH inhibitors is associated with several sui generis mechanisms that are not seen with other targeted therapies. One of these is the phenomenon of second site mutations in *trans* on the wild-type allele. Sequencing of samples from patients heterozygous for *IDH1* or *IDH2* mutations with acquired resistance to IDH inhibitors revealed that relapse is associated with acquisition of mutations in the wild-type allele that do not confer neomorphic IDH function but rather inhibit binding of the inhibitor to the IDH–dimer interface and are pathogenic only in combination with the neomorphic R140Q mutation (Intlekofer et al. 2018). Isoform switching is another unique mechanism of resistance to IDH1/IDH2 inhibition, in which a patient with *IDH1*-mutated AML treated with ivosidenib may relapse with an *IDH2* mutation or vice versa (Harding et al. 2018). Both mechanisms illustrate the selective pressure to maintain 2HG production in IDH-mutated AML. This resistance mechanism is conceptually similar to MAPK reactivation due to secondary *RAS* and *PTPN11* mutations in *FLT3*-mutated

AML. It has been suggested that concurrent inhibition of IDH1 and IDH2 may preclude isoform-switching metabolism. The safety and efficacy of coinhibitors of IDH1 and IDH2 are under investigation in early phase clinical trials (**https://www.clinicaltrials.gov/** identifiers NCT02492737, NCT04764474, and NCT04603001). It has also been observed that dependence on 2HG production in *IDH*-mutated AML is accompanied by increased dependence on fatty acid oxidation and OXPHOS and that targeting these processes may sensitize patients who are resistant to IDH inhibition (Stuani et al. 2021).

EMERGING TARGETS: MENIN AND CD33

Menin

KMT2A is a gene encoding a lysine methyltransferase whose locus on chromosome 11q23 is targeted by recurrent translocations in AML and acute lymphocytic leukemia (ALL). KMT2A fusion proteins and wild-type *KMT2A* in the setting of *NPM1* mutation are targeted to histones in the promoters of *HOX* genes and *MEIS1* by the oncogenic cofactor menin, which results in chromatin remodeling and expression of a stem-like gene expression program with consequent differentiation block (Brien et al. 2019). A phase 1 study of the menin inhibitor revumenib in patients with *KMT2A*-rearranged or *NPM1*-mutated R/R AML and ALL reported an encouraging objective response rate (ORR) of 53% and a CR rate of 30% with a favorable toxicity profile (Issa et al. 2023). A concurrently reported analysis of patients with acquired resistance to revumenib and other menin inhibitors after initial response showed selection for mutations in *MEN1* at the menin–revumenib interface, which prevent inhibitor binding and maintain menin occupancy of chromatin (Perner et al. 2023). While this is the first report of acquired mutations mediating resistance to treatment with inhibitors targeting chromatin remodeling, the mechanisms responsible for primary resistance to such agents remain unclear.

CD33

Gemtuzumab ozogamicin (GO) is an antibody–drug conjugate: a monoclonal antibody targeting the cell surface protein CD33 that is conjugated to a genotoxic compound of the calicheamicin class. Upon antibody binding to CD33, the toxin is internalized and trafficked to the nucleus, where it causes DNA double-strand breaks and apoptosis (Jabbour et al. 2021). GO is approved for treatment in young patients with CD33-positive AML, for whom it is incorporated into induction regimens. Primary resistance is associated with splice-site polymorphism, leading to a an alternative splice isoform that lacks exon 2, which encodes the IgV domain recognized by GO (Lamba et al. 2017). This mechanism of resistance appears to be most common in children (Gale et al. 2018, Short et al. 2020).

Another polymorphism has been reported to decrease GO efficacy by reducing CD33 internalization and therefore reducing delivery of the ozogamicin payload to the leukemia cell (Gbadamosi et al. 2021). Polymorphisms in the P-glycoprotein gene that lead to increased drug efflux and a multidrug resistance phenotype are also associated with primary resistance (Rafiee et al. 2019, Walter et al. 2007). Loss of CD33 expression due to degradation of CD33 by SOCS3 is also associated with decreased response to GO (Orr et al. 2007). Acquired resistance may be mediated by compensatory upregulation of downstream pathways, including PI3K/AKT (Rosen et al. 2013). Sensitivity to GO is predicted by high expression of CD33 as well as the presence of mutations that activate signaling pathways (e.g., FLT3 and RAS), which are thought to correlate with high expression of CD33, though a causative link between hypermorphic signaling mutations and increased expression of CD33 remains speculative (Fournier et al. 2020).

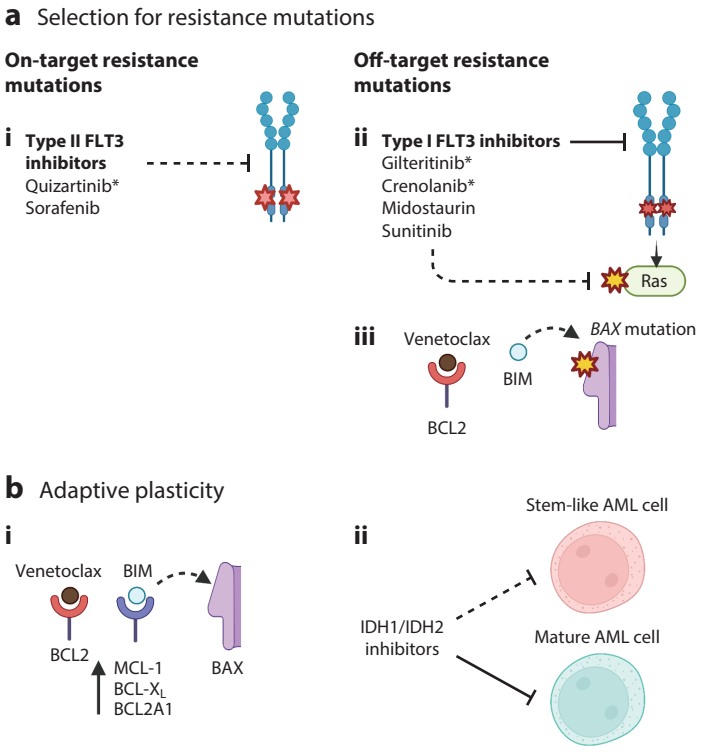

Figure 3

Summary of mechanisms of resistance to targeted therapy. We have grouped mechanisms of resistance into two broad categories: (*a*) Selection for resistance mutations, which includes on-target mutations such as (*i*) *FLT3* mutations that decrease the effect of type II inhibitors and off-target mutations such as (*ii*) downstream Ras mutations seen with type I FLT3 inhibitors or (*iii*) *BAX* mutations seen with venetoclax. (*b*) Adaptive plasticity, which includes upregulation of compensatory pathways such as (*i*) increased expression of BCL2 family proteins with venetoclax or change in cell state such as (*ii*) resistance to IDH1/IDH2 inhibition in AML cells with stem-like transcriptional profiles. Asterisks denote second-generation FLT3 inhibitors. Abbreviations: AML, acute myeloid leukemia; BCL2, B cell lymphoma 2; FLT3, FMS-like tyrosine kinase 3. Figure adapted from images created with BioRender.com.

CONCLUSION

The advent of genomic technologies has enabled personalized treatment aimed at the genetic drivers of AML. Many of these therapies are effective at achieving temporary responses, and in some cases even a temporary response is potentially lifesaving if it can serve as a bridge to curative hematopoietic stem cell transplant. However, acquired resistance to single-agent targeted therapy is virtually inevitable. A thorough understanding of the mechanisms of this resistance is important not only to optimize the currently available targeted therapies but also to empower the discovery of better treatments in the future. Several broad patterns of resistance are seen (**Figure 3**).

Selective Pressure in Heterogeneous Disease

Cancer is a disease of somatic evolution, with genetically related yet diverging clones competing in the face of selective pressure. When a targeted therapy is introduced, the relative fitness of clones bearing the targeted mutation plummets. Under the new selective pressure of targeted therapy,

either the targeted clones acquire new mutations that confer resistance, or parallel clones with different mutations enjoy new competitive advantages and expand over time. The expansion of treatment-resistant clones correlates with the clinical disease relapse. There are two major types of mutations observed at the time of relapse: on target and off target. On-target resistance mutations affect the coding sequence of the targeted gene and confer increased fitness by directly attenuating the pharmacodynamic effect of the drug, for example, *FLT3* mutations in residue D835 with quizartinib. Off-target resistance mutations result in activation of downstream or parallel signaling pathways that circumvent the effect of targeted therapy while preserving the pharmacodynamic inhibition of the target. Examples include activation of Ras/MAPK pathway mutations downstream of FLT3 in treatment with gilteritinib.

Adaptive Plasticity

We have also discussed mechanisms of resistance to targeted therapies outside of genetic selection that depend on cell state, differentiation, apoptotic dependencies, and metabolic factors. For example, monocytic AML is intrinsically less sensitive to BCL2 inhibition compared with AML with a primitive differentiation state due to increased dependency on MCL1 in monocytic AML. Although LSCs are not a genetically distinct population, their unique metabolic state with higher reliance on OXPHOS also results in decreased venetoclax and IDH inhibitor sensitivity. The large BEAT-AML study has observed that response to treatment with a broad panel of targeted inhibitors independently correlates with differentiation state (Bottomly et al. 2022), suggesting a context-dependent effect for many molecularly targeted therapies.

AML relapse after treatment with targeted therapy appears to be a daunting clinical challenge. The genetic and phenotypic heterogeneity within a single patient's AML combined with the complex adaptive mechanisms of myeloid cell physiology suggests that relying on targeting any single gene or pathway in AML is likely to end with resistance. Are we doomed to play whack-a-clone with AML, such that whenever one malignant clone is hit with targeted therapy, a resistant clone pops up in its place? On the contrary, there is reason for optimism that rational combination and sequencing of targeted therapies to sensitive cell states may yield improved responses.

DISCLOSURE STATEMENT

C.C.S. reports research funding from Abbvie, Inc., Erasca, Inc., and Revolution Medicines; has served as a consultant/advisory board member for Abbvie, Inc., Astellas, and Genentech; and has received research funding for conduct of clinical trials from Zentalis, Inc. and Celgene/Bristol-Meyers Squibb.

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