



Review Article

The Growing Role of the BH3 Mimetic Drug Venetoclax in the Therapy of Acute Myeloid Leukemia

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Abstract. Despite recent progress, acute myeloid leukemia (AML) remains a disease associated with poor prognosis, particularly in older AML patients unfit to tolerate intensive chemotherapy treatment. The development and introduction in the therapy of Venetoclax (VEN), a potent BH3 mimetic targeting the antiapoptotic protein BCL-2, inducing apoptosis of leukemic cells, has shown to be a promising treatment for newly diagnosed, relapsed, and refractory AML patients ineligible for induction chemotherapy. Combination treatments using Venetoclax and a hypomethylating agent (azacitidine or decitabine) or low-intensity chemotherapy have shown in newly diagnosed patients variable response rates, with highly responsive patients with *NPM1*, *IDH1-IDH2*, *TET2*, and *RUNX1* mutations and with scarcely responsive patients with *FLT3*, *TP53* and *ASXL1* mutations, complex karyotypes, and secondary AMLs. Patients with refractory/relapsing disease are less responsive to Venetoclax-based regimens.

However, in the majority of patients, the responses have only a limited duration, and the development of resistance is frequently observed. Therefore, understanding the resistance mechanisms is crucial for developing new strategies and identifying rational drug combination regimens. In this context, two strategies seem to be promising: (i) triplet therapies based on the combined administration of Venetoclax, a hypomethylating agent (or low-dose chemotherapy), and an agent targeting a specific genetic alteration of leukemic cells (i.e., FLT3 inhibitors in *FLT3*-mutated AMLs) or an altered signaling pathway; (ii) combination therapies based on the administration of two BH3 mimetics (i.e., BCL-2 + MCL-1 mimetics) and a hypomethylating agent.

Keywords: BH3; Acute myeloid leukemia.

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Introduction. Apoptosis is an important biological process in health and disease and is regulated by BCL-2 family proteins.

BCL-2 was identified as an oncogene resulting from a translocation between chromosomes 14 and 18 that promotes malignant lymphomagenesis. In the early '90s, BCL-2 was identified as a pro-survival protein preventing apoptotic cell death.

These proteins exert either a pro-apoptotic or anti-apoptotic effect, and their activity balance is crucial for

controlling cell viability.¹ The main activity of these proteins consists in controlling the activation of caspases, the proteolytic enzymes executioner of the apoptotic process.¹ BCL-2 is a member of the anti-apoptotic protein family expressing BCL-2-like homology domains 1-4, which includes, in addition to BCL-2, BCL-X_L, BCL-W, BCL2-A1, and MCL-1.¹ The BCL-2 family protein also comprises some proteins with pro-apoptotic activity, including the pro-apoptotic activators (BID, BIM, and PUMA), the pro-apoptotic effectors

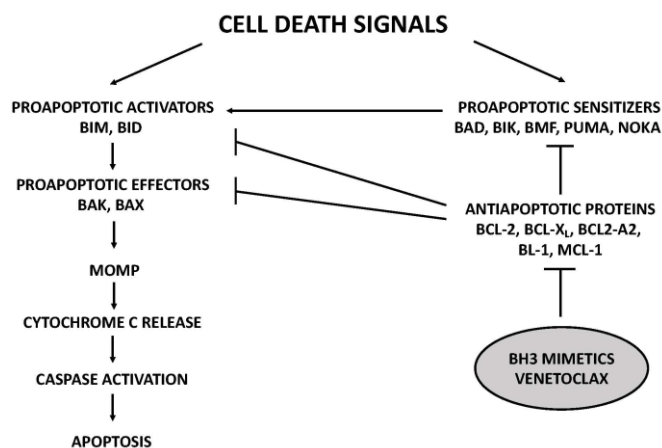


Figure 1. Members of the BCL-2 family subdivided according to their function in proapoptotic sensitizers, proapoptotic activators, proapoptotic effectors and antiapoptotic proteins and their role in the intrinsic apoptotic pathway. The effect of BH3 mimetics, such as Venetoclax, is also shown.

(BAK and BAX), and the sensitizer effector (NOXA).¹ All these proteins form the intrinsic apoptotic pathway. Intrinsic apoptosis is executed in response to cellular damage and most anti-cancer agents (**Figure 1**).

BH domains play a key role in controlling the activity of BCL-2 proteins and the apoptotic process. BH3 domains are expressed by all the members of the BCL-2 family; BAX and BAK proteins express all four BH domains; the activator (BIM and BID) and sensitizer (NOXA) proteins contain only BH3 domains. BH3 domain-mediated interactions between apoptotic and anti-apoptotic BCL-2 family members play an essential role in the control of apoptotic response: thus, the interaction of the sensitizer and anti-apoptotic BCL-2 family members triggers apoptosis by enabling activator proteins, not more bound to anti-apoptotic BCL-2 family members, to interact with BAK/BAX effectors on the outer mitochondrial membrane, resulting in the damage of this membrane with pore formation and membrane permeabilization, the release of cytochrome C from mitochondria, caspase activation and full induction of the apoptotic machinery.¹

Several BH3 mimetic drugs have been synthesized, including venetoclax (VEN, ABT-199), navitoclax (ABT-263), and ABT-737. These drugs selectively bind to the BH3 domain present on anti-apoptotic proteins and, through this mechanism, induce the release of bound proapoptotic proteins and apoptosis. Navitoclax binds to BCL-2, BCL-X_L, and BCL-W and, for this reason, induces *in vivo* platelet lowering; this side effect is not observed with VEN that selectively binds to BH3 expressed on BCL-2, thus sparing platelets.²

Preclinical studies have supported the clinical evaluation of VEN as a potential anti-leukemic drug. These studies have shown that AML bulk cells and leukemic stem cells (LSCs) depend on BCL for their survival and BCL-2 inhibition causes cell death in AML

cells.^{3,4} *In vitro* studies have shown that AML cell lines, primary patient AML samples, and primary murine xenografts are very sensitive to treatment with VEN, with induction of cell death.³ Furthermore, mitochondrial studies using BH3 profiling showed that VEN treatment acts at the mitochondrion level, correlating with leukemic cell cytotoxicity.³ Other studies have provided evidence that VEN's cytotoxic effect is also exerted at the level of LSCs, the cells that initiate and maintain the leukemic process.⁴ In fact, it was shown that LSCs are present in a condition of quiescence, with a low energy state and reactive oxygen species *ROS; these cells are thus dependent on oxidative phosphorylation, whose activity is dependent on oxidative phosphorylation and thus vulnerable to BCL/2 inhibition using VEN.⁴

In initial clinical studies, VEN was evaluated in monotherapy, but due to its limited effects, it was evaluated in association with current anti-leukemic treatments in subsequent studies.

Venetoclax in newly diagnosed AML. The use of venetoclax in the treatment of newly diagnosed AMLs (ND-AMLs) was mainly tested in patients with comorbidities precluding intensive chemotherapy or in those older than 65-70 years. In these studies, VEN was used in association with azacytidine (AZA) or decitabine (DEC), or low-dose Ara C (LDAC). All these regimens have shown a good safety profile and low 30-day mortality (**Table 1**).

Combination with hypomethylating agents. DiNardo and co-workers have explored two groups of ND elderly AMLs, one of 23 patients treated with 7-day AZA+VEN and the other of 22 patients treated with 5-day DEC+VEN: in both treatment arms, a CR+CRi, with incomplete count recovery (CRi), rate of 60% was observed.⁵ Subsequently, the same authors reported the exploration of a large number of patients (145 ND-AML patients at least 65 years old). In this group of patients, two VEN doses were explored, 400 mg and 800 mg, showing better results for the 400 mg dosage at the level of safety profile and therapeutic efficacy; using the 400 mg VEN dosage, a global CR+CRi rate of 73% was observed; patients with high-risk cytogenetics showed a CR+CRi rate of 60%. In addition, the median duration of CR+CRi was 11.3 months, and the mean OS was 17.5 months for all patients and was not reached for those treated with VEN at 400 mg.⁶

The low-dose Ara-C (LDAC) regimen based on LDAC and VEN was explored in a group of 82 older AML patients not eligible for IC (49% of these patients had s-AML and 32% had poor-risk cytogenetic features): 54% of these patients achieved a CR+CRi, with a median OS of 10.1 months and DOR of 8.1 months.⁷ In patients without previous hypomethylating agents (HMA) exposure, CR+CRi was 62%, with a mean overall

Table 1. Clinical studies evaluating Venetoclax-based regimens in newly diagnosed AML patients.

Reference	Trial Phase	Patient Number	Median Age (range)	Combination Therapy	Cytogenetic Risk for ELN	CR+Cri (%)	MRD Negativity (%)	Median OS (months)
DiNardo et al (2019) [8]	Ib/II	72	74 (65-86) 73 (64-86)	HMA (AZA 7d) HMA (DEC 5d)	Intermediate/Adverse Intermediate/Adverse	33-76 60-73	37 29	8.8-NR 14.2-NR
Wei et al (2019) [7]	Ib/II	82	74(63-90)	LDAC	Intermediate/Adverse	54	ND	10.1
Winters et al (2019) [11]	Out of trial	33	72 (33-85)	HMA (AZA 7d)	Adverse	63	28	12.7
DiNardo et al (2020) [73]	Ib/II	58 23	74 (62-87) 73 (66-78)	HMA (AZA 7d) LDAC	Intermediate/Adverse Intermediate/Adverse	69 52	ND	15.1 10.7
DiNardo et al (2020) [8]	III	286	76 (49-91)	HMA (AZA 7d)	Intermediate/Adverse	66	37	14.7
DiNardo et al (2020) [11]	II	85	72 (63-89)	HMA (DEC 10d)	Intermediate/Adverse	81	63	12.4
Pollyea et al (2020) [12]	Ib	84 31	75 (61-90) 72 (65-86)	HMA (AZA 7d) HMA (DEC 5d)	Intermediate/Adverse Intermediate/Adverse	71 74	ND ND	16.4 16.2
Wei et al (2020) [9]	III	143	76 (36-93)	LDAC	Intermediate/Adverse	34	6	8.4
Morsia et al (2020)	Out of trial	44	65 (18-79)	HMA	Intermediate/Adverse	50	NR	17
Chua et al (2020) [16]	II	51	72 (63-80)	5+2 AraC Ida	Intermediate/Adverse	94	80	11.2
DiNardo et al (2021) [17]	II	29	45 (20-65)	FLAG-Ida	Non-APL	90	96	NR 94% (1-yr OS)
Kadia et al (2022) [14]	II	60	68 (57-84)	Clad-LDAC/AZA	Any	93	84	NR

APL indicates acute promyelocytic leukemia; HMA hypomethylating agent; CR complete remission; Cri complete remission with incomplete hematological recovery; OS overall survival; LDAC low-dose adjuvant chemotherapy; ND not done; NR not reached.

survival of 13.5 months.⁷

Two randomized clinical trials showed the superiority of the combined 7-day AZA+VEN compared to 7-day AZA+placebo and LDAC+VEN compared to LDAC+placebo. Thus, a large phase III clinical trial (VIALE-A trial) involved the study of 431 elderly AML patients randomized to receive 7-day AZA+VEN or 7-day AZA+placebo: CR+CRi rate was 66% vs. 28%, and the median OS was 14.7 months vs. 9.6 months in 7-day AZA+VEN and 7-day AZA+placebo, respectively, thus showing a consistent benefit deriving from AZA+VEN administration compared to AZA alone.⁸ In the second trial, 211 elderly AML patients were randomized to receive LDACc+VEN or LDAC+placebo: CR+CRi were 48% and 13% for LDAC+VEN and LDAC+placebo, respectively; median OS was 7.2 months for LDAC+VEN compared to 4.1 months for LDAC+placebo.⁹ Interestingly, 164 patients of the VIALE-A trial with CR+CRi were explored for MRD status as assessed by multiparametric flow cytometry: with a cut-off of $<10^{-3}$, 41% of patients displayed an MRD-negative condition, and 59% were MRD-positive; in MRD-negative patients after a follow-up of 12 months DoR, EFS and OS were not reached, whereas in MRD-positive patients DoR, EFS and OS were 81%, 83% and 94%.¹⁰

Winters et al. have reported a “real-world” experience of VEN with AZA in 33 newly diagnosed AML patients; these patients received the same treatment as another group of AML patients enrolled in phase I/II study.¹¹ The

CR+CRi rate was 63% for out-trial patients, compared to 85% of the trial patients; the mOS was 381 days for out-trial patients, compared to 880 days for trial patients.¹¹ Prior exposure to hypomethylating agents was associated with poor outcomes. On 14 patients out-trial, the MRD was evaluated after treatment: 4/14 were MRD-negative and displayed sustained remission; 10/14 were MRD-positive, and 6 of these patients showed sustained remission, while the 4 other patients relapsed.¹¹

Pollyea et al. reported the results of a phase Ib study of VEN with azacitidine (84 patients) or decitabine (31 patients): the CR+CRi rate was 71% for VEN+AZA and 74% for VEN+DEC; the median duration of CR/Cri was 21.9 months and 15.0 months, and the median OS was 16.4 months and 16.2 months, respectively.¹²

In order to improve the rate and the duration of responses, more intensive treatments were associated with VEN. DiNardo et al. have explored the safety and the therapeutic impact of the administration of DEC 20mg/m² for 10 days and VEN 400 mg daily for induction, followed by DEC for 5 days with oral VEN 400 mg for consolidation in a group of 70 elderly ND-AML patients and 15 untreated s-AML patients: ORR was 89% and 80% for ND-AML and s-AML patients, respectively; OS was 18.1 months for ND-AML and 7.8 months for s-AML.¹³ A more comprehensive report on these patients, including 80 ND-AML and 20 untreated s-AML treated with 10-day DEC+VEN, explored the therapeutic responses in genomic subgroups of patients: patients bearing *NPM1*, *FLT3*, *IDH1/IDH2*, *TP53*,

RUNX1, *N/KRAS* mutations show CR+CRi rates ranging from 70 to 88%; patients with *ASXL1* and *TP53* mutations displayed 55% and 50% of CR+CRi.¹⁴ Median OS values for ND-AML patients were not-reached for *NPM1*-mutant, 29.6 months for *IDH1/IDH2*-mutant, 24.5 months for *FLT3*-mutant, 24.5 months for *ASXL1*-mutant, 16.1 for *RUNX1*-mutant, 12.1 months for *N/KRAS*-mutant and 5.4 months for *TP53*-mutant AMLs. A propensity score-matched analysis of DEC10-VEN vs. intensive chemotherapy stratified by risk of treatment-related mortality (TRM) showed that DEC10-VEN offers better outcomes compared to intensive chemotherapy in terms of CR+CRi rate, lower rate of relapse, and longer overall survival.¹⁵

Combination with Reduced Intensity Regimens. Kadia et al. have evaluated in a group of 60 older (≥ 60 years) AML patients a peculiar therapeutic regimen based on VEN added to cladribine (CLAD) plus LDAC, alternating with AZA; after treatment, 93% of patients had CR+CRi, 84% were MRD-negative, and after 22 months of follow-up the median OS and DFS were not reached.¹⁶ These results support the conclusion that VEN+CLAD/LDAC alternating with VEN+AZA is an effective regimen in older or unfit patients with ND-AML.

A recent study reported the initial evaluation of AZA+VEN in combination with the anti-CD47 mAb magrolimab in a small cohort of 17 ND-AML patients older/unfit or high-risk (14/17).¹⁷ 94% of treated patients achieved a CR+CRi condition, with 55% of MRD negativity. Although these observations involve few patients, the results observed in these patients are promising given their frequent *TP53* mutant status (50% of cases) and high-risk condition.

Combination with Intensive Chemotherapy. Other studies have explored the safety and efficacy of VEN administered with intensive chemotherapy. In this context, the first study by Chua et al. explored VEN in association with a modified intensive chemotherapy protocol (CAVEAT, an attenuated 7+3 regimen consisting of 5 days of cytarabine and 2 days of idarubicin, 5+2) in 51 AML patients with a median age of 72 years; the overall CR+CRi rate among both de novo and secondary AML (sAML) patients was 72%; CR+CRi rate was 97% in ND-AML.¹⁸ After a median follow-up of about 2 years, mOS for the overall study population was 11.2 months; markedly longer mOS was observed among de novo AMLs (31.3 months) compared to sAMLs (6.1 months).¹⁸ The safety profile for the patients receiving up to 400 mg VEN was usually good, with a number of infectious adverse events for their frequency and grade, expected for AML patients of this age undergoing intensive chemotherapy treatment; thus, these results showed that therapy with VEN in

combination with intensive chemotherapy is feasible in an elderly AML population.¹⁸ A phase I/II clinical study enrolled 29 ND-AML patients of a wide range of ages, suitable for intensive chemotherapy, who were treated with FLAG-IDA (fludarabine, cytarabine, G-CSF, and idarubicin) combined with VEN with an ORR of 97%, 90% of CR+CRi, 96% of MRD negativity; 69% of these patients proceeded to allo-HSCT.¹⁹ A recent study reported the results of an expanded cohort of ND-AML patients enrolled in the FLAG-IDA+VEN study; the results on response rate, CR rate, and MRD status confirmed those previously observed.²⁰ Estimated 24-months EFS and OS were 64% and 76%, respectively.²⁰ A post-hoc propensity score-matched analysis of prospective clinical trials in patients of the Texas University supported the conclusion that VEN plus intensive chemotherapy improved event-free survival; however, overall survival did not differ significantly compared to that observed in patients treated with intensive chemotherapy alone.²¹

Other studies have explored VEN in association with standard 7+3 induction chemotherapy. In an initial study, Stone et al. reported the preliminary results on 10 DN-AML patients treated with 7+3 chemotherapy (cytarabine at days 1-7 and daunorubicin at days 2-4) in association with VEN (400 mg was the maximum tolerated dose): the ORR was 100%, and 75% of the patients achieved MRD-negative remissions.^{22,23} Very recently, Wang et al. reported the results of phase II, a single-arm trial enrolling 33 ND-AML patients aged 18-60 years treated with 7+3 induction chemotherapy and VEN at 400 mg.²⁴ After one cycle of therapy, a CR rate of 91% was observed; 97% of these patients in CR had an MRD-negative status; after 11 months of follow-up, 97% of OS and 1-year EFS was 72%.²⁴

Recent studies have explored the combination of VEN with CPX-351; CPX-351 is a dual-drug liposomal encapsulation of cytarabine (ara-C) and daunorubicin at 5:1 molar ratio that is approved for the treatment of newly diagnosed therapy-related AML or AML with myelodysplasia-related changes. Drug synergism / additivity in preclinical studies provided a rationale for combining CPX-351 + VEN clinically. A first study based on only 5 newly diagnosed AML patients with adverse prognosis showed a CR/CRi rate of 80%; 80% of these patients were transitioned to hematopoietic stem cell transplantation.²⁵ The second study explored 31 patients with de novo AML, with a median age of 74 years, predominantly with poor-risk disease; CR+CRi was observed in 57 of patients; MRD-negativity was observed in 75% of patients who achieved CR or CRi; survival data are not yet mature.²⁶

Venetoclax in refractory/relapsing AMLs. About sixty percent of newly diagnosed patients with AML receiving frontline induction/consolidation chemotherapy achieve

Table 2. Clinical studies evaluating Venetoclax-based regimens in relapsing/refractory AML patients.

Reference	Trial Phase	Patient Number	Median Age (range)	Combination Therapy	Cytogenetic Risk for ELN	CR+CRi (%)	MRD Negativity (%)	Median OS (months)
Konopleva et al (2016) [27]	II	32	71 (19-84)	None	Intermediate/Adverse	19%	ND	4.7
DiNardo et al (2018) [28]	I/II	43	68 (25-83)	HMA (AZA) LDAC	Intermediate/Adverse	12%	ND	3
Aldoss et al (2018) [29]	Out of trial	33	62 (19-81)	HMA (AZA or DEC)	Intermediate/Adverse	51	53% in CR+CRi	NR
Aldoss et al (2019) [75]	Out of trial	90	59 (18-81)	HMA (AZA or DEC)	Intermediate/Adverse	46	64	16.6
Morsia et al (2020) [102]	Out of trial	42	65 (18-79)	HMA (AZA or DEC)	Intermediate/Adverse	33	ND	15
Wang et al (2020) [33]	Out of trial	40	63 (20-88)	HMA (AZA) or LDAC	Intermediate/Adverse	23	ND	NR
DiNardo et al (2020) [13]; Maiti et al (2021) [34]	II	83	72 (63-89)	HMA (DEC d10)	Intermediate/Adverse	41	51	6.8
DiNardo et al (2021) [19]	II	23	47 (22-66)	FLAG-Ida	Non-APL	61	79	NR; 68% 1-yr OS
Kim et al (2021) [25]	I/II	26	54 (26-72)	CPX-351	Intermediate/Adverse	46	73	7.1; 39% 1-yr OS

APL indicates acute promyelocytic leukemia; HMA hypomethylating agent; CR complete remission; CRi complete remission with incomplete hematological recovery; OS overall survival; LDAC low-dose adjuvant chemotherapy; ND not done; NR not reached.

a complete response, but 30-40% of these patients' relapse. Relapsed or refractory AMLs (R/R-AMLs) remain a population with very adverse prognosis and necessitate improved therapeutic options. The successful use of Venetoclax as frontline treatment supported the exploration of its possible use for the treatment also of R/R-AML patients (**Table 2**).

Combination with Hypomethylating Agents or Reduced-Intensity Chemotherapy. In an initial phase II study, VEN was tested as a single agent in 32 R/R-AML patients and produced a limited CR+CRi rate of 19%.²⁷

An initial study by DiNardo and co-workers reported the clinical results of treatment based on VEN+HMA or VEN+LDAC AML patients, using protocols like those used in elderly *de novo* AML patients, in 39 R/R-AML patients, showing objective responses in 21% of cases, including patients with *IDH1/2*, *RUNX1* and *TP53* mutations.²⁸ In another initial study, Aldoss et al. reported a "real-world" analysis of 33 R/R-AML patients treated with either VEN+AZA or VEN+DEC, reporting a CR+CRi rate of 51%.²⁹ 53% of CR+CRi responders were MRD-negative by multicolor flow cytometry.²⁹ Higher responses to the treatment were observed among patients with refractory *de novo* AMLs and therapy-related AMLs, compared to those with secondary AMLs.²⁹ The 1-year overall survival for all patients was 53% and was longer for patients with *de novo* than with secondary or therapy-related AMLs.²⁹

Subsequent studies have been performed using two different strategies: (i) some studies explored standard VEN-based regimens using this agent in combination with HMAs or with LDAC; (ii) other studies have

evaluated new VEN-based regimens. The first type of study involved, in most instances, the limited experience of single centers and did not imply controlled clinical trials. In this context, the study involving the largest number of R/R-AML patients (86) was performed by Stahl et al.³⁰ In this study, 86 R/R-AML patients were treated either with VEN+AZA or with VEN+LDAC: VEN+AZA resulted in higher response rates than VEN+LDAC (49% vs. 15%) and in a significantly longer OS (25 vs. 3.9 months). In addition, mutations in *NMP1* were associated with higher response rates, whereas adverse cytogenetics and mutations in *TP53*, *KRAS/NRAS*, and *SF3B1* were associated with worse OS.³⁰

Other studies largely confirmed these findings. Labrador et al. reported the results observed in 51 AML patients in the context of the PATHEMA group who were treated with either VEN+AZA or VEN+DEC, or VEN+LDAC: the frequency of responders (CR+PR) patients was higher for VEN+AZA (32%) compared to VEN+DEC (13%) or VEN+LDAC (0%).³¹ The patients enrolled in this study had very poor risk features and were heavily pre-treated.³¹ Feld et al. reported the results of their single-institution experience in the treatment of 39 R/R-AML patients treated with VEN+HMA; 39% of these patients achieved a CR/CRi, with an OS of 8.1 months; responders to treatment were enriched for *TET2*, *IDH1/IDH2* mutations, while non-responders were associated with *FLT3* and *RAS* mutations.³² Wang reported the results on 40 R/R AML patients treated with VEN-based therapy, showing 22% of CRs; patients with *RUNX1* mutations showed a significantly longer OS; patients with intermediate-risk cytogenetics had better

outcomes compared to those with adverse-risk cytogenetics.³³

Combination with New Therapeutic Regimens. The type 2 studies were based on the development of new therapeutic regimens more appropriate for the treatment of a high-risk AML population, such as R/R-AML. DiNardo et al. have developed a therapeutic regimen involving a longer 10-day administration of decitabine; it was hypothesized that VEN with 10-day DEC could lead to an enhanced therapeutic response in both ND-AMLs and R/R-AMLs.¹³ Thus, patients with R/R-AML received DEC 20mg/m² for 10 days with oral VEN 400 mg daily for induction, followed by DEC for 5 days with daily VEN consolidation. The overall response rate was 62%, with a median overall survival of 7.8 months and a duration of response of 16.8 months.¹³ The most significant rates of response and the longer OS were observed in patients with diploid cytogenetics, *NPM1*, and *IDH1/IDH2* mutations.¹⁴ Maiti et al. recently reported the long-term outcomes of major genomic subgroups of these RR-AML patients treated with DEC10-VEN: CR/CRi rates in patients with mutations of *NPM1* were 68%, of *IDH1/IDH2* 50%, of *FLT3* 42%, of *RUNX1* 45%, of *TP53* 30% and of *KRAS/NRAS* 26%; the longer OS was observed in patients with mutated *IDH1/IDH2* (16.9 months), *RUNX1* (13.7 months) and *NPM1* (12.4 months), but shorter in patients with mutant *ASXL1* (9.0 months), *FLT3* (6.4 months), *KRAS/NRAS* (6.0 months) and *TP53* (4.5 months).¹⁵ Maiti et al. have compared the outcomes of 64 R/R-AML patients treated with DEC10-VEN to a cohort of 130 patients comparable for age and other baseline characteristics treated with standard intensity chemotherapy regimens commonly used for these patients: DEC10-VEN displayed significantly higher responses compared to the IC cohort, including ORR (60% vs. 36%) (MRD negativity assessed by multiparametric flow cytometry (28% vs. 13%) and CR+CRi (19% vs. 6%). Multivariate analysis supported a longer median event-free survival (5.7 vs. 4.5 months) and median overall survival (6.8 months vs. 4.7 months) for DEC10+VEN compared to IC.³⁴

A phase I/II clinical study enrolled 39 R/R-AML patients suitable for intensive chemotherapy treatment who were treated with FLAG-IDA (fludarabine, cytarabine, G-CSF, and idarubicin) combined with VEN: an overall response rate of 70-75% and a CR+CRi rate of 61-75% was observed.¹⁹ After a median follow-up of 12 months, median OS was not reached; 46% of patients proceeded to allogeneic HSCT with a one-year survival post-HSCT of 78%.¹⁹ Wolach et al. have performed a real-world analysis of 24 R/R-AML patients undergoing treatment with FLAG-IDA+VEN and reported a CR+CRi rate of 72% (91% for patients post-HSCT) and with an OS of 50% at 12 months.³⁵ A registry-based study with FLAG-IDA+VEN corroborated the results observed in

the other studies with CR/CRi rate of 69%, MDR negativity in 22% of patients, and 6-months OS of 10.5 months.³⁶

A recent study reported the preliminary results of a phase I/II clinical trial based on the administration of AZA+VEN in association with an anti-CD47 mAb (Magrolimab) in older ND-AMLs and in R/R-AMLs. Phase II of the study involved 8 VEN-naïve R/R-AML patients and 13 VEN-treated R/R-AML patients: in the former, a CR of 63% and an OS not reached were observed; in the latter ones, a CR of 27% and an OS of 3.1 months were observed.¹⁷

Ravandi and co-workers reported the preliminary data on the safety and efficacy of combination therapy based on DEC+VEN+ASTX727 (cytidine deaminase inhibitor cedazuridine) in 13 R/R-AML patients: ORR was 45%, with 30% of CR+CRi and an OS of 7.2 months.³⁷

A recent study evaluated the safety and the clinical efficacy of CPX-351 in combination with VEN, using an approach like that adopted for newly diagnosed AML patients. 26 R/R-AML patients were treated with CPX-351 and VEN, achieving a CR+CRi rate of 46%, with an MRD negativity by flow cytometry of 75% and with an mOS of 7.1 months (in the responding patients, the mOS was 26.9 months) and a 1-year OS of 39%.²⁵ In addition, achieving measurable residual disease (MRD) absence was associated with better OS in these patients, with an mOS of 26.9 months in MRD-negative compared to 2.6 months in MRD-positive patients.²⁵

The treatment of IDH-mutant AMLs changed in the last years due to the introduction in the therapy of IDH1 and IDH2-specific pharmacologic inhibitors. A recent phase III trial in elderly *IDH1*-mutant AML patients, who were ineligible for intensive induction chemotherapy, showed a consistent clinical benefit deriving from the administration of ivosidenib (an inhibitor of mutant *IDH1*) and azacytidine, with a mean OS of 24 months.³⁸ Lachowicz et al. have performed a phase I/II clinical study involving the administration of ivosidenib with VEN, with or without AZA, to a group of either ND-AMLs or s-AMLs or R/R-AMLs.³⁹ The available results are relative to 8 R/R-AML patients: *IDH1* mutation clearance following treatment was achieved in 50% of these patients; at 24 months, 50% of these patients survived and MRD negativity correlated with improved survival.³⁹ A clinical study reported the results of the clinical activity of EC10-VEN on a small cohort of 11 *IDH2*-mutant R/R-AML patients, with an ORR of 82%, a CR-CRi rate of 54%, MRD-negativity as assessed by Flow cytometry of 54% and by PCR of 36%; after a follow-up of 21 months, 1-year OS was 59% and the mean overall survival 14.7 months.⁴⁰ A preliminary report on 7 *IDH2*-mutant R/R-AML patients showed the therapeutic efficacy of the triplet based on the administration of AZA+VEN+Enasidenib: 86% of these

patients displayed CR+CRi, including those with prior exposure to AZA or Enasidenib; the median OS was not reached, and the 1-year OS was 67%.⁴¹

Venetoclax as Maintenance Therapy. The possible role of venetoclax in the maintenance therapy remains undefined, and it remains unclear what is the optimal therapeutic strategy for AML patients responding to venetoclax-based treatments.

In this context, a recent study explored the possible consequences of ceasing venetoclax-based therapy in responding patients. Thus, Chua et al. have explored the effect of ceasing therapy in 13 patients ceasing venetoclax administration in a condition of remission for a minimum of 12 months, compared to 16 comparable patients continuing therapy. The median OS in the stop group of patients was 71.3 months, compared to 50.2 months in the group continuing the treatment.⁴² During the observation period (>5 years), 46% and 69% of patients relapsed in the STOP and CONT groups, respectively.⁴² Although based on a few patients, these observations support the option to stop venetoclax maintenance treatment after achieving at least 12 months of CR.

The benefit of allo-HSCT in patients achieving response to venetoclax-based treatment is uncertain. In this context, Pollyea et al. have explored a group of 119 ND-AML patients who received AZA/VEN as initial therapy: 21 of these patients underwent HSCT, while 31 additional patients were potentially eligible for HSCT but deferred transplantation.⁴³ Median OS was significantly better among patients undergoing HSCT compared to those HSCT eligible not undergoing HSCT.⁴³ Future studies will be required to define at the level of individual AML patients the criteria required for selecting patients for ceasing treatment or for HSCT based on prognostic disease criteria and response evaluations.

Few studies have explored the possible use of VEN as maintenance therapy. Kent et al. showed that VEN administration to 23 AML patients post-ASCT is tolerable without unexpected side effects.⁴⁴ A larger number of patients and a longer follow-up are required to assess the efficacy of VEN as maintenance therapy post-HSCT.⁴⁴ A phase II study based on AZA+VEN administration for AML patients in CR after intensive or low-dose chemotherapy as maintenance therapy: the 1-year OS was 93.8% in the intensive cohort and 53% in the low-dose cohort; of the seven patients with an MRD-positive status, 2 cleared their MRD on AZA+VEN maintenance therapy; MRD-positive patients had a median of molecular relapse-free survival (MRFs) of only 4 months, compared to not reached for MRD-negative patients.^{45,46} These observations suggest that AZA/VEN maintenance is effective and tolerable in patients not immediately eligible for HSCT after

intensive or low-dose chemotherapy induction.^{45,46}

Several ongoing trials are evaluating HMA-VEN after induction chemotherapy (NTC 04102020), after allo-SCT (NCT 04161885), and as MRD-directed therapy after allo-SCT (NCT 04809181).

Outcomes of Selected AML Subtypes Following Venetoclax-Based Therapy.

***NPM1*-mutated AMLs.** *NPM1* mutations occur in about 30% of AML patients; although typically associated with favorable prognosis, the beneficial impact of *NPM1* mutations decreases in the presence of some co-mutations and with increasing age in patients treated with intensive chemotherapy or with HMAs. The studies carried out HMAs in elderly AML patients with *NPM1* mutations showed that the HMA+VEN drug combination is highly effective compared with HMA alone or with intensive chemotherapy. Thus, a retrospective analysis carried out by Lachowicz et al. showed in AML patients of age >65 years treated with HMA+VEN a CR rate of 88%, 1-yr OS of 80%, and mOS not reached; in patients treated with standard induction chemotherapy, a CR rate of 56%, a 1-yr OS of 30% and mOS of 10.8 months and in those treated with HMA alone a CR rate of 28%, a 1-yr OS of 12 months and an mOS of 4.8 months.⁴⁷

In a clinical study involving the treatment of ND AML patients with DEC10-VEN, after a median follow-up of 25.4 months, treatment-naïve *NPM1*-mutated AMLs displayed an mOS not reached, the highest compared to other molecular subgroups.^{13,14} In newly diagnosed AML patients treated with VEN+AZA, a CR rate of 96% was observed, compared to 89% with intensive chemotherapy and 36% with AZA alone; at 4 years, the OS with AZA+VEN was longer than with AZA alone or with chemotherapy.^{8,15}

The efficacy of VEN in *NPM1*-mutated AMLs is further supported by the retrospective analysis of 12 *NPM1*-mutated AMLs: 5 with molecular persistence of *NPM1* mutations and 7 with molecular relapse/progression.⁴⁸ All patients with molecular persistence achieved durable molecular CR following treatment with VEN+low-intensity chemotherapy; 6/7 patients with molecular relapse/progression achieved CR, MRD-negative, after 1-2 cycles of VEN+low-intensity chemotherapy.⁴⁸ These observations suggest a promising efficacy of VEN-based therapy also in high-risk *NPM1*-mutant AML patients.⁴⁸

The reasons for the high sensitivity of *NPM1*-mut AMLs to VEN-based therapies remain to be determined. Studies performed in *de novo* elderly AML patients have shown anti-leukemic activity in about 60-70% of these patients, with *NPM1*-mut AMLs being the most responsive.⁷ One of the mechanisms through which AZA potentiates the pro-apoptotic effects of VEN is related to its capacity to downregulate the expression of MCL-1

and to enhance the expression of pro-apoptotic proteins NOXA and PUMA, thus increasing the dependence of leukemic cells on BCL-2 for their survival.⁴⁹

Another mechanism could be related to the capacity of VEN to target and kill intensive metabolically. Previous studies have shown that LSCs are characterized by a condition of quiescence and low energetic metabolism, mainly maintained by a low rate of oxidative phosphorylation, a process dependent on BCL-2, which can be inhibited by VEN.⁴ LSCs isolated from *de novo* AML patients are uniquely reliant on amino acid metabolism for oxidative phosphorylation and survival;⁵⁰ in cooperation with HMAs, VEN decreases amino acid uptake and, through this mechanism, induces LSC cytotoxicity.⁵¹ In contrast, LSCs isolated from relapsed AML patients are not reliant on amino acid metabolism due to their ability to compensate through two different mechanisms: increased fatty acid metabolism occurring as a consequence of RAS pathway mutations⁵² or increased nicotinamide levels resulting from increased nicotinamide uptake via NAMP transporter and synthesis through amino acid salvage pathway: the increased nicotinamide metabolism activates both amino acid metabolism and fatty acid oxidation driving oxidative phosphorylation.⁵³

Interestingly the preclinical studies evaluating *in vitro* drug sensitivity have shown consistent responsiveness of *NPM1*-mut AML primary leukemic blasts to VEN: VEN displayed an IC₅₀ of 289-486 nM vs. 4558-6539 nM for *NPM1*-mut and *NPM1*-WT specimens, respectively.⁵⁴ However, the sensitivity of *NPM1*-mut AMLs to VEN was heterogeneous, with the FAB (French American British) M1 class of these leukemias being sensitive and FAB M5B class (with monocytic features) being resistant; furthermore, the co-occurrence-in *NPM1*-mut AMLs of *TET2* and *PTPN11* mutations was associated with significantly reduced *in vitro* sensitivity to VEN.⁵⁴ Interestingly, this *in vitro* screening also showed that *RAD21*-mut AMLs are highly sensitive to VEN; this high sensitivity is extended to other mutations of cohesion genes, such as *SMC1A*, *SMC3*, and *STAG2*.⁵⁴

The high sensitivity of *NPM1*-mut AMLs could be related to the high expression of *HOX* genes in these AMLs: in fact, the high expression of *HOXA* genes is a marker of VEN sensitivity in primary AML samples.⁵⁵ These findings were recapitulated by the knockdown of the *FOXM1* transcription factor (*FOXM1* interacts with *NPM1*-mut protein and is vehiculated to the cytoplasm by the mutant *NPM1* protein), which induces sensitization to VEN and a pattern of *HOXA* gene overexpression comparable to that observed in *NPM1*-mut AMLs.⁵⁶

The elevated sensitivity of *NPM1*-mut AMLs could be related to the impaired mitochondrial function observed in these AMLs.⁵⁷

Despite the good initial responses, a significant

proportion of *NPM1*-mut patients treated with VEN+HMA develop resistance and eventually relapse. New drug combinations involving VEN with another drug that could inhibit *NPM1*-mut have been identified to bypass this problem. One of these approaches involves the association of VEN with a drug inhibiting nuclear export. The selective inhibitors of nuclear export, such as Selinexor and eltanexor, make part of a new class of molecules that target exportin-1 (XPO1), a protein essential for the nuclear export of major tumor suppressor proteins and of *NPM1*-mut protein. Preclinical studies using Selinexor have shown that XPO1 inhibition induces nuclear relocation of mutant *NPM1* and reduces *HOX* gene expression, cell differentiation, and growth arrest. Thus, there is a strong rationale for using these drugs to develop new therapeutic strategies for treating *NPM1*-mut AMLs.⁵⁸ Studies in leukemic cell lines showed that VEN response was enhanced by selective inhibitors of nuclear export compounds.⁵⁹ However, patients with *NPM1*-mut AMLs displayed only limited responses to Selinexor, which was also associated with a consistent number of adverse events. These observations support new clinical studies using eltanexor, a second-generation XPO1 inhibitor, inducing fewer adverse events in association with other anti-leukemic drugs, such as VEN.⁶⁰

The other approach implies the association of VEN with a menin inhibitor. Increased expression of *HOX* genes is a specific feature of two AML subsets, including *MLL*-rearranged and *NPM1*-mut AMLs. Preclinical studies have shown that menin inhibitors inhibit AMLs overexpressing *HOX* genes: these inhibitors block the interaction between menin and *MLL*, thereby altering the binding of *MLL* to a subset of its target genes, including *MEIS1*, a cofactor of *HOX* transcription factors.⁶¹⁻⁶² Phase III trials have shown that monotherapy with menin inhibitors is well tolerated and has achieved objective responses in patients previously treated with relapsed/refractory AML harboring *MLL* rearrangements or *NPM1* mutations. Furthermore, in mouse models of *NPM1-FLT3-ITD* AMLs, VEN+menin inhibitor exerted a more potent anti-tumor effect compared to menin inhibitor alone, eliminating leukemic cells, including LSCs; these effects involve a decreased expression of BCL-2 and BCL-X_L.⁶³ These results were confirmed in another recent study, thus supporting the development of clinical trials involving VEN+menin inhibitors for the treatment of *MLL1*-rearranged or *NPM1*-mut AMLs.⁶⁴

IDH1-IDH2 mutated AMLs. After *NPM1*-mutated AMLs, *IDH1*-2-mutated AMLs exhibit the most favorable outcomes following therapy with VEN+HMA. *IDH1*-mutant AML patients in frontline therapy display a CR rate ranging from 75% to 100%, with a median overall survival (mOS) not reaching and 1-yr OS of 72%,

while *IDH2*-mutant patients show a CR rate ranging from 75 to 86% with an mOS of 29.6 months.^{65,66}

In the salvage setting, the outcomes of *IDH1-2*-mutated AML patients treated with VEN-HMA were inferior, with a CR rate of 33% and 1-yr mOS of 66%; in *IDH1*-mutant AMLs and a CR rate of 54%, with an mOS of 14.7 months in *IDH2*-mutant AMLs.⁶⁵

Similarly, in the DE10-VEN study, a very high response rate was observed among patients with *IDH1-2* mutations.¹⁴ In treatment-naïve patients, an ORR of 92%, with an mOS of 29.6 months and mRFS not reached, and an MRD-negative status in 90% were observed; in previously treated patients, the ORR was 71%, with a mOS of 16.9 months and a rate of MRD negativity of 70%.¹⁴

A pooled analysis of the results reported in the phase Ib study⁶ and in the randomized phase III study⁸ was recently published and showed for *IDH1-2*-mutated AML patients a CR rate of 79% for VEN-AZA compared to 11% for AZA alone, and a median duration of remission of 29.5 months for the VEN-AZA group compared to 9.5 months for AZA alone, and a mOS of 24.5 months for VEN-AZA compared to 6.2 months for AZA alone.⁶⁷ In *IDH1-1* wild-type AML patients, CR rates were 63% with VEN-AZA and 31% with AZA alone, the mean duration of remission was 27.5 months for VEN-AZA and 10.3 months for AZA alone, and mOS 12.3 months for VEN-AZA and 10.1 months for AZA alone;⁶⁷ in *IDH1*-mutated patients, CR rates were 66.7% vs. 9.1% and mOS 15.2 months vs. 2.2 months; in *IDH2*-mutant patients, CR rates were 86% vs. 11% and mOS not reached for VEN-AZA vs. 11%.⁶⁷ *IDH1-2* wild-type AML with poor cytogenetics treated with VEN-AZA had inferior outcomes compared to equivalent patients with *IDH1-2* mutations; *IDH1-2*-mutated patients had a better outcome regardless of cytogenetic risk.⁶⁷

Analysis of the genetic determinants affecting the response of *IDH1-2*-mutated AMLs to VEN-HMA supported the conclusion that *IDH1-2* and *NPM1* co-mutations tend to have favorable outcomes, whereas *IDH1-1* and RAS pathway or *TP53* co-mutations have lower outcomes.⁶⁶

These observations strongly supported the development of clinical studies based on the combined administration of an HMA compound with VEN and a selective IDH inhibitor. In this context, it is important to underline that recent studies have supported both the safety and efficacy of combining a hypomethylating agent with an IDH inhibitor. Thus, a recent phase III trial in elderly *IDH1*-mutant AML patients, who are ineligible for intensive induction chemotherapy, showed a consistent clinical benefit deriving from the combined administration of ivosidenib (an inhibitor of mutant *IDH1*) and azacitidine, with a mOS of 24 months.⁶⁸ Recently, Botton et al. presented the findings of the

molecular analyses on newly diagnosed *IDH1*-mut AML patients enrolled in the above-mentioned AGILE phase III study comparing AZA+ivosidenib to AZA+placebo: 58 patients received AZA+IVO and 62 AZA+placebo.⁶⁹ *DNMT3A*, *SRSF2*, and *RUNX1* were the most frequent co-mutated genes in these patients; mutations in *DNMT3A*, *RUNX1*, *SRSF2*, and RTK pathway mutations were associated with improved outcomes.⁶⁹ Furthermore, a phase Ib and II study showed that combination therapy based on enosidenib plus azacitidine was well tolerated and significantly improved overall response rates compared with AZA alone, thus supporting that this therapeutic regimen may improve outcomes for elderly AML patients with *IDH2* mutant AML.⁷⁰

It is important to note that it was shown that about 90% of newly diagnosed AML patients with *IDH1-2* mutations achieve a MRD-negative status, as assessed by multiparametric flow cytometry; however, only 52% of these patients achieved a molecular MRD-negative condition as assessed by molecular evaluation of residual *IDH1-2* mutations.⁶⁶ There is hope that the triplet therapy VEN+IDH inhibitor+ HMA may augment the fraction of patients achieving a molecular MRD negativity.

Early results of a phase Ib/II study explored the triplet combination with ivosidenib, VEN with or without AZA in *IDH1*-mutated AML in newly diagnosed and R/R patients, reporting CR rates of 100% and 67%, respectively; the 1-yr overall survival in newly diagnosed AML was 100% and that in R/R patients 50%.⁷¹ Lachowicz et al. recently reported the preliminary results of a phase Ib/II clinical study involving the administration of ivosidenib with VEN, with or without AZA, to a group of either *de novo* AMLs or s-AMLs or RR-AMLs; the available results were relative only to 8 R/R AML patients.⁶⁶ *IDH1* mutation clearance following treatment was achieved in 50% of these patients; at 24 months, 50% of these patients survived, and MRD-negativity correlated with improved survival.⁷² In patients exhibiting massive leukemic cell lysis following treatment, the median OS was 42 months.⁷² All patients relapsing after *IDH1* mutant clearance showed no *IDH1*-mutant relapse.⁷²

Venugopal and co-workers explored the safety and efficacy of enasidenib (a specific *IDH2*-mutant inhibitor) and azacitidine in 26 AML patients: 7 newly diagnosed and 19 relapsed/refractory; the CR rate was 100% for newly diagnosed patients and 58% for R/R AMLs.⁷³ Interestingly, 7 R/R patients received the triplet ENA+AZA+Ven and showed a trend toward a better mOS than those treated with ENA+AZA.⁷³

Preliminary results of a phase Ib/II clinical trial (Enaven-AML trial) explored enasidenib in combination with VEN in a group of AML patients previously treated with at least two lines of treatment and mostly with relapsing or refractory disease.⁷⁴ A CR rate of 55% was observed, and all responders remained in remission

during the study.⁷⁴

Since *IDH* mutations induced inhibition of the TET2 enzyme, it seemed interesting to explore the effect of VEN-based therapies in *TET2*-mutated AMLs. The presence of *TET2* mutations seems to be associated with high responsiveness to VEN, particularly in relapsed/refractory AML patients, with complete remission rates of up to 86% compared to 39% in patients with wild-type *TET2*.⁷⁵

The molecular mechanism responsible for the high responsiveness of *IDH1/IDH2*-mutated AMLs to VEN remains largely undetermined and implies the dependency of these leukemias on BCL-2. It was suggested that this marked sensitivity to VEN could be related to a decrease of cytochrome C oxidase activity induced by enhanced levels of 2-hydroxyglutarate (2HG) present in *IDH2*-mutated AMLs, lowering the threshold for VEN-induced apoptosis.⁷⁶

FLT3-mutated AMLs. Several studies have retrospectively analyzed the response of *FLT3*-mutated AMLs to VEN-based treatments. In an initial phase Ib study, treatment with VEN and AZA or DEC showed a 72% CR rate, with a median duration of remission (mDoR) of 11 months among *FLT3*-mutated AMLs.⁶ These findings were validated and extended in the phase III VIALE-A trial showing that patients with *FLT3* mutations had a better response to the treatment with AZA+VEN compared to AZA+placebo: CR rates of 72.4% vs. 36.4% and mOS of 13.6 months vs. 8.6 months were observed.^{12,77}

A recent study further analyzed *FLT3*-mutated AML patients included in these studies for their response to VEN-AZA-based therapy compared to AZA alone.⁷⁸ The CR rates were 67% for the VEN-AZA group, compared to 36% for the AZA alone group; the mDoR was 18.4 months and mOS was 14.7 months for the VEN-AZA group to 13.4 months and 10.1 months, respectively for AZA alone group.⁷⁸ In patients treated with VEN+AZA, the responses were higher for *FLT3-TKD* than *FLT3-ITD*-mutated patients: CR rates 77% vs. 63%; mOS 19.2 months vs. 9.2 months, respectively.⁷⁸

A high rate of responses was observed among patients treated with ten-day decitabine with VEN (DEC10-VEN).¹⁴ In newly diagnosed AMLs, the CR rate to DEC10-VEN was 86%, with an MRD-negativity of 80% and a mOS of 24.5 months; in previously treated AML patients, the CR rate was 42%, with an MRD negativity of 70% and a mOS of 6.4 months.¹⁸ Among *FLT3-NPM1* co-mutated patients not previously treated, CR rates were 88%, MRD negativity 92%, and mOS not reached; in *FLT3-ITD* co-mutated patients previously treated, CR rates were 56%, with MRD negativity in 86% and mOS of 12.4 months.¹⁸

It is important to note that DiNardo et al. explored the molecular patterns of response and treatment failure in

58 AML patients treated with VEN+HMA and in 23 AML patients treated with VEN+LDAC.⁷⁹ Primary and adaptive resistance to venetoclax was associated with the enrichment or acquisition of leukemic clones activating signaling pathways, such as *FLT3* or *RAS*, or biallelically affecting the *TP53* gene.⁷⁹ Particularly, serial molecular analyses showed more frequently an increased *FLT3* clonal burden in some patients at the time of disease progression and, more rarely, the acquisition of new *FLT3-ITD* mutations; furthermore, single-cell sequencing studies showed in some instances, the clearance of some *FLT3-ITD*-bearing subclones and the outgrowth of a resistant *FLT3-ITD* subclone.⁷⁹ Studies in experimental models showed that *FLT3-ITD* causes dual resistance to both VEN and LDAC that can be bypassed by the concomitant addition of VEN and an *FLT3* inhibitor.⁷⁹

Studies in preclinical models of *FLT3-ITD* AML showed that *FLT3* inhibition (using *FLT3* inhibitors) combined with VEN showed a pronounced anti-tumor activity and strongly supported clinical trials using this drug combination.^{80,81} In addition, *BCL2* inhibitors and *FLT3* inhibitors synergize to induce the elimination of *FLT3-ITD* mutated leukemic cells through BIM activation.⁸²

At the clinical level, the association of gilteritinib, a potent *FLT3* inhibitor, with VEN showed a robust anti-leukemic activity with a CR rate of 86%, molecular MRD clearance in 69% of responders, and mOS of 10.5 months.⁸³ Daver and coworkers reported the study of 56 R/R AML patients with *FLT3* mutations (64% had received prior *FLT3* inhibitor therapy) treated with VEN-gilteritinib.⁸⁴ 75% of these patients achieved a CR following treatment, which had a similar rate in patients with or without prior *FLT3* inhibitor therapy (80% vs. 67%, respectively).⁸⁴ The mOS was 10.0 months, and molecular MRD negativity was reached in 60% of patients achieving a CR.⁸⁴

Recent studies have explored the triplet HMA, VEN, and *FLT3* inhibitors. Thus, in a recently published clinical study, a small cohort of older/unfit patients with newly diagnosed *FLT3* mutated AMLs was treated with a triplet regimen (HMA, VEN, and *FLT3* inhibitor): 11 of the 12 patients treated with this therapeutic regimen achieved CR, with MRD-negativity in 91% of these responding patients.⁸⁵ In a more recent study, the same authors reported a retrospective analysis of 87 older/unfit newly diagnosed AML patients with *FLT3*-mutated AMLs treated with this triplet regimen (VEN+decitabine+*FLT3* inhibitor) compared to 60 similar patients treated with low-intensity chemotherapy and an *FLT3* inhibitor.⁸⁶ This study showed that triplet therapy was associated with better clinical responses than doublet therapy: CR rate 67% vs. 32%, molecular MRD negativity 96% vs. 54%. After a median follow-up of 24 months, patients receiving the triplet regimen

displayed a longer mOS than those treated with the doublet regimen: not reached vs. 9.5 months, respectively.⁸⁶

Another recent study explored the triplet drug combination based on quizartinib (a second-generation FLT3 inhibitor), VEN and DEC in newly diagnosed and R/R patients with *FLT3-ITD* mutated AML.⁸⁷ Preclinical studies supported the rationale of the association of VEN with quizartinib.⁸⁰ In this preliminary report, the results on 13 R/R AML patients and 4 newly diagnosed AML patients were shown.⁸⁷ In the 13 R/R AML patients (85% of these patients received prior treatment with at least one FLT3 inhibitor), 69% of CRs were observed, with 4/9 of these patients achieving a molecular MRD negativity; in the four newly diagnosed AML patients, 100% of CRs were observed, with 100% of molecular MRD negativity.⁸⁷ With a follow-up of 7.2 months, the mOS was not reached in the frontline cohort and was 7.1 months in the R/R AML cohort.⁸⁷

A phase I/II study explored the triplet combination based on azacitidine, venetoclax and gilteritinib for *FLT3*-mutated AML patients with *de novo* (11 patients) or refractory/relapsing (15 patients) disease.⁸⁶ In ND AML patients, 82% of CRs were observed, with 18% of patients proceeding to HSCT; in R/R AML patients, 27% of CRs were observed.⁸⁸

New drug combinations involving VEN are under preclinical evaluation for the therapy of *FLT3*-mutated AMLs. Thus, VEN synergizes with the AXL/MER tyrosine kinase inhibitor ONO-7475 in inducing the killing of *FLT3-ITD*-mutated AML cells.⁸¹ ONO-7475 even alone exerts an inhibitory effect on *FLT3-ITD* leukemic cells, related to its capacity to inhibit ERK phosphorylation and expression of the anti-apoptotic protein MCL1.⁸⁹ Importantly, the drug combination VEN+ONO-7475 is able to overcome VEN resistance of *FLT3-ITD*-mutated AML cells.⁸⁹

Janssen and coworkers screened *in vitro* 654 anti-leukemic compounds in combination with VEN in 31 primary samples of high-risk AMLs and observed that gilteritinib exhibited the highest synergy with VEN in WT *FLT3* AMLs.⁹⁰ Importantly, the VEN+gilteritinib was active in inducing apoptosis of leukemic cell lines and primary AML cells resistant to VEN+AZA.⁹⁰ Mechanistically, the VEN+gilteritinib combination decreased phosphorylation of ERK and GSK3B via combined inhibition of *FLT3* and AXL, mediating suppression of the MCL1 antiapoptotic protein through induction of its proteasomal degradation.⁹⁰ These observations support the evaluation of VEN+gilteritinib as a potential therapeutic regimen for high-risk AML patients with *FLT3* WT.

Potential resistance mechanisms of *FLT3*-mutated AMLs may be represented by the inactivation of BAX expression mediated by constitutive *FLT3* activation and by enhanced expression of MCL-1 induced by *FLT3*-

ITD. Two preclinical studies have explored mechanisms driving the synergy between VEN and an *FLT3* inhibitor.^{82,91} Thus, it was shown that treatment with A *FLT3* inhibitor (midostaurin or gilteritinib) alone or in combination with VEN elicited a downmodulation of MCL-1 expression, seeming induced by simultaneous suppression of multiple signaling pathways, including STAT5, RAS-MAPK and PI3K-AKT.^{82,90} The effect of the two drugs was complementary: gilteritinib treatment reduced the binding of BIM to MCL-1 and increased the binding of BIM to BCL-2, while VEN increased the binding of BIM to MCL-1 but inhibited the binding of BIM to BCL-2.^{82,91} Importantly, co-treatment with VEN and gilteritinib increased the binding of BIM to BAX without increasing the binding of BIM to other BCL-2 anti-apoptotic proteins.⁸² Thus, the combination therapy decreased the binding of BIM to both BCL-2 and MCL-1, liberating BIM for interaction with BAX and induction of apoptosis.

AMLs with Spliceosome Mutations. A retrospective analysis at a single institution (The University of Texas, MD Anderson Cancer Center) analyzed 39 AML patients with spliceosome mutations and 80 WT AML patients for these mutations and treated with VEN in combination with hypomethylating agents.⁹² No significant difference in overall survival was observed between patients with spliceosome mutations and those without these mutations (35 vs. 14 months, respectively); 1-year overall survival was 63% in the spliceosome cohort and 53% in the WT cohort.⁹² For the various subtypes of spliceosome mutations, the OS for patients with *SRSF2*, *SF3B1*, and *U2AF1* was not reached at 35 months and 8 months, respectively.⁹² *IDH2* mutations were enriched in patients with *SRSF2* mutations and were associated with favorable outcomes; *RAS* mutations were enriched in patients with *U2AF1* mutations and were associated with poor outcomes.⁹²

TP53-mutated AMLs. The presence of *TP53* mutations was associated with resistance to VEN. Preclinical studies have shown that the *TP53* apoptotic network is a main mediator of resistance to BCL2 inhibition in AML cells.⁹³ In addition, knockout gene experiments have shown that the inactivation of genes such as *TP53*, *BAX*, and *PMAIP1* results in venetoclax resistance in AML cell lines.⁹³

The outcomes of AML patients with *TP53* mutations are poor, with median overall survival in newly diagnosed AML patients of about 5-10 months and in salvage settings of about 5 months.^{79,94} However, in the DEC10+Ven trial, *TP53*-mutant AML patients displayed a lower rate of CRs compared to patients without these mutations (35% vs. 57%, respectively) and a lower rate of MRD negativity (19% vs. 52%) and a markedly lower mOS (5.2 months vs. 19.4 months).⁹⁵

Pollyea et al. recently reported the retrospective analysis of the high-risk AML patients reported in the phase Ib⁶ and phase III⁸ studies involving VEN+AZA administration to older, newly diagnosed AML patients. Particularly, the outcomes of poor risk cytogenetics+*TP53*-mut AML patients were compared to those of poor risk cytogenetics+*TP53*-WT: in poor risk cytogenetics+*TP53*-mut patients, VEN+AZA improved remission rates but not DoR and mOS compared to AZA alone; in poor risk cytogenetics+*TP53*-WT patients a higher remission rates and longer DoR and mOS than AZA alone, with outcomes similar to those observed in intermediate-risk AML patients undergoing similar treatment were found.⁹⁶ These observations support the conclusion that among high-risk AML patients, those *TP53*-WT exhibit a better benefit than those *TP53*-mut following treatment with VEN+HMA.

In a retrospective analysis performed on 81 AML patients treated with VEN+HMA or VEN+LDAC, none of the 18 patients with *TP53* mutations displayed durable remission; some displayed primary resistance, and others rapidly relapsed after an initial remission.⁷⁹ Individual serial molecular analyses of some of these patients showed an expansion of the size of the *TP53*-mutant clones with biallelic *TP53* defects under therapeutic pressure; polyclonal selection of clones with biallelic *TP53* mutation was observed at relapse, with the appearance also of additional *TP53* variants.⁷⁹

Very interestingly, an ongoing clinical trial involving the administration of VEN+AZA+Magrolimab (anti-CD47 mAb) to older/unfit AML patients reported in 7 newly diagnosed *TP53*-mutated AML patients a CR rate of 86%, with MRD negativity in 57% of cases and complete cytogenetic response in 3 patients.²¹

Preliminary results on a very limited number of *TP53*-mutated AML patients showed that weekly VEN with low-dose DEC results in a high rate of clinical and molecular responses.⁹⁷ However, these observations need to be confirmed on a larger cohort of *TP53*-mut AML patients.

***RUNX1*-mutated AMLs.** *RUNX1*-mutated AMLs represent a particular subtype of AMLs (about 10% of newly-diagnosed AMLs), being almost exclusive of AMLs with recurrent genetic alterations.⁹⁸ These leukemias frequently co-occur with genetic mutations involving epigenetic modifiers, such as *ASXL1*, *IDH2*, *KMT2A*, and *EZH2*, components of the spliceosome complex, such as *SRSF2* and *SF3B1*, *STAG2*, *PHF6* and *BCOR*; these AMLs usually have an immature phenotype and frequently are sAMLs evolving from MDS.⁹⁸ Since a significant proportion of *RUNX1*-mutated AMLs evolves from a pre-existing MDS syndrome, it is fundamental to distinguish *de novo* cases from those evolving from MDS (sAML). The analysis of

de novo *RUNX1*-mutated AMLs showed that these AMLs, compared to *RUNX1*-WT AMLs, displayed a higher frequency of *SRSF2* and *ASXL1* mutations of normal karyotype and absent *NPM1* mutations.⁹⁹ *De novo* *RUNX1*-mutated AMLs showed an overall survival similar to that observed for *RUNX1*-WT AMLs, thus indicating that the poor prognosis of *RUNX1*-mutated AMLs is not due to the mutation itself but is attributable to pre-existent MDS.⁹⁹

Few studies explored the response of *RUNX1*-mutated AMLs to VEN+HMA as frontline therapy. DiNardo et al. reported in a retrospective analysis that 33% of *RUNX1*-mutated AML patients exhibit durable remission after VEN-HMA therapy, 13% remission then relapse, and 45% primary resistance, thus supporting the existence of a consistent heterogeneity of these leukemias to frontline therapy with VEN+HMA.⁷⁹ Cherry and coworkers have retrospectively analyzed 143 *de novo* AMLs who received VEN-AZA and 149 who received intensive chemotherapy treatment; the presence of *RUNX1* mutations in these patients was associated with better outcomes for VEN-AZA compared to intensive chemotherapy.¹⁰⁰ The benefit deriving from the VEN-AZA regimen over intensive chemotherapy was particularly evident for patients with *RUNX1* mutation and an age >65 years.¹⁰⁰ Venogopal et al. have retrospectively analyzed 907 AML patients, including 137 patients with newly diagnosed mut*RUNX1* AML who underwent first-line treatment based either on intensive chemotherapy (IC), low-intensity therapy (LIT) or LIT+VEN: there was no significant difference in outcomes between *RUNX1*mut and *RUNX1*wt AMLs, regardless of therapy received; among patients who received LIT+VEN there was a trend towards better survival with mut*RUNX1* AML compared to those without mut*RUNX1* (25.1 vs. 11.3 months of overall survival with a 2-year overall survival of 54% vs. 33%).¹⁰¹ Furthermore, in patients without other adverse-risk cyto-molecular features, the presence of mut*RUNX1* conferred inferior overall survival in patients who received IC or LIT but not in those treated with LIT+VEN.¹⁰¹

In addition to the studies on *de novo* *RUNX1*-mutated AMLs, studies on refractory relapsing patients support good responsiveness to VEN+HMA. Wang et al. explored the factors predictive for response among 40 relapsing/refractory AML patients treated with VEN-based regimens: patients harboring *NPM1*, *RUNX1*, or *SRSF2* mutations seemed to have higher complete remission rates, and mOS was significantly longer in *RUNX1*-mutated AMLs.²⁹ DiNardo reported a retrospective analysis in 43 refractory/relapsing AML patients treated with VEN-based regimens and observed 50% of clinical responses among *RUNX1*-mutated AML patients; interestingly, the *TP53*-mutated patients who responded to treatment had concurrent *RUNX1*

mutations and, similarly, of the 15% of responding patients with adverse cytogenetics, all had concurrent *RUNX1* mutations.²⁵ Other studies in relapsing/refractory AML patients bearing *RUNX1* mutations have shown a rate of objective responses from 35% to 75%.¹⁰²

The sensitivity of some AMLs bearing *RUNX1* mutations to VEN may be related to the differentiation stages of these AMLs and to some peculiar effects induced by *RUNX1* mutations at the level of the hematopoietic stem cell compartment. Mutations in *RUNX1* reduced ribosome biogenesis, metabolism, and sensitivity for induction of apoptosis in hematopoietic stem cells, thus creating resistance to endogenous and genotoxic stress.¹⁰³ The impaired ribosomal biogenesis is a condition that renders *RUNX1*-mutated AMLs more sensitive to the protein translational inhibitor hemaharringtonine (omacetaxine) and to VEN: hemaharringtonine treatment reduced the levels of c-Myc, c-Myb, MCL-1, and BCL-X_L and, consequently, synergized with VEN in inducing apoptosis of AML cells expressing mutant *RUNX1*.¹⁰⁴ This combination treatment improved the survival of immunodepleted mice engrafted with AML cells bearing mutant *RUNX1*.¹⁰⁴ The sensitivity of *RUNX1*-mutated AMLs to VEN could also be related to their arrest at an early stage of hematopoietic differentiation. In fact, AMLs harboring *RUNX1* mutations or *inv(3)* are among the different AMLs blocked at the earliest stage of hematopoietic stem cell/progenitor-like differentiation.¹⁰⁵

Secondary AMLs. Secondary AMLs (sAMLs) derive from the leukemic transformation of preceding myeloid neoplasia, either a myelodysplastic syndrome (MDS) or of myeloproliferative neoplasms (primary myelofibrosis, polycythemia vera, or essential thrombocytosis). These AMLs are poorly responsive to standard treatments and have poor prognosis.

Unfortunately, the studies with VEN-based therapies have also shown a limited response in patients with sAML. A single-center evaluated VEN-based combinations (either with HMA or with chemotherapy) in 14 ND and 17 R/R patients developing AML post-myeloproliferative neoplasms.¹⁰⁰ In frontline patients, CRs were observed in 54% of patients, while no objective responses were observed in R/R patients; the median duration of response among newly diagnosed patients was 6.4 months.¹⁰⁶ Data pooled from the VIALE-A study showed that patients with sAML evolving from preceding MDS or MPN demonstrated superior response rates and overall survival when treated with AZA+VEN compared to AZA alone: CRs 66% vs. 27%; mDoR 15.9 vs. 10.1 months.¹⁰⁷

Short et al. have reported the retrospective analysis of 562 patients who developed AML from preceding

myelodysplastic syndrome (MDS) or chronic myelomonocytic leukemia (CMML); these patients were stratified according to frontline therapy: intensive chemotherapy (IC, 271 patients), low-intensity chemotherapy without VEN (LIT, 237 patients) and VEN+HMA (54 patients).¹⁰² Compared to IC or LIT, VEN+HMA induced a higher CR rate (39% vs. 25%) and a better overall survival (1-year OS 34% vs. 17%).¹⁰⁷ Importantly, the benefit deriving from VEN+HMA treatment was restricted to patients with non-adverse karyotype, with a mOS of 13.7 months and 1-year OS of 54%.¹⁰⁸ In addition, patients who underwent subsequent hematopoietic stem cell transplantation displayed a superior 3-year OS compared to those not transplanted (33% vs. 8%).¹⁰⁸

A recent study explored the stem cell architecture of MDSs progressing to AMLs and identified some properties of myelodysplastic cells predicting response to VEN.¹⁰⁹ The bone marrow samples of one group of MDS patients (52% of total) displayed an abnormal differentiation pattern characterized by increased frequency of common myeloid progenitors (CMP) and the other group by increased frequency of granulomonocytic progenitor (GMP); these two MDS differentiation patterns did not derive from the expansion of either the CMP and GMP populations but were the consequence of the marked decrease of the frequency of the other two respective progenitor populations: GMPs and megakaryocytic progenitors (MEPs) in CMP-pattern MDS and CMPs and MEPs in GMP-pattern MDSs.¹⁰⁹ At the HSC level, the CMP pattern was associated with an expansion of LT-HSCs and MPPs, while the GMP pattern was associated with an expansion of lymphoid-primed multipotent progenitors (LMPPs). These two MDS architectures are driven by different genetic alterations: *TP53* and *EZH2* mutations are significantly associated with the CMP pattern, while *RUNX1*, *DNMT3A*, *BCOR*, and *STAG2* mutations are enriched with the GMP pattern.¹⁰⁹ These two MDS patterns are maintained in MDS patients undergoing treatment with HMs alone. Importantly, during disease progression to AML, these two different MDS patterns undergo the expansion of distinct stem cells that activate specific survival pathways: the BCL-2 pathway in the CMP pattern and the nuclear factor-kappa B-mediated survival in the GMP pattern; in line with these findings, VEN-based therapy selectively targets HSCs from CMP-pattern MDS at blast progression after HMA therapy failure.¹⁰⁹

Interestingly, a recent report showed a high response rate in 44 patients with MDS undergoing treatment with VEN+HMA, with an objective response rate of 75% in HMA-naïve patients, 62% in previously HMA-exposed patients, and 44% in patients after HMA failure.¹¹⁰ Importantly, this treatment also led to high allogeneic stem cell transplantation rates performed in 62% of

responding patients.¹¹⁰ Factors associated with poor overall survival were represented by those previously identified in AML patients, including *TP53* mutations and complex karyotypes.¹¹⁰

Safety Profile of Venetoclax. The numerous studies carried out using VEN have allowed to evaluate of the safety profile of this drug in the various drug combinations in which it was used, showing that VEN administration is clinically feasible when administered in combination with hypomethylating agents (AZA or DEC), low-intensity chemotherapy, intensive chemotherapy and in the context of “triplet” regimens in which this drug is administered in combination with low-intensity chemotherapy or a hypomethylating agent and a drug molecular targeting a leukemic genetic abnormality (i.e., an IDH inhibitor in an *IDH*-mutated AML).¹¹¹⁻¹¹²

The most relevant information concerning the safety profile of VEN derives from the two phase III studies discussed above.^{8,9} The VIALE-A study comparing VEN+AZA to AZA+placebo showed that the VEN+AZA safety profile was consistent with the side-effect profiles of these two drugs when used in monotherapy, and the observed adverse events are those expected in a population of elderly AML patients.⁸ The most common adverse events in the two groups of patients were hematologic and gastrointestinal, with a higher frequency of neutropenia and febrile neutropenia in the group of patients treated with VEN+AZA; furthermore, a higher incidence of dose interruptions but not the discontinuation of treatment or reduction in doses, to allow hematological recovery in patients treated with VEN+AZA.⁸ These results supported the conclusion that VEN+AZA administration is feasible in a population of older AML patients and that monitoring and management of myelosuppression are important clinical issues in AML patients treated with VEN+AZA.⁸ The findings of the other phase III study involving the comparison of VEN-LDAC to LDAC+placebo confirmed the findings of the VIALE study, showing that among the hematological toxicities, only neutropenia but not febrile neutropenia, thrombocytopenia and anemia, were more frequent in VEN-LDAC patients compared to LDAC+placebo patients.⁹

Tumor lysis syndrome (TLS) is observed in some AML patients treated with VEN-based regimens due to rapid and massive lysis of tumor cells. The incidence of laboratory TLS was reported ranging from 1% to 6% among patients undergoing treatments with lower-intensity VEN-based regimens and from 0% to 6% in those treated with VEN+chemotherapy; however, the incidence of clinical TLS was lower, ranging from 0% to 2.7% and this is seemingly due to the use in clinics of therapeutic measures to reduce WBC count less than 10×10^9 before to start the therapy with VEN

combinations.¹¹³ These estimates of TLS were based on the evaluation of patients treated in the context of clinical trials; however, for patients treated outside of clinical trials, the estimate of the frequency of TLS is around 5%.¹¹⁴

The analysis of AML patients treated with VEN+chemotherapy regimens, such as FLG-IDA or CLIA, resulted in similar myelosuppression compared to other intensive chemotherapy treatments used in AML patients and required standard antimicrobial prophylaxis.¹¹²

The good tolerability of VEN-based regimens is also supported by two recent case reports in extremely vulnerable patients with concurrent COVID-19 infection and AML: one patient with concurrent severe COVID-19 pneumonia and AML was first treated for infectious pneumonia and when the patient's conditions related to pneumonia improved was treated with VEN+AZA achieving complete remission and was now potentially available for hematopoietic stem cell transplantation;¹¹⁵ the other patients with COVID-19 infection had concurrent t-AML evolved from chronic myelomonocytic leukemia, achieving complete remission when sequentially treated with VEN+AZA, with the abrogation of both mutant clones associated with AML evolution and pre-existing to leukemia progression.¹¹⁶

Venetoclax Metabolism and Drug Interactions.

Venetoclax is primarily metabolized by cytochrome P450 isoform 3A4 (CYP3A4) and is predominantly cleared by the liver, as shown by *in vitro* studies. In fact, *in vitro* studies of drug-drug interaction with ketoconazole, Posaconazole, and rifampin have supported the metabolism of VEN by CYP3A4.¹¹⁷⁻¹¹⁸ Studies of drug metabolism in normal volunteers using a single dose of [¹⁴]-Venetoclax showed that all the administered drug was excreted with feces, with only a minimal contribution (0.1%) of the urinary tract; the extent of drug absorption was around 65%.¹¹⁹ VEN was primarily cleaved by hepatic metabolism (66% of the administered dose); 33% of the administered drug was recovered as the parent drug and its nitro reduction metabolite M30; M27 is a major drug metabolite and is primarily formed by CYP3A4.¹¹⁹

The pharmacokinetics of Ven was characterized in chronic lymphocytic leukemia and lymphoma patients after a single oral dose, with VEN plasma concentrations peaking at 6 to 8 hours after administration and with a terminal phase elimination half-life of approximately 19 hours.¹²⁰⁻¹²¹

Venetoclax is a substrate of CYP3A4; antifungal agents used to prevent systemic fungal infections in neutropenic hematologic patients are inhibitors of CYP3A4: Posaconazole or voriconazole are strong inhibitors, while isavuconazole or fluconazole are

moderate CYP3A4 inhibitors. Pharmacokinetic studies have shown that the concomitant administration of an antifungal agent, such as posaconazole, with VEN, requires a VEN dose reduction by at least 75%; both the 50- and 100-mg VEN doses administered with Posaconazole were well tolerated, in spite the 400-mg that represents the optimal VEN dose without concomitant antifungal agents.¹²² Using robust pharmacokinetic models for the drug Posaconazole, it was estimated that the recommended dose of VEN is 70 mg in the presence of Posaconazole up to 500 mg doses.¹²³

The clinical experience showed that the combination of VEN and antifungal azoles results in prolonged cytopenias, namely, thrombocytopenia, compared to using VEN without an azole.¹²⁴⁻¹²⁵ However, this effect did not result in higher rates of febrile neutropenia, infections, or duration of hospitalization, thus indicating that the concomitant use of VEN and antifungal azoles represents a clinically safe and effective therapeutic regimen, after adjustment of VEN dosage.¹²⁴⁻¹²⁵

Interestingly, grapefruit, star fruit, and oranges can potentially increase VEN plasma concentrations if taken concomitantly; this effect is related to the capacity of these fruits to act as moderate CYP3A4 inhibitors. According to these findings, it is recommended to avoid

eating these fruits during VEN-based treatment.

Recent studies suggest that antifungal agents enhance VEN-AUC not only through inhibition of CYP3A4 but also through inhibition of the transporter OATP1B1, involved in the elimination of VEN.¹²⁶

Venetoclax Resistance. The most consistent limitation of the therapy of AMLs with VEN is the short duration and the development of resistance.¹²⁷ Therefore, understanding the mechanisms of resistance to this drug is of fundamental importance for the development of new strategies able to bypass these therapeutic blocks and for the definition of new drug combinations.¹²⁷ Various mechanisms of resistance to VEN-based regimens have been identified in AML cells. However, the two most important are represented by dependencies on alternative anti-apoptotic BCL-2 family members and the selection of activating kinase mutations.¹²⁷

The mechanism related to the development of dependencies on alternative anti-apoptotic BCL-2 proteins seems to be particularly relevant. These alternative dependencies include different anti-apoptotic mediators, such as BCL-2-A1, MCL-1, and BCL-X_L and are involved in primary and adaptive VEN resistance mechanisms (**Figure 2**).

In this context, a role particularly relevant is played

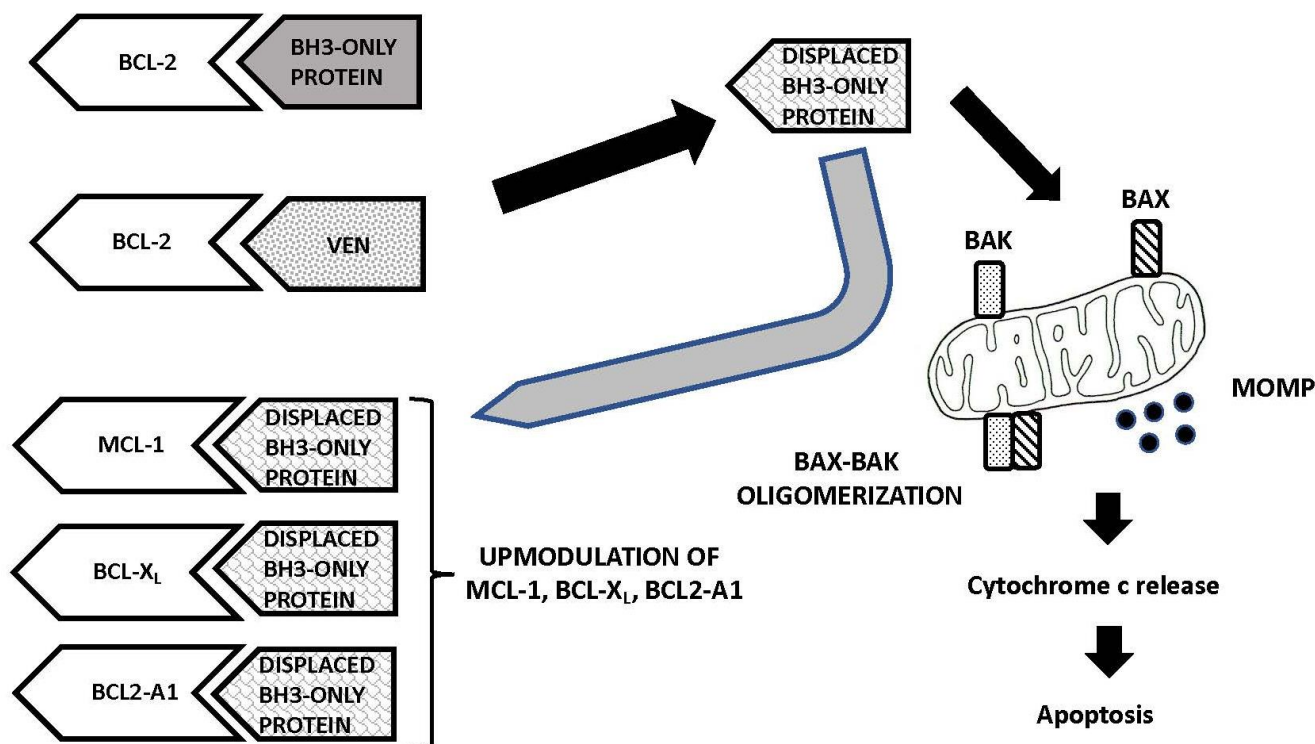


Figure 2. Mechanism of action of and resistance to Venetoclax. In the absence of overexpression of MCL-1, BCL-X_L and BCL2-A1, binding of the BH3 mimetic Venetoclax to BCL-2 determines the release of bound BH3-only proteins, such as BIM, inducing the interaction between these displaced BH3-only proteins and BAK/BAX with consequent activation of a BAK/BAX complex (BAK/BAX oligomerization); this activated BAK/BAX complex determines an increase of the mitochondrial outer membrane permeability (MOMP) with consequent release of cytochrome c from the mitochondria into the cytoplasm and activation of the apoptotic process through caspase activation. In the presence of increased levels of the antiapoptotic proteins MCL-1, BCL-X_L and BCL2-A1, the proapoptotic effect of Venetoclax is inhibited through a mechanism involving sequestration of displaced BH3-only proteins, thus preventing the capacity of these proteins to bind and to activate BAK/BAX and thus blocking the proapoptotic effect of Venetoclax.

by MCL-1 as the main mechanism of VEN resistance. A fundamental study by Bhatt and co-workers explored the mechanisms of acquired resistance to BH3 mimetic antagonists of BCL-2 and MCL-1 using AML patient-derived xenograft (PDX) models: these models involved AML cells that have acquired resistance to VEN following treatment with this drug and AML cells that were constitutively resistant to VEN.¹²⁸ BH3 profiling studies showed that BH3 mimetic resistance is characterized by decreased mitochondrial apoptotic priming, as measured both in primary AML samples and in PDX models, due to alterations at the level of BCL-2 family proteins, variable from one case to another.¹²⁶ In VEN-resistant AML cells, BCL-2 sequestration of pro-apoptotic proteins, necessary to confer BCL-2 dependence, was reduced. This phenomenon was not due to a decreased expression of BCL-2 or BIM after the acquisition of resistance but to the sequestration of BIM at the level of MCL-1; an opposite phenomenon is observed in cells resistant to MCL-1 antagonists, with BIM sequestration at the level of BCL-2.¹²⁸ This finding has considerable implications for therapy based on combinations of BCL-2 and MCL-1 antagonists since it implies that the concurrent rather than the sequential administration of these inhibitors is more effective.¹²⁸

Two initial studies showed that MCL-1 upregulation renders leukemic cells resistant to VEN. Thus, Pan et al. provided evidence that a pan-BCL-2 inhibitor (-)BI97D6 potently induced apoptosis through intrinsic pathway activation by disrupting MCL-1/BIM and BCL-2/BAX interactions; importantly, this pan-BCL-2 inhibitor, as a single agent, effectively overcame AML cell apoptosis resistance mediated by MCL-1 in AML cells.¹²⁹

The exploration of VEN-resistant AML cell lines derived through chronic exposure to VEN showed that the upregulation of MCL-1 and BCL-X_L drives drug resistance; targeting MCL-1 and/or BCL-X_L restored the sensitivity of these leukemic cells to VEN.¹³⁰

A consistent number of studies supported the role of MCL-1 in VEN resistance mechanisms.

Ewold and coworkers reported a side-by-side comparison of three different BH3-mimetics targeting BCL-2 or MCL-1, or BCL-X_L; they drew the conclusion that MCL-1 may be a more prevalent therapeutic target than BCL-2 in AML. Interestingly, MCL-1 BH3-mimetics induced displacement of the BH3-only protein BIM and BAK, resulting in BAK-dependent apoptosis; in contrast, VEN-induced cell death was mediated by BAX rather than BAK.¹³¹ This finding supports distinct non-redundant molecular functions of BCL-2 and MCL-1 in AML cells.

The analysis *in vitro* of a large panel of AML cell lines and of primary AML samples co-cultured with bone marrow mesenchymal stromal cells showed that inhibition of MCL-1, whose expression is weak

compared to that of BCL-2, induces apoptosis of AML cells and strongly synergizes with VEN.¹³²

VU661013 is a selective and potent MCL-1 inhibitor that destabilizes the association between BIM and MCL-1, induces AML cells apoptosis, and is active in VEN-resistant and patients-derived xenografts.¹³³ Importantly, BH3 profiling of patient samples and *ex vivo* drug-sensitivity assays predicted sensitivity to BCL-2 or MCL-1 inhibitors and showed the benefit deriving from the combination of the two types of inhibitors.¹³³

Studies in pre-clinical models of AMLK supported the double targeting of MCL-1 and BCL-2. Particularly, the analysis of primary AML samples, including those with poor risk genotypes, showed the efficacy of the contemporaneous MCL-1 and BCL-2 targeting in the induction of leukemic cell apoptosis.¹³⁴ Furthermore, co-targeting of MCL-1 and BCL-2 was more effective against the leukemic compartment compared to normal hematopoietic stem/progenitor cells, thus supporting a therapeutic window of activity and a tolerable safety profile at the level of the hematopoietic system.¹³⁴ Importantly, BCL-2 and MCL-1 co-targeting prolonged animal survival in xenograft models of AML and suppressed patient-derived leukemia but not normal hematopoietic cells in the bone marrow of engrafted animals.¹³⁴

A recent study showed that co-targeting of BCL-2 and MCL-1 was highly effective in inhibiting leukemic stem cells derived from AMLs resistant to VEN or relapsed after VEN-based therapy, irrespective of genetic alterations and cytogenetic alterations.¹²⁷ Interestingly, enhanced antileukemia activity was also observed in a PDX model of monocytic leukemia, a leukemia subtype known to be resistant to VEN-based therapy.¹³⁵

Importantly, a recent study provided evidence that the BCL-2 and MCL-1 co-targeting was effective not only against VEN-resistant AML cells but also against chemotherapy-resistant cells. Accordingly, S63845, an MCL-1 inhibitor, in combination with VEN, effectively inhibits AML cells that have acquired resistance to cytarabine, thus supporting an evaluation of this drug combination in relapsed/refractory AML patients.¹³⁶

Proteasome inhibition may represent a new strategy to antagonize MCL-1 activity in cancer cells via transactivation of the MCL-1 antagonist NOXA.¹³⁷ Interestingly, proteasome inhibitors strongly synergize with VEN in inducing apoptosis of cancer cells.¹³⁷ However, this synergistic combination was not yet evaluated in preclinical models of AML.

The increased dependency on MCL-1 observed constitutively in some AMLs or following VEN treatment may be related to activation of the MAPK signaling pathway, which induces stabilization of MCL-1 protein and prevents its degradation.¹³⁸⁻¹³⁹ RAS-MAPK activation is a major mechanism of acquired VEN

resistance, mediated by MCL-1 upmodulation and that can be abrogated by MCL-1 inhibition; rapid clonal selection of RAS-mutated clones was observed in some AML patients treated with VEN-containing regimens and represented an important mechanism underlying development of VEN resistance.¹⁴⁰

RAS pathway mutations, such as those occurring at the level of *KRAS* and *PTPN11* genes, induce VEN resistance to AML cells, related to a mechanism involving MCL-1 and BCL-X_L upmodulation; these AML subtypes are sensitive to MCL-1 inhibitors.¹³⁸ Studies in AML cell lines have shown that VEN resistance was reproduced through transduction of *G12D KRAS* or *A72D PTPN11* mutants; *G12D KRAS*-transduced cells displayed decreased BCL-2 and BAX levels, associated with increased MCL-1 and BCL2-A1 levels.¹³⁸ Only MCL-1 inhibitors, but not BCL-2 or BCL-X_L inhibitors, could reduce the viability of these leukemic cells.¹³⁸ The combination of VEN+MCL-1 inhibitors showed synergy in inducing apoptosis of leukemic cells.¹³⁸

RAS pathway activation, observed in RAS- and PTPN11-mutated cases, induces the activation of multiple sources of energy for cell survival, including fatty acid and amino acid metabolism, glycolysis and upregulation of OXPHOS.¹⁴¹ Furthermore, AMLs harboring a *PTPN11* mutation frequently display a monocytic differentiation phenotype,¹⁴² usually associated with dependency on MCL-1 and thus scarcely sensitive to VEN-mediated BCL-2 inhibition.¹⁴³

In line with these findings, inhibition of the MAPK signaling pathway using MEKK/MEK2 inhibitors synergizes with VEN to induce apoptosis of AML cells.¹⁴⁴ Furthermore, this drug combination induced downregulation of MCL-1 levels and disrupted the binding of BIM to both MCL-1 and BCL-2, thus releasing BIM that was able to initiate the apoptotic process.¹⁴⁴ The observations provided a rationale for the combinatorial blockade of MEK and BCL-2 pathways in AML subsets characterized by *KRAS*, *NRAS*, *PTPN11*, and *FLT3-ITD* mutations.

Ongoing phase I clinical trials are evaluating different MCL-1 inhibitor-based treatments for AML patients, including the combination therapies with VEN (NCT03672695, NCT02979366, and NCT04629443).

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Interestingly, a recent phase II study showed that VEN with low-dose Navitoclax was well tolerated and had promising efficacy in patients with relapsed/refractory acute lymphoblastic leukemia or lymphoblastic lymphoma.¹⁴⁵ Therefore, an ongoing clinical study is evaluating the combination of VEN, Navitoclax, and Decitabine in relapsed/refractory AML patients previously treated with VEN (NCT05222984).

Conclusions. The advent of VEN as a potent BCL-2 inhibitor has transformed the treatment of AML, particularly for elderly patients. Multiple VEN-based combination therapies have been developed based on understanding the mechanisms underlying VEN responsiveness and resistance (primary and adaptive). Numerous studies carried out in the last years have shown that *de novo*-treated AML patients respond to these treatments better than refractory/relapsing patients.

Retrospective studies involving molecular characterization of treated patients have shown that the most responding patients are represented by those with *NPM1*, *IDH1-IDH2*, and *TET2* mutations; *RUNX1* mutated patients, both in frontline and in relapsing/refractory status, seem to be more responsive to VEN-based regimens than to intensive chemotherapy; *FLT3*-mutant AMLs shows a reduced sensitivity to VEN+HMA or VEN+LDAC regimens, but could be more responsive to “triplet” regimens based on VEN+FLT3 inhibitors+HMA (or LDAC); AML patients harboring *TP53*, *RAS*, or *PTPN11* mutations, monocytic AML, secondary AML and AML cases pre-treated with HMAs show reduced sensitivity to VEN-based therapies.

The ongoing development of “triplet” therapies based on the administration of VEN+HMA (or LDAC) + a drug targeting a specific molecular alteration or a signaling pathway could significantly improve the rates and the duration of the clinical responses. However, the development of these “triplet” therapies could be hampered in some instances by the occurrence of not tolerable toxicities.

Another important development will consist in the association of VEN with another BH3 targeting drug, such as MCL-1 or BCL-XL inhibitor, to extend the number of responding patients and bypass VEN primary and adaptive resistance.

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