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Review article

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Apoptosis-targeting BH3 mimetics: transforming treatment for patients with acute myeloid leukaemia

Antonino Glaviano[1], Ellen Weisberg[2,3], Hiu Y. Lam[4,5], Donavan J. J. Tan[4,5,6], Andrew J. Innes[7,8], Yubin Ge[9], Catherine E. Lai[10], Wendy Stock[11], Christina Glytsou[12,13], Linda Smit[14], Tatsushi Yoshida[15], Tian Y. Zhang[16], Vanessa E. Kennedy[17], B. Douglas Smith[18], Thomas Mercher[19], Stéphane de Botton[20], Patrizia Diana[1], Marina Konopleva[21], Michael J. Mauro[22], James D. Griffin[2,3], Courtney D. DiNardo[23] ✉ & Alan P. Kumar[4,5] ✉

Abstract

Acute myeloid leukaemia (AML) remains a challenging haematological malignancy, with most patients developing resistance to standard-of-care (SOC) treatments. This resistance is often attributed to the overexpression of anti-apoptotic BCL-2 family proteins, which regulate the intrinsic apoptotic pathway by inhibiting pro-apoptotic effector proteins such as BAX and BAK. AML cells exploit this imbalance to evade apoptosis and sustain survival, necessitating the development of novel therapeutic strategies. BH3 mimetics are small-molecule inhibitors targeting the pro-survival BCL-2 family proteins and have emerged as promising agents in patients with AML who are unable to receive high-intensity induction chemotherapy. Co-treatment with the BCL-2-specific inhibitor venetoclax and various SOC therapies has been proven effective, with several combinations now approved by the US Food and Drug Administration for adults with AML who are ≥75 years of age and/or are ineligible for intensive induction chemotherapy, on the basis of improved response rates and survival outcomes compared with the previous SOC. In this Review, we highlight the transformative potential of BH3 mimetics in AML therapy, including ongoing studies investigating novel combination regimens and efforts to further refine treatment strategies, with the ultimate goal of improving outcomes for patients with AML.

Sections

A full list of affiliations appears at the end of the paper. ✉e-mail: cdinardo@mdanderson.org; apkumar@nus.edu.sg

Key points

- Resistance to standard treatments for acute myeloid leukaemia (AML) is often attributed to the overexpression of anti-apoptotic BCL-2 family proteins, which inhibit apoptosis and sustain cancer cell survival, posing a major therapeutic challenge.
- BH3 mimetics, which target pro-survival BCL-2 proteins, have demonstrated substantial anticancer activity, effectively overcoming resistance in patients with AML and improving therapeutic outcomes, especially when combined with hypomethylating agents or low-dose cytarabine.
- Venetoclax, a potent BCL-2-specific BH3 mimetic, has revolutionized the management of patients with AML owing to improved efficacy when combined with standard therapies, leading to US Food and Drug Administration approval of this agent for specific groups of patients with AML.
- Innovative sequential or combinatorial approaches targeting BCL-xL and MCL-1 dependencies will be crucial to addressing the current challenges, including thrombocytopenia and the limited ability to target resistant BCL-2 family proteins.
- Techniques such as BH3 profiling, mitochondrial profiling and gene and/or protein expression profiling are transforming the management of patients with AML by enabling precise, personalized therapies and enhancing the optimization of therapeutic regimens, leading to improved patient outcomes.
- The future treatment of patients with AML lies in developing novel drug combinations, optimizing BH3 mimetic dosages, mitigating adverse effects and exploring dual-action therapies.

Introduction

Homeostasis relies on a balance between cell division and cell death[1]. Among the different types of cell death (including autophagy, pyroptosis, necroptosis and ferroptosis)[2], apoptosis is the most widely investigated and understood form of programmed cell death[3,4]. Caspase-dependent apoptosis is also the most commonly occurring regulated cell death pathway in eukaryotic cells[5], having critical roles in development, homeostasis[6] and other important processses, including immune responses[7]. Apoptotic cells undergo a series of morphological changes such as membrane blebbing and nucleus shrinkage, as well as loss of mitochondrial function, cleavage of intracellular structures and DNA fragmentation[8]. Caspase-dependent apoptosis is a tightly regulated process occurring via two distinct signalling pathways with specific initiation steps: (1) the extrinsic apoptotic signalling, or death receptor signalling pathway, which is activated via binding of death receptors at the cell membrane; and (2) the intrinsic apoptotic signalling, or mitochondrial apoptotic pathway, directed by the B cell lymphoma-2 (BCL-2) family of proteins[9–14]. These two signalling axes interconnect and culminate in activation of the executioner caspases before finally leading to cell death. Evasion of apoptosis is a notorious hallmark of cancer[15,16]. Proteins of the BCL-2 family have a crucial role in regulating apoptosis[17,18], and aberrant overexpression of pro-survival BCL-2 family members is associated with haematological malignancies such as acute myeloid leukaemia (AML). This dependence has led to earnest efforts to understand whether these proteins can be targeted to eradicate cancer cells. The BH3 mimetic venetoclax is thus far the only US Food and Drug Administration (FDA)-approved BCL-2 inhibitor, initially for patients with chronic lymphocytic leukaemia (CLL)[19,20] and later for those with AML[21]. Beyond the FDA-approved combinations of venetoclax with hypomethylating agents (HMAs) or low-dose cytarabine (LDAC), other venetoclax-based regimens are currently under clinical investigation. Meanwhile, a wide variety of other BH3 mimetics have emerged as promising candidates.

In this Review, we highlight the therapeutic potential of inhibiting pro-survival BCL-2 family members in adult patients with AML and explore the promise of BH3 mimetic-based therapeutic regimens. We also aim to provide a focused and up-to-date overview of the current landscape and future directions of BH3 mimetics in the clinical management of AML.

Regulation of apoptosis by BCL-2 family proteins

The BCL-2 family proteins are defined by the presence of BCL-2 homology (BH) domains and can be divided into groups based on their structure and function[22,23]. The pro-apoptotic group includes proteins with one to three BH domains. This group comprises two distinct categories: (1) multidomain proteins, named BCL-2 homologous antagonist killer (BAK) and BCL-2-associated X protein (BAX)[18], which consist of three BH domains (BH1–BH3) and a C-terminal transmembrane domain (TMD) warranting associations with intracellular membranes including the mitochondrial outer membrane (MOM), the endoplasmic reticulum (ER) and nuclear membranes[24–26]; and (2) BH3-only proteins, which include only one BH domain (BH3), such as BCL-2 associated death promoter (BAD), BCL-2-interacting mediator of cell death (BIM), BH3-interacting domain death agonist (BID), BCL-2-interacting killer (BIK), p53-upregulated modulator of apoptosis (PUMA), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1 or NOXA), BCL-2-modifying factor (BMF) and Harakiri (HRK)[18]. Activation of this second group depends on several heterogeneous stress signals, including antigen receptor signalling, cytokine deprivation, DNA damage, anoikis, activation of oncogenes, chemotherapy and exposure to ultraviolet light and/or γ-rays[27]. The anti-apoptotic group includes proteins containing three to four BH domains and comprises the four-domain (BH1–BH4) and C-terminal TMD-containing proteins BCL-2, B cell lymphoma-extra large (BCL-xL), B cell lymphoma-w (BCL-w) and BCL-2-related gene expressed in fetal liver-1 (BFL-1), as well as the three domain-containing (BH1–BH3 domains) and C-terminal TMD-containing protein myeloid cell leukaemia-1 (MCL-1)[23] (Supplementary Information).

Apoptosis is initiated through two well-characterized pathways: the extrinsic and intrinsic pathways[9–14] (Fig. 1). In the intrinsic apoptotic pathway, the BCL-2 family proteins have been proposed to regulate BAX/BAK activation via the direct or indirect activation model, or a combination of both[28,29] (Fig. 2). In the direct activation model, the BH3-only proteins are classified into two groups: (1) activators (such as BIM, BID and PUMA), which can directly activate BAX/BAK but are sequestered by pro-survival BCL-2 family proteins (such as MCL-1 and BCL-2); and (2) sensitizers (such as BAD, NOXA and HRK), which suppress the pro-survival proteins and, in turn, release the activators to activate BAX/BAK. In the indirect activation model, BAX/BAK are constitutively active but are sequestered by the pro-survival proteins; in response to a death stimulus, BH3-only proteins (such as BAD, NOXA and HRK)

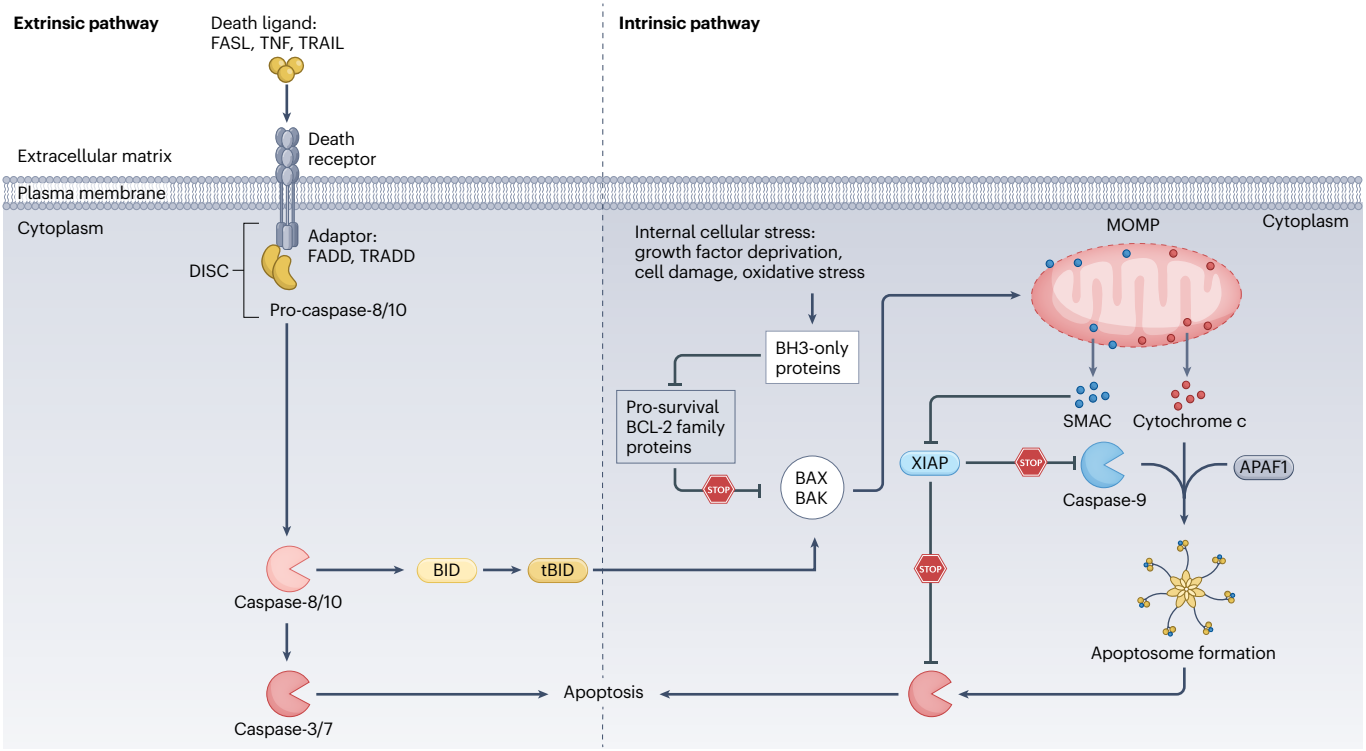

Fig. 1 | The extrinsic and intrinsic apoptotic pathways. The extrinsic apoptotic pathway is initiated by the binding of cell surface death receptors with death ligands, whereas the intrinsic pathway is activated by internal cellular stress. On induction of apoptosis via the extrinsic pathway, the BH3-only protein BH3-interacting domain death agonist (BID) is cleaved by caspase-8 and caspase-10 to give rise to truncated BH3-interacting domain death agonist (tBID). tBID facilitates BAX/BAK activation within the intrinsic apoptotic pathway, therefore serving as a critical link between the two apoptotic signalling cascades. On activation, BAX/BAK undergo oligomerization, leading to mitochondrial outer membrane permeabilization (MOMP). The efflux of mitochondrial proteins as a result of MOMP, namely cytochrome c and second mitochondria-derived activator of caspases (SMAC), promotes caspase activation, thereby facilitating the execution of apoptosis. APAF1, apoptotic protease-activating factor 1; DISC, death-inducing signalling complex; FADD, FAS-associated death domain protein; FASL, Fas ligand; TNF, tumour necrosis factor; TRADD, tumour necrosis factor receptor type 1-associated death domain protein; TRAIL, TNF-related apoptosis-inducing ligand; XIAP, X-linked inhibitor of apoptosis protein.

inhibit the pro-survival proteins and release the sequestered BAX/BAK. However, whether the BH3-only proteins can directly induce BAX/BAK activation remains a subject of ongoing debate. One study provided evidence demonstrating that BAX/BAK activation can occur in the absence of all known BH3-only proteins[30]. This study outlined a membrane (lipid)-mediated spontaneous model in which the MOM activates BAX/BAK. In this model, the BH3-only proteins function only to inhibit the pro-survival BCL-2 family members; subsequently, BAX/BAK associate with the MOM via free diffusion. This membrane association then induces the spontaneous activation and oligomerization of BAX/BAK.

Classification of AML

AML is a heterogeneous disease in terms of morphology, the underlying molecular genetic/cytogenetic abnormalities, immunophenotypes, response to treatment and patient outcomes. AML is characterized by an increase in immature myeloid blasts in the bone marrow, extramedullary tissues and blood. The growth of these blast cells outpaces that of haematopoiesis, resulting in vulnerability to infection, bleeding, fever and anaemia. Over the past years, a better understanding of AML, accompanied by remarkable improvements in measurement technology, resulting in a wide array of instruments and techniques used to analyse and quantify biological processes, biochemical reactions and biomolecules, and the approval of novel therapies have revamped the diagnosis, prognosis and therapeutic landscape[31].

AML is classified according to the World Health Organization (WHO), International Consensus Classification (ICC) and European Leukaemia Network (ELN) criteria[32–34]. The WHO 2022 classification divides AML into AML-defining genetic abnormalities (DGAs) and AML defined by differentiation[33,35]. This classification also broadens the number of different *KMT2A* and *MECOM* rearrangements[33]. AML with myelodysplasia-related changes (AML-MR) now incorporates somatic mutations (such as those in *ASXL1*, *EZH2* and *SF3B1*), replacing the previous morphological dysplasia-based criteria[33]. The latest WHO classification also removed the blast cut-off for all AML-DGAs apart from AML with BCR–ABL1, AML with *CEBPA* mutations and AML-MR, while the 20% blast cut-off is still in place for AML defined by differentiation in order to discriminate from myelodysplastic syndrome (MDS)[33,35]. The ICC builds on the WHO classification but also introduces several notable distinctions[36]. The blast threshold for AML-DGAs is lowered to 10% rather than entirely removed, whereas MDS/AML is introduced as a new category for patients with 10–19% blasts lacking in DGAs[36]. Unlike that provided by the WHO, the ICC classification recognizes

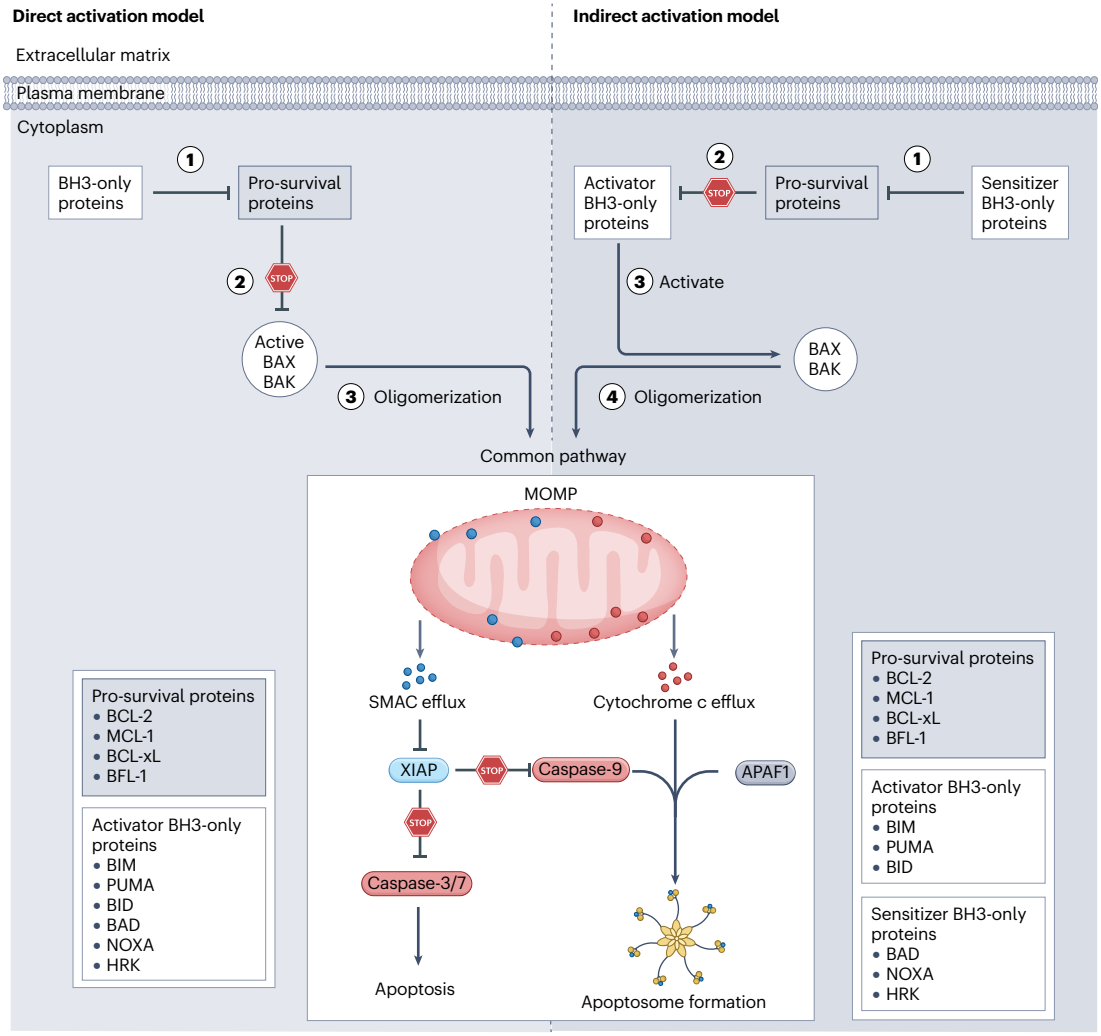

Fig. 2 | BAX/BAK activation. The activation of BAX/BAK is proposed to follow two distinct models: the direct and indirect activation models. On BAX/BAK activation and oligomerization, both models converge on a common pathway in which mitochondrial outer membrane permeabilization (MOMP) is induced and pores are formed on the mitochondrial outer membrane (MOM). These changes result in cytochrome c and second mitochondria-derived activator of caspases (SMAC) efflux, triggering caspase activation and ultimately inducing apoptosis. APAF1, apoptotic protease-activating factor 1; XIAP, X-linked inhibitor of apoptosis protein.

AML with mutated *TP53* as a distinct entity[36]. The latest ICC classification narrows the criteria for *KMT2A* and *MECOM* rearrangements by specifying partner genes[36] and separates AML-MR into two subgroups: myelodysplasia-related mutations and myelodysplasia-related cytogenetic abnormalities[36]. Notably, single-gene mutations or fusions take precedence over myelodysplasia-related mutations and cytogenetic abnormalities when classifying AML[36]. The ELN 2022 guidelines, meanwhile, focus on risk stratification, grouping AML into favourable, intermediate and adverse risk categories based on the presence of certain molecular markers[37]. Within the favourable *CEBPA*-mutated risk group, the ELN 2022 guidelines only include bZIP in-frame mutated *CEBPA*, despite the 2017 iteration of these guidelines also considering biallelic *CEBPA* mutations[37]. The ELN 2022 guidelines also add other *MECOM*-rearrangements and myelodysplasia-related-defining mutations to the category of adverse risk-defining genetic abnormalities[37]. These systems integrate molecular, morphological and cytogenetic insights to better classify the heterogeneity of AML and facilitate a more personalized treatment approach.

Risk stratification in AML is largely based on the findings of conventional karyotyping, whereas the targeted investigation of specific recurrent genetic anomalies involves fluorescence in situ hybridization analysis and PCR with reverse transcription (RT-PCR)[31,38,39]. AML can be further stratified using next-generation sequencing with molecular profiling based on the presence or absence of mutations associated with its onset, including alterations in genes encoding tumour suppressors, master haematopoietic transcription factors, epigenetic regulators and cell signalling pathways[40,41]. Upstream of the intrinsic apoptotic pathway, genomic inactivation of *TP53* is present in approximately 10–15% of AMLs[42]. Notably, alterations in *TP53* in AML are related to definitive biological and/or genomic characteristics, including

augmented genomic instability and complex karyotypes, which are associated with an inferior prognosis[43]. Consequently, patients with AML harbouring *TP53* mutations and/or chromosomal aneuploidy are identified as separate prognostic subgroups[44]. Likewise, *TP53* alterations are infrequent in AML without a complex karyotype, although inactivation of p53 is necessary for transformation[45]. Genes involved in apoptosis, such as *CDKN2A* and *ATM*, are rarely altered in AML. Similarly, *MDM2* amplifications are also less common in AML, although overexpression is associated with wild-type *TP53* status and loss of p21WAF1/CIP1 expression, supporting the implication that such alterations enable evasion of apoptosis[42]. Moreover, genes encoding proteins located downstream of the intrinsic apoptotic pathway are also rarely altered in AML. This lack of alterations might reflect deregulated expression induced by alterations in genes encoding epigenetic regulators, leading to deregulated apoptotic signalling pathways without relevant genetic alterations as seen in AML[45]. BCL-2 is overexpressed in 71% of patients with AML (varying from 34% to 87% depending on specific characteristics) and is associated with resistance to chemotherapy and/or targeted therapies[46,47]. MCL-1 and BCL-xL overexpression also have important roles in the pathogenesis of AML[48]. The cytogenetic and molecular alterations detected during the diagnostic process have implications for prognosis and, importantly, have been used to subtype patients with AML into favourable, intermediate or adverse prognostic risk categories[37,49]. Importantly, the effectiveness of targeted therapies depends on the presence of specific genomic lesions as well as the fitness and age of the patients.

Overexpression of pro-survival BCL-2 family proteins

BCL-2 is expressed by $CD34^+$ AML progenitor cells and promyelocytes, but not by their corresponding non-malignant cells; thus, first-line induction chemotherapy leads to the selective survival of leukaemic $CD34^+$ cells with high levels of BCL-2 expression[47]. Accordingly, the presence of BCL-2 overexpression in $CD34^+$ AML cells is associated with an inferior prognosis and resistance to chemotherapy[50]. BCL-2 expression is also required for disease maintenance in mouse models of leukaemia[51,52]. BCL-2 expression is upregulated both in patients with newly diagnosed AML (84%) and in those with relapsed AML (95%). High levels of BCL-2 expression are correlated with an inferior prognosis, with lower complete remission (CR) rates, shorter survival durations and resistance to chemotherapy[53–55]. Patients with high levels of BCL-2 expression are also reported to have an increased number of peripheral blasts, and are associated with CD34 and CD117 positivity[46,56,57]. Among French–American–British (FAB) subtypes, BCL2 overexpression is associated with FAB-M0/M1, whereas a lack of expression is associated with FAB-M5 (ref. 46). Several BCL-2 family proteins are implicated in the genesis of AML[58]. On induction chemotherapy, BCL-2, BCL-xL and BAD proteins are upregulated in most AML stem and progenitor cell populations, compared with non-malignant haematopoietic stem and progenitor cells[59,60]. The MCL-1 protein, which is often highly expressed in patients with AML, is associated with higher relapse rates[61] and the survival of cancer stem cells, particularly those harbouring internal tandem duplication (ITD) of *FLT3* (encoding FMS-like tyrosine kinase 3)[62]. In line with this observation, *MCL-1* depletion results in the death of AML cells and extends the survival of mouse models of AML, whereas overexpression of BCL-2 or MCL-1 prevents AML cell death[61,63,64]. Data from several studies highlight that AML cells are often addicted to either *BCL-2*, *MCL-1* or both genes, and that this characteristic depends on the genomic landscape of the disease in each patient at the time of diagnosis[63,65].

An improved understanding of intrinsic apoptosis has led to the development of novel small molecules (protein–protein interaction inhibitors) that mimic the BH3 domain found in pro-apoptotic BCL-2 family proteins. These BH3 mimetics are designed to mimic the action of pro-apoptotic BH3-only proteins (such as BAD or BID) by binding with the BH3-binding domains of anti-apoptotic proteins (such as BCL-2) with consequent displacement of native BH3-only proteins and activation of apoptosis[60].

BH3 mimetics

Various different methodologies have been implemented in an attempt to reveal the anti-apoptotic dependencies of haematological malignancies, contributing to the development of BH3 mimetics[24,66]. This new class of small-molecule antagonists of the anti-apoptotic BCL-2 proteins mimics the action of BH3-only proteins. Biochemically, BH3 mimetics exert their inhibitory functions by binding to the hydrophobic clefts of anti-apoptotic BCL-2 proteins at the BH3-binding groove, leading to activation of intrinsic apoptosis[67]. The small molecule ABT-737 is a BH3 mimetic designed to inhibit BCL-2, BCL-w and BCL-xL[68]. Owing to insufficient oral bioavailability of ABT-737, an orally bioavailable inhibitor targeting the same group of pro-survival BCL-2 family proteins, navitoclax, was subsequently developed[69]. This compound has shown promising activity as monotherapy in patients with relapsed and/or refractory CLL with a partial response rate of 35%, albeit with grade 3–4 thrombocytopenia in 28% of patients, reflecting the essential role of BCL-xL in platelet survival[70]. Preclinical data also demonstrate that BCL-xL inhibition can induce transient thrombocytopathy, potentially compromising the haemostatic functions of platelets[71]. These clinically serious adverse events related to BCL-xL inhibition have since been resolved by the development of the highly BCL-2-specific inhibitor venetoclax, with a reduced risk of thrombocytopenia[72,73]. The FDA approved venetoclax for patients with 17p-deleted CLL in 2016 (ref. 19), in combination with rituximab in patients with CLL or small lymphocytic lymphoma (SLL) with or without 17p deletions who had received at least one prior line of therapy in 2018 (ref. 74), in combination with obinutuzumab for patients with previously untreated CLL/SLL in 2019 (ref. 20), and in combination with HMAs or LDAC for adults with newly diagnosed AML who either are ≥75 years of age or have comorbidities precluding intensive induction chemotherapy in 2020 (ref. 21). Nonetheless, resistance to venetoclax has emerged as a near-universal phenomenon in patients with AML. Approximately 30% of patients fail to respond to venetoclax-based regimens, and almost all responders will eventually have disease relapse[75]. Even if BCL-2 is not the principal pro-survival BCL-2 family member in AML, alternative BH3 mimetics might be considered for future clinical studies (Supplementary Information). BH3 mimetics are also being investigated in preclinical studies involving models of various advanced-stage solid tumours (Supplementary Information).

Venetoclax for the treatment of AML

Anticancer activity of venetoclax. AML blasts are more sensitive to BH3 mimetics than non-malignant myeloid blasts or other tissues owing to an altered balance between pro-death and pro-survival proteins. AML cells typically express higher levels of endogenous BH3-only proteins, which are counterbalanced by the expression of pro-survival proteins that neutralize the apoptotic signals arising from oncogene activation, priming them for apoptosis in response to venetoclax.[76] Conversely, non-malignant tissues have a decreased level of BH3-only protein priming and, thus, greater tolerance of BH3 mimetics[77].

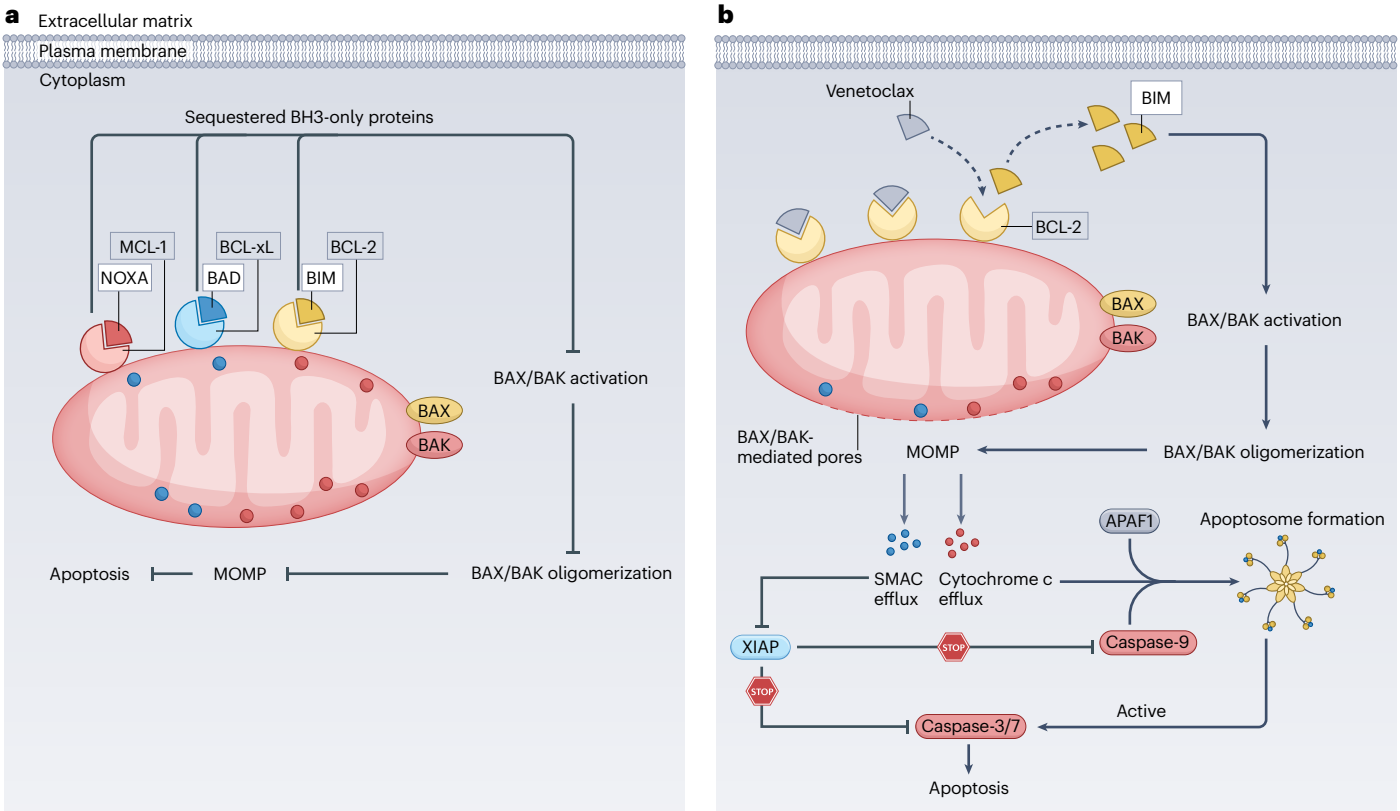

Fig. 3 | Mechanisms of venetoclax-induced apoptosis. Venetoclax induces the release of pro-apoptotic BIM sequestered by the anti-apoptotic BCL-2 protein, leading to apoptosis. **a**, In cancer cells primed for death, pro-apoptotic BH3-only proteins (such as NOXA, BIM and BAD) bind to and are sequestered by anti-apoptotic BCL-2 family proteins (such as MCL-1, BCL-2 and BCL-xL). This inhibits the activation of BAX/BAK, thereby suppressing apoptosis. **b**, Venetoclax triggers apoptosis by displacing BIM from BCL-2, thus enabling BAX/BAK oligomerization, mitochondrial outer membrane permeabilization (MOMP), efflux of cytochrome c and second mitochondria-derived activator of caspases (SMAC) and consequent caspase activation. APAF1, apoptotic protease-activating factor 1; XIAP, X-linked inhibitor of apoptosis protein.

Venetoclax-mediated release of BCL-2 from sequestration by BIM and resulting in apoptosis is thought to be the dominant mechanism of action[78] (Fig. 3). Unlike chemotherapy, venetoclax induces cell death both in dividing cells and in senescent cancer cells. This observation is supported by the ability of venetoclax to induce deep molecular remission, as indicated by data from minimal residual disease (MRD) evaluations in patients with AML[79]. However, venetoclax-induced apoptosis of leukaemic cells can result in clinically serious adverse effects such as tumour lysis syndrome, particularly in patients with a high disease burden[80]. Despite the potent antitumour activity of venetoclax in *TP53*-deleted and/or *TP53*-mutated cancer cells, clinical data indicate that p53-mediated tumour suppressor function is necessary for sustained responses to venetoclax in patients across various leukaemias[81]. Accordingly, regimens combining venetoclax with HMAs are less effective in patients with *TP53*-mutated AML compared with those with *TP53*-wild-type disease[82].

Non-canonical effects of venetoclax. Venetoclax has also demonstrated non-canonical anticancer activities. Indeed, venetoclax-mediated BCL-2 inhibition can directly reduce the extent of oxidative phosphorylation, especially in leukaemia stem cells (LSCs), resulting in their selective eradication[83]. In addition, co-inhibition of BCL-2 and mitochondrial complex I synergistically induces apoptosis and determines the extent of activity against AML cells reliant on this pathway as the primary source of energy. Furthermore, resistance to the mitochondrial complex I inhibitor IACS-010759 often involves retention of cytochrome c in mitochondria, an effect that can be abolished by venetoclax, leading to caspase-dependent apoptosis[84]. Interestingly, venetoclax-mediated metabolic reprogramming can occur independent of BCL-2 expression[85]. These off-target metabolic alterations can potentially regulate the cytotoxicity of venetoclax and might result in unexpected drug interactions[86]. High levels of reactive oxygen species (ROS) can be toxic, although intermediate levels can act as a source of signalling messengers capable of supporting T cell activation and differentiation. Accordingly, venetoclax can also augment the cytotoxicity of T cells by promoting the overproduction of ROS, which are generated through the suppression of the respiratory chain supercomplexes. Indeed, therapeutic concentrations of venetoclax are able to enhance T cell effector function without a reduction in viability. Ex vivo T cells from patients receiving venetoclax also have increased levels of ROS. Exposure to ROS augments the localization of the transcription factor nuclear factor of activated T cells, thus promoting the expression of genes correlated with T cell activation and potentially explaining the role of increased ROS in augmented T cell activity[86,87].

The clinical development of venetoclax

Considerable progress has been made in the treatment of patients with AML over the past decade[88]. Preclinical studies testing venetoclax alone and in combination with various other therapies have been carried out since 1993, ultimately leading to successful large international phase III trials that provided improvements in patient care. These efforts have spurred further preclinical research into combinations of BH3 mimetics with additional standard-of-care (SOC) therapies and successive promising trials[24,89–91] (Fig. 4). Venetoclax has demonstrated the ability to kill target cells in various mouse xenograft models of AML, and especially in xenografts generated using AML cells harbouring *MLL* fusions, a large and diverse group of leukaemia drivers arising from the ample molecular heterogeneity of C-terminal fusion partners of *MLL*[92], and in acute promyelocytic leukaemia cell lines[93]. Notably, venetoclax is also particularly active against AML cells and xenografts harbouring isocitrate dehydrogenase 1 and 2 (*IDH1*/*IDH2*) mutations[94]. Accordingly, in a phase II trial testing venetoclax in patients with relapsed and/or refractory AML, patients without *IDH1/IDH2* mutations receiving venetoclax had an overall response rate (ORR) of 19%, with 4 of 12 patients with *IDH1/IDH2*-mutant disease having a CR[95]. Venetoclax is also particularly effective in patients with AML harbouring *NPM1*, *TET2* and *RUNX1* mutations[96]. Conversely, patients with *TP53*, *FLT3*, *RAS* or *PTPN11* mutations have decreased sensitivity to venetoclax-based regimens[97]. Patients receiving the combination of venetoclax plus cytarabine and/or idarubicin have enhanced BCL-2 expression in the $CD34^+$ compartment[47,93].

Most patients with AML treated with venetoclax will ultimately experience disease relapse; therefore, developing and evaluating novel combination strategies is essential. Indeed, novel venetoclax-based therapies specifically for patients with relapsed and/or refractory AML following venetoclax plus an HMA remain scarce[98]. Current preclinical studies are investigating novel approaches that might overcome the diminished sensitivity of AML cells to venetoclax, such as regimens involving MCL-1, FLT3, MEK1/2 and mitochondrial complex inhibitors[97]. Further research into BH3-mimetic therapies for patients with AML is uncovering innovative treatment approaches with the potential to improve efficacy and overcome resistance. Given that MCL-1 is a vital survival protein in AML cells with resistance to BCL-2 inhibition, MCL-1 inhibitors are emerging as a key strategy to counteract venetoclax resistance[78,99,100]. Other preclinical studies are attempting to develop novel drug delivery systems, such as nanoparticle-based drug delivery, which aims to improve the effectiveness of BH3-mimetics[99]. Among the most noteworthy developments is DT2216, a BCL-xL-specific degrader developed using proteolysis-targeting chimaera (PROTAC) technology. By selectively degrading BCL-xL, DT2216 induces apoptosis in AML cells and particularly those reliant on this anti-apoptotic protein, demonstrating durable antileukaemic effects in most models of post-myeloproliferative neoplasm AML[101]. These innovations, along with novel combination regimens, hold immense potential to overcome the limitations of current therapies and offer the potential for more effective, personalized treatments for patients with AML. These innovations have been described in detail elsewhere[101].

Mechanisms of resistance to venetoclax

Despite high initial response rates among patients with AML receiving venetoclax-based combination therapies, long-term follow-up data indicate that most patients will have disease relapse[102]. Several mechanisms can contribute to resistance to venetoclax in patients with AML (Fig. 5).

Upregulation of non-BCL-2 pro-survival proteins. AML cells can become resistant to the pro-apoptotic effects of venetoclax by upregulating the expression of non-BCL-2 anti-apoptotic proteins (such as MCL-1, BCL-xL and BFL-1), which maintain binding to BIM proteins, thereby contributing to resistance to venetoclax[78,103] (Fig. 6). AML cell lines that acquire resistance to venetoclax typically have upregulation of MCL-1 and/or BCL-xL with reduced dependency on BCL-2[104,105]. In a phase II open-label, single-arm, multicentre trial patients with MCL-1 or BCL-xL-dependent AML with disease relapse on at least one previous line of therapy had less-durable responses to venetoclax[95]. In line with this observation, there is growing research interest in the development of MCL-1 inhibitors, which have shown synergy with BCL-2 inhibitors in venetoclax-resistant AML cell lines and xenograft models[105,106]. Some of these agents have also been tested in clinical trials[100,107,108].

Conversely, the clinical development of the BCL-2/BCL-xL/BCL-w inhibitor navitoclax has been hampered by on-target thrombocytopenia owing to the essential role of BCL-xL in platelet survival[70]. A transcriptomic analysis of samples from patients with AML revealed differential expression of *BFL-1* in venetoclax-resistant cells[109,110]. Interestingly, data from a separate study demonstrate that upregulation of *BFL-1* expression is associated with a larger area under the curve (AUC)[111] of venetoclax and reduced apoptosis after treatment with venetoclax either with or without cytarabine and azacitidine. Notably, *BFL-1* knockdown decreased the extent of cell growth and restored apoptosis in the venetoclax-resistant AML cells, without substantially affecting the $CD34^+$ haematopoietic stem and progenitor cell populations. Thus, there is potential for synergy between venetoclax and BFL-1 inhibitors, particularly in selected subgroups of patients with AML[99,109]. Future studies testing venetoclax alongside other novel apoptosis-targeting therapies, particularly MCL-1 and BFL-1 inhibitors, hold the promise to improve response durations and delay the onset of acquired resistance in patients with AML[99,112].

Activating KRAS and PTPN11 mutations. Somatic mutations in activating kinases such as *KRAS* or *PTPN11* can also confer resistance to venetoclax in patients with AML (Fig. 7). An analysis of samples from the Beat Acute Myeloid Leukaemia (BEAT) AML database[109] estimating the AUC[111] of venetoclax based on in vitro analyses along with genomic data, reported higher AUCs in samples harbouring *KRAS* or *PTPN11* alterations. In the same study, RT-PCR and immunoblot analysis revealed lower levels of BAX and BCL-2 expression, with upregulation of BFL-1 and MCL-1 in cells harbouring $KRAS^{G12D}$ and augmented BCL-xL and MCL-1 levels in those harbouring $PTPN11^{A72D}$. Furthermore, treatment with the MCL-1 inhibitor AZD5991 reduced the viability of cells expressing $KRAS^{G12D}$. Nevertheless, neither BCL-2 inhibition nor concurrent suppression of BCL-2, BCL-xL and BCL-w by navitoclax and ABT737 had the same effects. These results demonstrated that MCL-1 mediated resistance to venetoclax is dependent on the presence of *KRAS* mutations. Exposure to the MCL-1 inhibitor AZD5991 also suppressed *PTPN*-mutant cells, whereas only partial responses were observed with the BCL-2/BCL-xL dual inhibitors navitoclax and ABT-737 in cells harbouring $PTPN11^{A72D}$, emphasizing a partial dependence of PTPN-induced venetoclax resistance on MCL-1 and BCL-xL. The combination of venetoclax plus the MCL-1 inhibitor AZD5991 demonstrated synergistic activity in these models, including the ability to rescue mutant cell lines from *KRAS*-induced and *PTPN11*-induced resistance, suggesting that this combination might be effective in patients with AML harbouring these specific mutations[99,109].

1993: High BCL-2 expression found to be associated with hyperleukocytosis, monoblastic subtype (M4 and M5) and a low CR rate after intensive chemotherapy in samples from patients with newly diagnosed AML; in vitro survival of leukaemic blasts found to correlate with a higher proportion of BCL-2$^+$ cells[240]

1995: BCL-2 antisense oligonucleotides directed against BCL-2 mRNA found to augment the sensitivity of AML cells induced by the chemotherapeutic cytarabine[241]

BCL-2 expression detected in 87.3% of patients at AML onset and in 100% at relapse[242]

1997: BCL-2 is variably expressed on the blasts of patients with AML and high levels of BCL-2$^+$ AML blast expression found to correlate with CD34 positivity while predicting failure to achieve a CR after standard chemotherapy[56]

1999: BCL-2 is upregulated in AML progenitor cells compared with their non-malignant counterparts[47]

2000: A cell-permeable BCL-2-binding peptide, cell-permeable moiety-1285, is found to induce apoptosis of human AML cells with minimal effects on non-malignant peripheral blood lymphocytes[243]

A small molecule and non-peptidic ligand of a BCL-2 surface pocket, HA14-1, induces apoptosis of AML cells[244]

2005: ABT-737, an inhibitor of BCL-2, BCL-xL and BCL-w, is described for the first time but found to lack sufficient oral bioavailability[68]

2008: An orally bioavailable inhibitor of BCL-2, BCL-xL and BCL-w, ABT-263 (navitoclax), is described for the first time[69]

2012: Thrombocytopenia due to inhibition of BCL-xL-dependent platelets by navitoclax is found to be the major dose-limiting toxicity in patients with CLL in a phase I trial (NCT00481091)[71]

2013: An orally bioavailable inhibitor, ABT-199 (venetoclax), is described for the first time and found to inhibit the growth of CLL tumours in vivo, while sparing platelets[72]

Beginning of a phase II trial (NCT01994837) testing venetoclax as monotherapy in patients with AML

2014: Venetoclax found to induce cell death in AML cell lines, primary patient samples and mouse xenograft models[245]

ABT-737 demonstrates more-potent activity and sensitization to the HMA 5-azacytidine in most AML-derived cell lines[158]

Beginning of a phase Ib trial (NCT02203773) testing venetoclax in combination with the HMAs azacitidine or decitabine in patients with AML

Beginning of a phase I/II trial (NCT02287233) testing venetoclax plus LDAC in patients with AML

2015: Ex vivo, venetoclax and ABT-737 found to be similarly potent in sensitizing AML samples to azacitidine[159]

2016: FDA grants Breakthrough Therapy Designation for venetoclax in combination with decitabine or azacitidine in patients ≥65 years of age with treatment-naive AML who are ineligible to receive standard high-dose induction chemotherapy[246]

Combining venetoclax with cytarabine or daunorubicin leads to synergistic induction of apoptosis in primary AML cell lines and patient samples[147]

In a phase II trial (NCT01994837) testing venetoclax monotherapy, 19% of patients with high-risk relapsed and/or refractory AML have a CR or CR with incomplete blood count recovery[95]

Beginning of the phase III VIALE-A trial (NCT02993523) testing venetoclax in combination with azacitidine in patients with AML

2017: Beginning of the phase III VIALE-C trial (NCT03069352) testing venetoclax in combination with LDAC in patients with AML[150]

2018: In a phase Ib trial (NCT02203773), 61% of enrolled patients ≥65 years of age with treatment-naive AML had a CR or CR with incomplete marrow recovery following treatment with venetoclax plus decitabine or azacitidine[127]

Activating FLT3 mutations. A breakthrough in the pathophysiology of AML came from the discovery of mutations in *FLT3*, located on chromosome 13q12 (ref. 113). Activation of intracellular signalling pathways by FLT3-mutant proteins has been related to venetoclax resistance[114]. *FLT3*-ITD mutations, which occur in approximately 30% of adults with AML[115], can promote survival through the induction of PI3K–AKT,

Fig. 4 | Timeline of the development of venetoclax. Preclinical studies from the early 1990s initially identified the pro-survival BCL-2 protein as a promising therapeutic target in patients with AML. This discovery led to the development of ABT-737, although clinical application of this compound was hindered by poor oral bioavailability. To overcome this, the orally bioavailable ABT-263 (navitoclax) was developed; however, its clinical utility was constrained by thrombocytopenia arising from BCL-xL inhibition. This experience spurred the development of ABT-199 (venetoclax), which demonstrated promising results in both preclinical and clinical studies, culminating in US Food and Drug Administration (FDA) approval for patients with AML. AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CR, complete remission; HMA, hypomethylating agent; LDAC, low-dose cytarabine[240–246].

MAPK–ERK and STAT5 signalling pathways[116]. Activation of these pathways leads to higher levels of MCL-1 and BCL-xL expression, thereby contributing to venetoclax resistance[117] (Fig. 7). Various preclinical studies have investigated the combination of FLT3 inhibitors with venetoclax in *FLT3*-ITD$^+$ cell lines, xenograft models and samples from patients with AML. Co-administration of the first-generation type 1 FLT3 inhibitor midostaurin or second-generation type 1 FLT3 inhibitor gilteritinib with venetoclax has synergistically triggered apoptosis in both *FLT3*-ITD$^+$ AML cell lines and patient samples[118,119]. In particular, the combination of gilteritinib with venetoclax demonstrated improved activity in suppressing the proliferation of *FLT3*-ITD$^+$ cells, and also reduced the disease burdens of *FLT3*-ITD$^+$ PDX and resistant MOLM13 xenograft models, compared with either gilteritinib or venetoclax[116,117]. In addition, co-administration of the second-generation type 2 FLT3 inhibitor quizartinib and venetoclax resulted in prolonged antitumour activity by delaying disease recurrence for up to 3 months after treatment, compared with each agent alone in a xenograft model[119,120]. Notably, the combination of gilteritinib and venetoclax augmented the binding of BIM to BAX without enhancing the binding of BIM to other BCL-2 anti-apoptotic proteins (such as BCL-xL). Thus, the co-administration of a FLT3 inhibitor plus venetoclax can reduce the extent to which BIM binds both MCL-1 and BCL-2 in vitro, enabling BIM to interact with BAX, and consequently trigger apoptosis[116]. Collectively these preclinical data provide a strong rationale for the clinical use of regimens comprising venetoclax plus a FLT3 inhibitor in patients with *FLT3*-ITD$^+$ AML. Clinical investigations in this area are already underway with a phase Ib multicentre clinical trial (NCT03625505) testing venetoclax and gilteritinib in patients with relapsed and/or refractory AML, and a phase Ib/II trial (NCT03735875) which tested venetoclax plus quizartinib in patients with relapsed and/ or refractory FLT3-mutated AML, albeit with results suggesting an unacceptable safety profile. Thus far, co-administration of venetoclax and gilteritinib has demonstrated a high response rate, with blast clearance observed in 90% of patients with *FLT3*-mutant AML. The combination has shown a generally favourable safety profile, with the most common treatment-related adverse events being febrile neutropenia (47%), anaemia (27%), thrombocytopenia (7%) and neutropenia (7%). Dose interruptions were required to manage myelosuppression. Preliminary data on efficacy indicate a modified composite CR rate of 75%, with similar modified composite CR rates regardless of previous exposure to FLT3 inhibitors[121]. Finally, preliminary data from a phase I/II trial testing the triplet combination of venetoclax, gilteritinib and azacitidine led to CRs or CR with incomplete haematological recovery (CRi) in virtually all patients (96%), as well as deep *FLT3* molecular responses and encouraging survival outcomes (18-month relapse-free survival (RFS) and overall survival (OS) 71% and 72%), in patients with newly diagnosed *FLT3*-mutated AML. The most frequent grade ≥3 non-haematological adverse events were infection (62%) and febrile neutropenia (38%), which were more common in the relapsed/refractory cohort. Myelosuppression was described as manageable with mitigative dosing strategies[122]. These ongoing trials are expected to provide important information on the activity and safety of venetoclax in combination with FLT3 inhibitors in patients with AML[117].

Inactivating mutations affecting BAX, NOXA and TP53. Inactivating mutations affecting the expression and/or function of *BAX*[90], leading to a loss of and/or inactivation of *NOXA*[75], and mutations/multihit inactivation of *TP53* have all been reported to contribute to venetoclax resistance[81,123,124]. Gene enrichment and protein–protein interaction analyses have identified three genes with a central role in the intrinsic apoptotic pathway whose inactivation can confer resistance to venetoclax in patients with AML: *BAX*, *NOXA* and *TP53* (refs. 99,103,105,123). BAX and BAK, as well as various BH3-only proteins, such as NOXA and PUMA, are all p53 target genes[125,126]. In line with this consideration, lower levels of BAK, NOXA and PUMA have been detected in *TP53*-knockout cell lines. Notably, transcriptional alterations have been identified outside of p53 target genes with an enhanced ratio of BCL-xL:BCL-2, which might confer further resistance to venetoclax in *TP53*-knockout cells[123]. Data from numerous in vitro studies have shown that *TP53*-mutated AML cells and AML xenograft models are resistant to monotherapy with venetoclax[123] or MCL-1 inhibitors[81]. In the absence of *TP53*, delayed BAX/BAK-induced apoptosis upon treatment with BH3 mimetics was supposed to reduce the efficacy of these agents by lifting the initial threshold for apoptosis. Notably, co-inhibition of BCL-2 and MCL-1 has been beneficial in terms of overcoming this delayed apoptosis and prolonging antitumour activity, thus reproducing the synergy observed in earlier preclinical studies[123]. Interestingly, data from a trial combining venetoclax and DNA methyltransferase inhibitors have identified lower response rates in patients with *TP53*-mutant AML (47%) than in the overall cohort (70%). In fact, patients in the *TP53*-mutant subgroup had the worst response rates compared with those of any other molecularly defined subgroup, and the presence of such alterations was the only variable for which a significant correlation with inferior response was demonstrated[127].

Mitochondria. Disruption of mitochondrial homeostasis with enhanced oxidative phosphorylation caused by upregulated amino acid and/or fatty acid oxidation is another mechanism of resistance to venetoclax in patients with AML[128,129]. Interestingly, the combination of venetoclax and the oxazolidinone-class antibiotic tedizolid results in augmented integrated stress responses, a related decrease in the extent of oxidative phosphorylation and diminished glycolysis, leading to rapid exhaustion of ATP and cell death in mouse xenograft models of treatment-resistant AML[130]. Moreover, mitophagy, the selective degradation of mitochondria via autophagy, has been associated with resistance to various BH3 mimetics including venetoclax[91]. Integration of data from various genome-wide CRISPR–Cas9 screens has demonstrated that loss of mitophagy regulators can sensitize AML cells to venetoclax. Indeed, overexpression of the mitophagy regulator MFN2 is sufficient to induce resistance to these agents in patients with AML. In the same study, a lack of responsiveness to BH3 mimetics was followed by augmented mitochondria–ER interactions and enhanced mitophagic

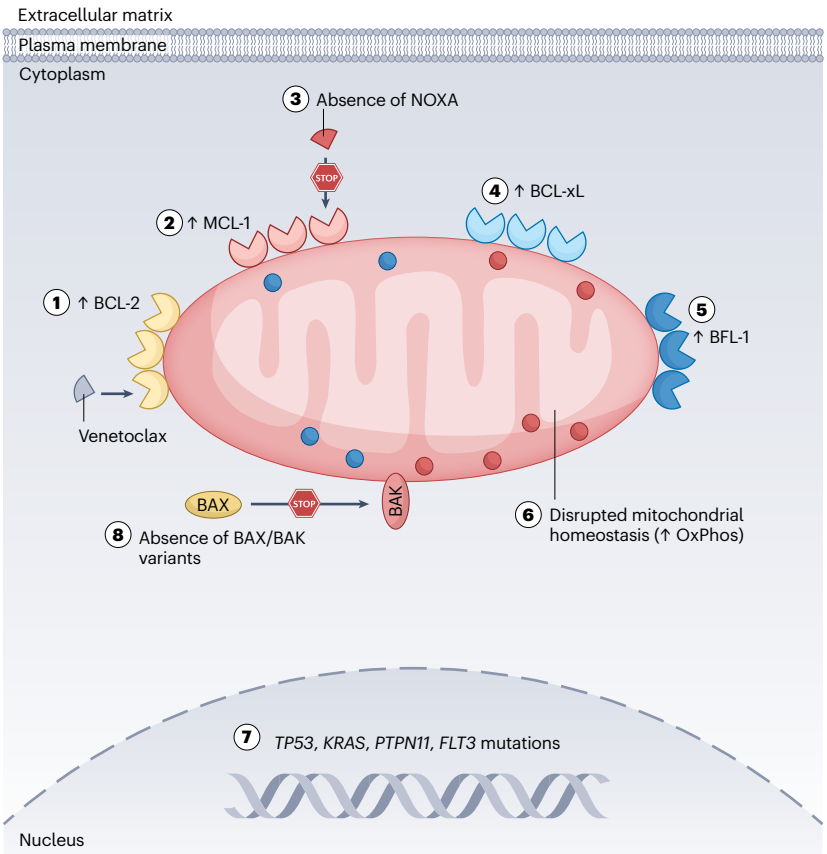

Fig. 5 | Mechanisms of resistance to venetoclax in AML. The major mechanisms contributing to venetoclax resistance in acute myeloid leukaemia (AML). (1) Overexpression of BCL-2, but generally not mutations in *BCL-2*. (2) Overexpression of MCL-1. (3) Absence of NOXA. (4) Overexpression of BCL-xL. (5) Overexpression of BFL-1. (6) Disruption of mitochondrial homeostasis with enhanced oxidative phosphorylation (OxPhos). (7) Mutations in *TP53*, *KRAS*, *PTPN11* and *FLT3*. (8) Absence of BAX/BAK variants.

flux, which acted as a pro-survival mechanism and reduced the extent of damage to the mitochondria[91]. Consistent with this observation, agents targeting MFN2 can synergize with BH3 mimetics by specifically hampering the clearance of damaged mitochondria and increasing the extent of apoptosis. Thus, suppressing the activity of MFN2 and mitochondrial clearance through mitophagy might be a powerful strategy to overcome venetoclax resistance in patients with AML.

Data on mitochondrial biology indicate a role of aberrant mitochondrial structures in the apoptotic response to venetoclax[103]. Thus, targeting the mitochondrial architecture could provide a promising strategy to overcome resistance to venetoclax in patients with AML[75]. Interestingly, CRISPR–Cas9 screens have revealed a negative association between mitochondrial chaperonin caseinolytic peptidase B protein homologue (CLPB), which regulates the structure of the mitochondrial cristae as well as cellular metabolism, and venetoclax resistance[78]. Indeed, overexpression of CLPB has been reported in samples from patients with AML, which results in a tighter mitochondrial cristae lumen. Conversely, loss of CLPB in AML cells leads to wider cristae and activation of the mitochondrial stress response, which in turn induces cell-cycle arrest, and lowers the mitochondrial threshold for activation of apoptosis[99,103,131]. In line with this observation, the combination of venetoclax with CLPB deletion has been shown to rescue venetoclax resistance in in vitro models of *TP53*-mutant AML[103]. Another genomic CRISPR–Cas9 knockout screen has revealed negative selection of *RBFA*, *MRPL17*, *MRPL54* and *DAP3*, which are integral components of the mitochondrial translation machinery[132–134]. In one of these studies, a decrease in AML cell viability was detected only in the presence of venetoclax plus tedizolid, but not with either agent alone[134]. This observation probably reflects an increase in the integrated stress response and a related reduction in oxidative phosphorylation and glycolytic capacity with co-administration of both agents, leading to ATP consumption and cell death. These findings might be immediately clinically relevant given the availability of FDA-approved antibiotics with defined inhibitory effects on mitochondrial translation[130].

Monocytic AML. The responses of patients with AML to venetoclax-based therapies can vary depending on the differentiation stage of the blasts. Ex vivo investigations of drug sensitivity in samples from patients with AML have demonstrated a continuous decline in sensitivity to venetoclax from the most primitive phase of AML maturation (M0) to monocytic cell maturation (M5)[65,135]. This progressive reduction in sensitivity primarily results from a loss of BCL-2 expression and an increased reliance on MCL-1 to support oxidative phosphorylation and cell survival, characterized by a distinct transcriptomic profile[135]. This differential sensitivity probably reflects the selective outgrowth of monocytic subpopulations at the time of relapse. Similarly, evidence from the BEAT AML dataset suggests a greater venetoclax AUC, indicating reduced sensitivity, in leukaemic blasts with high levels of *CLEC7A* and *CD14* expression, which are typically present in monocytic AML of the M4/M5 subtypes. These observations emphasize the propensity of monocytic or myelomonocytic AML cells for resistance to venetoclax[109], which might reflect a lineage-associated switch to MCL-1 as the mediator

of oxidative phosphorylation[65,135]. Furthermore, augmented BFL-1 expression and *KRAS* mutations have also been detected in patients with M4/M5 AML[65,109,135]. These observations suggest that venetoclax-resistant AML with myelomonocytic differentiation might be driven by mutant *KRAS*-mediated upregulation of BFL-1 (refs. 99,109,135).

Various novel therapeutic regimens have been explored in an attempt to address these challenges. Notably, the triplet nucleoside regimen incorporating alternating cycles of cladribine (CLAD), LDAC and azacitidine plus venetoclax has demonstrated remarkable efficacy[136]. This combination demonstrated CR/CRi rates of 93%, with 84% of patients having MRD-negative disease as measured using flow cytometry[137]. The 24-month OS was 72.9% at a median follow-up duration of 22 months[137]. Moreover, a post-hoc analysis comparing CLAD, LDAC and venetoclax with an HMA plus venetoclax demonstrated superior CR/CRi rates of 73% versus 45% in patients with *NRAS*/*KRAS*-mutated AML[138]. Updated phase II data indicate a median OS of 25 months for patients with intermediate-risk AML, highlighting the promise of this regimen in addressing disease harbouring active signalling mutations[136]. A prospective phase II trial evaluating this regimen in patients with monocytic AML or AML harbouring mutations associated with active oncogenic signalling is currently recruiting patients (NCT06504459)[136].

Interestingly, post-venetoclax investigations of matched samples obtained from patients with AML at diagnosis and relapse demonstrate the concurrent existence of primitive and monocytic features at diagnosis, suggesting the existence of heterogenicity within the developing leukaemic blast population[65]. Under the selective pressures created by venetoclax plus azacitidine, monocytic clones are able to successively expand with the concomitant disappearance of primitive populations at the time of disease relapse[65]. Notably, some of the relapsed monocytic subclones also had increased expression of HOXA9, which was not observed in their parental clones[65]. Nevertheless, monocytic clones maintained their dependency on MCL-1 both at diagnosis and following disease relapse[65]. These results suggest a potential clinical role of MCL-1 inhibitors.

Others. A reduction in mitochondrial priming has been observed in both primary AML cells and patient-derived xenografts[139]. Unlike in CLL, *BCL-2* mutations are generally not observed at the time of acquired resistance to venetoclax in patients with AML[140]. This observation may reflect the influence of cellular context on mutational activity, as well as the rapidly progressive nature of AML, resulting in fewer patients being exposed to long-term (6–12 months) venetoclax monotherapy[90]. The effects of epigenetic alterations on carcinogenesis have also emerged as a mechanism contributing to venetoclax resistance. In fact, alterations in methylation during RNA transcription have been related to the development of venetoclax resistance in haematological malignancies. In particular, changes in DNA methylation can affect the expression of genes involved in apoptosis, such as PUMA, and other genes that regulate cell survival and proliferation. These changes can result in the downregulation of pro-apoptotic proteins as well as the upregulation of anti-apoptotic proteins, therefore reducing the sensitivity of tumour cells to venetoclax[78,141,142]. Alterations in the expression of tumour suppressor genes, cell cycle dysregulation and various other processes affecting resistance to venetoclax resistance are all areas of ongoing research interest[78,143].

FDA-approved venetoclax-based regimens for patients with AML

Venetoclax plus cytarabine

Cytarabine, also known as cytosine arabinoside, is a nucleotide analogue chemotherapeutic drug[144,145]. Intracellularly, cytarabine is converted to its active form, cytosine arabinoside triphosphate, which is suggested to compete with deoxycytidine triphosphate for incorporation into the growing DNA strands of dividing cells, in which it can act as a relative chain terminator that leads to premature termination of DNA synthesis[146].

This mechanism of action makes cytarabine a valuable agent in targeting rapidly dividing tumour cells by promoting cell-cycle arrest during S phase. Cytarabine is currently the backbone of the SOC induction

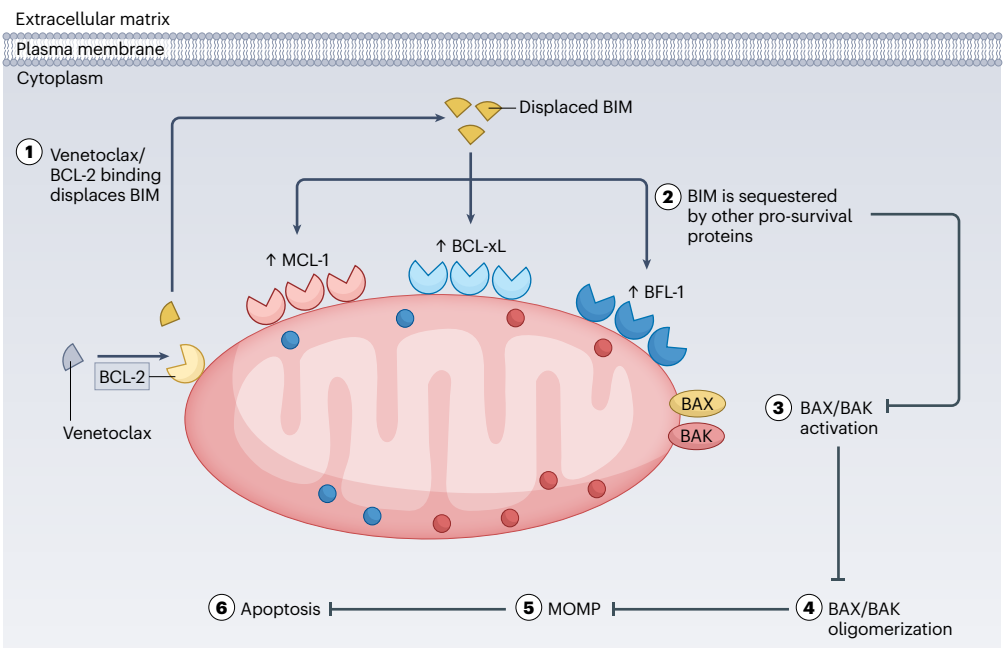

Fig. 6 | Role of other anti-apoptotic BCL-2 proteins in resistance to venetoclax. Increased dependencies on other members of the anti-apoptotic BCL-2 family, rather than the BCL-2 protein, are a key contributor to venetoclax resistance in patients with acute myeloid leukaemia (AML). BIM, which is displaced from BCL-2 by venetoclax, can be sequestered by other overexpressed anti-apoptotic BCL-2 family members, such as MCL-1, BCL-xL and BFL-1. This suppresses the activation and oligomerization of BAX/BAK, mitochondrial outer membrane permeabilization (MOMP), cytochrome c and second mitochondria-derived activator of caspases (SMAC) efflux, and caspase activation, thereby inhibiting venetoclax-mediated apoptosis of AML cells.

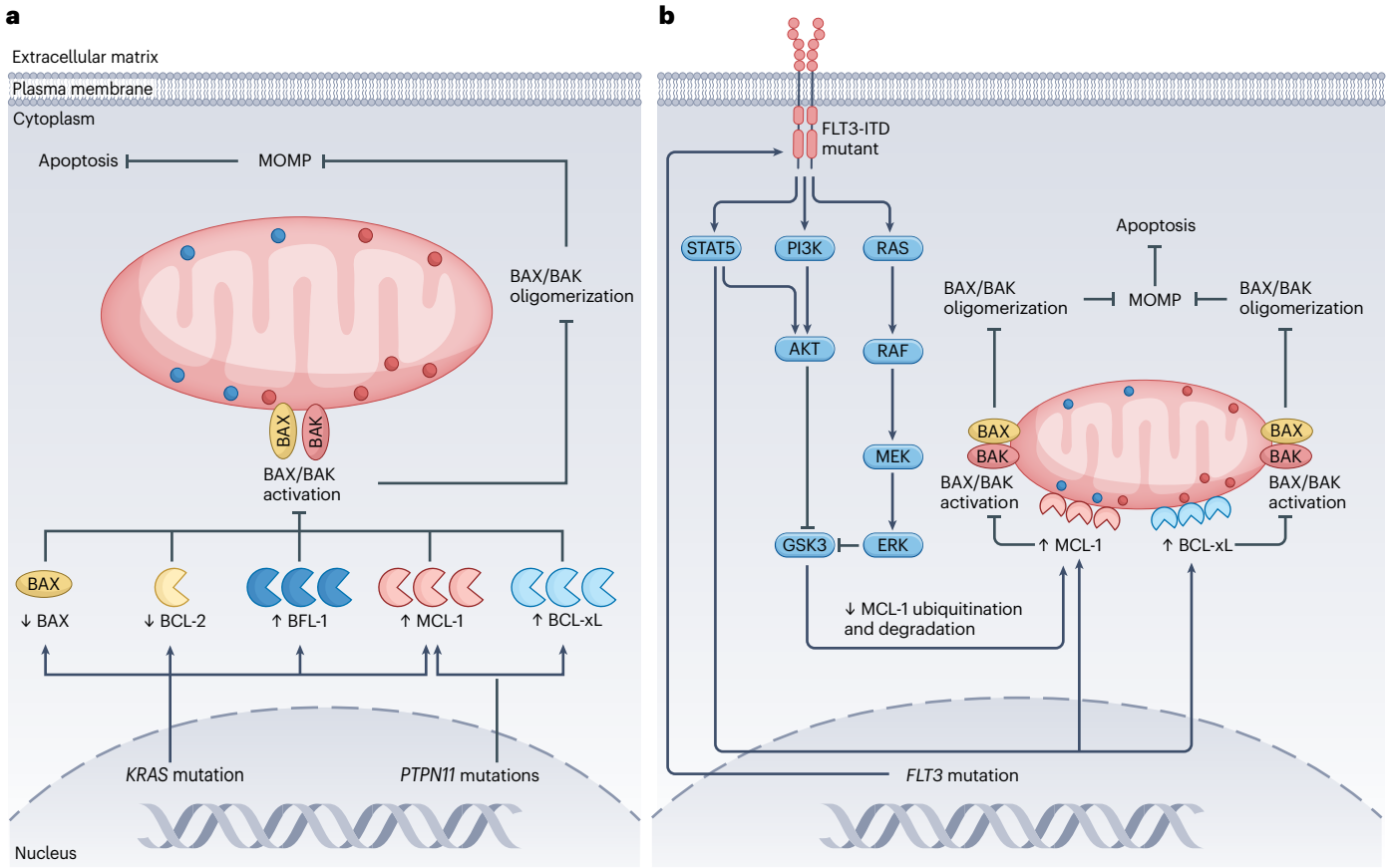

Fig. 7 | The role of genomic instability in the development of resistance to venetoclax. a, Mutations in *KRAS* and *PTPN11* dysregulate the expression of BAX and the anti-apoptotic proteins BCL-2, BFL-1, MCL-1 and BCL-xL, thereby suppressing venetoclax-mediated apoptosis. **b**, Mutant FLT3-ITD proteins upregulate anti-apoptotic MCL-1 and BCL-xL expression via activation of downstream STAT5, PI3K–AKT and MAPK–ERK signalling pathways. Similarly, this dysregulation inhibits apoptosis and contributes to venetoclax resistance. MOMP, mitochondrial outer membrane permeabilization.

chemotherapy regimens for patients with AML and acute lymphoblastic leukaemia (ALL)[146]. Interestingly, cytarabine-induced reductions in MCL-1 expression can synergize with BCL-xL inhibition and/or BCL-2 inhibition[93] with synergistic cytotoxic effects demonstrated for the combinations of venetoclax or obatoclax plus cytarabine in AML cells in vitro[147,148]. Similar synergistic effects, including reduced MCL-1 expression, have been described in primary AML cells from patient samples, in comparison with the effects of venetoclax alone[147]. Consistent with these observations, combining the experimental BCL-2, BCL-xL and MCL-1 inhibitor obatoclax with cytarabine leads to BAX activation, which is not seen after exposure to each drug individually[148]. This combination leads to a loss of mitochondrial membrane potential followed by the early induction of DNA double-stranded breaks, which precede a reduction in MCL-1 expression, as well as BCL-2, BCL-xL and MCL-1 nuclear translocation, and finally apoptosis[148]. Co-administration of venetoclax with LDAC in a phase I/II trial resulted in a 54% CR rate and a median OS of 10.1 months in patients with AML ≥60 years of age who are ineligible for intensive chemotherapy[149]. These outcomes improved to CRs in 62% of patients and a median OS of 13.5 months, respectively, among patients who had not previously received an HMA. Venetoclax plus LDAC had an acceptable safety profile, albeit with grade ≥3 adverse events including febrile neutropenia (in 42% of patients), thrombocytopenia (in 38%) and reduced white blood cell count (in 34%). Early (1-month) mortality was low for this population at 6% (ref. 149). In the large-cohort international phase III VIALE-C trial, patients with AML who were ineligible for intensive chemotherapy receiving venetoclax plus LDAC had a greater CR rate (48% versus 13%) and longer median OS (7.2 months versus 4.1 months) compared with those receiving placebo plus LDAC. A manageable safety profile was again identified, although key grade ≥3 adverse events such as febrile neutropenia, neutropenia and thrombocytopenia were all more prevalent in the venetoclax plus LDAC group (32%, 47% and 45%, respectively) compared with the placebo plus LDAC group (29%, 16% and 37%, respectively)[150]. Owing to these significant improvements in efficacy, this combination was approved in 2020 for patients with treatment-naive AML who are unable to receive standard intensive chemotherapy[151–153] (Table 1).

Venetoclax plus hypomethylating agents

Epigenetic alterations, including DNA methylation, are important therapeutic targets in many haematological malignancies[154]. Aberrant DNA methylation at CpG islands within promoter regions can result in the silencing of tumour suppressor genes related to critical

Table 1 | Clinical trials testing venetoclax-based combinations in patients with AML

Trial	Intervention and patient population	Outcomes	Adverse events	Ref.
Venetoclax plus a hypomethylating agent				
NCT02203773 (phase Ib)	Venetoclax plus decitabine or azacitidine (n=145)[a]	CR/CRi in 67% of patients (all doses); 73% in venetoclax 400 mg+HMA cohort; mOS 17.5 months (95% CI 12.3–NR) (all patients), NR for venetoclax 400 mg cohort	Common events (>30%) included nausea, diarrhoea, constipation, febrile neutropenia, fatigue, hypokalaemia, anorexia and reduced WBC	160
NCT02203773 (phase Ib)	Venetoclax plus decitabine (n=23) versus venetoclax plus azacitidine (n=22) versus venetoclax plus decitabine plus posaconazole (n=12)[a]	CR/CRi 61% versus 59% versus 67%; mOS 15.2 months versus 14.2 months versus NR	Common grade 3–4 events included thrombocytopenia (39% versus 59% versus 42%), febrile neutropenia (48% versus 45% versus 33%) and neutropenia (52% versus 36% versus 25%)	127
VIALE-A NCT02993523 (phase III)	Venetoclax plus azacitdine (n=286) versus placebo plus aazacitidine (n=145)[b]	CR/CRi 66.4% versus 28.3%, (P<0.001); mOS 14.7 versus 9.6 months (HR 0.66, 95% CI 0.52–0.85, P<0.001)	Any-grade nausea (44% versus 35%), grade ≥3 thrombocytopenia (45% versus 38%), grade ≥3 neutropenia (42% versus 28%), febrile neutropenia (42% versus 19%), any-grade infection (84% versus 67%), SAEs (83% versus 73%)	162
ENHANCE-3 NCT05079230 (phase III)	Venetoclax plus magrolimab plus azacitidine (n=189) versus venetoclax plus placebo plus azacitidine (n=189)[d]	CR within 6 cycles 41.3% versus 46.0%; mOS 10.7 versus 14.1 months (HR 1.18, 95% CI 0.85–1.64)	Fatal AEs (19.0% versus 11.4%) including infections (11.1% versus 6.5%) and fatal respiratory events (2.6% versus 0%); trial stopped early owing to futility	213
Venetoclax plus LDAC				
NCT02287233 (phase Ib/II)	Venetoclax plus LDAC (n=82)[c]	CR/CRi 54%; mOS 10.1 months	Grade 3–4 febrile neutropenia (42%), thrombocytopenia (38%), neutropenia (27%) and anaemia (27%)	149
VIALE-C NCT03069352 (phase III)	Venetoclax plus LDAC (n=143) versus placebo plus LDAC (n=68)[d]	CR/Cri 48% versus 13%; mOS 8.4 versus 4.1 months (HR 0.70, 95% CI 0.50–0.99, P=0.04)	Grade ≥3 febrile neutropenia in 32% versus 29%, grade ≥3 neutropenia in 47% versus 16%, grade ≥3 thrombocytopenia in 45% versus 37%	150
Venetoclax plus MCL-1 inhibitors				
NCT02670044 (phase Ib)	Venetoclax plus idasanutlin in patients with R/R AML who are unfit for cytotoxic chemotherapy (n=56)[d]	CR/CRi 26%; mOS 5.1 months (95% CI 3.4–7.3)	Common AEs (≥40%) included diarrhoea (87.3%), nausea (74.5%), vomiting (52.7%), hypokalaemia (50.9%) and febrile neutropenia (45.5%)	239
NCT03441555 (phase Ib)	Venetoclax plus alvocidib in patients with R/R AML (n=35)[d]	CR/CRi 11.4%	Grade ≥3 AEs included febrile neutropenia (45.7%), diarrhoea (31.4%) and hypokalaemia (28.6%)	189
NCT03862157 (phase I/II)	Venetoclax plus azacitidine plus pevonedistat (n=32)[c]	CR/CRi 66%; mOS 8.1 months	Common grade 3–4 AEs included infection (35%), febrile neutropenia (25%) and hypophosphataemia (23%)	190
NCT04588922 (phase IIa)	Venetoclax/azacitidine plus SLS009 in patients with R/R AML (n=30)	CR/CRi 17%; mOS for the trial NR. At the first dose level in which 8/10 patients died, mOS was 5.5 months	Any-grade AEs included nausea (23%), diarrhoea (13%), hyperphosphataemia (10%), pyrexia (7%) and white blood cell count decrease (7%)	181
Venetoclax plus FLT3 TKIs				
NCT04140487 (phase I/II)	Venetoclax plus azacitidine and gilteritinib in patients with newly diagnosed *FLT3*-mutated AML who were unfit for intensive chemotherapy (n=30)[d]	CR/CRi 96%, 18-month RFS 71%, 18-month OS 72%	Most common grade ≥3 non-haematological AEs included infection (62%) and febrile neutropenia (38%)	122
	Venetoclax plus azacitidine and gilteritinib in patients with R/R FLT3-mutated AML (n=22)[d]	CR/CRi 27%		
NCT03625505 (phase Ib)	Venetoclax plus gilteritinib (n=56 *FLT3*-mutant, n=5 *FLT3* wild type)[d]	CR/CRi 22%, mOS 10.0 months (95% CI 6.3–12.3)	Grade 3–4 cytopenias (80%); SAEs in 75% included febrile neutropenia (44%), pneumonia (13%).	121
NCT03661307m (phase I)	Venetoclax plus decitabine plus quizartinib as first-line therapy (n=4) or R/R AML (n=13) who received a median of 3 prior therapies; 85% had previously received a FLT3 inhibitor	CR/CRi (frontline versus R/R AML) 100% versus 69%, mOS (frontline versus R/R AML) not reached versus 7.1 months	Grade 3–5 non-haematological toxicities included lung infections (53%) and neutropenic fever (35%). Dose-limiting toxicities with 40 mg quizartinib included prolonged cytopenia (n=2)	203

Table 1 (continued) | Clinical trials testing venetoclax-based combinations in patients with AML

Trial	Intervention and patient population	Outcomes	Adverse events	Ref.
Venetoclax plus BCL-2, BCL-w, BCL-xL inhibitors				
NCT03181126 (phase I)	Venetoclax plus navitoclax plus chemotherapy (paediatric and adult patients with R/R AML or lymphoblastic lymphoma) (*n*=47)	CR/CRi 59.6%, mOS 7.8 months	Most common grade 3–4 AEs included febrile neutropenia (46.8%), neutropenia (38.3%) and thrombocytopenia (25.5%); SAEs (78.7%) included febrile neutropenia (27.7%) and sepsis (17.0%)	194
Venetoclax plus p53 function-restoring agents				
NCT04214860 (phase I)	Venetoclax plus eprenetapopt versus venetoclax plus eprenetapopt plus azacitidine in patients with AML harbouring *TP53* mutations (*n*=49)[d]	CR/CRi 38%	Common grade ≥3 AEs included febrile neutropenia (47%), thrombocytopenia (37%), leukopenia (25%) and anaemia (22%); treatment-related SAEs in 27%; and 1 treatment-related death (sepsis)	201

[a]In patients ≥65 years of age who were ineligible for standard induction therapy. [b]In patients ≥75 years of age and/or who were ineligible for standard induction therapy. [c]In patients ≥60 years of age and ineligible for standard induction therapy. [d]In patients ≥18 years of age. AEs, adverse events; AML, acute myeloid leukaemia; CDK9, cyclin-dependent kinase 9; CI, confidence interval; CR, complete remission; CRi, complete remission with incomplete blood count recovery; FLT3, FMS-like tyrosine kinase-3; HMA, hypomethylating agent; HR, hazard ratio; LDAC, low-dose cytarabine; mOS, median overall survival; NR, not reached; RFS, relapse-free survival; R/R, relapsed/refractory; SAEs, serious adverse events; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitor.

signalling pathways such as those involved in cell-cycle regulation and DNA repair in malignant cells[155]. Reversing these modifications to reexpress silenced tumour suppressor genes has led to the introduction of HMAs, such as azacitidine and decitabine which are now routinely administered to patients with AML[156]. Data from preclinical studies have demonstrated synergistic cytotoxic effects by combining either venetoclax or ABT-737 with azacitidine in AML cell lines and samples from patients[112,157–159]. However, the molecular vulnerabilities underlying these synergistic effects have remained ambiguous. Interestingly, RNA-interference screens have identified pro-apoptotic BCL-2 family members as potential targets for which inhibition might augment the efficacy of azacitidine[158].

These encouraging findings have led to clinical trials testing regimens combining BCL-2-targeting BH3 mimetics with azacitidine in patients with myeloid leukaemias. For example, the combination of venetoclax plus the HMAs decitabine or azacitidine has been tested in treatment-naive older (>65 years of age) patients with AML[127,160]. Results from this study demonstrate a composite CR (CR or CRi) of 73% in patients who received the recommended phase II dose of 400 mg venetoclax plus an HMA. The median duration of composite CR was 11.3 months, and the median OS was 17.5 months. This combination was well tolerated in older patients; any-grade adverse events (>30%) included nausea, fatigue, diarrhoea, constipation, febrile neutropenia, hypokalaemia, reduced white blood cell count and decreased appetite. In light of these findings, this combination was designated as an FDA Breakthrough Therapy for this population in 2016 (refs. 127,160,161). The phase III VIALE-A trial tested venetoclax plus azacitidine compared with placebo plus azacitidine in previously untreated patients with AML who were unable to tolerate standard-dose chemotherapy and/or were ≥75 years of age. At an interim analysis, both median OS and composite CR increased with venetoclax plus azacitidine (OS 14.7 months versus 9.6 months and composite CR 66.4% versus 28.3%) in comparison with the control group. Key grade ≥3 adverse events in the venetoclax plus azacitidine and in the placebo plus azacitidine groups, respectively, included thrombocytopenia (in 45% versus 38% of patients), neutropenia (42% versus 28%) and febrile neutropenia (42% versus 19%); whereas any-grade nausea occurred in 44% versus 35% and any-grade infections in 84% versus 67% (ref. 162). Venetoclax plus either an HMA (azacitidine or decitabine) or LDAC received full FDA approval in 2020 as first-line therapy for patients with AML who are unable to receive induction chemotherapy[153,163] (Table 1).

In an attempt to enhance prognostic precision for patients receiving venetoclax plus azacitidine, researchers developed a molecular prognostic risk signature (mPRS) using data from the VIALE-A trial[164]. This mPRS incorporates *KRAS*-, *NRAS*- and *FLT3*-ITDs, as well as *TP53* mutations to classify patients into higher, intermediate and lower benefit groups. This simple four-gene approach demonstrated superior accuracy compared with that of the ELN 2022 risk classification in segregating patients into distinct cohorts with varying response rates and survival outcomes. The ELN 2024 risk classification criteria refine prognostic stratification for patients with AML receiving less intensive therapies. By integrating genetic markers such as alterations in *IDH1*/*IDH2*, *KRAS*, *NRAS*, *DDX41*, *TP53* and *FLT3*-ITDs, these criteria provide a more tailored approach to differentiate favourable, intermediate and adverse risk groups[165]. These revised criteria address the specific needs of older adults and those who are ineligible for intensive chemotherapy and complement the mPRS in guiding personalized treatment decisions and improving prognostic precision for these patients[165].

Venetoclax-based regimens under clinical investigation in AML

Venetoclax and MCL-1 inhibitors

MCL-1 mediates resistance to venetoclax via various upstream genomic/epigenetic mediators. Thus, direct or indirect targeting of MCL-1 provides a rational approach that might restore sensitivity to venetoclax[100,105,118,147]. Direct MCL-1 inhibitors have demonstrated various limitations in the clinical trials conducted thus far, mainly reflecting that the binding sites of these inhibitors are less flexible and, thus, less likely to adapt to different ligands, compared with those of BCL-2 or BCL-xL[166–169]. In addition, translating compounds with promising results in in vitro or in vivo studies to the clinic revealed unexpected toxicities including troponin leak and cardiotoxicity, perhaps reflecting the role of MCL-1 in the regulation of mitochondrial homeostasis[63,170,171]. However, substantial progress has been made in the development of these agents, some of which have already entered clinical trials. At least five therapeutic agents with activity against MCL-1, S64315 (ref. 172), AMG176 (ref. 173), AMG397 (ref. 174), AZD5991 (ref. 175) and PRT1419 (ref. 107) have been tested as monotherapies in phase I trials involving patients with AML (NCT02979366, NCT02675452, NCT04543305, NCT03465540 and NCT03218683, of which the latter two were terminated by the sponsor). Several of these agents

(S64315, AMG-176 and AZD5991) have also been investigated in combination with venetoclax (NCT03672695, NCT03797261 and NCT03218683)[63,100,176]. AZD5991 is a potent and selective BH3 mimetic with nanomolar potency against human MCL-1 and >5,000-fold selectivity over other pro-survival BCL-2 family member proteins. In a phase I trial, systemic administration of AZD5991 either as monotherapy or in combination with venetoclax resulted in limited clinical activity across patients with different haematological malignancies, apart from in those with MDS. This lack of activity was accompanied by troponin elevations of uncertain clinical significance across all dose levels. These issues precluded further clinical development of AZD5991 (ref. 108). AMG-176 has also been administered in combination with azacitidine (NCT02675452). With ongoing clinical developments aiming to improve the therapeutic window of these agents, MCL-1 selective inhibitors might provide a novel future class of anti-AML drugs.

Over the past decade, several strategies involving indirect MCL-1 inhibition have been investigated, including CDK9 inhibitor-mediated suppression of MCL-1 transcription, NEDD8-activating enzyme (NAE) inhibitor-mediated upregulation of NOXA to enhance MCL-1 neutralization, and MEK inhibitor-mediated targeting of MAPK–ERK signalling to promote MCL-1 degradation[177–180]. Based on evidence of synergistic effects in preclinical studies, several completed or ongoing trials have tested and/or are testing venetoclax plus these innovative small-molecule inhibitors[99]. For example, SLS009, a highly selective CDK9 inhibitor, has been evaluated in combination with azacitidine and venetoclax in a phase IIa trial, showing promising clinical activity in patients with relapsed and/or refractory AML after prior venetoclax-based therapy. This combination demonstrated a favourable safety profile with no dose-limiting toxicities and manageable adverse events such as any-grade nausea (in 23% of patients) and diarrhoea (in 13%). The investigators hypothesized that SLS009 could overcome venetoclax resistance by targeting MCL-1, a key mediator of resistance. These findings highlight the potential of CDK9 inhibitors, such as SLS009, to overcome intrinsic mechanisms of resistance to apoptosis-inducing agents and thus improve the outcomes of patients with AML[181].

Inhibition of CDK9 downregulates the transcription of *c-MYC* and *MCL-1*, which are involved in cell survival[182]. CDK9 inhibitors such as alvocidib[183], AZD4573 (ref. 184), CYC065 (ref. 185) and dinaciclib[186], which are able to indirectly inhibit MCL-1, have shown encouraging activity in preclinical models of AML and are being tested in several early-phase trials. However, data from several studies suggest that inhibiting MCL-1 might cause cardiotoxicities[170,187,188]. Alvocidib (also known as flavopiridol) is a flavonoid alkaloid CDK9 inhibitor that is currently under clinical development that has demonstrated promising clinical activity in patients with relapsed and/or refractory AML. Alvocidib has also shown encouraging results when administered in combination with fludarabine, cytarabine and mitoxantrone in patients with relapsed and/or refractory AML[182]. The combination of venetoclax plus alvocidib has been reported to regulate the proportion of BCL-2 family members by activating mechanisms that favour apoptosis and thus provides a promising therapy that might overcome intrinsic mechanisms of resistance to BH3 mimetics[178]. In a phase Ib trial, co-administration of venetoclax plus alvocidib resulted in a modest composite CR rate (11.4%) in patients with relapsed and/or refractory AML. This combination had a favourable safety profile, with no maximum-tolerated dose reached, albeit with grade ≥3 adverse events in 94.3% of patients. Adverse events including any grade nausea (in 77.1% of patients), diarrhoea (88.6%) and vomiting (62.9%) were all frequent; otherwise, the overall incidence of toxicities was consistent with the safety profile of both drugs[189]. In a phase I/II trial, the combination of venetoclax, the NEDD8-activating enzyme inhibitor pevonidostat plus azacitidine demonstrated encouraging activity, with a composite CR rate of 66% (CRs in 50% of patients and CRis in 16%), a median OS of 8.1 months and an ORR of 75% in the subgroup with newly diagnosed secondary AML, who traditionally have a very poor prognosis. This triplet combination also had a favourable safety profile, albeit with grade 3–4 adverse events such as infection (in 35% of patients), febrile neutropenia (25%) and hypophosphataemia (23%)[190]. In a phase I trial (NCT02670044), the combination of venetoclax plus the MEK inhibitor cobimetinib resulted in an ORR of 18% in patients with relapsed and/or refractory AML[191]. Gastrointestinal adverse effects such as diarrhoea, nausea and vomiting might limit the clinical utility of this combination[191] (Table 1).

Venetoclax and BCL-2/BCL-w/BCL-xL inhibitors

The BCL-2 and BCL-xL inhibitor navitoclax has been tested clinically in patients with advanced-stage solid tumours and lymphoid malignancies, although clinical tolerability has been limited by thrombocytopenia, with several patients having dose-limiting forms of this on-target adverse event in early-phase trials[70,192,193]. Nonetheless, the triplet combination of venetoclax, low-dose navitoclax and chemotherapy, has demonstrated promising efficacy (CR rate 59.6%) and a favourable safety profile (grade 3–4 adverse events possibly related to venetoclax or navitoclax in 74.5% of patients) in the setting of relapsed and/or refractory ALL or lymphoblastic lymphoma[194,195].

Venetoclax and p53 function-restoring agents

The apoptotic network of the tumour suppressor gene *TP53* contributes to resistance to BH3 mimetics in AML cells. These alterations, supported by changes in mitochondrial homeostasis and cellular metabolism, are the main drivers of resistance to venetoclax in patients with AML[123]. Indeed, *TP53* mutations are known to confer resistance to venetoclax and are related to inferior outcomes among patients with AML, suggesting that venetoclax-containing combination regimens might be most effective in patients without these characteristics[196].

MDM2 promotes the rapid degradation of p53 (ref. 197); therefore, MDM2 inhibitors might reactivate wild-type p53 via disruption of p53–MDM2 interactions, thereby preventing proteasomal p53 degradation and reducing p53 nuclear export[177,198]. Nonetheless, MDM2 inhibitors require wild-type *TP53* to exert apoptotic activity[199]. Eprenetapopt is a novel first-in-class small molecule, with a mechanism of action that purportedly involves restoring the functions of wild-type p53 in *TP53*-mutated cell lines. Indeed, eprenetapopt activates p53-dependent apoptosis by covalently binding with the p53-mutated protein after conversion to its reactive electrophilic form, methylene quinuclidinone[200]. In a dose-finding and expansion phase I trial[201], the combination of venetoclax, eprenetapopt and azacitidine demonstrated encouraging efficacy (ORR 64%) and an acceptable safety profile albeit with grade ≥3 febrile neutropenia (in 47% of patients), thrombocytopenia (in 37%) and leukopenia (in 25%), providing support for further frontline evaluations of this triplet for patients with *TP53*-mutated AML, which unfortunately did not prove successful. Although the incidence of grade ≥3 adverse events in this trial suggests an acceptable safety profile, the failure of other trials testing eprenetapopt might reflect an increased risk of toxicities. Indeed, in this trial, 27% of patients had clinically serious adverse events, and 2% had treatment-related deaths, owing to sepsis[201] (Table 1).

Venetoclax and FLT3 TKIs

Feasible combination partners for administration alongside BCL-2 inhibitors can include FLT3 inhibitors. Data from both preclinical and clinical studies suggest that combination regimens involving FLT3 inhibitors plus venetoclax might have synergistic effects and thus ameliorate the outcomes of patients with AML. The combination of venetoclax with various FLT3 tyrosine kinase inhibitors (TKIs) is currently being tested in various trials[202–204]. In a phase Ib trial, the co-administration of venetoclax and gilteritinib resulted in a 90% ORR in patients with *FLT3*-mutated relapsed and/or refractory AML, with a corresponding high ORR among patients who had previously had disease progression on a TKI[202]. Early data from an ongoing phase I/II trial testing triplet therapy with venetoclax, quizartinib and decitabine have also been encouraging, including a 69% composite response rate and a median OS duration of 7.1 months in patients with *FLT3*-mutated relapsed and/or refractory AML, whereas median OS has not been reached in the frontline setting. No patients developed dose-limiting toxicities at 30 mg/day quizartinib, which was therefore selected as the recommended phase II dose for inclusion in the triplet. Grade 3–5 non-haematological toxicities included lung infections (53%) and neutropenic fever (35%)[203] (Table 1). Similarly, the triplet regimen of azacitidine, venetoclax and gilteritinib resulted in both reasonable tolerability (with grade ≥3 infections in 62% of patients and febrile neutropenia in 38%) as well as efficacy (CR/CRi in 96% of patients, 18-month OS 72%), especially in patients who were able to undergo stem cell transplantation (NCT04140487)[122].

Venetoclax and CD47-targeting agents

Over the past decade, various immunotherapies have been developed and tested clinically in patients with AML. The majority of these approaches have focused on activating the adaptive immune system (such as T cells)[205], although other strategies targeting innate immune cells (such as macrophages) have also been investigated[206–208]. The dominant macrophage checkpoint, CD47, is a 50-kDa cell-surface glycoprotein that provides a potent 'do not eat me' signal by binding with signal regulatory protein α (SIRPα) and is often overexpressed by AML cells as a method of evading innate immune system-mediated phagocytosis[119,209]. Owing to high levels of expression on LSCs, CD47 has become a potential therapeutic target. This potential is supported by the observation that higher levels of CD47 expression are associated with inferior OS in patients with AML[210]. CD47 blockade has also been shown to permit the phagocytosis of AML LSCs and also suppresses their in vivo engraftment in mouse models[210]. Among various agents developed to inhibit the interaction between CD47 and SIRPα, the anti-CD47 antibody magrolimab (previously known as Hu5F9-G4) is the first to be tested clinically[209]. In a phase Ib trial involving patients with AML or MDS, magrolimab plus azacitidine was well tolerated with 53% of previously untreated patients having a CR or CRi with evidence of a reduction in the size of, or elimination of the LSC fraction in most responders. These results suggest that magrolimab plus other cytotoxic agents (such as venetoclax) could plausibly have synergistic antitumour activity[209,211,212]. Clinical trials using magrolimab as well as other CD47 agents such as TTI-622 (NCT03530683), a fully human recombinant fusion protein that inhibits the CD47–SIRPα axis by binding to human CD47 and augmenting phagocytosis of malignant cells, have been evaluated for anticancer efficacy and safety. However, this trial was terminated early owing to the sponsor's business priorities, with no evidence of safety concerns or requests from regulatory authorities at that time. Nonetheless, magrolimab is not currently under ongoing development in AML in light of the findings of futility and an increased risk of treatment-related death from respiratory failure and infection in the phase III ENHANCE-3 trial[213]. These findings are supported by phase III data from patients with higher-risk MDS (ENHANCE, NCT04313881) and *TP53*-mutated AML (ENHANCE-2, NCT04778397). This experience highlights the difficulties in improving the outcomes of patients with AML who are ineligible for intensive chemotherapy.

Venetoclax and CXCR4–CXCL12 pathway suppression

The chemokine receptor CXCR4, which is specific for CXCL12, is expressed in over 23 different tumour types[214,215]. The CXCR4–CXCL12 signal transduction pathway has been recognized as a plausible therapeutic target in cancer owing to the ability to promote the differentiation of cancer stem cells as well as various oncogenic signalling pathways, including MAPK–ERK and SAPK–JNK[216]. Despite many patients receiving venetoclax having a response, most will ultimately have disease relapse owing to the presence of MRD. The extent of post-treatment MRD and LSC survival in the AML bone marrow is influenced by the CXCL12–CXCR4 signalling pathway[217]. The AML bone marrow provides survival signals and promotes the development of features of stemness in therapy-resistant AML cells[218,219]. Interestingly, the LSC-expressed adhesion molecule CD44, which has a crucial role in AML development, has been reported to regulate venetoclax resistance by activating CXCR4–CXCL12 signalling. Preclinical data demonstrate that CXCL12-mediated resistance to venetoclax can be abolished by CD44 knockdown, by *CD44* knockout or using anti-CD44 antibodies[219]. Thus, CD44 can be considered a future target for attempts to restore the sensitivity of AML cells to venetoclax-based regimens. With the emergence of several small-molecule CD44 inhibitors[216], future approaches combining venetoclax-based regimens and CD44 or agents capable of suppressing CXCR4–CXCL12 offer a compelling potential treatment strategy.

BH3 mimetic-based combinations in preclinical investigation

Venetoclax has improved tolerability compared with intensive induction chemotherapy, and response rates to venetoclax are higher compared with SOC therapies without the addition of venetoclax, such as HMAs. Nonetheless, the OS durations of most patients remain modest (median OS ~15 months). Hence, alternative strategies are being explored to overcome this refractoriness. Apart from venetoclax, other BH3 mimetics have emerged as potential inducers of apoptosis and thus provide plausible therapeutic options for patients with AML[89]. The positive clinical outcomes observed with venetoclax plus HMAs and/or LDAC have resulted in further research interest in new combinations of diverse BH3 mimetics with other established therapeutic agents. Daunorubicin is a DNA-intercalating chemotherapeutic agent that can activate sphingomyelin hydrolysis and ceramide synthesis[220,221]. However, BCL-2 overexpression precludes daunorubicin-mediated apoptosis in AML cell lines via degradation of AKT and suppression of XIAP[222]. Removing this BCL-2-induced protection against daunorubicin using either venetoclax or ABT-737 confers synergistic induction of apoptosis and inhibition of the growth of various AML cell lines and samples obtained from patients[93,147,223].

More than 30% of patients with AML have been reported to have *FLT3* mutations. Notably, the presence of *FLT3*-ITDs is related to inferior treatment responsiveness because AML cells can become hyperproliferative and resistant to apoptosis following constitutive induction of *FLT3* signalling[224]. The apoptotic response to midostaurin, a natural product-derived first-generation FLT3 multikinase inhibitor,

is augmented in the presence of venetoclax in *FLT3*-ITD-positive AML cell lines and *FLT3*-ITD-positive primary samples from patients with AML[225]. The presence of a *FLT3*-ITD also upregulates MCL-1 via activation of the AKT signalling pathway and STAT5 (ref. 226). In line with these observations, venetoclax together with *FLT3* suppression abolishes the preservation of MCL-1 and BCL-2, thereby rendering cancer cells more sensitive to apoptosis[204,227,228]. Moreover, the multikinase inhibitor sorafenib, which inhibits FLT3, VEGFR2, PDGFRB, RAF and KIT, induces apoptosis via activation of pro-apoptotic BIM[229] and downregulation of MCL-1 in AML cells[230]. Indeed, sorafenib upregulates BIM, BAX, BAK and BAD, and decreases the expression of MCL-1, XIAP and survivin, resulting in activation of the intrinsic apoptotic pathway[229]. Accordingly, BH3 mimetics can further sensitize AML cells to sorafenib-induced apoptosis via upregulation of BIM, as observed with the combination of the BH3 mimetic navitoclax and sorafenib[93,231]. Despite considerable interest in combining BH3 mimetics with other targeted agents, such efforts are often limited by toxicities[24,89,232], although this issue might be addressable with careful selection of specific agents. For example, the combination of the MCL-1 inhibitor S63845 with the BCL-2 inhibitor S55746 prolongs survival as well as suppressing malignant, but not non-malignant, cell engraftment in mouse xenograft models of AML. This strategy suggests a promising clinical combination therapy with selective activity against AML cells and limited toxicity to non-malignant haematopoietic precursors compared with chemotherapy[232]. Importantly, co-administration of BH3 mimetics also provides the opportunity to attenuate the ability of AML cells to switch their reliance on different semiredundant pro-survival BCL-2 family members, which is a common mechanism of resistance to BCL-2 suppression[104,167,233].

BH3 profiling

There is an urgent need for strategies that enable the personalized use of BH3 mimetics, given the heterogeneous responses observed with these agents. Nowadays, prominent techniques such as BH3 profiling[234], mitochondrial profiling[235] and gene and/or protein expression profiling[236] can effectively serve as essential high-throughput methods to assess susceptibility to BH3 mimetics (Supplementary Information). These methods involve defined ex vivo exposure of samples from patients with AML and require only a minimal number of cells, making them feasible for use with limited patient samples[93,237]. BH3 profiling has been successfully incorporated into clinical studies to assess the extent of mitochondrial priming and predict therapeutic responses in patients with AML and in those with other haematological malignancies[238]. However, certain limitations also exist. These techniques all require specialized expertise and equipment, and their reliance on functional assays might not fully account for the complexities of the tumour microenvironment, such as interactions with stromal cells or immune components[238]. Therefore, although BH3 profiling is a powerful tool, results provided by such investigations should be interpreted alongside those of other diagnostic investigations as well as clinical data to provide a comprehensive understanding of AML biology and to support informed therapeutic decision-making[237].

Conclusions

AML is a complex and heterogeneous haematological malignancy. The outcomes of patients with myeloid leukaemias have improved over the past decade, although a deeper understanding of cancer biology and patient characteristics is required to continue to advance towards more effective treatments. Dysregulation of pro-survival BCL-2 proteins is involved in both the oncogenesis and resistance to treatment of several types of haematological malignancy, including myeloid leukaemias. Substantial gains have been made in our understanding of the dysregulation and targeting of pro-survival BCL-2 family proteins in patients with AML. Innovative small-molecule BH3 mimetics have been effective at inhibiting pro-survival BCL-2 proteins, leading to a balancing rectification of the apoptotic signalling pathway, and, as a result, have shown considerable anticancer activity, both as single agents and in combination with different targeted or conventional agents in patients with myeloid leukaemias. Thus, exploiting the full therapeutic potential of BH3 mimetics is an important goal for improving the outcomes of patients with haematological malignancies. BH3 mimetics activate BAX/BAK-dependent apoptosis but do not require induction of DNA damage signalling pathways, thereby allowing the elimination of non-dividing malignant cells with complex karyotypic alterations, *TP53* defects and inactivation of other critical tumour suppressor genes. In addition to their established pro-apoptotic activities, clinical data from the past 5 years have revealed critical non-canonical anticancer functions of pro-survival BCL-2 proteins such as the modulation of oxidative phosphorylation.

Venetoclax, a potent BCL-2-selective BH3 mimetic is effective when administered in combination with HMAs or LDAC and is FDA-approved for adult patients with newly diagnosed AML who are ≥75 years of age and/or who are ineligible for intensive induction chemotherapy. Indeed, venetoclax has transformed the management of AML and numerous combination regimens either have been or are being developed on the basis of insights into mechanisms of sensitivity and resistance to BH3 mimetics. The ongoing clinical trials testing venetoclax plus other drugs or combinations not only have the potential to improve the efficacy of BH3 mimetics but might also establish the optimal duration of treatment, lead to improved safety profiles and enable the identification of reliable biomarkers of resistance. Considering how technologies have improved the solubility, delivery and efficacy of therapeutics, the initial limitations related to, for example, ABT-737, including a lack of solubility and bioavailability, can now be circumvented and have enabled the emergence of BH3 mimetics as a successful class of therapies. However, further investigations will be needed. For example, owing to difficulties in directly targeting MCL-1 and BCL-xL, novel therapeutic approaches will be required, including sequential/alternating treatment approaches to allow sufficient lengths of time for a complete recovery of the non-malignant tissues and/or the administration of effective co-treatments that enable considerable reductions in the doses of BH3 mimetics. In the future, dependencies on specific BCL-2 family members should be identified in point-of-care settings to improve the selection of both clinically approved and investigational treatments.

Current techniques such as BH3 profiling, mitochondrial profiling, and gene and/or protein expression profiling can serve as high-throughput methods of providing valuable diagnostic information while requiring only limited amounts of material from patients. The widespread availability of molecular profiling will undoubtedly improve the personalization of treatment plans, particularly the selection of firstline regimens, and before adjusting a therapy upon MRD persistence or disease refractoriness or relapse. BH3 profiling has already been incorporated into certain phase Ib/phase II trials as a prognostic marker and/or determinant of response and resistance in patients with AML (NCT03214562 and NCT03471260). Investigating (1) whether the success of venetoclax in combination with LDAC or HMAs can be extended to other combinatorial approaches or strategies

involving other SOC therapies; (2) whether clinical use of BCL-xL and MCL-1 inhibitors in patients with AML might be an effective therapeutic strategy; and (3) whether the presence of BH3 mimetic-related adverse effects, such as thrombocytopenia, which are major source of concern, can be somehow minimized through molecular refinements or novel targeting strategies (such as the development of the PROTAC DT2216) and/or complementary approaches (such as the use of improved prophylaxis) will be important areas of research in the coming years. The multitude of ongoing studies aimed at addressing these questions is expected to yield important clinical insights and drive further improvements in patient outcomes. Research in this area will undoubtedly also benefit from advances in technology that could enable the future development of novel single, combined or dual action therapies, with personalized medicine at the forefront of such efforts.

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References

1. Melino, G. The Sirens' song. *Nature* **412**, 23 (2001).
2. Kroemer, G. et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* **16**, 3–11 (2009).
3. Cotter, T. G. Apoptosis and cancer: the genesis of a research field. *Nat. Rev. Cancer* **9**, 501–507 (2009).
4. Vitale, I. et al. Apoptotic cell death in disease-current understanding of the NCCD 2023. *Cell Death Differ.* **30**, 1097–1154 (2023).
5. Kayagaki, N., Webster, J. D. & Newton, K. Control of cell death in health and disease. *Annu. Rev. Pathol.* **19**, 157–180 (2024).
6. Newton, K., Strasser, A., Kayagaki, N. & Dixit, V. M. Cell death. *Cell* **187**, 235–256 (2024).
7. Gregory, C. D. Hijacking homeostasis: regulation of the tumor microenvironment by apoptosis. *Immunol. Rev.* **319**, 100–127 (2023).
8. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
9. Plati, J., Bucur, O. & Khosravi-Far, R. Apoptotic cell signaling in cancer progression and therapy. *Integr. Biol.* **3**, 279–296 (2011).
10. Xu, G. & Shi, Y. Apoptosis signaling pathways and lymphocyte homeostasis. *Cell Res.* **17**, 759–771 (2007).
11. Mustafa, M. et al. Apoptosis: a comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications. *Cells* **13**, 1838 (2024).
12. Jin, Z. & El-Deiry, W. S. Overview of cell death signaling pathways. *Cancer Biol. Ther.* **4**, 139–163 (2005).
13. Tian, X. et al. Targeting apoptotic pathways for cancer therapy. *J. Clin. Invest.* **134**, e179570 (2024).
14. Wani, A. K. et al. Targeting apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. *Biomolecules* **13**, 194 (2023).
15. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
16. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
17. Mohammad, R. M. et al. Broad targeting of resistance to apoptosis in cancer. *Semin. Cancer Biol.* **35**, S78–S103 (2015).
18. Kaloni, D., Diepstraten, S. T., Strasser, A. & Kelly, G. L. BCL-2 protein family: attractive targets for cancer therapy. *Apoptosis* **28**, 20–38 (2023).
19. US Food and Drug Administration. FDA approves new drug for chronic lymphocytic leukemia in patients with a specific chromosomal abnormality. *fda.gov* https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-chronic-lymphocytic-leukemia-patients-specific-chromosomal-abnormality (2016).
20. US Food and Drug Administration. FDA approves venetoclax for CLL and SLL. *fda.gov* https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-venetoclax-cll-and-sll (2019).
21. US Food and Drug Administration. FDA grants regular approval to venetoclax in combination for untreated acute myeloid leukemia. *fda.gov* https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-regular-approval-venetoclax-combination-untreated-acute-myeloid-leukemia (2020).
22. Warren, C. F. A., Wong-Brown, M. W. & Bowden, N. A. BCL-2 family isoforms in apoptosis and cancer. *Cell Death Dis.* **10**, 177 (2019).
23. Qian, S. et al. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Front. Oncol.* **12**, 985363 (2022).
24. Klener, P., Sovilj, D., Renesova, N. & Andera, L. BH3 mimetics in hematologic malignancies. *Int. J. Mol. Sci.* **22**, 10157 (2021).
25. Gajkowska, B., Motyl, T., Olszewska-Badarczuk, H. & Godlewski, M. M. Expression of BAX in cell nucleus after experimentally induced apoptosis revealed by immunogold and embedment-free electron microscopy. *Cell Biol. Int.* **25**, 725–733 (2001).
26. Nutt, L. K. et al. Bax-mediated Ca^{2+} mobilization promotes cytochrome c release during apoptosis. *J. Biol. Chem.* **277**, 20301–20308 (2002).
27. Roufayel, R., Younes, K., Al-Sabi, A. & Murshid, N. BH3-only proteins Noxa and Puma are key regulators of induced apoptosis. *Life* **12**, 256 (2022).
28. Chen, M. et al. Eltrombopag directly activates BAK and induces apoptosis. *Cell Death Dis.* **14**, 394 (2023).
29. Gonzalo, Ó et al. Study of the Bcl-2 interactome by BiFC reveals differences in the activation mechanism of Bax and Bak. *Cells* **12**, 800 (2023).
30. O'Neill, K. L., Huang, K., Zhang, J., Chen, Y. & Luo, X. Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane. *Genes. Dev.* **30**, 973–988 (2016).
31. Shimony, S., Stahl, M. & Stone, R. M. Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. *Am. J. Hematol.* **98**, 502–526 (2023).
32. Wang, X. et al. The latest edition of WHO and ELN guidance and a new risk model for Chinese acute myeloid leukemia patients. *Front. Med.* **10**, 1165445 (2023).
33. Huber, S. et al. AML classification in the year 2023: how to avoid a Babylonian confusion of languages. *Leukemia* **37**, 1413–1420 (2023).
34. Jung, J. et al. Perspectives on acute myeloid leukemia diagnosis: a comparative analysis of the latest World Health Organization and the International Consensus Classifications. *Leukemia* **37**, 2125–2128 (2023).
35. Salman, H. Comparative analysis of AML classification systems: evaluating the WHO, ICC, and ELN frameworks and their distinctions. *Cancers* **16**, 2915 (2024).
36. Arber, D. A. et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood* **140**, 1200–1228 (2022).
37. Dohner, H. et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* **140**, 1345–1377 (2022).
38. Li, Q. et al. FISH improves risk stratification in acute leukemia by identifying KMT2A abnormal copy number and rearrangements. *Sci. Rep.* **12**, 9585 (2022).
39. Rausch, C. et al. Validation and refinement of the 2022 European LeukemiaNet genetic risk stratification of acute myeloid leukemia. *Leukemia* **37**, 1234–1244 (2023).
40. Matos, S. et al. Screening a targeted panel of genes by next-generation sequencing improves risk stratification in real world patients with acute myeloid leukemia. *Cancers* **14**, 3236 (2022).
41. Krizsán, S. et al. Next-generation sequencing-based genomic profiling of children with acute myeloid leukemia. *J. Mol. Diagn.* **25**, 555–568 (2023).
42. Cassier, P. A., Castets, M., Belhabri, A. & Vey, N. Targeting apoptosis in acute myeloid leukaemia. *Br. J. Cancer* **117**, 1089–1098 (2017).
43. Rücker, F. G. et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood* **119**, 2114–2121 (2012).
44. Qin, G. & Han, X. The prognostic value of TP53 mutations in adult acute myeloid leukemia: a meta-analysis. *Transfus. Med. Hemother.* **50**, 234–244 (2023).
45. Papaemmanuil, E. et al. Genomic classification and prognosis in acute myeloid leukemia. *N. Engl. J. Med.* **374**, 2209–2221 (2016).
46. Mehta, S. V., Shukla, S. N. & Vora, H. H. Overexpression of Bcl2 protein predicts chemoresistance in acute myeloid leukemia: its correlation with FLT3. *Neoplasma* **60**, 666–675 (2013).
47. Andreeff, M. et al. Expression of Bcl-2-related genes in normal and AML progenitors: changes induced by chemotherapy and retinoic acid. *Leukemia* **13**, 1881–1892 (1999).
48. Glaser, S. P. et al. Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia. *Genes. Dev.* **26**, 120–125 (2012).
49. Lachowiez, C. A. et al. Comparison and validation of the 2022 European LeukemiaNet guidelines in acute myeloid leukemia. *Blood Adv.* **7**, 1899–1909 (2023).
50. Karakas, T. et al. High expression of bcl-2 mRNA as a determinant of poor prognosis in acute myeloid leukemia. *Ann. Oncol.* **9**, 159–165 (1998).
51. Letai, A., Sorcinelli, M. D., Beard, C. & Korsmeyer, S. J. Antiapoptotic BCL-2 is required for maintenance of a model leukemia. *Cancer Cell* **6**, 241–249 (2004).
52. Gorombei, P. et al. BCL-2 inhibitor ABT-737 effectively targets leukemia-initiating cells with differential regulation of relevant genes leading to extended survival in a NRAS/BCL-2 mouse model of high risk-myelodysplastic syndrome. *Int. J. Mol. Sci.* **22**, 10658 (2021).
53. Moon, J. H. et al. BCL2 gene polymorphism could predict the treatment outcomes in acute myeloid leukemia patients. *Leuk. Res.* **34**, 166–172 (2010).
54. Saygin, C. & Carraway, H. E. Emerging therapies for acute myeloid leukemia. *J. Hematol. Oncol.* **10**, 93 (2017).
55. Tiribelli, M. et al. BCL-2 expression in AML patients over 65 years: impact on outcomes across different therapeutic strategies. *J. Clin. Med.* **10**, 5096 (2021).
56. Lauria, F. et al. High bcl-2 expression in acute myeloid leukemia cells correlates with CD34 positivity and complete remission rate. *Leukemia* **11**, 2075–2078 (1997).
57. Zhou, J. D. et al. BCL2 overexpression: clinical implication and biological insights in acute myeloid leukemia. *Diagn. Pathol.* **14**, 68 (2019).
58. Lee, C. et al. Transcriptional signatures of the BCL2 family for individualized acute myeloid leukaemia treatment. *Genome Med.* **14**, 111 (2022).
59. Konopleva, M. et al. The anti-apoptotic genes Bcl-X(L) and Bcl-2 are over-expressed and contribute to chemoresistance of non-proliferating leukaemic CD34+ cells. *Br. J. Haematol.* **118**, 521–534 (2002).
60. Wei, Y. et al. Targeting Bcl-2 proteins in acute myeloid leukemia. *Front. Oncol.* **10**, 584974 (2020).

61. Carter, B. Z. et al. Targeting MCL-1 dysregulates cell metabolism and leukemia-stroma interactions and resensitizes acute myeloid leukemia to BCL-2 inhibition. *Haematologica* **107**, 58–76 (2022).
62. Qiu, Y. et al. The GSK3β/Mcl-1 axis is regulated by both FLT3-ITD and Axl and determines the apoptosis induction abilities of FLT3-ITD inhibitors. *Cell Death Discov.* **9**, 44 (2023).
63. Wei, A. H. et al. Targeting MCL-1 in hematologic malignancies: rationale and progress. *Blood Rev.* **44**, 100672 (2020).
64. Chin, H. S. & Fu, N. Y. Physiological functions of Mcl-1: insights from genetic mouse models. *Front. Cell Dev. Biol.* **9**, 704547 (2021).
65. Pei, S. et al. Monocytic subclones confer resistance to venetoclax-based therapy in patients with acute myeloid leukemia. *Cancer Discov.* **10**, 536–551 (2020).
66. Diepstraten, S. T. et al. Putting the STING back into BH3-mimetic drugs for TP53-mutant blood cancers. *Cancer Cell* **42**, 850–868 (2024).
67. Ferrarini, I., Rigo, A. & Visco, C. The mitochondrial anti-apoptotic dependencies of hematologic malignancies: from disease biology to advances in precision medicine. *Haematologica* **107**, 790–802 (2022).
68. Oltersdorf, T. et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
69. Tse, C. et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* **68**, 3421–3428 (2008).
70. Roberts, A. W. et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **30**, 488–496 (2012).
71. Schoenwaelder, S. M. et al. Bcl-xL-inhibitory BH3 mimetics can induce a transient thrombocytopathy that undermines the hemostatic function of platelets. *Blood* **118**, 1663–1674 (2011).
72. Souers, A. J. et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* **19**, 202–208 (2013).
73. Coutre, S. et al. Venetoclax for patients with chronic lymphocytic leukemia who progressed during or after idelalisib therapy. *Blood* **131**, 1704–1711 (2018).
74. US Food and Drug Administration. FDA approves venetoclax for CLL or SLL, with or without 17 P deletion, after one prior therapy *fda.gov* https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-venetoclax-cll-or-sll-or-without-17-p-deletion-after-one-prior-therapy (2018).
75. Garciaz, S., Hospital, M. A., Collette, Y. & Vey, N. Venetoclax resistance in acute myeloid leukemia. *Cancers* **16**, 1091 (2024).
76. Wei, A. H. & Roberts, A. W. BCL2 inhibition: a new paradigm for the treatment of AML and beyond. *Hemasphere* **7**, e912 (2023).
77. Lessene, G., Czabotar, P. E. & Colman, P. M. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug. Discov.* **7**, 989–1000 (2008).
78. Liu, J., Chen, Y., Yu, L. & Yang, L. Mechanisms of venetoclax resistance and solutions. *Front. Oncol.* **12**, 1005659 (2022).
79. Vazquez, R. et al. Venetoclax combination therapy induces deep AML remission with eradication of leukemic stem cells and remodeling of clonal haematopoiesis. *Blood Cancer J.* **11**, 62 (2021).
80. Esparza, S. et al. Venetoclax-induced tumour lysis syndrome in acute myeloid leukaemia. *Br. J. Haematol.* **188**, 173–177 (2020).
81. Thijssen, R. et al. Intact TP-53 function is essential for sustaining durable responses to BH3-mimetic drugs in leukemias. *Blood* **137**, 2721–2735 (2021).
82. Kim, K. et al. Outcomes of TP53-mutant acute myeloid leukemia with decitabine and venetoclax. *Cancer* **127**, 3772–3781 (2021).
83. Lagadinou, E. D. et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell* **12**, 329–341 (2013).
84. Liu, F. et al. Cotargeting of mitochondrial complex I and Bcl-2 shows antileukemic activity against acute myeloid leukemia cells reliant on oxidative phosphorylation. *Cancers* **12**, 2400 (2020).
85. Roca-Portoles, A. et al. Venetoclax causes metabolic reprogramming independent of BCL-2 inhibition. *Cell Death Dis.* **11**, 616 (2020).
86. Lee, J. B. et al. Venetoclax enhances T cell-mediated antileukemic activity by increasing ROS production. *Blood* **138**, 234–245 (2021).
87. Sena, L. A. et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **38**, 225–236 (2013).
88. Gong, X. et al. Venetoclax-based therapy for relapsed or refractory acute myeloid leukemia: latest updates from the 2023 ASH annual meeting. *Exp. Hematol. Oncol.* **13**, 17 (2024).
89. Cerella, C., Dicato, M. & Diederich, M. BH3 mimetics in AML therapy: death and beyond? *Trends Pharmacol. Sci.* **41**, 793–814 (2020).
90. Moujalled, D. M. et al. Acquired mutations in BAX confer resistance to BH3-mimetic therapy in acute myeloid leukemia. *Blood* **141**, 634–644 (2023).
91. Glytsou, C. et al. Mitophagy promotes resistance to BH3 mimetics in acute myeloid leukemia. *Cancer Discov.* **13**, 1656–1677 (2023).
92. Eldeeb, M. et al. A fetal tumor suppressor axis abrogates MLL-fusion-driven acute myeloid leukemia. *Cell Rep.* **42**, 112099 (2023).
93. Parry, N., Wheadon, H. & Copland, M. The application of BH3 mimetics in myeloid leukemias. *Cell Death Dis.* **12**, 222 (2021).
94. Chan, S. M. et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat. Med.* **21**, 178–184 (2015).
95. Konopleva, M. et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. *Cancer Discov.* **6**, 1106–1117 (2016).
96. Othman, J. et al. Molecular MRD is strongly prognostic in patients with NPM1-mutated AML receiving venetoclax-based nonintensive therapy. *Blood* **143**, 336–341 (2024).
97. Griffioen, M. S., de Leeuw, D. C., Janssen, J. J. W. M. & Smit, L. Targeting acute myeloid leukemia with venetoclax; biomarkers for sensitivity and rationale for venetoclax-based combination therapies. *Cancers* **14**, 3456 (2022).
98. Garciaz, S., Saillard, C., Hicheri, Y., Hospital, M. A. & Vey, N. Venetoclax in acute myeloid leukemia: molecular basis, evidences for preclinical and clinical efficacy and strategies to target resistance. *Cancers* **13**, 5608 (2021).
99. Ong, F., Kim, K. & Konopleva, M. Y. Venetoclax resistance: mechanistic insights and future strategies. *Cancer Drug. Resist.* **5**, 380–400 (2022).
100. Roberts, A. W., Wei, A. H. & Huang, D. C. S. BCL2 and MCL1 inhibitors for hematologic malignancies. *Blood* **138**, 1120–1136 (2021).
101. Wang, Z. et al. Efficacy of a novel BCL-xL degrader, DT2216, in preclinical models of JAK2-mutated post-MPN AML. *Blood* https://doi.org/10.1182/blood.2024027117 (2025).
102. Pratz, K. W. et al. Long-term follow-up of VIALE-A: venetoclax and azacitidine in chemotherapy-ineligible untreated acute myeloid leukemia. *Am. J. Hematol.* **99**, 615–624 (2024).
103. Chen, X. et al. Targeting mitochondrial structure sensitizes acute myeloid leukemia to venetoclax treatment. *Cancer Discov.* **9**, 890–909 (2019).
104. Lin, K. H. et al. Targeting MCL-1/BCL-XL forestalls the acquisition of resistance to ABT-199 in acute myeloid leukemia. *Sci. Rep.* **6**, 27696 (2016).
105. Zhang, Q. et al. Activation of RAS/MAPK pathway confers MCL-1 mediated acquired resistance to BCL-2 inhibitor venetoclax in acute myeloid leukemia. *Signal. Transduct. Target. Ther.* **7**, 51 (2022).
106. Ramsey, H. E. et al. A novel MCL1 inhibitor combined with venetoclax rescues venetoclax-resistant acute myelogenous leukemia. *Cancer Discov.* **8**, 1566–1581 (2018).
107. Wang, H., Guo, M., Wei, H. & Chen, Y. Targeting MCL-1 in cancer: current status and perspectives. *J. Hematol. Oncol.* **14**, 67 (2021).
108. Desai, P. et al. A phase 1 first-in-human study of the MCL-1 inhibitor AZD5991 in patients with relapsed/refractory hematologic malignancies. *Clin. Cancer Res.* **30**, 4844–4855 (2024).
109. Zhang, H. et al. Integrated analysis of patient samples identifies biomarkers for venetoclax efficacy and combination strategies in acute myeloid leukemia. *Nat. Cancer* **1**, 826–839 (2020).
110. Bisaillon, R. et al. Genetic characterization of ABT-199 sensitivity in human AML. *Leukemia* **34**, 63–74 (2020).
111. Hanley, J. A. & McNeil, B. J. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* **143**, 29–36 (1982).
112. Samra, B., Konopleva, M., Isidori, A., Daver, N. & DiNardo, C. Venetoclax-based combinations in acute myeloid leukemia: current evidence and future directions. *Front. Oncol.* **10**, 562558 (2020).
113. Nakao, M. et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia* **10**, 1911–1918 (1996).
114. Garciaz, S. & Hospital, M. A. FMS-like tyrosine kinase 3 inhibitors in the treatment of acute myeloid leukemia: an update on the emerging evidence and safety profile. *Onco Targets Ther.* **16**, 31–45 (2023).
115. Stuani, L. et al. Mitochondrial metabolism supports resistance to IDH mutant inhibitors in acute myeloid leukemia. *J. Exp. Med.* **218**, e20200924 (2021).
116. Zhu, R. et al. FLT3 tyrosine kinase inhibitors synergize with BCL-2 inhibition to eliminate FLT3/ITD acute leukemia cells through BIM activation. *Signal. Transduct. Target. Ther.* **6**, 186 (2021).
117. Singh Mali, R. et al. Venetoclax combines synergistically with FLT3 inhibition to effectively target leukemic cells in FLT3-ITD+ acute myeloid leukemia models. *Haematologica* **106**, 1034–1046 (2021).
118. Ma, J. et al. Inhibition of Bcl-2 synergistically enhances the antileukemic activity of midostaurin and gilteritinib in preclinical models of FLT3-mutated acute myeloid leukemia. *Clin. Cancer Res.* **25**, 6815–6826 (2019).
119. Short, N. J. et al. Advances in the treatment of acute myeloid leukemia: new drugs and new challenges. *Cancer Discov.* **10**, 506–525 (2020).
120. Chyla, B. et al. Genetic biomarkers of sensitivity and resistance to venetoclax monotherapy in patients with relapsed acute myeloid leukemia. *Am. J. Hematol.* **93**, E202–E205 (2018).
121. Daver, N. A.-O. X. et al. Venetoclax plus gilteritinib for FLT3-mutated relapsed/refractory acute myeloid leukemia. *J Clin Oncol* (2022).
122. Short, N. J. et al. Azacitidine, venetoclax, and gilteritinib in newly diagnosed and relapsed or refractory FLT3-mutated AML. *J. Clin. Oncol.* **42**, 1499–1508 (2024).
123. Nechiporuk, T. et al. The TP53 apoptotic network is a primary mediator of resistance to BCL2 inhibition in AML cells. *Cancer Discov.* **9**, 910–925 (2019).
124. DiNardo, C. D. et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood* **135**, 791–803 (2020).
125. Fischer, M. Census and evaluation of p53 target genes. *Oncogene* **36**, 3943–3956 (2017).
126. Aubrey, B. J., Kelly, G. L., Janic, A., Herold, M. J. & Strasser, A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ.* **25**, 104–113 (2018).

127. DiNardo, C. D. et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol.* **19**, 216–228 (2018).
128. Shi, X. et al. Nuclear NAD+ homeostasis governed by NMNAT1 prevents apoptosis of acute myeloid leukemia stem cells. *Sci. Adv.* **7**, eabf3895 (2021).
129. Stevens, B. M. et al. Fatty acid metabolism underlies venetoclax resistance in acute myeloid leukemia stem cells. *Nat. Cancer* **1**, 1176–1187 (2020).
130. Sharon, D. et al. Inhibition of mitochondrial translation overcomes venetoclax resistance in AML through activation of the integrated stress response. *Sci. Transl. Med.* **11**, eaax2863 (2019).
131. Thevarajan, I., Zolkiewski, M. & Zolkiewska, A. Human CLPB forms ATP-dependent complexes in the mitochondrial intermembrane space. *Int. J. Biochem. Cell Biol.* **127**, 105841 (2020).
132. Cavdar Koc, E. et al. A new face on apoptosis: death-associated protein 3 and PDCD9 are mitochondrial ribosomal proteins. *FEBS Lett.* **492**, 166–170 (2001).
133. Wang, F., Zhang, D., Li, P. & Gao, Y. Mitochondrial protein translation: emerging roles and clinical significance in disease. *Front. Cell Dev. Biol.* **9**, 675465 (2021).
134. Xu, Y. & Ye, H. Progress in understanding the mechanisms of resistance to BCL-2 inhibitors. *Exp. Hematol. Oncol.* **11**, 31 (2022).
135. Kuusanmäki, H. et al. Phenotype-based drug screening reveals association between venetoclax response and differentiation stage in acute myeloid leukemia. *Haematologica* **105**, 708–720 (2020).
136. Lachowiez, C. A. & DiNardo, C. D. Mutation- and MRD-informed treatments for transplant-ineligible patients. *Hematol. Am. Soc. Hematol. Educ. Program* **2024**, 168–177 (2024).
137. Kadia, T. M. et al. Phase II study of venetoclax added to cladribine plus low-dose cytarabine alternating with 5-azacitidine in older patients with newly diagnosed acute myeloid leukemia. *J. Clin. Oncol.* **40**, 3848–3857 (2022).
138. Rivera, D. et al. Implications of RAS mutational status in subsets of patients with newly diagnosed acute myeloid leukemia across therapy subtypes. *Am. J. Hematol.* **97**, 1599–1606 (2022).
139. Bhatt, S. et al. Reduced mitochondrial apoptotic priming drives resistance to BH3 mimetics in acute myeloid leukemia. *Cancer Cell* **38**, 872–890.e876 (2020).
140. Zhang, X. et al. Not BCL2 mutation but dominant mutation conversation contributed to acquired venetoclax resistance in acute myeloid leukemia. *Biomark. Res.* **9**, 30 (2021).
141. Thomalla, D. et al. Deregulation and epigenetic modification of BCL2-family genes cause resistance to venetoclax in hematologic malignancies. *Blood* **140**, 2113–2126 (2022).
142. Guirguis, A. A. et al. RNA methylation: where to from here for hematologic malignancies? *Exp. Hematol.* **143**, 104694 (2024).
143. Calderon, A., Han, C., Karma, S. & Wang, E. Non-genetic mechanisms of drug resistance in acute leukemias. *Trends Cancer* **10**, 38–51 (2024).
144. Kufe, D. W., Munroe, D., Herrick, D., Egan, E. & Spriggs, D. Effects of 1-beta-D-arabinofuranosylcytosine incorporation on eukaryotic DNA template function. *Mol. Pharmacol.* **26**, 128–134 (1984).
145. Hiddemann, W. Cytosine arabinoside in the treatment of acute myeloid leukemia: the role and place of high-dose regimens. *Ann. Hematol.* **62**, 119–128 (1991).
146. Wang, X. et al. Chemotherapy-induced differential cell cycle arrest in B-cell lymphomas affects their sensitivity to Wee1 inhibition. *Haematologica* **103**, 466–476 (2018).
147. Niu, X. et al. Binding of released bim to Mcl-1 is a mechanism of intrinsic resistance to ABT-199 which can be overcome by combination with daunorubicin or cytarabine in AML cells. *Clin. Cancer Res.* **22**, 4440–4451 (2016).
148. Xie, C. et al. Obatoclax potentiates the cytotoxic effect of cytarabine on acute myeloid leukemia cells by enhancing DNA damage. *Mol. Oncol.* **9**, 409–421 (2015).
149. Wei, A. H. et al. Venetoclax combined with low-dose cytarabine for previously untreated patients with acute myeloid leukemia: results from a phase Ib/II study. *J. Clin. Oncol.* **37**, 1277–1284 (2019).
150. Wei, A. H. et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. *Blood* **135**, 2137–2145 (2020).
151. Gozzo, L. et al. Off-label use of venetoclax in patients with acute myeloid leukemia: single center experience and data from pharmacovigilance database. *Front. Pharmacol.* **12**, 748766 (2021).
152. Wei, A. H. et al. Long-term follow-up of VIALE-C in patients with untreated AML ineligible for intensive chemotherapy. *Blood* **140**, 2754–2756 (2022).
153. Alsouqi, A., Geramita, E. & Im, A. Treatment of acute myeloid leukemia in older adults. *Cancers* https://doi.org/10.3390/cancers15225409 (2023).
154. Baylin, S. B. & Jones, P. A. A decade of exploring the cancer epigenome - biological and translational implications. *Nat. Rev. Cancer* **11**, 726–734 (2011).
155. Robertson, K. D. DNA methylation and human disease. *Nat. Rev. Genet.* **6**, 597–610 (2005).
156. Stomper, J., Rotondo, J. C., Greve, G. & Lübbert, M. Hypomethylating agents (HMA) for the treatment of acute myeloid leukemia and myelodysplastic syndromes: mechanisms of resistance and novel HMA-based therapies. *Leukemia* **35**, 1873–1889 (2021).
157. Tsao, T. et al. Concomitant inhibition of DNA methyltransferase and BCL-2 protein function synergistically induce mitochondrial apoptosis in acute myelogenous leukemia cells. *Ann. Hematol.* **91**, 1861–1870 (2012).
158. Bogenberger, J. M. et al. BCL-2 family proteins as 5-Azacytidine-sensitizing targets and determinants of response in myeloid malignancies. *Leukemia* **28**, 1657–1665 (2014).
159. Bogenberger, J. M. et al. Ex vivo activity of BCL-2 family inhibitors ABT-199 and ABT-737 combined with 5-azacytidine in myeloid malignancies. *Leuk. Lymphoma* **56**, 226–229 (2015).
160. DiNardo, C. D. et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood* **133**, 7–17 (2019).
161. Male, H. J. & Lin, T. L. The approach of HMA plus VEN with or without BMT for all patients with AML. *Hematol. Am. Soc. Hematol. Educ. Program* **2023**, 186–191 (2023).
162. DiNardo, C. D. et al. Azacitidine and Venetoclax in previously untreated acute myeloid leukemia. *N. Engl. J. Med.* **383**, 617–629 (2020).
163. Ucciero, A. et al. Venetoclax with hypomethylating agents in newly diagnosed acute myeloid leukemia: a systematic review and meta-analysis of survival data from real-world studies. *Cancers* **15**, 4618 (2023).
164. Bataller, A. et al. Prognostic risk signature in patients with acute myeloid leukemia treated with hypomethylating agents and venetoclax. *Blood Adv.* **8**, 927–935 (2024).
165. Dohner, H. et al. Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations. *Blood* **144**, 2169–2173 (2024).
166. Wan, Y., Dai, N., Tang, Z. & Fang, H. Small-molecule Mcl-1 inhibitors: emerging anti-tumor agents. *Eur. J. Med. Chem.* **146**, 471–482 (2018).
167. Hormi, M. et al. Pairing MCL-1 inhibition with venetoclax improves therapeutic efficiency of BH3-mimetics in AML. *Eur. J. Haematol.* **105**, 588–596 (2020).
168. Carter, B. Z. et al. Combined inhibition of BCL-2 and MCL-1 overcomes BAX deficiency-mediated resistance of TP53-mutant acute myeloid leukemia to individual BH3 mimetics. *Blood Cancer J.* **13**, 57 (2023).
169. Aid, Z. et al. High caspase 3 and vulnerability to dual BCL2 family inhibition define ETO2::GLIS2 pediatric leukemia. *Leukemia* **37**, 571–579 (2023).
170. Rasmussen, M. L. et al. MCL-1 inhibition by selective BH3 mimetics disrupts mitochondrial dynamics causing loss of viability and functionality of human cardiomyocytes. *iScience* **23**, 101015 (2020).
171. Bolomsky, A. et al. MCL-1 inhibitors, fast-lane development of a new class of anti-cancer agents. *J. Hematol. Oncol.* **13**, 173 (2020).
172. Szlávik, Z. et al. Structure-guided discovery of a selective Mcl-1 inhibitor with cellular activity. *J. Med. Chem.* **62**, 6913–6924 (2019).
173. Yi, X. et al. AMG-176, an Mcl-1 antagonist, shows preclinical efficacy in chronic lymphocytic leukemia. *Clin. Cancer Res.* **26**, 3856–3867 (2020).
174. Hird, A. W. & Tron, A. E. Recent advances in the development of Mcl-1 inhibitors for cancer therapy. *Pharmacol. Ther.* **198**, 59–67 (2019).
175. Tron, A. E. et al. Discovery of Mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. *Nat. Commun.* **9**, 5341 (2018).
176. Yue, X., Chen, Q. & He, J. Combination strategies to overcome resistance to the BCL2 inhibitor venetoclax in hematologic malignancies. *Cancer Cell Int.* **20**, 524 (2020).
177. Pan, R. et al. Synthetic lethality of combined Bcl-2 inhibition and p53 activation in AML: mechanisms and superior antileukemic efficacy. *Cancer Cell* **32**, 748–760 (2017).
178. Bogenberger, J. et al. Combined venetoclax and alvocidib in acute myeloid leukemia. *Oncotarget* **8**, 107206–107222 (2017).
179. Knorr, K. L. et al. MLN4924 induces Noxa upregulation in acute myelogenous leukemia and synergizes with Bcl-2 inhibitors. *Cell Death Differ.* **22**, 2133–2142 (2015).
180. Han, L. et al. Concomitant targeting of BCL2 with venetoclax and MAPK signaling with cobimetinib in acute myeloid leukemia models. *Haematologica* **105**, 697–707 (2020).
181. Zeidner, J. F. et al. Phase 2a study of SLS009, a highly selective CDK9 inhibitor, in combination with azacitidine and venetoclax for relapsed/refractory acute myeloid leukemia after prior venetoclax treatment. *Blood* **144**, 2877–2877 (2024).
182. Lee, D. J. & Zeidner, J. F. Cyclin-dependent kinase (CDK) 9 and 4/6 inhibitors in acute myeloid leukemia (AML): a promising therapeutic approach. *Expert. Opin. Investig. Drugs* **28**, 989–1001 (2019).
183. Zeidner, J. F. & Karp, J. E. Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia. *Leuk. Res.* **39**, 1312–1318 (2015).
184. Cidado, J. et al. AZD4573 is a highly selective CDK9 inhibitor that suppresses MCL-1 and induces apoptosis in hematologic cancer cells. *Clin. Cancer Res.* **26**, 922–934 (2020).
185. Chantkran, W. et al. Interrogation of novel CDK2/9 inhibitor fadraciclib (CYC065) as a potential therapeutic approach for AML. *Cell Death Discov.* **7**, 137 (2021).
186. Baker, A. et al. The CDK9 inhibitor dinaciclib exerts potent apoptotic and antitumor effects in preclinical models of MLL-rearranged acute myeloid leukemia. *Cancer Res.* **76**, 1158–1169 (2016).
187. Wang, X. et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. *Genes. Dev.* **27**, 1351–1364 (2013).
188. Thomas, R. L. et al. Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. *Genes. Dev.* **27**, 1365–1377 (2013).
189. Jonas, B. A. et al. A phase 1b study of venetoclax and alvocidib in patients with relapsed/refractory acute myeloid leukemia. *Hematol. Oncol.* **41**, 743–752 (2023).
190. Short, N. J. et al. A phase 1/2 study of azacitidine, venetoclax and pevonedistat in newly diagnosed secondary AML and in MDS or CMML after failure of hypomethylating agents. *J. Hematol. Oncol.* **16**, 73 (2023).
191. Daver, N. et al. Preliminary results from a phase Ib study evaluating BCL-2 inhibitor venetoclax in combination with MEK inhibitor cobimetinib or MDM2 inhibitor idasanutlin in patients with relapsed or refractory (R/R) AML. *Blood* **130**, 813 (2017).
192. Puglisi, M. et al. A phase I study of the safety, pharmacokinetics and efficacy of navitoclax plus docetaxel in patients with advanced solid tumors. *Future Oncol.* **17**, 2747–27580 (2021).

193. Nor Hisam, N. S. et al. Combination therapy of navitoclax with chemotherapeutic agents in solid tumors and blood cancer: a review of current evidence. *Pharmaceutics* **13**, 1353 (2021).
194. Pullarkat, V. A. et al. Venetoclax and navitoclax in combination with chemotherapy in patients with relapsed or refractory acute lymphoblastic leukemia and lymphoblastic lymphoma. *Cancer Discov.* **11**, 1440–1453 (2021).
195. Marinoff, A. E. et al. Venetoclax in combination with chemotherapy as treatment for pediatric advanced hematologic malignancies. *Pediatr. Blood Cancer* **70**, e30335 (2023).
196. Stahl, M. et al. Clinical and molecular predictors of response and survival following venetoclax therapy in relapsed/refractory AML. *Blood Adv.* **5**, 1552–1564 (2021).
197. Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **387**, 296–299 (1997).
198. Khurana, A. & Shafer, D. A. MDM2 antagonists as a novel treatment option for acute myeloid leukemia: perspectives on the therapeutic potential of idasanutlin (RG7388). *Onco Targets Ther.* **12**, 2903–2910 (2019).
199. Wang, H., Guo, M., Wei, H. & Chen, Y. Targeting p53 pathways: mechanisms, structures, and advances in therapy. *Signal. Transduct. Target. Ther.* **8**, 92 (2023).
200. Zhang, Q., Bykov, V. J. N., Wiman, K. G. & Zawacka-Pankau, J. APR-246 reactivates mutant p53 by targeting cysteines 124 and 277. *Cell Death Dis.* **9**, 439 (2018).
201. Garcia-Manero, G. et al. Eprenetapopt combined with venetoclax and azacitidine in TP53-mutated acute myeloid leukaemia: a phase 1, dose-finding and expansion study. *Lancet Haematol.* **10**, e272–e283 (2023).
202. Perl, A. E. et al. Venetoclax in combination with gilteritinib in patients with relapsed/refractory acute myeloid leukemia: a phase 1b study. *Blood* **134**, 3910 (2019).
203. Yilmaz, M. et al. Quizartinib with decitabine and venetoclax (triplet) is highly active in patients with FLT3-ITD mutated acute myeloid leukemia (AML). *J. Clin. Oncol.* **39**, e19019 (2021).
204. Milnerowicz, S., Maszewska, J., Skowera, P., Stelmach, M. & Lejman, M. AML under the scope: current strategies and treatment involving FLT3 inhibitors and venetoclax-based regimens. *Int. J. Mol. Sci.* **24**, 15849 (2023).
205. Daver, N., Alotaibi, A. S., Bücklein, V. & Subklewe, M. T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments. *Leukemia* **35**, 1843–1863 (2021).
206. Miari, K. E., Guzman, M. L., Wheadon, H. & Williams, M. T. S. Macrophages in acute myeloid leukaemia: significant players in therapy resistance and patient outcomes. *Front. Cell Dev. Biol.* **9**, 692800 (2021).
207. Li, W., Wang, F., Guo, R., Bian, Z. & Song, Y. Targeting macrophages in hematological malignancies: recent advances and future directions. *J. Hematol. Oncol.* **15**, 110 (2022).
208. Perzolli, A., Koedijk, J. B., Zwaan, C. M. & Heidenreich, O. Targeting the innate immune system in pediatric and adult AML. *Leukemia* **38**, 1191–1201 (2024).
209. Chao, M. P. et al. Therapeutic targeting of the macrophage immune checkpoint CD47 in myeloid malignancies. *Front. Oncol.* **9**, 1380 (2019).
210. Majeti, R. et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* **138**, 286–299 (2009).
211. Sallman, D. A. et al. The first-in-class anti-CD47 antibody Hu5F9-G4 is active and well tolerated alone or with azacitidine in AML and MDS patients: initial phase 1b results. *J. Clin. Oncol.* https://doi.org/10.1200/JCO.2019.37.15_suppl.7009 (2019).
212. Donio, M. J. et al. Pre-clinical combination of AO-176, a highly differentiated clinical stage CD47 antibody, with either azacitidine or venetoclax significantly enhances DAMP induction and phagocytosis of acute myeloid leukemia. *Blood* **136**, 9–10 (2020).
213. Daver, N. G. et al. The ENHANCE-3 study: venetoclax and azacitidine plus magrolimab or placebo for untreated AML unfit for intensive therapy. *Blood* https://doi.org/10.1182/blood.2024027506 (2025).
214. Teicher, B. A. & Fricker, S. P. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin. Cancer Res.* **16**, 2927–2931 (2010).
215. López-Gil, J. C., Martin-Hijano, L., Hermann, P. C. & Sainz, B. The CXCL12 crossroads in cancer stem cells and their niche. *Cancers* **13**, 469 (2021).
216. Zhou, W., Guo, S., Liu, M., Burow, M. E. & Wang, G. Targeting CXCL12/CXCR4 axis in tumor immunotherapy. *Curr. Med. Chem.* **26**, 3026–3041 (2019).
217. Krause, D. S. Evading eviction: leukemic stem cell squatters. *Blood* **138**, 1007–1008 (2021).
218. Konopleva, M., Tabe, Y., Zeng, Z. & Andreeff, M. Therapeutic targeting of microenvironmental interactions in leukemia: mechanisms and approaches. *Drug. Resist. Updat.* **12**, 103–113 (2009).
219. Yu, X. et al. CD44 loss of function sensitizes AML cells to the BCL-2 inhibitor venetoclax by decreasing CXCL12-driven survival cues. *Blood* **138**, 1067–1080 (2021).
220. Jaffrézou, J. P. et al. Daunorubicin-induced apoptosis: triggering of ceramide generation through sphingomyelin hydrolysis. *EMBO J.* **15**, 2417–2424 (1996).
221. Al-Aamri, H. M. et al. Time dependent response of daunorubicin on cytotoxicity, cell cycle and DNA repair in acute lymphoblastic leukaemia. *BMC Cancer* **19**, 179 (2019).
222. Kim, Y. H., Park, J. W., Lee, J. Y., Surh, Y. J. & Kwon, T. K. Bcl-2 overexpression prevents daunorubicin-induced apoptosis through inhibition of XIAP and Akt degradation. *Biochem. Pharmacol.* **66**, 1779–1786 (2003).
223. Dariushnejad, H. et al. ABT-737, synergistically enhances daunorubicin-mediated apoptosis in acute myeloid leukemia cell lines. *Adv. Pharm. Bull.* **4**, 185–189 (2014).
224. Daver, N., Schlenk, R. F., Russell, N. H. & Levis, M. J. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia* **33**, 299–312 (2019).
225. Tecik, M. & Adan, A. Therapeutic targeting of FLT3 in acute myeloid leukemia: current status and novel approaches. *Onco Targets Ther.* **15**, 1449–1478 (2022).
226. Yoshimoto, G. et al. FLT3-ITD up-regulates MCL-1 to promote survival of stem cells in acute myeloid leukemia via FLT3-ITD-specific STAT5 activation. *Blood* **114**, 5034–5043 (2009).
227. Seipel, K., Marques, M. A. T., Sidler, C., Mueller, B. U. & Pabst, T. MDM2- and FLT3-inhibitors in the treatment of. *Haematologica* **103**, 1862–1872 (2018).
228. Yilmaz, M. et al. Hypomethylating agent and venetoclax with FLT3 inhibitor "triplet" therapy in older/unfit patients with FLT3 mutated AML. *Blood Cancer J.* **12**, 77 (2022).
229. Zhang, W. et al. Sorafenib induces apoptosis of AML cells via Bim-mediated activation of the intrinsic apoptotic pathway. *Leukemia* **22**, 808–818 (2008).
230. Rahmani, M., Davis, E. M., Bauer, C., Dent, P. & Grant, S. Apoptosis induced by the kinase inhibitor BAY 43-9006 in human leukemia cells involves down-regulation of Mcl-1 through inhibition of translation. *J. Biol. Chem.* **280**, 35217–35227 (2005).
231. Rahmani, M. et al. Inhibition of Bcl-2 antiapoptotic members by obatoclax potently enhances sorafenib-induced apoptosis in human myeloid leukemia cells through a Bim-dependent process. *Blood* **119**, 6089–6098 (2012).
232. Moujalled, D. M. et al. Combining BH3-mimetics to target both BCL-2 and MCL1 has potent activity in pre-clinical models of acute myeloid leukemia. *Leukemia* **33**, 905–917 (2019).
233. Lin, V. S., Xu, Z. F., Huang, D. C. S. & Thijssen, R. BH3 mimetics for the treatment of B-cell malignancies-insights and lessons from the clinic. *Cancers* **12**, 3353 (2020).
234. Olesinski, E. A. et al. BH3 profiling identifies BCL-2 dependence in adult patients with early T-cell progenitor acute lymphoblastic leukemia. *Blood Adv.* **7**, 2917–2923 (2023).
235. Ishizawa, J. et al. Mitochondrial profiling of acute myeloid leukemia in the assessment of response to apoptosis modulating drugs. *PLoS ONE* **10**, e0138377 (2015).
236. Soderquist, R. S. et al. Systematic mapping of BCL-2 gene dependencies in cancer reveals molecular determinants of BH3 mimetic sensitivity. *Nat. Commun.* **9**, 3513 (2018).
237. Pacchiardi, K. et al. Prospective feasibility of a minimal BH3 profiling assay in acute myeloid leukemia. *Cytom. B* **108**, 86–94 (2025).
238. Iyer, P., Jasdanwala, S. S., Wang, Y., Bhatia, K. & Bhatt, S. Decoding acute myeloid leukemia: a clinician's guide to functional profiling. *Diagnostics* **14**, 2560 (2024).
239. Daver, N. A.-O. X. et al. Venetoclax and idasanutlin in relapsed/refractory AML: a nonrandomized, open-label phase 1b trial. *Blood* **141**, 1265–1276 (2023).
240. Campos, L. et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood* **81**, 3091–3096 (1993).
241. Keith, F. J., Bradbury, D. A., Zhu, Y. M. & Russell, N. H. Inhibition of bcl-2 with antisense oligonucleotides induces apoptosis and increases the sensitivity of AML blasts to Ara-C. *Leukemia* **9**, 131–138 (1995).
242. Bensi, L. et al. Bcl-2 oncoprotein expression in acute myeloid leukemia. *Haematologica* **80**, 98–102 (1995).
243. Wang, J. L. et al. Cell permeable Bcl-2 binding peptides: a chemical approach to apoptosis induction in tumor cells. *Cancer Res.* **60**, 1498–1502 (2000).
244. Wang, J. L. et al. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proc. Natl Acad. Sci. USA* **97**, 7124–7129 (2000).
245. Pan, R. et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. *Cancer Discov.* **4**, 362–375 (2014).
246. Venetoclax receives 3rd breakthrough therapy designation from the FDA for the combination treatment of patients with untreated acute myeloid leukemia not eligible for standard induction chemotherapy. *AbbVie* https://news.abbvie.com/2016-01-28-Venetoclax-Receives-3rd-Breakthrough-Therapy-Designation-from-the-FDA-for-the-Combination-Treatment-of-Patients-with-Untreated-Acute-Myeloid-Leukemia-not-Eligible-for-Standard-Induction-Chemotherapy (2016).

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Author contributions

A.G., E.W., H.Y.L., D.J.J.T., M.J.M., J.D.G., C.D.D. and A.P.K. researched data for the manuscript, A.G., E.W., H.Y.L., D.J.J.T., A.J.I., Y.G., C.E.L., W.S., C.G., L.S., T.Y., T.Y.Z., V.E.K., B.D.S., T.M., M.J.M., J.D.G., C.D.N. and A.P.K. wrote the manuscript, and all authors made a significant contribution to discussions of content and edited and/or reviewed the manuscript before submission.

Competing interests

B.D.S. is a consultant and adviser of Servier. M.K. is a consultant of AbbVie, Adaptive, AmMax, Curis, Janssen, Kyowa Kirin, Menarini/Stemline Therapeutics, Novartis, Sanofi Aventis, Servier and Vincerx and is an adviser of AbbVie, Auxenion GmbH, Dark Blue Therapeutics, Legend, MEI Pharma, Menarini/Stemline Therapeutics, Novartis and Syndax and receives research funding from AbbVie, Janssen and Klondike Biopharma. C.D.D. is a consultant and/or adviser of Abbvie, Astellas, AstraZeneca, BMS, Daiichi Sankyo, GenMab, GSK, Rigel, Ryvu, Schrodinger, Servier and Solu Therapeutics and has received research funding from Abbvie, Astex, Beigene, BMS, Jazz, ImmuneOnc, Remix, Servier, Schrodinger and Systimmune. A.P.K. serves on the advisory board for AUM Biosciences. The other authors declare no competing interests.

Additional information

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[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy. [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. [3]Department of Medicine, Harvard Medical School, Boston, MA, USA. [4]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore. [5]NUS Center for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore. [6]School of Chemical and Life Sciences, Singapore Polytechnic, Singapore Polytechnic, Singapore, Singapore. [7]Centre for Haematology, Department of Immunology and Inflammation, Faculty of Medicine, Imperial College London, London, UK. [8]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK. [9]Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA. [10]Division of Hematology/Oncology, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [11]Department of Hematology and Oncology, Medical Center, University of Chicago, Chicago, IL, USA. [12]Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ, USA. [13]Department of Pediatrics, Robert Wood Johnson Medical School, Rutgers University, The State University of New Jersey, New Brunswick, NJ, USA. [14]Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, VU Medical Center, Amsterdam, Netherlands. [15]Department of Biochemistry and Molecular Biology, Kyoto Prefectural University of Medicine, Kyoto, Japan. [16]Department of Medicine, Division of Hematology, Stanford Cancer Institute, Stanford University, Stanford, CA, USA. [17]Department of Medicine, Division of Blood and Marrow Transplantation and Cellular Therapy, Stanford University, Stanford, CA, USA. [18]Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA. [19]OPALE Carnot Institute, Gustave Roussy Cancer Campus, Université Paris-Saclay, Paris, France. [20]Department of Hematology, Gustave Roussy Cancer Campus, Université Paris-Saclay, Paris, France. [21]Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York City, NY, USA. [22]Myeloproliferative Neoplasms Program, Memorial Sloan Kettering Cancer Center, New York City, NY, USA. [23]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.