Supplementary Methods

Patients and Treatment

Patients must have completed all alvocidib dosing and at least 80% of venetoclax dosing or have experienced dose-limiting toxicity (DLT) within the first cycle to be considered evaluable for dose-escalation decisions. Table S1 shows the dose escalation decision rule for the BOIN design with target toxicity rate of 0.30 and optimal interval of (0.236, 0.359). When the decision rule indicated escalation, either the venetoclax dose or the alvocidib dose was to be escalated. A DLT was defined as a Grade ≥ 3 non-hematological toxicity not clearly resulting from the underlying leukemia, that did not improve to baseline grade, or ≤ Grade 1 if not present at baseline, with treatment interruption and maximal medical therapy, except for the following: alopecia, fatigue, asthenia, fever, anorexia, or constipation; nausea, vomiting, or diarrhea not requiring tube feeding, total parenteral nutrition, or prolonged hospitalization; aspartate aminotransferase or alanine aminotransferase that resolves to < Grade 2 within 14 days; electrolyte abnormalities that resolve to < Grade 2 levels in 72 hours; TLS that is successfully managed clinically and resolves within 7 days without end-organ damage; Grade 4 neutropenia or thrombocytopenia continuing 42 days from the start of therapy for patients who are without residual morphologic leukemia and who had ≤ Grade 3 neutropenia or thrombocytopenia at baseline.

For venetoclax, the lowest target dose level was 400 mg, and the highest was 800 mg, as further escalation did not previously result in additional clinical benefit.[1] The highest dose of alvocidib administered was 30 mg/m^2 as a 30-minute loading dose followed immediately by 60 mg/m^2 as a 4-hour IV infusion.[2, 3] Patients continued treatment until disease progression, unacceptable toxicity, withdrawal of consent, or beginning other treatment. Patients who completed a 28-day treatment cycle without evidence of significant treatment-related toxicity or progressive disease continued to receive treatment at the same dose level.

Pharmacokinetic analysis

Plasma concentrations of venetoclax and alvocidib were determined using a validated liquid/liquid extraction followed by a high-performance liquid chromatography-tandem mass spectrometric method.[4, 5] PK samples for venetoclax were gathered when co-administered with alvocidib (Cycle 2 Day 3) and when administered alone (Cycle 1 Day 22). Values for the PK parameters, including the maximum observed plasma concentration (C_{max}), the time to C_{max} (T_{max}), and the area under the plasma concentration-time curve from time 0 to 24 hours post-dose (AUC_{24}) were determined using noncompartmental methods. PK samples for alvocidib

were gathered on Cycle 2 Day 3. Values for the PK parameters, including the C_{max}, T_{max}, terminal phase elimination half-life, AUC from time 0 to the time of the last measurable concentration (AUC_t), AUC from time 0 to infinite time (AUC_{inf}), and clearance were determined using noncompartmental methods.

Biomarker Analyses

Bone marrow aspirates were collected at baseline, and the presence of AML-associated mutations was determined using the next-generation sequencing panel MyAML (Invivoscribe, San Diego, CA, USA). BCL-2 (*BCL2*) and BCL-xL (*BCL2L1*) gene expression were defined centrally by quantitative polymerase chain reaction (qPCR) using the deltaCt (ΔCt) method (Roche Molecular Systems, Pleasanton, CA, USA). *BCL2* and *BCL2L1* expression were normalized to a reference gene. Peripheral blood was collected at baseline, and the dependency of tumor cells on BCL-2, BCL-xL, MCL-1, and BCL-w were determined using the BH3 assay using multiple BH3 mimetics. This assay was performed as described in detail above, with certain modifications.[6-8] In brief, a modified MS1 peptide (for probing MCL-1 dependence) and inhibitors for BCL-2 and BCL-xL were used to detect the dependence on BH3 family member survival proteins. BH3 priming cut-off for determination of BCL-2, BCL-xL, and BCL-w dependence was set at 25%, and MCL-1 dependence was set at 40%.

Statistical analyses

All patients that received at least one dose of the study drug were included in all analyses. Demographics were summarized by descriptive statistics. Remission rates were summarized in counts and proportions. OS and DoR were evaluated by the Kaplan–Meier methodology. The 95% confidence intervals (CIs) for time to event endpoints were estimated using the binomial distribution.

For pharmacokinetic analysis, the PK parameter values of venetoclax and alvocidib were tabulated for each patient and each dose level combination by visit, and summary statistics were computed for each parameter. To assess alvocidib's effect on venetoclax PK, a repeated measures analysis was performed for T_{max}, the apparent terminal phase elimination rate constant, and the natural logarithms of dose-normalized C_{max} and AUC_{24}. The relative bioavailability of the combination regimen (in Cycle 2 Day 3) to that of the venetoclax alone regimen (in Cycle 1 Day 22) was assessed by 90% CIs for the difference of the least square means obtained from the repeated measures analyses of $\ln(C_{max})$ and ln(AUC).

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Table S1. Dose Escalation Decision Rules

Action	# of Patients Treated at Current Combination							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Escalate if # of patients with DLT ≤	0	0	1	1	1	1	2	2
Stay at current combination if # of patients with DLT =	1	1	-	2	2	2	3	3
De-escalate if # of patients with DLT ≥	2	2	2	3	3	3	4	4
Eliminate[a] if # of patients with DLT ≥	3	3	4	4	5	5	5	6

DLT = dose-limiting toxicity

[a]Eliminate current combination and higher combinations (ie venetoclax and alvocidib dose ≥ current dose

Table S2. Pharmacokinetic Parameters of Venetoclax Given Alone and in Combination with Alvocidib

	Ven 400mg				Ven 600mg		Ven 800mg	
	Alvo 45mg/m^2		Alvo 60mg/m^2		Alvo 60mg/m^2		Alvo 60mg/m^2	
Parameter (Unit)	**Ven Alone (n=9)**	**Ven + Alvo (n=4)**	**Ven Alone (n=7)**	**Ven + Alvo (n=3)**	**Ven Alone (n=3)**	**Ven + Alvo (n=3)**	**Ven Alone (n=4)**	**Ven + Alvo (n=3)**
T_{max} (h)[a]	8.0 (0.0-9.0)	5.0 (4.0-8.0)	8.0 (2.0-24.0)	6.0 (4.0-24.0)	8.0 (8.0-74.9)	11.0 (7.0-24.0)	6.0 (6.0-10.3)	4.0, 6.0[b,c]
C_{max} (µg/mL)	1.32 (1.46, 47)	0.744 (0.816,53)	1.82 (1.95,39)	0.639 (0.696,50)	2.10 (2.41, 63)	0.585 (0.698, 59)	2.13 (2.55, 78)	0.629, 1.30[b,c]
AUC_{24} (µg•h/mL)	21.4 (22.7, 35)	12.7 (14.9, 69)	28.0 (29.3,31)	10.0 (11.6,60)	29.6, 70.2[b]	14.0, 19.0[b]	29.5 (37.3, 89)	4.58, 21.1[b,c]
Dose normalized C_{max} (ng/mL)/mg	3.31 (3.64,47)	1.86 (2.04, 53)	4.56 (4.89,39)	1.60 (1.74, 50)	3.50 (4.01, 63)	0.975 (1.16,59)	2.67 (3.18, 78)	2.02 (2.96, 104)
Dose-Normalized AUC_{24} (ng•h/mL)/mg	53.5 (56.6, 35)	3.18 (37.3, 69)	70.1 (73.2, 31)	25.1 (29.1, 60)	49.3, 117[b]	23.4, 31.6[b]	36.9 (46.6, 89)	25.5 (47.1, 116)

AUC_{24}, area under the plasma concentration-time curve from time 0 to 24 hours; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; T_{max}, time to C_{max}, peak time.
Values are presented as the geometric mean (mean, %CV) unless indicated otherwise
a. T_{max} presented as median (range).
b. Individual values are presented where n ≤ 2.
c. One patient is not included due to venetoclax dose reduction.

Table S3. Relative bioavailability of venetoclax co-administered with alvocidib

	Ven Alone	**Ven + Alvo**	**Relative Bioavailability (90% CI)**
Dose-normalized C_{max} (ng/mL)/mg	3.74	3.05	0.814 (0.441–1.50)
Dose-normalized AUC_{24} (ng•h/mL)/mg	61.1	50.0	0.818 (0.562–1.19)

AUC_{24}, area under the plasma concentration-time curve from time 0 to 24 hours; CI, confidence interval; C_{max}, maximum observed plasma concentration

Table S4. Pharmacokinetic Parameters of Alvocidib in combination with Venetoclax

	Alvo 45mg/m^2	**Alvo 60mg/m^2**		
Parameter (unit)	**Ven 400mg (n=4)**	**Ven 400mg (n=3)**	**Ven 600mg (n=4)**	**Ven 800mg (n=3)**
T_{max} [a] (h)	0.5 (0.5-4.0)	4.0 (0.5-6.0)	5.0 (2.0-6.0)	0.7 (0.5-4.0)
C_{max} (µg/mL)	0.679 (0.722, 42)	0.811 (0.822, 20)	1.41 (1.83, 93)	1.13 (1.29, 54)
AUC_{24} (µg•h/mL)	4.40 (4.71, 41)	5.95 (6.43, 48)	8.63 (8.97,29)	6.10 (6.50, 39)
AUC_{inf} (µg•h/mL)	4.62 (4.92, 40)	4.14[c]	10.5, 5.33[c]	6.22 (6.65, 40)
CL (L/h)	9.74 (10.4, 40)	14.5[c]	5.74, 11.3[c]	9.65 (10.4, 51)
$t_{1/2}$ (h)[b]	5.95 ± 1.29	7.59[c]	5.65, 4.69[c]	3.96 ± 1.39
Dose normalized C_{max} (ng/mL)/(mg/m^2)	15.1 (16.0, 42)	13.5 (13.7, 20)	23.5 (30.4, 93)	18.8 (21.6, 54)
Dose-Normalized AUC_{24} (ng•h/mL)/(mg/m^2)	97.7 (105, 41)	99.2 (107, 48)	144 (149, 29)	102 (108, 39)

AUC_{inf}, area under the plasma concentration-time curve from time 0 to infinite time; AUC_{24}, area under the plasma concentration-time curve from time 0 to 24 hours; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; T_{max}, time to C_{max}, peak time; $t_{1/2}$, terminal phase elimination half-life.
Values are presented as the geometric mean (mean, %CV) unless indicated otherwise
a. Tmax presented as median (range).
b. $t_{1/2}$ presented as harmonic mean ± pseudo standard deviation
c. Individual values are presented where n ≤ 2.

Table S5. Median BCL2 and BCL2L1 expression in bone marrow blasts by prior lines of therapy

	BCL2 mRNA (2–ΔCt) Median (95%CI)	**BCL2L1 mRNA (2–ΔCt) Median (95%CI)**
Evaluable N = 14	0.48 (0.34, 0.70)	17.46 (8.03, 37.96)
Prior Lines of therapy ≥ 4 n = 7	0.45 (0.30, 0.68)	25.00 (9.28, 67.30)
Prior Lines of therapy < 4 n = 7	0.52 (0.25, 1.12)	12.20 (2.85, 52.21)

Table S6. BH3 profiling of patient tumor dependencies at baseline and best response during study

	BCL2, MCL1, BCL-xL	**BCL2, BCLxL**	**BCL2**	**MCL-1, BCL-xL**	**MCL-1, BCL-W**	**BCL-xL**	**BCL-W**	**Resistant**
Tumor cell dependency, n (%)	1 (4.8)	1 (4.8)	1 (4.8)	6 (28.6)	1 (4.8)	4 (19.0)	4 (19.0)	3 (14.2)
CR/CRi, n (%)	1 (100.0)	0	0	0	0	1 (25.0)	0	0
MLFS	0	0	0	2 (33.3)	0	0	0	1 (33.3)
PD or RD	0	1 (100.0)	0	2 (33.3)	1 (100.0)	2 (50.0)	2 (50.0)	2 (66.7)
SD	0	0	1 (100.0)	2 (33.3)	0	1 (25.0)	2 (50.0)	0

CR, Complete Response; CRi, CR with incomplete hematologic recovery; PR, partial response; MLFS, morphologically leukemia-free state; RD, resistant disease; PD, progressive disease; SD, stable disease