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The Intriguing Clinical Success of BCL-2 Inhibition in Acute Myeloid Leukemia

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Abstract

Over the past several decades numerous preclinical and clinical studies have pursued new approaches for the treatment of acute myeloid leukemia (AML). While some degree of clinical response has been demonstrated for many therapies, for the most part, fundamental changes in the treatment landscape have been lacking. Recently, the use of the BCL-2 inhibitor venetoclax has emerged as a potent therapy for a majority of newly diagnosed AML patients. Venetoclax regimens have shown broad response rates with deep and durable remissions, with a superior toxicity profile compared with traditional intensive chemotherapy agents. Numerous ongoing studies are now using venetoclax in combination with a wide range of other agents as investigators seek even more effective and well-tolerated regimens. Notably, however, while the empirical results of BCL-2 inhibition are encouraging, the mechanisms that have led to these successful clinical outcomes remain unclear. Intriguingly, the activity of venetoclax in AML patients appears to go beyond simply modulating canonical antiapoptosis mechanisms; in addition, the efficacy of venetoclax is linked to its combined use with conventional low-intensity backbone therapies. This article will evaluate the state of the field, provide a summary of key considerations, and propose directions for future studies.

INTRODUCTION

Thirty-five years ago, a seminal publication by Tsujimoto et al. (1985) showed that the t(14;18) translocation commonly observed in follicular lymphoma resulted in overexpression of the newly described gene *BCL-2* (B cell lymphoma 2). Subsequent studies revealed that *BCL-2* overexpression did not result in cell proliferation, as expected, but instead resulted in impaired cell death (Hockenbery et al. 1990; Nunez et al. 1990; Tsujimoto 1989a,b). Since this critical discovery, the field has gone on to describe multiple BCL-2 family members (Danial & Korsmeyer 2004, Gross et al. 1999). The canonical role of these proteins has been extensively described and involves a complex set of heterologous interactions that either promote or inhibit apoptosis (Danial & Korsmeyer 2004, Gross et al. 1999). Importantly, however, BCL-2 has also been associated with several other cellular activities. Indeed, as a membrane protein in both mitochondria and the endoplasmic reticulum, BCL-2 has the potential to influence a variety of pathways (Bonneau et al. 2013, Gross & Katz 2017). As described herein, considerable evidence indicates that the intriguing clinical activity of venetoclax in AML arises as a consequence of both the canonical and noncanonical roles of BCL-2.

Due to the nearly universal overexpression of BCL-2 in lymphoid malignancies (as well as many other cancers), and the role of BCL-2 as an inhibitor of apoptosis, therapeutic targeting of BCL-2 has long been a logical endeavor in oncology drug development. Indeed, multiple strategies have been described, and inhibition of Bcl-2 has shown broad activity in preclinical models (Leber et al. 2010, Lessene et al. 2008).

Venetoclax is the most specific BCL-2 inhibitor, and by far the furthest developed in the clinical setting. Venetoclax-based regimens have been shown to result in deep and durable remissions in the majority of newly diagnosed AML patients who receive this therapy. Importantly, the regimen is considerably less toxic than conventional chemotherapy regimens, thereby permitting treatment for many AML patients who are otherwise unable to tolerate standard therapy. Notably, the molecular properties of venetoclax suggest that it has the ability to eradicate leukemia stem cells (LSCs), the recalcitrant disease population inefficiently eradicated by traditional chemotherapy and the source of relapse when the disease recurs. The initial success of venetoclax led to US Food and Drug Administration (FDA) approval for acute myeloid leukemia (AML) in 2018, in combination with one of three conventional agents in the setting of untreated AML patients who are older or unfit for intensive chemotherapy (Leverson et al. 2017).

SCIENTIFIC UNDERPINNINGS OF VENETOCLAX-BASED THERAPY

Single-Agent Venetoclax

Inhibition of BCL-2 has been pursued in the AML field for many years (Yogarajah & Stone 2018). Forerunners to venetoclax (e.g., ABT-737 and ABT-263) showed potent activity in multiple preclinical studies, including analyses focused on AML stem cell populations (Konopleva et al. 2006, Lagadinou et al. 2013). Via mechanisms that have previously been extensively described, venetoclax-mediated inhibition of BCL-2 can directly induce apoptosis in AML cells (Pan et al. 2014). Furthermore, studies by Letai and colleagues (Vo et al. 2012) have shown that primary AML cells can demonstrate so-called "mitochonodrial priming," whereby the expression pattern of various BH3 family proteins makes cells particularly sensitive to BCL-2 inhibition. Notably, the degree of priming among patient specimens can vary and is largely independent of underlying genetic mutations, suggesting that prescreening patients for the primed phenotype might identify individuals more responsive to BCL-2 inhibition.

In addition to direct apoptosis, several studies have suggested that BCL-2 inhibition may induce cell death through alternate noncanonical mechanisms. Indeed, analysis of BCL-2-mediated

events, as well as related studies, have strongly pointed to inhibition of mitochondrial metabolism as a potent means to induce AML cell death (X. Chen et al. 2019, Cole et al. 2015, Mirali et al. 2020, Sharon et al. 2019, Skrtic et al. 2011, Sriskanthadevan et al. 2015). In particular, venetoclax was shown to inhibit mitochondrial uptake/catabolism of amino acids, an activity that leads to inhibition of oxidative phosphorylation (OXPHOS) in AML stem/progenitor populations (Jones et al. 2018). In addition, recent CRISPR-based synthetic lethal screens also show a link to mitochondrial function (X. Chen et al. 2019). To date, most of these studies converge on OXPHOS as the central component of mitochondrial function that mediates AML-selective cell death. Of particular interest, functionally defined AML stem cells appear to be particularly sensitive to inhibition of OXPHOS, suggesting that clinical efficacy may be at least partially associated with improved targeting of the AML stem cell population (Pollyea et al. 2018). The preferential reliance of primitive AML cells on OXPHOS appears to derive from an intrinsic deficiency in regulating energy metabolism. Upon inhibition of OXPHOS, AML stem/progenitor cells are unable to upregulate compensatory energy production via glycolysis. This is in contrast to normal hematopoietic stem/progenitor cells, which do upregulate glycolysis as needed and thereby circumvent the toxicity associated with inhibition of OXPHOS (Lagadinou et al. 2013).

Single-Agent Hypomethylating Agents

The three backbone therapies most extensively studied in combination with venetoclax include azacitidine, decitabine, and low-dose cytarabine (LDAC). These well-described nucleoside analogs are chemically similar but have notable differences in structure and activity. Azacitidine and decitabine are known to act as DNA hypomethylating agents (HMAs), whereas cytarabine does not. In addition, azacitidine is unique in that it inhibits ribonucleotide reductase and is incorporated into RNA as a nucleoside analog, thereby also acting as an inhibitor of RNA synthesis (Aimiuwu et al. 2012). Early studies indicated a high response rate for venetoclax when added to all three agents (DiNardo et al. 2019, Wei et al. 2019); however, a recent report that venetoclax with LDAC does not prolong overall survival (OS) compared to LDAC alone has led to more enthusiasm for venetoclax with HMAs (Wei et al. 2020). The recent finding (DiNardo et al. 2020) that venetoclax with azacitidine did result in an OS benefit compared with azacitidine alone will likely enshrine azacitidine as the standard-of-care backbone therapy with venetoclax in newly diagnosed AML patients.

Azacitidine and decitabine can function both as traditional nucleoside analogs and as HMAs. Presumably, incorporation into DNA (and RNA for azacitidine) would yield relatively fast impairment of viability for actively cycling cells. In contrast, antitumor activity as a consequence of epigenetic changes is likely a slower process that occurs with continued treatment over several weeks or months. Arguably, both mechanisms contribute to the overall efficacy observed with venetoclax and azacitidine or decitabine.

Venetoclax-Based Combination Therapies

In the context of combination therapy with HMAs, it is tempting to speculate that HMAs act to prime cells to be more receptive to cell death via BCL-2 inhibition. Indeed, it has been reported that azacitidine increases expression of NOXA, which in turn primes AML cells for venetoclax-mediated apoptosis (Jin et al. 2020), supporting this hypothesis. Furthermore, RNA interference screens identified antiapoptotic BCL-2 family members as azacitidine-sensitizing targets (Bogenberger et al. 2014). However, currently there is no evidence that this occurs in AML patients treated with this regimen.

It is important to consider that venetoclax, with HMAs, may trigger noncanonical BCL-2-related events. For example, a recent study described the uptake and catabolism of amino acids as a key metabolic requirement of primitive AML cells (Jones et al. 2018). Specifically, AML stem/progenitor cells appear to be reliant on the catabolism of amino acids (rather than glucose or fatty acids) as a means to drive OXPHOS. Amino acid metabolism appears to be necessary to maintain OXPHOS through at least two related mechanisms in AML stem cells. First, amino acids are catabolized to synthesize citric acid cycle intermediates (Jones et al. 2018). Second, amino acids including cysteine are required for the synthesis of glutathione, which mediates the activity of electron transport chain II through glutathionylation of succinate dehydrogenase A (Jones et al. 2019, Pollyea et al. 2018). Surprisingly, treatment of AML cells with venetoclax and azacitidine inhibited amino acid metabolism, leading to decreased OXPHOS and leukemia-specific cell death. This observation was demonstrated using primary AML cultures and corroborated in an analysis of serial specimens obtained from AML patients undergoing treatment with venetoclax and azacitidine (Pollyea et al. 2018). These findings support several other studies that suggest venetoclax is acting at least in part through modulation of mitochondrial activities (X. Chen et al. 2019, Nechiporuk et al. 2019, Sharon et al. 2019).

PROPOSED MODELS FOR VENETOCLAX INTERACTION WITH HYPOMETHYLATING AGENTS

In considering how venetoclax may be interacting with HMAs, we note three distinct possibilities with regard to eradication of clinical disease: (*a*) activity of venetoclax as a single agent, (*b*) activity of HMAs as single agents, or (*c*) combinatorial activity of venetoclax with HMAs. Furthermore, we propose that one or more of these scenarios are potentially occurring simultaneously given the results observed in clinical treatment of AML. Indeed, given the significant intrapatient heterogeneity of primary AML tumors (Ding et al. 2012, van Galen et al. 2019), the possibility of multiple overlapping mechanisms seems likely. In addition, we note that AML patients also display substantial interpatient heterogeneity (Cancer Genome Atlas Res. Netw. 2013, Papaemmanuil et al. 2016). Thus, specific mechanisms of tumor eradication may vary from patient to patient. Bringing the considerations noted above together, we postulate there are least three specific scenarios in which the combination of venetoclax with HMAs might demonstrate antileukemic activity (**Figure 1**).

Single-Agent Temporal Model

In the single-agent temporal model, each agent could be acting independently on distinct subpopulations of cells (stem/progenitor subpopulation versus bulk tumor). This hypothesis is supported by temporal observations of tumor reduction. We observed extremely rapid clearance of peripheral blast cells in patients treated with venetoclax plus azacitidine, with major reductions in peripheral tumor burden in as little as 24–48 hours (Pollyea et al. 2018). For a tumor with significant BH3 priming towards BCL-2, this immediate apoptosis may have been primarily due to the single-agent activity of venetoclax. Similarly, the antileukemic activity of single-agent HMAs through their ability to block proliferation of cycling cells would also be expected to be fast. However, in these same patients, an epigenetic effect of HMAs would likely take much longer. As noted above, treatment with these agents typically takes several months. Thus, one could easily envision a two-staged process in which an immediate induction of bulk tumor killing by venetoclax or HMAs is followed by further tumor suppression at later times mediated by the long-term epigenetic effect of HMAs.

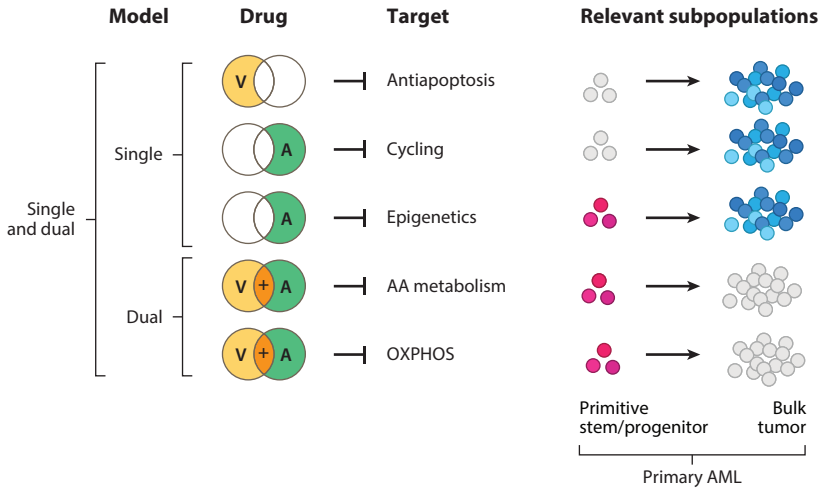

Figure 1

Single- and dual-drug activities for venetoclax-based regimens. Venetoclax or azacitidine can potentially impact multiple cellular mechanisms, as indicated. Furthermore, the relative importance of each mechanism varies as a function of AML subpopulations. Abbreviations: A, azacitidine; AA, amino acid; AML, acute myeloid leukemia; OXPHOS, oxidative phosphorylation; V, venetoclax.

Dual-Agent Model

As described above, preclinical data support the hypothesis that venetoclax and azacitidine act together to enhance the death of primitive AML cell subpopulations via mechanisms that target key metabolic pathways, and we have observed similar events in AML patients treated with venetoclax plus azacitidine (Pollyea et al. 2018). This combined activity may be sufficient to explain tumor eradication in at least some patients. Similarly, HMAs may increase sensitivity to canonical BCL-2-mediated apoptosis via the induction of BH3 priming.

Single- and Dual-Agent Model

Of course, some combination of both the single- and dual-agent models is possible. Particularly appealing is the hypothesis that the rapid killing of bulk tumor cells may be induced via a direct apoptosis induction mechanism (i.e., canonical BCL-2 inhibition), and that more specific targeting of primitive AML stem/progenitor cells is facilitated by the combined action of venetoclax and azacitidine, which targets metabolic properties of primitive cells. We note that all three of the proposed models may have varying degrees of relevance/activity as a function of both intra- and interpatient heterogeneity.

FACTORS THAT CONTRIBUTE TO THERAPY RESPONSE/RESISTANCE

While venetoclax-based regimens are highly active, these treatments are not a panacea. Thus far, clinical findings show that 20–30% of patients are refractory and that the majority of patients who initially achieve remission ultimately relapse. As outlined in **Figure 1**, the intrinsic heterogeneity of primary AML tumors suggests that varying responses to single- versus dual-drug combinations are possible. Moreover, preclinical data strongly support differential activity of venetoclax-based regimens as a function of the following parameters.

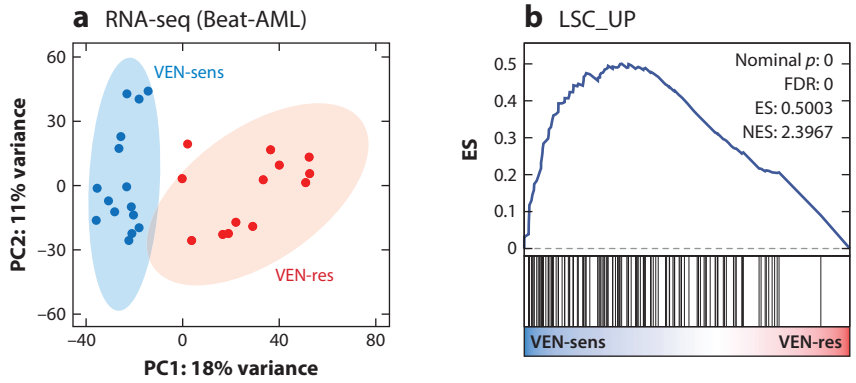

Figure 2

Enrichment of LSC gene expression signatures in venetoclax-sensitive AML. (*a*) Principal components analysis of the RNA-seq data of the selected venetoclax-resistant ($n = 13$) and venetoclax-sensitive ($n = 15$) primary AML specimens from the Beat-AML® clinical trial. (*b*) Gene set enrichment analysis showing enrichment of LSC signatures from Eppert et al. (2011) (LSC_UP). Panel created using data from Tyner et al. (2018). Abbreviations: ES, enrichment score; FDR, false discovery rate; LSC, leukemia stem cell; NES, normalized ES; PC1/2, principal component 1/2; RNA-seq, RNA sequencing; VEN-res, venetoclax-resistant; VEN-sens, venetoclax-sensitive.

Intrapatient Heterogeneity

As reported by Lagadinou et al. (2013), BCL-2 inhibition is preferentially more effective in phenotypically primitive AML cell types than bulk AML cells within the same specimens. Selective targeting of primitive cells was corroborated in subsequent analysis of the Beat-AML® data set (Tyner et al. 2018), where AML stem cell gene expression profiles (Eppert et al. 2011) were strongly correlated with increased sensitivity to venetoclax (**Figure 2**). Furthermore, recent studies have demonstrated that venetoclax plus azacitidine is more active and more durable for phenotypically primitive AML cells, in comparison to more mature monocytic-differentiated cells, within the same patient (Kuusanmaki et al. 2019, Pei et al. 2020). From a mechanistic perspective, drug resistance in cells with a more monocytic phenotype is associated with loss of dependence on BCL-2 and a concomitant increase in reliance on MCL-1. Intriguingly, the apparently intrinsic reliance on MCL-1 in monocytic cells may confer an increase in metabolic flexibility, where multiple forms of catabolism can feed into energy production via OXPHOS. Together, these findings strongly suggest that intrapatient heterogeneity contributes to varying degrees of drug response within a given AML tumor.

Interpatient Heterogeneity

AML can arise from multiple combinations of mutations, and the consequent biology of such tumors displays a range of properties (Cancer Genome Atlas Res. Netw. 2013, J. Chen et al. 2019, de Boer et al. 2018, Papaemmanuil et al. 2016, Shlush et al. 2014). Aside from the well-documented spectrum of myeloid developmental characteristics, AML tumors commonly display heterogeneous phenotypes that can differ quite dramatically among patients. Differing phenotypes are clearly linked to varying degrees of drug responsiveness.

Bringing the considerations outlined above into a model, the schematic illustration in **Figure 3** describes how inter- and intrapatient heterogeneity of AML patients may dictate response to venetoclax plus azacitidine. In this example, patient 1 harbors a phenotypically primitive AML with

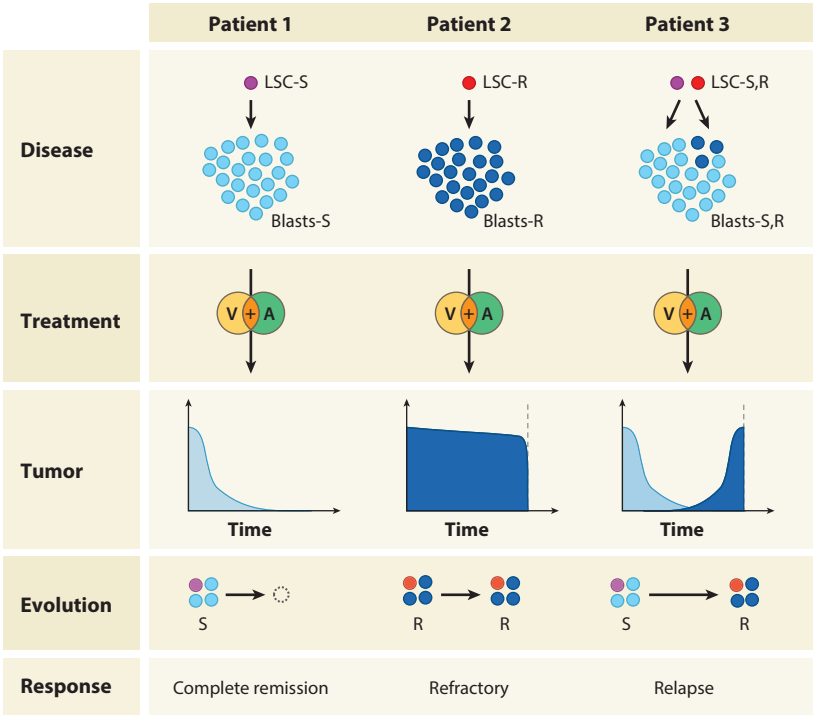

Figure 3

Inter- and intrapatient heterogeneity drives differential responses to venetoclax plus azacitidine therapy. Abbreviations: S, venetoclax-sensitive; R, venetoclax-resistant; A, azacitidine; LSC, leukemia stem cell; V, venetoclax.

high reliance on BCL-2. Patient 2 represents a phenotypically monocytic AML patient or a patient whose underlying mutations (e.g., *RAS*, *TP53*) drive resistance to venetoclax-based therapy (DiNardo et al. 2020, Nechiporuk et al. 2019). Patient 3 represents the most common scenario, in which intrinsic intrapatient heterogeneity is present, and tumor subpopulations have varying degrees of BCL-2 reliance. For this patient, clinical response is evident, but the selective pressure of venetoclax plus azacitidine drives outgrowth of drug-resistant subclones.

Disease Pathogenesis

Clinical findings reported to date show a major difference in the efficacy of venetoclax-based therapies between newly diagnosed and relapsed/refractory AML patients (Aldoss et al. 2018; DiNardo et al. 2018, 2019). These data clearly indicate that the stage of disease pathogenesis is a significant factor. Interestingly, findings to date suggest both genetic and nongenetic mechanisms may play a role in this context. First, data from preclinical and clinical studies show that *TP53* mutations may predispose AML patients to venetoclax resistance (DiNardo et al. 2020, Nechiporuk et al. 2019). Thus, specific genetic features appear to affect therapeutic responses. Second, an initial report has shown changes in metabolic properties of AML cells at relapse. Specifically, as mentioned above, newly diagnosed patients show a reliance on amino acid metabolism in the AML stem/progenitor population. In contrast, relapsed patients display increased metabolic flexibility and can utilize fatty acid oxidation as an alternate pathway to drive OXPHOS (Jones et al. 2018). This property

permits relapsed cells to circumvent induction of cell death in response to venetoclax plus azacitidine treatment. Consequently, various metabolic properties (which may be linked to underlying mutational status) appear to also dictate drug responsiveness. Lastly, from a developmental and biological perspective, it has been reported that the frequency and diversity of AML stem cells are dramatically increased at relapse, further underscoring the iatrogenic nature of chemotherapy and the challenge in creating effective therapies for relapsed/refractory patients (Ho et al. 2016).

IMPROVING USE OF VENETOCLAX-BASED REGIMENS

While venetoclax-based regimens have provided an important new option for AML, particularly in older or less fit patients who cannot tolerate the toxicity of intensive chemotherapy, the approach is generally not curative. Consequently, the focus of many investigators is currently aimed at determining how to use venetoclax as a backbone in building even more effective therapies. Notably, preclinical data suggest that the addition of venetoclax to nearly any agent improves anti-AML activity, a relatively unusual finding in the experimental therapeutics field (Kurtz et al. 2017). However, in order for any such approach to improve clinical outcomes, we posit that the biological features of primary AML tumors should be considered. Such approaches may include the following.

Targeting Monocytic Subtypes

Initial data suggest that AML cells with more monocytic features may be less reliant on BCL-2, and consequently less responsive to venetoclax-based therapies (Kuusanmaki et al. 2019, Pei et al. 2020). Importantly, the majority of primary AML specimens consist of a heterogeneous mixture of tumor cells. As shown in **Figure 4**, two easily distinguishable subpopulations are commonly observed (primitive and monocytic). While the relative abundance of these two populations can vary substantially (as shown for patient 1 versus patient 2), the majority of AML patients display some level of both, similar to patient 3. Moreover, the developmental biology we have reported for patients that relapse following venetoclax plus azacitidine therapy suggests that distinct AML stem cell populations can exist in the primitive and monocytic subpopulations. Consequently, it seems

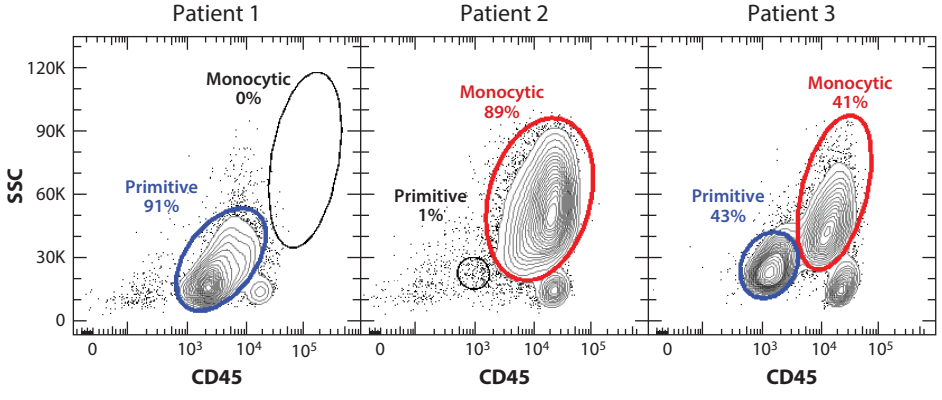

Figure 4

Examples of bulk tumor analysis from three newly diagnosed AML patients. Levels of primitive versus monocytic subpopulations can vary substantially from patient to patient. Figure adapted with permission from Pei et al. (2020), copyright 2020 AACR. Abbreviation: SSC, side scatter.

an attractive option to target monocytic cell types as a means to complement the activity of venetoclax. Potential strategies include use of monocyte-specific immune therapies (antibody-based therapy, CAR T cells, etc.), small molecules that inhibit monocytic-specific signaling pathways, and therapies that target monocytic metabolic dependencies such as MCL1-dependent energy metabolism (Pei et al. 2020).

Targeting Metabolic Flexibility

A characteristic associated with drug resistance in AML is adaptive or compensatory events that alter how energy metabolism is regulated. This has been observed in the context of relapse following either conventional chemotherapy or venetoclax plus azacitidine (Jones et al. 2018, Pei et al. 2020). Consequently, one potential strategy to better suppress the outgrowth of venetoclax-resistant cells would be to augment regimens with agents that target such mechanisms. Data thus far suggest that fatty acid oxidation is a key pathway to consider. At diagnosis, most AML patients appear to rely primarily on catabolism of amino acids to drive OXPHOS. However, at relapse, patients frequently acquire the ability to catabolize fatty acids as an alternate source of metabolic fuel (Jones et al. 2018). Based on this observation, preclinical studies have tested use of SSO (sorbitan sesquioleate), an inhibitor of CD36, which is a receptor that mediates uptake of fatty acids. As expected, SSO alone has little effect on AML stem/progenitor cell populations, but the addition of SSO to venetoclax plus azacitidine treatment restores drug sensitivity to AML cells derived from relapsed patients (Jones et al. 2018). Furthermore, recent studies have shown that use of the fatty acid oxidation inhibitor etomoxir (which inhibits CPT1a) can resensitize relapsed AML cells to venetoclax plus azacitidine treatment (Stevens et al. 2020). Similarly, researchers of another study utilized the BCL-2 inhibitor ABT-737 in combination with etomoxir and observed enhanced targeting of AML cells (Samudio et al. 2010). Hence, the concept of augmented metabolic targeting has been demonstrated in laboratory models and may represent a viable clinical strategy.

Clinical Strategies and Considerations

Due to the prominence of mutation-targeting therapies in AML, one obvious strategy for the development of venetoclax is to pair it with inhibitors of FLT3, IDH (isocitrate dehydrogenase), and others that are in clinical development. However, patients bearing these types of mutations often respond well to current venetoclax-based therapies, so determining when/how to most effectively add a targeted agent to a venetoclax regimen continues to be challenging. Similarly, because of the long history of intensive chemotherapy use in AML, there is also an instinct to pair venetoclax with this traditional backbone therapy. These studies will need to be well designed to ascertain the ways in which the additional therapies are adding benefit, perhaps with respect to response duration or OS, without increasing the toxicity to an unmanageable degree.

It is possible that additional therapies added on to venetoclax-based regimens will not improve outcomes in any meaningful way. If that is the case, there are two important directions the field must take. The first direction would be to expand the use of the current regimen to a wider swath of the AML patient population. The current FDA label for venetoclax includes newly diagnosed patients 75 years or older or those with comorbidities that preclude the use of intensive induction chemotherapy. However, the venetoclax clinical trials included patients 65 and older and did not show significantly different outcomes for younger patients compared with older patients (DiNardo et al. 2019, 2020). The continued assumption that patients who are fit for intensive induction chemotherapy should receive it, regardless of disease biology, quality of life considerations, or the availability of potentially safer alternatives, is outdated. Based on currently available

data, venetoclax-based regimens should be considered in younger age subsets and in those who may be fit for intensive induction chemotherapy. The second direction would be to make considerable efforts to identify those disease features that are associated with suboptimal responses to venetoclax-based regimens and offer different treatments for these patients.

In addition, as an LSC-targeting therapy that is well-tolerated, venetoclax could be used as a maintenance therapy for patients in remission (with or without allogeneic bone marrow transplantation), or potentially to eradicate measurable residual disease. Finally, strategies that could more successfully incorporate venetoclax into regimens for relapsed AML patients would be of very high value.

CONCLUSIONS

The development and approval of venetoclax represent an important change in the treatment landscape of AML. This effective and well-tolerated therapy has helped to energize the AML therapy field and provides an alternate approach that is not based on mutation-specific targeting. While the original rationale for use of BCL-2 inhibitors like venetoclax came from an extensive base of preclinical data, the mechanisms underlying the clinical success thus far remain somewhat unclear. Formally, some combination of single- and dual-agent activities may contribute to overall clinical responses. Furthermore, varying mechanisms may pertain to distinct AML subpopulations that reside within individual patients. Ongoing efforts to characterize drug resistance and the pathogenesis of relapse will likely yield additional insights. Perhaps most importantly, the promising initial results with venetoclax have spurred a huge number of additional studies that seek to refine and improve the use of BCL-2 inhibition as a backbone for AML therapy. Thus, enhanced regimens and better outcomes for AML patients seem likely in the near future.

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