

Augmenting Venetoclax Activity Through Signal Transduction in AML

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Abstract

Venetoclax, a small-molecule B-cell lymphoma 2 (BCL-2) inhibitor, selectively eradicates leukemic stem cells (LSCs). While venetoclax has revolutionized the treatment of acute myeloid leukemia (AML), treatment failure and disease relapse are common. Mechanisms underlying venetoclax resistance are surprisingly heterogeneous. Venetoclax resistance encompasses a spectrum of genetic and epigenetic changes, with numerous pathways contributing to the upregulation of additional anti-apoptotic proteins. In this review, we address the mechanisms of venetoclax resistance in the context of signal transduction. We emphasize how aberrant cell signaling impairs apoptosis and predisposes to venetoclax failure.

Commonly activated pathways, such as FLT3, PI3K/AKT/mTOR, and RAS, contribute to upregulated anti-apoptotic mediators and are frequently responsible for refractory disease or disease relapse. We highlight novel combination strategies aimed at disabling constitutively active signal transduction to augment response and overcome venetoclax resistance.

Keywords: Acute myeloid leukemia, Akt pathway, Apoptotic pathways, B-cell lymphoma 2, FMS-like tyrosine kinase 3, Isocitrate dehydrogenase 1, Isocitrate dehydrogenase 2, MAPK pathway cell signaling, Signal transduction cascades

Abbreviations: AML: Acute Myeloid Leukemia; BCL-2: B-cell Lymphoma 2; IDH1: Isocitrate Dehydrogenase 1; IDH2: Isocitrate Dehydrogenase 2; FLT3: FMS-Like Tyrosine kinase 3; LSC: Leukemic Stem Cell; MAPK: Mitogen-Activated Protein Kinase; mTOR: Mammalian Target of Rapamycin; RAS: Rat Sarcoma; PI3K: Phosphatidylinositol-3-kinase

Introduction

Acute myeloid leukemia is a heterogeneous hematologic malignancy characterized by a maturation arrest of hematopoietic precursors [1]. Because AML is primarily a disease of older adults occurring at a median age of 68 years, many are ineligible for intensive chemotherapy [2,3]. Intensity strategies offer a survival benefit in this population. Low-dose cytarabine or hypomethylating agents, such as azacitidine or decitabine, remain a mainstay of lower-intensity treatment

with a median survival of 5 – 9 months, varying by cytogenetic and molecular risk factors [4-6]. There is a persistent interest in intensifying lower-intensity treatment strategies to improve survival in the elderly population. The addition of venetoclax, a BCL-2 inhibitor, to a hypomethylating agent or low-dose cytarabine improves outcomes in patients ineligible for intensive chemotherapy, and the use of venetoclax-based regimens is now routine [5]. The race is ongoing to further characterize subsets of AML associated with improved survival when treated with venetoclax-based strategies.

Specific molecular subsets respond favorably to venetoclax augmentation. For example, AML with mutated isocitrate dehydrogenase (*IDH1* or *IDH2*) is associated with a survival benefit when treated with venetoclax and azacitidine compared to azacitidine alone (hazard ratio for death, 0.34; 95% CI, 0.20 - 0.60) [5]. In contrast, other molecular subsets — commonly those with mutations in signal transduction — are more likely to be resistant to treatment. AML with mutated FMS-like tyrosine kinase 3 (*FLT3*), rat sarcoma (*RAS*), or tyrosine-protein phosphatase non-receptor type 11 (*PTPN11*) are associated with decreased survival and higher rates of relapse following treatment with lower-intensity venetoclax-based strategies [7-11].

An emerging topic of interest is the modulation of signal transduction to circumvent venetoclax resistance. The FDA-approved *FLT3* inhibitors — midostaurin and gilteritinib — are being evaluated in clinical trials in combination with venetoclax and a hypomethylating agent to reduce the incidence of relapse and improve survival [12,13]. Similarly, the use of mitogen-activated protein kinase (MAPK) pathway inhibitors, such as trametinib or cobimetinib, with venetoclax-based strategies is emerging; triplet combinations, however, are limited by toxicity [14-16]. Dose-limiting toxicities have prompted interest in developing more active, specific inhibitors of *FLT3* and pathways downstream of *PI3K* and *RAS* to reduce venetoclax resistance and improve treatment tolerability. Consequently, numerous novel venetoclax-based combinations are surfacing. Following an overview of apoptosis, this review summarizes strategies to augment venetoclax response and reduce resistance, emphasizing the modulation of the commonly implicated signal transduction pathways: *FLT3*, *PI3K*, and *RAS*.

Regulation and Modulation of Apoptosis

Regulation of apoptosis

Apoptosis is a tightly regulated process of cell death, and aberrations of apoptosis are central to LSC persistence [17]. Understanding strategies that eradicate LSCs requires understanding the critical regulators of apoptosis — the BCL-2 family proteins. The BCL-2 family proteins are involved in a delicate interplay of mediators that moderate the mitochondrial (or intrinsic) apoptotic pathway. They are broadly categorized into pro-apoptotic and anti-apoptotic mediators.

Two prominent pro-apoptotic BCL-2 proteins are BAX and BAK. Activation of BAX and BAK and subsequent dimer formation results in pore-forming subunits in the mitochondria, consequently increasing the mitochondrial outer membrane permeability [18,19]. Due to increased mitochondrial permeability, egress of apoptotic effectors from the mitochondria into the cytosol occurs — with the most notable effector being cytochrome *c* [20]. Cytochrome

c then initiates the intrinsic pathway through the activation of caspase 9 and caspase 3, culminating in the cleavage of cytosolic and nuclear proteins and the procession of cell death [21].

Additional BCL-2 family proteins potentiate apoptosis; they contain homologous structures to BAX and BAK, known as BCL-2 homology (BH) motifs. There are four BCL-2 homology motifs: BH1, BH2, BH3, and BH4 [22]. These additional subsets of BCL-2 family proteins share the BH3 homology domain and indirectly sensitize apoptosis (BAD, BIK, HRK, NOXA) or directly activate apoptosis (BIM, BID, PUMA) [23-25]. After binding these apoptotic sensitizers and activators, the anti-apoptotic proteins release bound BAX and BAK, allowing apoptosis to proceed. Additionally, BH3 apoptotic activators (BIM, BID, PUMA) directly activate BAX and BAK.

In contrast to the pro-apoptotic mediators, the anti-apoptotic BCL-2 proteins bind BAX and BAK, rendering them unable to drive the intrinsic pathway. The anti-apoptotic proteins include BCL-2, BCL-X_L, BCL-w, BFL-1, and MCL-1. By binding to BAX and BAK, BCL-2 and BCL-X_L inhibit apoptosis and block the subsequent formation of the mitochondrial pore-forming subunits [18,26,27]. In addition, anti-apoptotic proteins sequester the BH3-only sensitizing and activating proteins, further reducing the procession of the mitochondrial apoptotic pathway [22]. An overview of these apoptotic regulators is provided in **Figure 1**.

Modulation of apoptosis: BCL-2 inhibition

Promoting leukemic cell death by activating apoptosis is an attractive therapeutic strategy. Owing to the overexpression of BCL-2 in LSCs, selective inhibition of BCL-2 leads to the sequestration of BAX and BAK and induces the eradication of quiescent LSCs [17]. This discovery led to the development of the BH3 mimetics — which free the pro-apoptotic mediators by disrupting the binding of the BH3 motif to anti-apoptotic proteins, allowing apoptosis to proceed.

Navitoclax was one of several BH3 mimetics investigated shortly after the discovery of BCL-2 inhibition as a therapeutic strategy. Navitoclax selectively targets BCL-2, BCL-w, and BCL-X_L [22]. However, BCL-X_L is a platelet pro-survival protein, and inhibition of BCL-X_L by navitoclax resulted in dose-limiting thrombocytopenia [28]. Rates of high-grade thrombocytopenia reduced the enthusiasm for further clinical development in hematological malignancies. This led to the investigation of venetoclax in clinical trials, which binds to BCL-2, but not to BCL-X_L. Soon afterward, the landmark VIALE-A trial demonstrated the superiority of venetoclax and 7-day azacitidine compared to azacitidine alone, and cemented the use of combination therapy in patients with AML ineligible for intensive induction [5].

There is, however, a critical caveat to the use of venetoclax.

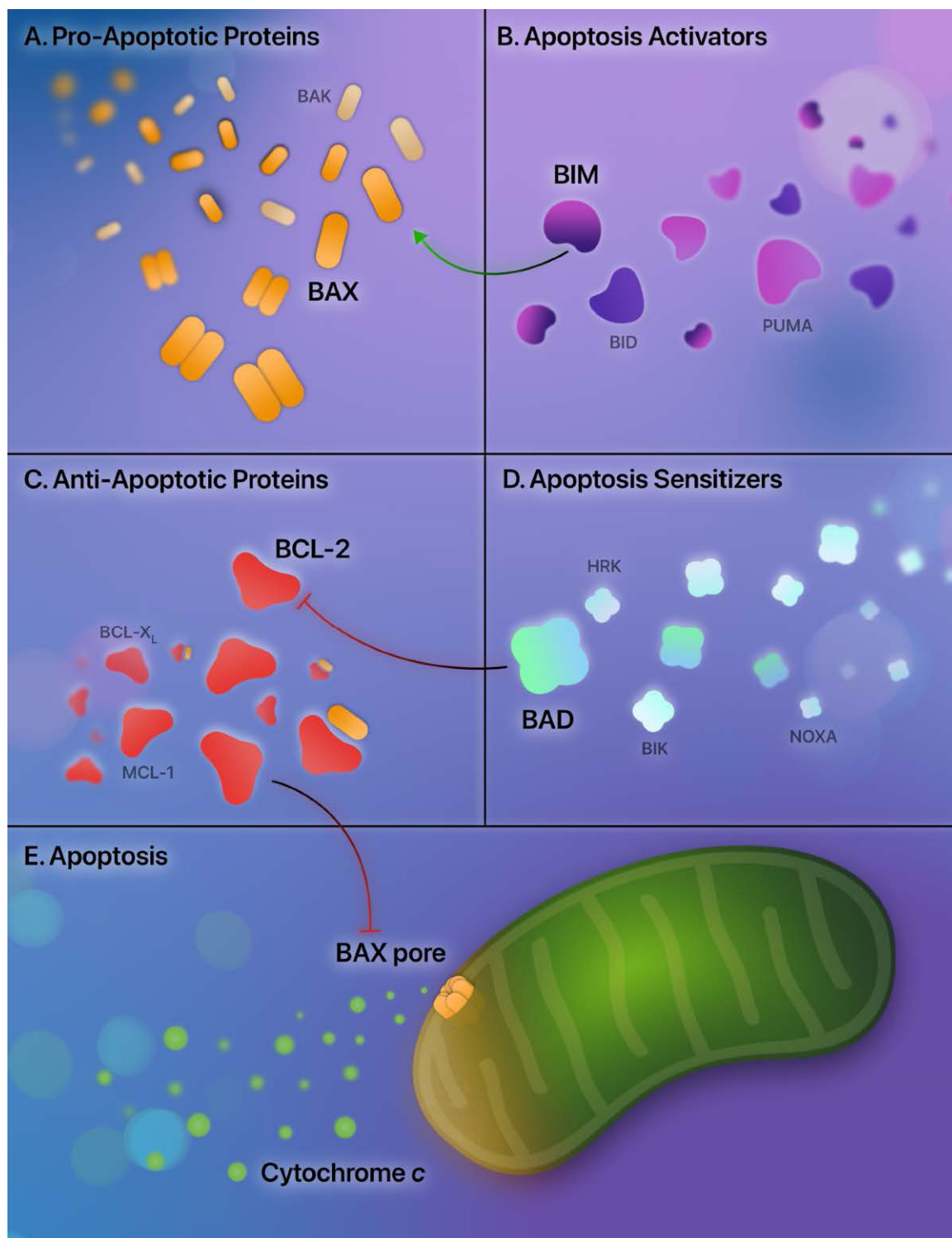


Figure 1. The apoptotic mediators. **A.** The primary pro-apoptotic proteins are BAX and BAK. In pro-apoptotic states, dimer formation and subsequent oligomerization occur, resulting in the initial steps of the formation of a pore subunit. **B.** The apoptosis activators are BIM, BID, and PUMA, which directly activate BAX and BAK. **C.** The anti-apoptotic proteins are BCL-2, MCL-1, BCL-X_L, as well as BCL-w and BFL-1 (not pictured). These proteins bind BAX and BAK and result in the inhibition of apoptosis. **D.** The apoptotic sensitizers are BAD, BIK, HRK, and NOXA; they bind to the anti-apoptotic proteins and release bound BAX and BAK, allowing apoptosis to proceed. **E.** Apoptosis proceeds through the formation of a mitochondrial outer membrane pore, increasing mitochondrial permeability and allowing the escape of cytochrome c.

Like navitoclax, venetoclax does not inhibit the anti-apoptotic mediator MCL-1; it also does not inhibit BFL-1 or BCL-X_L. Indeed, the upregulation of these anti-apoptotic proteins contributes directly to venetoclax resistance, and the downregulation of MCL-1 or BCL-X_L restores venetoclax sensitivity [29]. The upregulation of MCL-1 is common in leukemic cells following venetoclax exposure, and elevations in MCL-1 subside with venetoclax discontinuation [30]. The reduction in MCL-1 following venetoclax cessation forms the basis of a strategic molecular rationale to reduce venetoclax resistance: stopping or shortening the duration of venetoclax after a maximal response is reached.

Venetoclax resistance is remarkably heterogeneous. Elevation of anti-apoptotic mediators is a frequent accompaniment of venetoclax-resistant disease. Many paths lead to persistent cell survival following venetoclax exposure, including aberrations in epigenetics, gene amplifications, or BCL-2 family mutations [31,32] — but perhaps the path most appropriate to target is constitutively active signal transduction.

FLT3

Overview of FLT3

FMS-like tyrosine kinase 3 (FLT3) is among the most commonly mutated and well-understood signaling pathways in AML. FLT3 is a ligand-activated transmembrane tyrosine kinase that feeds into downstream pathways, including PI3K, RAS, and STAT5, promoting cell proliferation and survival [33]. The FLT3 receptor shares a high degree of homology with KIT, FMS, and PDGFR receptors — all of which regulate hematopoietic maturation and differentiation [34].

The two most commonly observed activating mutations in *FLT3* are an internal tandem duplication (*FLT3*-ITD) of the juxtamembrane domain and tyrosine kinase domain mutations (*FLT3*-TKD) in the activation loop, although many other *FLT3* mutations exist [35]. *FLT3*-ITD and *FLT3*-TKD lead to constitutive activation of FLT3 kinase, driving leukemic proliferation and survival. As *FLT3*^{mut} AML has an increased risk of relapse and shorter overall survival — particularly for *FLT3*-ITD [36,37] — there has been relentless interest in improving outcomes in this cohort of AML.

Sorafenib, a multi-kinase inhibitor with activity against *FLT3*-ITD but not *FLT3*-TKD [38], significantly prolonged event-free survival when combined with standard-of-care chemotherapy [39]. Subsequently, midostaurin, a first-generation multi-kinase inhibitor active against *FLT3*-ITD and *FLT3*-TKD, improved overall survival when combined with an anthracycline and cytarabine [40]. Midostaurin, however, lacks specificity for *FLT3*-ITD, which led to the development of more specific and potent next-generation FLT3 inhibitors, including quizartinib, crenolanib, and gilteritinib [41]. The most notable of these is gilteritinib, which resulted in significantly longer

overall survival and higher remission rates than salvage chemotherapy in *FLT3*^{mut} AML [42].

Outcomes of *FLT3*^{mut} AML are being investigated in the context of venetoclax-based induction without a FLT3 inhibitor. A recent subgroup analysis of the VIALE-A trial analyzed 42 patients with *FLT3* mutations treated with venetoclax and azacitidine. The median overall survival of *FLT3*^{mut} AML was shorter at 12.5 months compared to 14.7 months for wild-type *FLT3* [43], suggesting some degree of *FLT3*-mediated venetoclax resistance. Additionally, new *FLT3* mutations emerge following venetoclax treatment in patients without a *FLT3* mutation at diagnosis, suggesting that *FLT3* may be responsible for resistance by an adaptive mechanism [44]. These observations provided the groundwork to refine outcomes of *FLT3*^{mut} AML treated with venetoclax-based regimens.

Overcoming *FLT3* Resistance

Venetoclax resistance in *FLT3* mutants occurs through several mechanisms in addition to the emergence of new *FLT3* mutations. As venetoclax selectively inhibits BCL-2, upregulation of anti-apoptotic proteins other than BCL-2 is a primary cause of resistance. Indeed, constitutive FLT3 signaling upregulates the anti-apoptotic mediator MCL-1 [45]; siRNA-mediated MCL-1 inhibition restores the sensitivity of *FLT3*-ITD-AML to therapy [45].

Additional avenues of *FLT3*-mediated venetoclax resistance are similar to MCL-1 upregulation. The activity of another anti-apoptotic mediator, BCL-X_L, is maintained in *FLT3*-ITD, resulting in leukemic stem cell persistence and survival. Targeting *FLT3*-ITD results in BCL-X_L reduction [46]. Together, the upregulation of MCL-1 and BCL-X_L appears to be an indirect consequence of downstream activation of PI3K, RAS, and STAT5 [7,46,47]. Therefore, as multiple parallel pathways are activated, the most efficacious way to overcome *FLT3*-mediated resistance is by targeting FLT3 rather than its downstream effectors [46]. A summary of the interplay between FLT3 and the apoptotic pathway is provided in **Figure 2**.

The observation that *FLT3* mutations upregulate alternative anti-apoptotic mediators led to the design of several studies investigating the efficacy of venetoclax and FLT3 inhibition. Venetoclax with sorafenib, midostaurin, or gilteritinib led to synergistic apoptosis in *FLT3*^{mut} AML through the downregulation of MCL-1 [48,49]. These observations support the hypothesis that the downregulation of pro-survival mediators other than BCL-2 may overcome venetoclax resistance. The pre-clinical demonstration of synergistic efficacy between FLT3 and BCL-2 inhibition prompted the trial design of intensifying azacitidine and venetoclax with FLT3 inhibitors.

Early phase trials of gilteritinib with 7-day azacitidine and

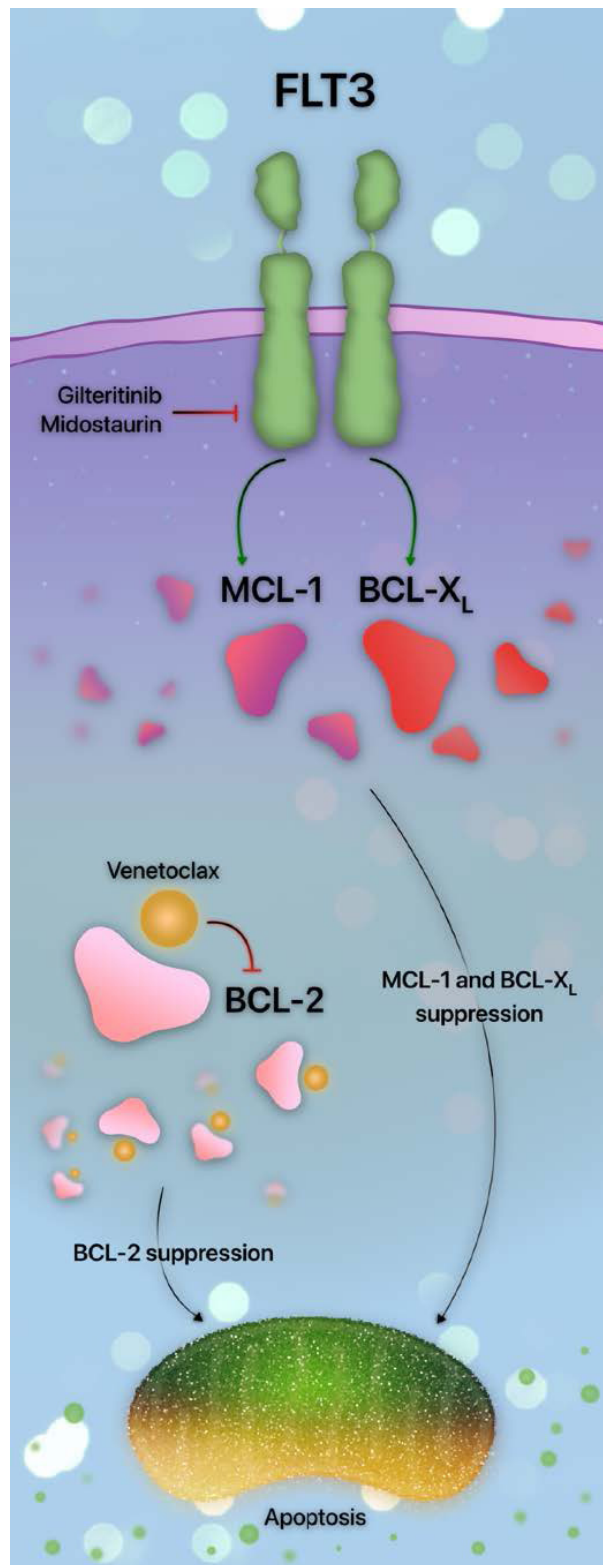


Figure 2. The FLT3 signaling pathway in apoptosis. Activation of FLT3 results in increased MCL-1 and BCL-X_L, which are reversed with FLT3 inhibition with agents such as gilteritinib or midostaurin. BCL-2 inhibition with venetoclax synergizes with FLT3 inhibitor-mediated MCL-1 and BCL-X_L suppression, culminating in potent apoptosis.

venetoclax demonstrated an overall response rate of 100% in newly diagnosed *FLT3*^{mut} AML, with no relapses observed during the follow-up period [13]. In the relapsed or refractory setting, the overall response rate was 67%, with 44% proceeding to allogeneic stem cell transplant [13]. Prolonged myelosuppression was the dose-limiting toxicity, suggesting that strategies to refine the dose or duration of drug exposure may minimize treatment-related complications.

A similar study of sorafenib, midostaurin, or gilteritinib with 10-day decitabine and venetoclax yielded a composite complete remission rate of 92% in newly diagnosed AML, and 56% were negative for measurable residual disease (MRD). The overall survival was not reached at a median follow-up time of 14.5 months [12]. The composite complete remission rate was 62% in the relapsed or refractory setting, although the median overall survival was shorter at 6.8 months [12]. Further trials are ongoing to determine the optimal *FLT3* inhibitor, hypomethylating agent backbone, and duration of drug exposure to balance efficacy and toxicity. Nevertheless, preliminary results in the front-line setting are impressive. In relapsed or refractory AML, the combination approach is more problematic. The emergence of new *FLT3* mutations frequently hampers outcomes through clonal evolution or constitutive activation of downstream parallel signaling pathways, such as PI3K or RAS.

Overview of PI3K

Overview of PI3K/AKT/mTOR

Cell signaling through the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) is one of the most central intracellular pathways in cancer. Mutations in receptor tyrosine kinases — such as *FLT3* — or GTPases frequently lead to upregulation of the PI3K pathway and are associated with inferior overall survival in AML [50]. PI3K is a plasma-associated lipid kinase that converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 recruits lipid-binding proteins to the cell membrane and localizes AKT, activating mTOR and additional downstream pathways, culminating in cell growth, proliferation, and survival [51].

AKT-mediated cell survival occurs directly through interactions with the apoptosis-regulating proteins. AKT, a protein kinase, phosphorylates numerous substrates, including the apoptotic sensitizer BAD. Phosphorylated BAD frees BCL-2 and BCL-X_L, allowing these anti-apoptotic proteins to block the progression of mitochondrial outer membrane permeability and subsequent cell death [52,53]. Therefore, cell survival is the net effect of PI3K/AKT signaling on apoptosis.

PI3K inhibition is unimpressive as monotherapy, and trials involving this class of agents have been limited by excessive toxicity. Gedatolisib, a dual PI3K/mTOR inhibitor, was

evaluated in relapsed or refractory AML, and no objective response or clinical benefit was observed [54]. Consequently, there has been growing interest in modulating other effectors downstream from PI3K. Triciribine, an AKT activation inhibitor, was evaluated in an early phase study, demonstrating reduction of BAD and induction of cell death [55]. Clinical trials combining triciribine with chemotherapy or additional targeted agents are ongoing or planned.

Inhibitors of mTOR were evaluated in AML. A caveat of mTOR inhibition is the resultant increased phosphorylation of AKT [50]. To circumvent this drawback, dual PI3K and mTOR inhibitors have been developed. Dactolisib, a dual PI3K/mTOR inhibitor, reduced cell growth and induced apoptosis in AML cells without affecting normal stem cell function [56]. The impact of combination therapy across the spectrum of PI3K/AKT/mTOR inhibitors is being elucidated. An overview of several PI3K pathway inhibitors is provided in **Figure 3**.

There is a relative paucity of data on outcomes of patients with AML harboring a PI3K pathway mutation treated with venetoclax-based strategies. The clinical impact of aberrations of PI3K is inferred from upstream effectors, such as *FLT3* or *KIT*. These upstream kinase-activating mutations are associated with reduced survival when treated with venetoclax-based strategies, suggesting additive mechanisms of primary or adaptive resistance [7,50].

Overcoming PI3K pathway resistance

Venetoclax resistance in AML with constitutively activated PI3K directly results from the upregulation of multiple anti-apoptotic mediators. In addition to AKT-dependent increases in BCL-2 and BCL-X_L, a third anti-apoptotic protein, MCL-1, is also upregulated by the PI3K pathway [57]. Therefore, constitutive PI3K signaling promotes multiple avenues for venetoclax resistance through the upregulation of alternative anti-apoptotic mediators, facilitating cell survival despite BCL-2 inhibition.

To combat multiple resistance mechanisms mediated through PI3K, apitolisib, a dual PI3K/mTOR inhibitor, was studied in AML cells undergoing treatment with concurrent BCL-2 inhibition. Venetoclax and apitolisib induced rapid AML cell apoptosis with MCL-1 downregulation and spared normal hematopoietic cells [58]. The combination of venetoclax and apitolisib was also effective in venetoclax-resistant cells. These findings support the notion that the downregulation of MCL-1 is a consequence of targeting PI3K and BCL-2 rather than a result of cell death. The success of co-targeting PI3K and BCL-2 reinforced the results of additional studies that demonstrated resistant AML cell lines can be re-sensitized to venetoclax [29,59].

Activation of the PI3K pathway also plays a unique role in *FLT3*^{mut} AML undergoing treatment with *FLT3* inhibition.

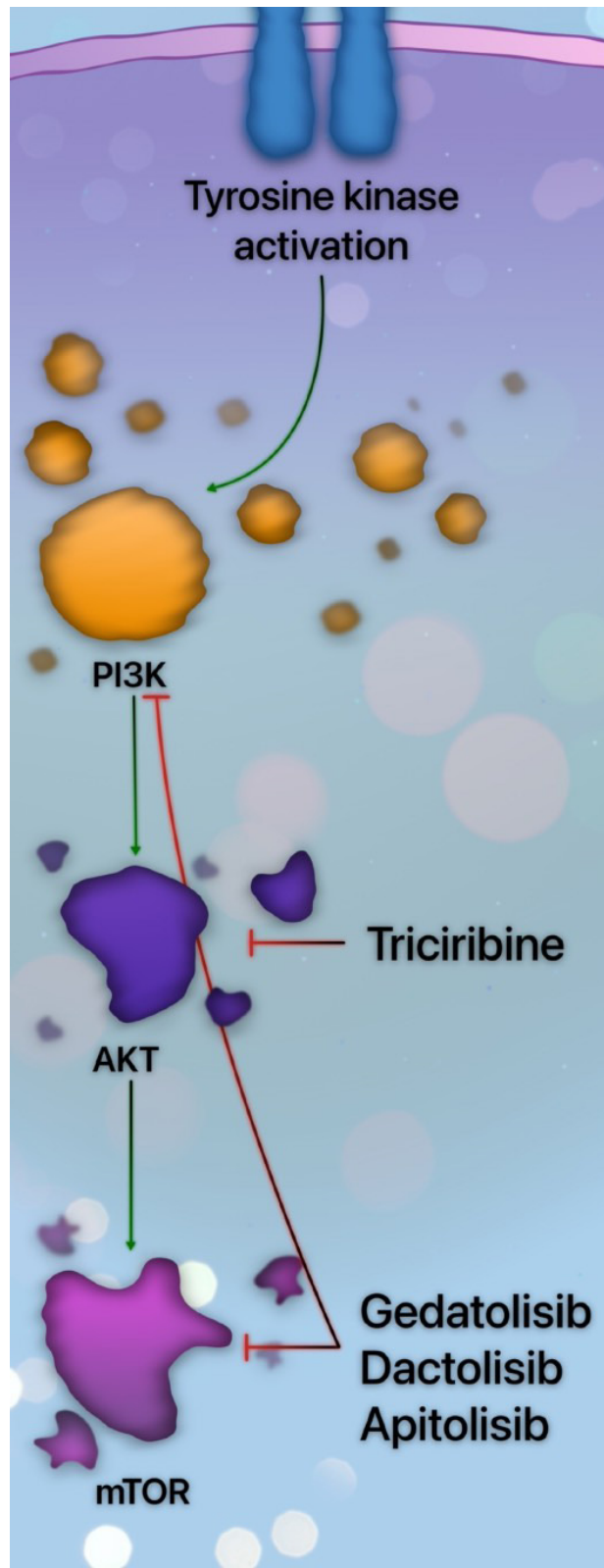


Figure 3. The PI3K pathway inhibitors in AML. Activation of a tyrosine kinase, such as FLT3 or KIT, leads to PI3K pathway activation. PI3K then phosphorylates AKT, which then sequentially activates mTOR.

FLT3-ITD AML cell lines resistant to sorafenib are enriched in the PI3K/mTOR pathway [60]. Gedatolisib blocked cell proliferation, induced apoptosis in resistant cell lines, and extended overall survival in PDX models following sorafenib exposure. These findings suggest that PI3K inhibition may be a subsequent therapeutic avenue to overcome resistance in patients initially treated with novel triplet regimens, such as a *FLT3* inhibitor, venetoclax, and a hypomethylating agent. Further clinical trials should explore the efficacy of targeting the PI3K pathway to overcome or delay therapeutic resistance in patients treated with venetoclax-based strategies.

RAS

Overview of RAS

The RAS proteins exhibit substantial crosstalk with the PI3K pathway and stimulate cell proliferation and survival through the cascade of RAF, MEK, and ERK, which constitute the RAS pathway [53]. RAS pathway mutations commonly emerge as cooperating mutations in leukemogenesis — dance partners to additional mutations which accelerate subclonal evolution and promote resistance to therapy [61,62].

Similar to PI3K, constitutively activated RAS signaling frequently occurs in the context of upstream *FLT3* activity, with downstream RAS activation contributing to *FLT3* inhibitor resistance [63,64]. In addition, as RAS activity is guanine-5-triphosphate (GTP)-dependent, mutations that inactivate RAS GTPases will also result in constitutive RAS activation [65].

In AML, RAS mutations confer a robust proliferative advantage and skew differentiation toward the myelomonocytic line [66,67]. Patients with *RAS*^{mut} AML have a significantly longer overall survival than *RAS* wild-type, perhaps partly due to a higher likelihood of association with favorable-risk cytogenetics [68]. In particular, *RAS*^{mut} AML demonstrated significantly longer overall survival when treated with intensive cytarabine-containing induction regimens. Intensive chemotherapy with venetoclax did not significantly improve response rates in *RAS*^{mut} AML compared to intensive chemotherapy without venetoclax. In contrast, response rates, but not survival, were significantly lower in patients harboring a *RAS* mutation treated with a hypomethylating agent and venetoclax [68]. These findings support the hypothesis of RAS-mediated venetoclax resistance when treated in combination with a hypomethylating agent.

Clinical trials evaluated multiple inhibitors of the RAS pathway. Selumetinib, a MEK inhibitor, was associated with modest single-agent activity in relapsed or refractory AML, but no patient with *FLT3*-ITD responded [69]. Resistance to MEK inhibition in *FLT3*-ITD AML suggests that the activation of parallel signaling pathways contributes to disease refractoriness, warranting the investigation of combination strategies. Indeed, MEK inhibition with trametinib enhanced

the response to *FLT3* inhibitors, improving survival in mouse models while sparing normal CD34⁺ cells [70]. Therefore, the notion that RAS pathway-targeting combination strategies may improve survival while reducing treatment resistance is gaining momentum.

Overcoming RAS resistance

AML with myelomonocytic differentiation is implicated in venetoclax resistance, raising the hypothesis that RAS activation may be a driving factor [71]. Similar to constitutive *FLT3* signaling, the RAS pathway contributes to MCL-1 stabilization through post-translational modification, ultimately resulting in venetoclax resistance [11,72,73]. This discovery prompted the investigation of combination strategies evaluating the efficacy of venetoclax with inhibitors of RAS or MEK.

Further studies in AML cell lines evaluated the combination of venetoclax and cobimetinib. Using this strategy, MCL-1 was downregulated following MEK inhibition, which sensitized cells to venetoclax and culminated in reduced leukemia cell burden in mouse models [14]. Next, MEK inhibitors were evaluated in venetoclax-resistant cells. Upon exposure of venetoclax-resistant cells to cobimetinib, the level of MCL-1 decreased only minimally, suggesting activation of pathways upstream of MEK [11].

Subsequently, *RAS* mutations and MAPK activation were discovered to stabilize MCL-1, resulting in persistent cell survival. To confirm the role of MCL-1 in RAS-mediated resistance to venetoclax, co-treatment with venetoclax and an MCL-1 inhibitor, AZD-5991, significantly reduced AML blasts in mice [11]. These findings formed the foundation for clinical trials co-targeting BCL-2 and the RAS pathway in *RAS*^{mut} AML.

Based on this data, a clinical trial evaluated trametinib with venetoclax and 7-day azacitidine in relapsed or refractory *RAS*^{mut} AML. Sixteen patients received a median of four lines of prior therapy, and 81% received a prior hypomethylating agent with venetoclax. While 67% of patients that did not receive venetoclax-based treatment responded, only 15% of patients with previous venetoclax exposure had a response [15]. The median overall survival was 2.4 months, and high-grade adverse events occurred in half of those treated [15], resulting in premature study closure due to low efficacy and high toxicity.

Therefore, more specific and less toxic inhibitors of the RAS pathway are needed to improve survival in this cohort.

Conclusion

Venetoclax revolutionized the treatment of AML and ushered in new waves of questions. The most glaring question is readily apparent: how can we overcome or delay venetoclax

resistance? Resistance to venetoclax has been consistently observed across molecular cohorts, primarily through the upregulation of MCL-1 and BCL-X_L. Signaling pathways commonly activated in AML frequently result in the elevation of these alternative anti-apoptotic mediators, culminating in treatment failure and disease relapse. These biological contributors of resistance are directly implicated in the observation of decreased overall survival in patients treated with venetoclax harboring constitutively activated signaling mutations [7].

Among the three commonly activated signaling pathways in AML — FLT3, PI3K, and RAS — the most promising therapeutic approach appears to be targeting FLT3 with a triplet regimen. The pre-clinical observation that FLT3 inhibitor-mediated MCL-1 downregulation is synergistic with BCL-2 inhibition illuminated the path to impressive early phase clinical trial results. Incorporating gilteritinib into triplet therapy with venetoclax offers a promising therapeutic advancement, particularly in the first-line setting. In this context, FLT3 inhibition may delay venetoclax resistance. In contrast, the optimal strategy at the time of disease relapse following exposure to a FLT3 inhibitor and venetoclax is unclear. Two therapeutic avenues can be envisioned: targeting the newly emerged mutation contributing to resistance or adopting a novel strategy. Innovative FLT3-directed therapies offer alternative methods of attack: FLT3 bispecific T-cell engagers (BiTEs) and FLT3-directed chimeric antigen receptor (CAR) T-cell therapy [74,75].

Specific, efficacious, and potent inhibition of PI3K and RAS has yet to be fully realized in AML, and the optimal combinations and targets remain to be explored. This leads to a second question: what are the optimal targets for these pathways, and how should they be built into existing treatment strategies for patients who are not candidates for intensive chemotherapy? While venetoclax-based triplet combinations are compelling, clinical trials have not yet supported this approach for AML with mutated *PI3K* or *RAS*. Sequencing targeted therapy after the emergence of activating *PI3K* or *RAS* mutations following venetoclax exposure may improve survival and reduce toxicity. Alternatively, novel approaches hold promise — such as RAS-directed CAR T-cell therapy [76].

The development of new therapies in AML has accelerated rapidly in recent years. Despite this, novel agents are still sorely needed and eagerly anticipated. Alternatively, direct inhibition of MCL-1 or other anti-apoptotic mediators may provide additional avenues for overcoming signaling pathway-mediated resistance. Regardless, it is an exciting era in the treatment of AML — one with many new therapeutic combination strategies waiting to be explored.

Conflicts of Interest

I.M. Bouligny, K.R. Maher, and S. Grant have no conflicts of interest to disclose.

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Author Contributions Statement

I.M. Bouligny prepared the manuscript. K.R. Maher and S. Grant contributed equally to revisions.

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