Supplementary Materials and Methods

Supplementary Methods

Efficacy assessments

Overall survival was the number of days from the date of first treatment to death from any cause. Event-free survival was defined as the date of first treatment to treatment failure, relapse from composite complete remission (complete remission, complete remission with incomplete count recovery, or complete remission with incomplete platelet recovery) or death from any cause, whichever occurred first.

Biomarker assessments

Minimal residual disease

Assessment of bone marrow (BM) aspirate minimal residual disease (MRD) was performed by multi-color flow cytometry, at LabCorp Central Laboratory (Burlington, NC, USA), to evaluate response depth in patients with complete remission (CR)/complete remission with incomplete count recovery (CRi)/complete remission with incomplete platelet recovery (CRp). MRD was assayed using a 5-tube, 8-color flow-cytometry panel: CD45, CD34, HLA-DR, CD13 as a backbone in all 5 tubes, with CD123, CD117, CD71, CD64, CD56, CD38, CD33, CD19, CD15, CD14, CD11b, CD7, CD4, and CD2 assessed across the remaining tubes. These markers align with the recent recommendations of the European LeukemiaNet consensus document for flow cytometry-based MRD assessments in acute myeloid leukemia.[1] The integrated leukemia-associated immunophenotypes and different than normal procedures were used.[1,2] The assay validation established the MRD panel analytical sensitivity at the upper limit of 0.0037% and a lower limit of 0.0027%. Patients were considered not evaluable for MRD if they had missing BM samples, were deemed a technical failure, or if the BM samples contained <100,000 CD45+ leukocytes.

Single cell DNA sequencing

Single Cell DNA Sequencing (scDNA-seq) was performed using the Tapestri platform (Mission Bio, South San Francisco, CA, USA), per the manufacturer's instructions. In short, a custom myeloid scDNA-seq panel targeting 107 genes with 396 amplicons covering relevant cancer hotspots in myeloid disorders were designed and manufactured by Mission Bio. Cryopreserved patient samples were thawed, washed with phosphate-buffered saline, quantified using a

Countess II (Thermo Fischer Scientific, Waltham, MA, USA) and diluted to a concentration of 4000 cells per µL in Cell Buffer (Mission Bio). Next, 35 µL of cell suspension was loaded onto a microfluidics cartridge, and cells were encapsulated on the Tapestri instrument followed by the cell lysis and protease digestion on a thermal cycler within the individual droplet. The cell lysate was reintroduced onto the cartridge and barcoded such that each cell had a unique molecular identifier.

Amplification of the targeted DNA regions was performed by incubating the barcoded DNA emulsions in a thermocycler as follows: 98°C for 6 min (4°C per s); ten cycles of 95°C for 30 s, 72°C for 10 s, 61°C for 9 min, 72°C for 20 s (1°C per s); ten cycles of 95°C for 30 s, 72°C for 10 s, 48°C for 9 min, 72°C for 20 s (1°C per s); and 72°C for 6 min (4°C per s). Emulsions were broken, and DNA was digested and purified with 0.72X AMPure XP beads (Beckman Coulter, Brea, CA, USA). The beads were pelleted and washed with 80% ethanol, and the DNA targets were eluted in nuclease-free water. Indexed Illumina libraries were generated by amplifying DNA libraries with Mission Bio V2 Index Primers in the thermocycler using the following program: 95°C for 3 min; 10 cycles of 98°C for 20 s, 62°C for 20 s, 72°C for 45 s; and 72°C for 2 min. Final libraries were purified with 0.69X AMPure XP beads (Beckman Coulter). All libraries were sized and quantified using an Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA) and pooled for sequencing on a NovaSeq6000 (Illumina, San Diego, CA, USA) with 150 base-paired ending multiplexed runs.

FASTQ files generated by sequencers were processed using the Tapestri Pipeline V2 (Mission Bio) and included adapter trimming, sequence alignment (BWA), barcode correction, cell finding and variant calling (GATK v4/Haplotype caller). Loom and h5 files were then processed with Tapestri Insights v2.2 (Mission Bio) and the Python-based Mosaic package (GitHub, San Francisco, CA, USA). Tapestri Insights analysis used default filter criteria (for example, genotype quality ≥30 and reads per cell per target ≥10) and annotation-based information (for example, ClinVar and DANN). Only cells with complete genotype information for all variants (previously detected in bulk sample) were included for downstream processing.

Macrophage inhibitory cytokine-1 (MIC-1)

The concentration of MIC-1 in human serum was determined by enzyme-linked immunosorbent assay (ELISA) using the “Quantikine® ELISA Human GDF-15 ELISA Immunoassay” from R&D Systems (distributed by bio-techne, Wiesbaden, Germany; catalogue number: DGD150). This kit utilizes the quantitative sandwich ELISA technique. Microtiter plates were pre-coated with a

monoclonal antibody specific for human MIC-1/GDF-15. Standards, quality controls and samples were pipetted into the wells and any MIC-1/GDF-15 present was bound by the immobilized capture antibody. After washing away any unbound substances, an enzyme-linked polyclonal detection antibody specific for human MIC-1/GDF-15 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a tetramethylbenzidine substrate solution was added to the plate and color developed in proportion to the amount of GDF-15 bound in the initial step. The color development was stopped, and the intensity of the color was measured with an optical density plate reader.

References

1. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood.* 2018;131(12):1275-1291.
2. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129(4):424-447.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.

Supplementary Results

Supplementary Tables

Supplemental Table 1. Reasons for treatment discontinuations for ven and idasa. Treatment continued until disease progression, unacceptable toxicity, pregnancy, patient non-compliance/loss to follow-up, death, or physician decision. Patients could continue with the second drug alone, if any of the individual drugs were permanently discontinued due to toxicity.

n, (%)	ven-idasa, n = 55
Discontinued ven*	**55 (100)**
AE	11 (20.0)
Death	4 (7.3)
Disease relapse	2 (3.6)
Lack of efficacy	9 (16.4)
Physician decision	3 (5.5)
Progressive disease	22 (40.0)
Withdrawal by subject	4 (7.3)
Discontinued idasa*	**55 (100.0)**
AE	12 (21.8)
Death	4 (7.3)
Disease relapse	2 (3.6)
Lack of efficacy	9 (16.4)
Physician decision	3 (5.5)
Progressive disease	21 (38.2)
Withdrawal by subject	4 (7.3)

*Twelve AEs led to treatment discontinuation of ven and/or idasa: BM failure, gastrointestinal hemorrhage, mucosal inflammation, hemophagocytic lymphohistiocytosis, pneumonia, cellulitis, *Pneumocystis jirovecii* pneumonia, sepsis, septic shock, sinusitis, blood bilirubin increased, and muscular weakness; each in one patient. Two of 12 patients were in marrow remission at the time of discontinuation due to AE (1 patient with AE *of Pneumocystis jirovecii* pneumonia, and 1 patient with BM failure).
AE, adverse event; BM, bone marrow; idasa, idasanutlin; ven, venetoclax.

Supplemental Table 2. Prior therapies in ≥1 patient.

n (%)	ven-idasa, n = 55	
	Dose escalation, n = 49	Dosing schedule optimization, n = 6
3+7 (anthracycline cytarabine)	13 (26.5)	5 (83.3)
Azacitidine	14 (28.6)	3 (50.0)
Decitabine	9 (18.4)	0 (0.0)
High-dose cytarabine	4 (8.2)	1 (16.7)
Intermediate-dose cytarabine	5 (10.2)	0 (0.0)
Low-dose cytarabine	4 (8.2)	0 (0.0)
FLAG	1 (2.0)	0 (0.0)
Sorafenib	1 (2.0)	0 (0.0)

FLAG, fludarabine, cytarabine, granulocyte-colony stimulating factor and idarubicin; idasa, idasanutlin; ven, venetoclax.

Supplemental Table 3. Baseline *TP53* mutations and patient characteristics.

Patient	Best response	DoR (mo)	OS (mo)	*TP53* mutation	*TP53* VAF baseline	*TP53* VAF EoT	*TP53* mono/ bi-allelic	*TP53* mutation impact	Cytogenetics	Other baseline mutations	AML type*	Relapsed/ Refractory	t-AML
1	CRp	6.05	8.44	S240R	35.6	N/D	mono	LOF	none reported†	*IDH1, PDCD11, FLT3, IGF1R, SRFS2, SETBP1, DOT1L, ASXL1, PBRM1, RELN, BRAF, MED12*	sAML, AML-MRC	Refractory	no
2	CRp	2.3	17.08	F109S	13.09	<1	mono	LOF	none reported†	*RUNX1, FAT3, CDH1, CD79B, S1PR2, PASK, FBXO11, DUSP2, RAD50, PDGFRB, RICTOR, MSH3, HIST1H1D, IRF4, EZH2, PRKDC, GATA1*	sAML, t-AML	Relapse	yes
3	MLFS	1.18	5.65	C141Y	15.9	<1	BI	LOF	-7, complex karyotype (-17)	*TSC2, AXIN1, PLCG2, SUZ12, EP300, INPP5D, HDAC4, NSD1, ZNF703, ZMYM3*	de novo, AML-MRC	Relapse	no
4	SD	-	4.37	S166* V143M	4.17 4.96	27.7 23.76	mono	LOF LOF	none reported†	*MAF, ERBB2, ERBB2, NOTCH2, DNMT3A, DNMT3A, ESR1*	de novo, AML NOS	Relapse	no
5	SD	-	1.77	Y163C	91.95	99.07	BI	LOF	complex karyotype	*RASGEF1A, FANCM, LRP1B, CAD, FOXP1, TET2, SMO, JAK2, BCORL1*	sAML, AML NOS	Refractory	no
6	SD	-	6.34	D259V	12.82	88.34	BI	LOF	-5, complex karyotype (-17)	*FAT3, HNF1A, AKT1, ERBB2, TCF3, MCL1, ALK, MSH2, GATA2, APC, MAP3K1, CRLF2*	de novo, AML NOS	Refractory	no
7	SD	-	2.96	H179Q	80.13	88.99	BI	LOF	unable to evaluate	*NCOR2, KDM5A, IRS2, NTRK1, MAPK1, LRP1B, MSH6, PDGFRB, MSH3, MLL3,*	sAML	Refractory	no

										MYST3, BCORL1, ZRSR2			
8	RD	-	0.76	H214R Y205N	30.08 29.74	N/D	mono	LOF LOF	-5, -7	*HDAC7, MLL2, ERBB3, AXIN1, TCF3, NOTCH2, RAD54L, ASXL1, FANCD2, HIST1H1D, GPR124, DKC1*	de novo, AML NOS	Refractory	no
9	ND	-	0.89	C141Y	5.8	N/D	mono	LOF	none reported†	*MKI67, EXOSC6, RAD51C, MAP3K6*	de novo, AML NOS	Refractory	no
10	ND	-	0.46	A159P	1.24	N/D	mono	LOF	cytogenetics not classified as favorable or adverse	*SMC3, TLL2, MLL2, FANCM, CDH1, SETBP1, NRAS, NRAS, TNFRSF14, MAP3K6, MAP3K6, FANCD2, TET2, HIST1H1E, EGFR, TRRAP, KDM4C, PHF6*	de novo, AML NOS	Relapse	no

*AML type = de novo or secondary, World Health Organization 2016 classification[3]. †'None reported' means cytogenetics were collected but no abnormalities were identified.

#, number of; AML, acute myeloid leukemia; AML-MRC, AML with myelodysplasia-related changes; CRp, CRp, complete remission with incomplete platelet recovery; DoR, duration of antileukemic response; EoT, end of treatment; LOF, loss of function; MLFS, morphologic leukemia-free state; mo, months; N/D, not done; NOS, not otherwise specified; OS, overall survival; RD, residual disease; sAML, secondary AML; SD, stable disease; t-AML, therapy-related AML; VAF, variant allele frequency.

Supplemental Table 4. Serious AEs occurring in ≥2 patients.

MedDRA preferred term, n (%)	ven-idasa, n = 55
Febrile neutropenia	20 (36.4)
Sepsis	9 (16.4)
Pneumonia	8 (14.5)
Cellulitis	4 (7.3)
Septic shock	4 (7.3)
Pneumonia fungal	3 (5.5)
Diarrhea	2 (3.6)
Pyrexia	2 (3.6)
Escherichia bacteremia	2 (3.6)
Escherichia sepsis	2 (3.6)
Fungal infection	2 (3.6)
Ejection fraction decreased	2 (3.6)
Acute kidney injury	2 (3.6)
Respiratory failure	2 (3.6)
Pleural effusion	2 (3.6)

AE, adverse event; idasa, idasanutlin; MedDRA, Medical Dictionary for Regulatory Activities; ven, venetoclax.

Supplemental Table 5. Causes of death* on study and for 30- and 60-day mortality estimates.

	Ven-idasa, n = 56		
	On study overall (n)	**30-day mortality (n)***	**60-day mortality (n)***
Cause of death	Disease progression (34) AE (8)[†] Other (2)	Respiratory failure (1)[‡¶] Nervous system failure (1)[‡§] Progressive disease (1)	Pneumonia bacterial (1)[‡] Progressive disease (5)

*None of the deaths occurred in the setting of marrow remission or response. [†]AEs included: Cardiorespiratory arrest (related to ven-idasa, n = 1), hemophagocytic lymphohistiocytosis (unrelated to ven-idasa, n = 1), pneumonia (related to ven-idasa, n = 1), sepsis (related to ven-idasa, n = 1), fungal infection (unrelated to ven-idasa, n = 1), pneumonia bacterial (unrelated to ven-idasa, n = 1), nervous system disorder (unrelated to ven-idasa, n = 1), respiratory failure (unrelated to ven-idasa, n = 1). [¶]Respiratory failure occurred in the setting of multifocal pneumonia. [‡]Assessed as unrelated to ven-idasa. [§]Nervous system failure was described as worsening agitation, with decline in consciousness, in the setting of ongoing prostatitis, urinary tract infection, mucosal inflammation, and *Klebsiella* sepsis.

AE, adverse event; idasa, idasanutlin; ven, venetoclax.

Supplemental Table 6. Efficacy for ven-idasa amongst the intent-to-treat population including details of cohort B1.2 (ven 600 mg + idasa 150 mg) and B2 (ven 600 mg + idasa 200 mg) reported separately.

	Dose escalation					Dosing optimization	Total, n = 56
n (%)	ven 400 mg + idasa 200 mg, n = 6	ven 600 mg + idasa 150 mg, n = 13	ven 600 mg + idasa 200 mg, n = 22	ven 400 mg + idasa 400 mg, n = 9	Total, n = 50	ven 600 mg (D1–21) + idasa 150 mg, n = 6	
Antileukemic responders (CRc/PR/MLFS)	1 (16.7)	8 (61.5)	9 (40.9)	2 (22.2)	20 (40.0)	2 (33.3)	22 (39.3)
CRc (CR/CRi/CRp)	1 (16.7)	5 (38.5)	7 (31.8)	0 (0.0)	13 (26.0)	2 (33.3)	15 (26.8)
CR	0 (0.0)	1 (7.7)	2 (9.1)	0 (0.0)	3 (6.0)	1 (16.7)	4 (7.1)
CRi	0 (0.0)	0 (0.0)	1(4.5)	0 (0.0)	1 (2.0)	0 (0.0)	1 (1.8)
CRp	1 (16.7)	4 (30.8)	4 (18.2)	0 (0.0)	9 (18.0)	1 (16.7)	10 (17.9)
PR	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	1 (2.0)	0 (0.0)	1 (1.8)
MLFS	0 (0.0)	3 (23.1)	2 (9.1)	1 (11.1)	6 (12.0)	0 (0.0)	6 (10.7)
Time to best CRc response, median (range)	1.3 (1.3–1.3)	2.1 (1.0–4.3)	1.3 (0.8–2.7)	NE	1.4 (0.8–4.3)	3.2 (1.6–4.8)	1.6 (0.8–4.8)
Median duration of response (CRc), median (range)	NE (0.7*–0.7*)	4.2 (2.2–12.5)	3.9 (1.4–9.7)	NE	3.9 (0.7*–12.5)	NE (1.0–1.2*)	3.0 (0.7*–12.5)
Median follow-up, median (range)	2.3 (1.6–21.9)	5.3 (1.4–17.7)	5.4 (0.0–23.3)	2.8 (0.4–14.4)	3.9 (0.0–23.3)	4.4 (3.0–5.8)	4.0 (0.0–23.3)

*Censored, response is ongoing before patient dropout.
CR, complete remission; CRc, composite complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; D, day; idasa, idasanutlin; MLFS, morphologic leukemia-free state; NE, not evaluable; PR, partial response; ven, venetoclax.

Supplemental Table 7. Summary of pharmacokinetic data of ven and idasa.

	Cohort B1, n = 6	Cohort B4, n = 8	Cohort B1.2.1, n = 5	Cohort B1.2, n = 11	Cohort B2, n = 19
ven (400–600 mg)	ven 400 mg	ven 400 mg	ven 600 mg	ven 600 mg	ven 600 mg
Mean C_{trough} (range) µg/mL	0.299 (0.0531–0.799)	0.485 (0.0459–1.56)	0.52 (0.167–0.884)	0.462 (0.145–0.929)	0.455 (0.152–1.21)
Mean C_{max} (range) µg/mL	1.11 (0.26–1.88)	1.04 (0.22–1.95)	1.45 (0.74–2.00)	1.47 (0.69–3.54)	1.15 (0.34–2.09)
idasa (150–400 mg)	idasa 200 mg	idasa 400 mg	idasa 150 mg	idasa 150 mg	idasa 200 mg
Mean C_{trough} (range) µg/mL	2.24 (0.488–4.52)	3.89 (1.19–9.19)	2.07 (0.821–2.84)	2.17 (0.704–4.20)	2.72 (0.815–5.90)
Mean C_{max} (range) µg/mL	3.77 (1.16–8.74)	7.11 (1.39–16.3)	3.22 (1.86–3.93)	3.76 (1.63–7.42)	4.13 (1.51–7.37)

C_{max}, observed maximum plasma drug concentration at steady state; C_{trough}, observed minimum plasma drug concentration at steady state; idasa, idasanutlin; ven, venetoclax.

Supplemental Table 8. Antileukemic response rates in mutation subsets.

	n	CRc, n (%)	MLFS+, n (%)	mDoR	mOS	EoT *TP53* evaluable	EoT *TP53* emergence
Evaluable	52	14 (26.9)	20 (38.5)	2.96	3.95	36	12 (33.3)
IDH1/2	14	7 (50.0)	11 (78.6)	4.86	7.64	11	4 (36.4)
RUNX1	20	9 (45.0)	12 (60.0)	5.30	6.45	18	6 (33.3)
IDH1/2 and/or RUNX1	28	14 (50.0)	18 (64.3)	5.17	6.89	23	7 (30.4)
TP53	10	2 (20.0)	3 (30.0)	2.3	3.67	N/A	N/A
N/KRAS	15	0	3 (20.0)	2.89	2.76	12	5 (41.7)
FLT3 (ITD and TKD)	10	1 (10.0)	2 (20.0)	9.84	2.68	7	2 (28.6)
CBL, NF1, PTPN11	15	4 (26.67)	4 (26.7)	7.26	4.62	11	2 (18.2)
Any signaling mut*	30	5 (16.67)	8 (26.7)	5.46	3.6	22	6 (27.2)
2+ signaling muts*	10	1 (10.0)	2 (20.0)	12.52	3.98	9	3 (33.3)

TP53 outgrowth indicates the number of patients that had 1 or more *TP53* mutations emerge (not detected >1% VAF at baseline) on therapy.

*****Signaling mutations include *FLT3*, *NRAS, KRAS, CBL, NF1* and *PTPN11.*

cCR, composite complete response; DoR, duration of response; EoT, end of treatment; m, median; MLFS+, morphologic leukemia-free state (antileukemic response rate); mut, mutation; OS, overall survival.

Supplemental Table 9. Emergent *TP53* mutations and co-occurring mutations.

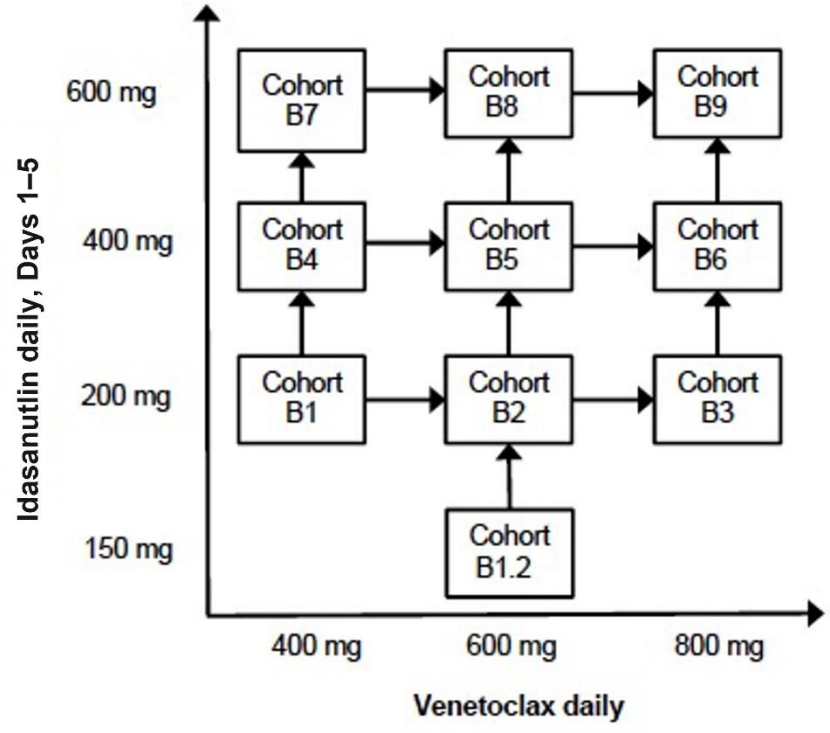
Mutation(s) DNA	Mutation(s) AA	*TP53* mutation impact	Baseline detected in BAM (VAF)	Baseline detected by DS (VAF)	DS sensitivity*, #*TP53* muts	Rate of outgrowth DVAF/day	Baseline mutations (irradicated† muts in bold)	EoT mutations (emergent muts in bold)	Best response	DoR (mo)	OS (mo)
733G>A	G245S	LOF	No	Yes (0.011%)	.0111%, 30	0.120	***FLT3, IDH2,*** *CBL, PTPN11, ETV6, CTCF, SRSF2, CEBPA, NRAS, YY1AP1, HDAC1, ASXL1, BARD1, NSD1, ROS1, SMO, PTCH1*	***TP53,*** *CBL, PTPN11, ETV6, CTCF, SRSF2, YY1AP1, HDAC1, ASXL1, BARD1, NSD1, ROS1, SMO, PTCH1*	SD	N/A	2.56
827C>G	A276G	LOF	Yes (0.0019%)	Yes (0.004%)	.0167%, 30	0.009	*MRE11A, ARID2, TYK2, CD22, ZNF217, EPHA5, ARHGAP26, ZNF703*	***TP53, TP53, EXOSC6****, MRE11A, ARID2, TYK2, CD22, ZNF217, EPHA5, ARHGAP26, ZNF703,*	CRp	1.41	15.51
646G>A	V216M	LOF	Yes (0.0017%)	Yes (0.005%)		0.017					
535C>G	H179D	LOF	No	Yes (0.014%)	.04%, 12	0.608	***NRAS,*** *NRAS, ETV6, SRSF2, AKT2, ASXL1, ERG, CASP8, HDAC4, TRRAP, RAD21, FANCG, STAG2, DKC1*	***TP53, CBL, PTPN11,*** *ETV6, SRSF2, AKT2, NRAS, ASXL1, ERG, CASP8, HDAC4, TRRAP, RAD21, FANCG, STAG2, DKC1*	ND	N/A	1.51
712T>A	C238S	LOF	No	Yes (0.067%)	.0278%, 55	0.016	***MAF****, TP53, TP53, ERBB2, ERBB2, NOTCH2, DNMT3A, DNMT3A, ESR1*	***TP53, MPL, ASXL1, EZH2****, TP53, TP53, ERBB2, ERBB2, NOTCH2, , DNMT3A, ESR1, EZH2*	SD	N/A	4.37
818G>A	R273H	LOF	Yes (0.18%)	Yes (0.204%)	.0128%, 75	0.004	***ASXL1****, MKI67, HDAC7, IDH2, BLM, SRSF2, CALR, CIC, NTRK1, RUNX1, LRP1B, FBXO11, FANCD2, CSF1R, JAK2, PHF6*	***TP53, TP53, PBRM1, RUNX1,*** *RUNX1, MKI67, HDAC7, IDH2, BLM, SRSF2, CALR, CIC, NTRK1, LRP1B, FBXO11, FANCD2, CSF1R, JAK2, PHF6*	CR	8.11	17.58
711G>A	M237I	LOF	No	Yes (0.040%)		0.013					
470T>G	V157G	LOF	Yes (0.07%)	No		0.004					
844C>T	R282W	LOF	Yes (0.0012%)	No	.0119%, 93	0.012	*FLT3, IDH2, SETBP1, TYK2, DOT1L, U2AF2, NRAS, ASXL1, RUNX1, PASK, ALK, FBXO11, NSD1, FANCE, EGFR, STAG2, ASMTL*	***TP53, TP53, TP53, TP53, SETBP1, STAG2****, SETBP1, STAG2, FLT3, IDH2, TYK2, DOT1L, U2AF2, NRAS, ASXL1, RUNX1, PASK, ALK, FBXO11, NSD1, FANCE, EGFR, ASMTL*	MLFS	13.63	14.36
746G>C	R249T	LOF	Yes (0.0008%)	Yes (0.008%)		0.010					
589G>C	V197L	Partial LOF	Yes (0.0006%)	Yes (0.003%)		0.063					
332T>A	L111Q	LOF	No	Yes (0.090%)		0.013					
701A>G	Y234C	LOF	Yes (0.0016%)	N/D	N/D	0.204	***IDH2, RUNX1, FGF14, ASXL1, SRSF2, CEBPA,***	***TP53, RICTOR,*** *TSC2, NOTCH2, PASK, FANCD2,*	MLFS	2.89	3.81

							CEBPA, CEBPA, NRAS, *TSC2, NOTCH2, PASK, FANCD2, KLHL6, FGF12, EBF1, FLT4, CD36*	*KLHL6, FGF12, EBF1, FLT4, CD36*			
659A>G	Y220C	LOF	Yes (0.09%)	No	.0278%, 33	0.025	*MKI67, LRRK2, FLT1, BRCA2, TYK2, PPP2R1A, RUNX1, WHSC1, POT1, FAM123B*	***TP53, TP53, PIK3C2G, PMS2, PHF6, DOT1L,*** *MKI67, LRRK2, FLT1, BRCA2, TYK2, PPP2R1A, RUNX1, WHSC1, POT1, FAM123B*	CRp	8.77	23.26
527G>A	C176Y	LOF	No	No		N/D					
772G>A	E258K	LOF	Yes (0.4%)	N/D	N/D	0.472	***EP300,*** *FGFR2, PTPRO, SRSF2, PIK3R2, PPP2R1A, RUNX1, DNMT3A, TET2, TET2, ZNF703*	***TP53, TP53, EPHA3, FOXO3, NOTCH4,*** *FGFR2, PTPRO, SRSF2, PIK3R2, PPP2R1A, RUNX1, DNMT3A, TET2, TET2, ZNF703*	CRp	3.02	9.76
527G>A	C176Y	LOF	Yes (0.1%)			0.024					
838A>G	R280G	LOF	Yes (0.02%)	N/D	N/D	0.010	***RUNX1, IDH1, NPM1, SRSF2, FGF14, CEBPA,*** *MLL2, FANCM, TSHR, WDR90, SETBP1, RAD54L, JAK1, BAP1, TET2, NOTCH1, NOTCH1*	***TP53, TP53,*** *MLL2, FANCM, TSHR, WDR90, SETBP1, RAD54L, JAK1, BAP1, TET2, NOTCH1, NOTCH1*	CRp	0.72	21.95
644G>A	S215N	LOF	No			N/D					
659A>G	Y220C	LOF	Yes (0.0009%)	N/D	N/D	0.002	***CPS1, EZH2, EZH2, TET2, NF1,*** *PTPN11, WDR90, CCT6B, SF3B1, CSF1R, EBF1, RELN, PCLO, RUNX1T1, NOTCH1*	***TP53, TP53,*** *PTPN11, WDR90, CCT6B, SF3B1, CSF1R, EBF1, RELN, PCLO, RUNX1T1, NOTCH1*	CR	11.4	13.67
524G>A	R175H	LOF	Yes (0.0019%)			0.080					
743G>A	R248Q	LOF	Yes (0.0128%)	N/D	N/D	0.014	***MUTYH, XPO1,*** *TET2, TET2, FAT3, TBX3, MLL2, CREBBP, NRAS, MPL, FGFR3, NPM1*	***TP53, TP53, TP53, TP53, MDM4, TET2,*** *TET2, TET2, FAT3, MLL2, CREBBP, NRAS, MPL, FGFR3, NPM1*	SD	N/A	4.07
659A>C	Y220S	LOF	No			N/D					
581T>G	L194R	LOF	Yes (0.0006%)			0.009					
536A>G	H179R	LOF	Yes (0.0007%)			0.017					

*Duplex sequencing sensitivity is dependent on sequencing depth at a particular region and is sample specific. Sensitivity provided is mean sensitivity (95% power to detect a mutation) across *TP53* for each sample. #Total number of low level *TP53* mutations detected by duplex sequencing for each baseline sample. †Irradicated mutations are those detected at baseline but not at end of treatment. Orange = fast rate of growth (0.1–0.9 DVAF/day); yellow = intermediate rate of growth (0.01–0.09 DVAF/day); green = slow rate of growth (001–0.009 DVAF/day).
AA, amino acid; BAM, binary alignment map; CR, complete remission; CRp, complete remission with incomplete platelet count recovery; DoR, duration of antileukemic response; DS, duplex sequencing; EoT, end of treatment; LOF, loss of function; MLFS, morphologic leukemia-free state; mo, months; muts, mutations; N/A, not applicable; N/D, not done; ND, nodal disease; OS, overall survival; SD, stable disease; VAF, variant allele frequency.

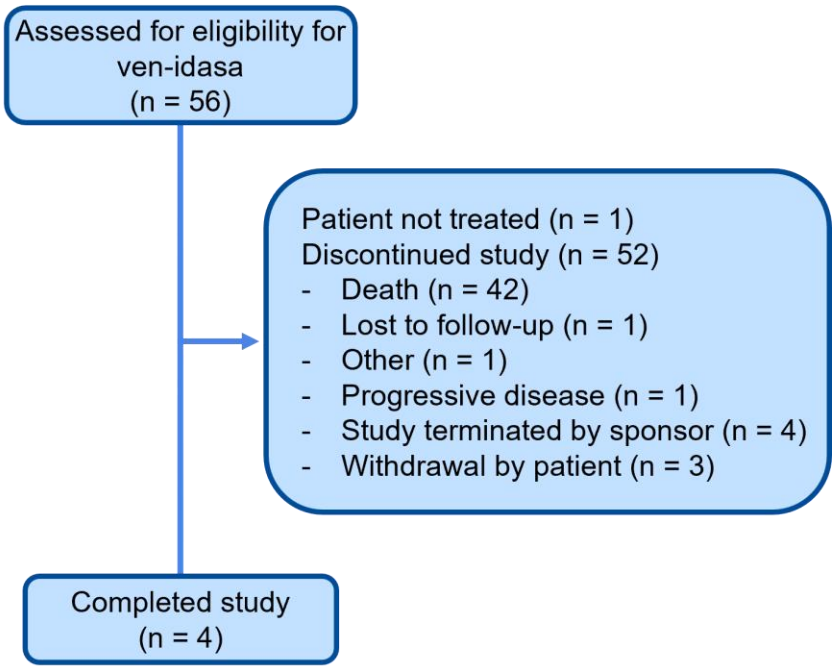
Supplementary Figures

Supplemental Figure 1. Dose-escalation plan for treatment with ven-idasa.



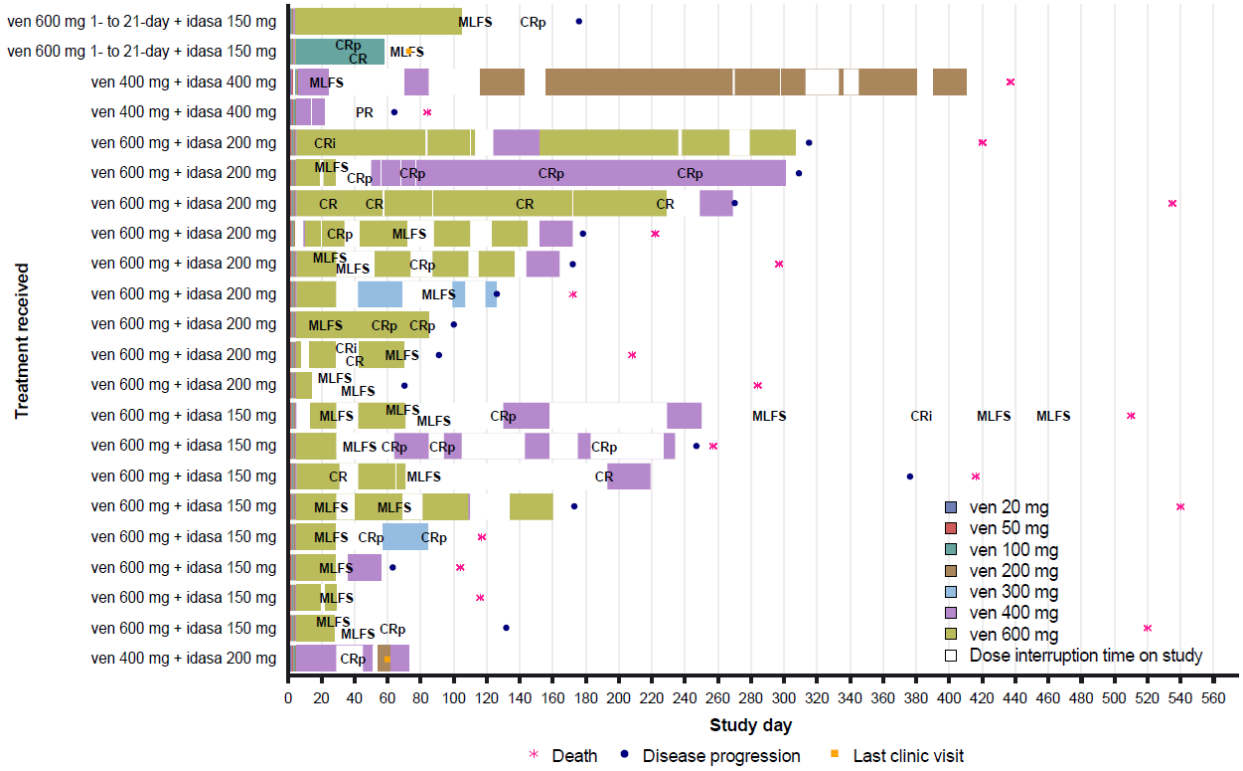
Note: ven doses listed represent the target doses after ramp-up period.
Actual numbers of patients who received doses in the DE stage were: ven 400 mg + idasa 200 mg, n = 6; ven 600 mg + idasa 150 mg, n = 13; ven 600 mg + idasa 200 mg, n = 21; ven 400 mg + idasa 400 mg, n = 9. In the DSO stage, 6 patients received ven 600 mg (days 1–21) + idasa 150 mg.
DE, dose escalation; DSO, dose scheduling optimization; idasa, idasanutlin; ven, venetoclax.

Supplemental Figure 2. Patient flow (CONSORT) diagram.



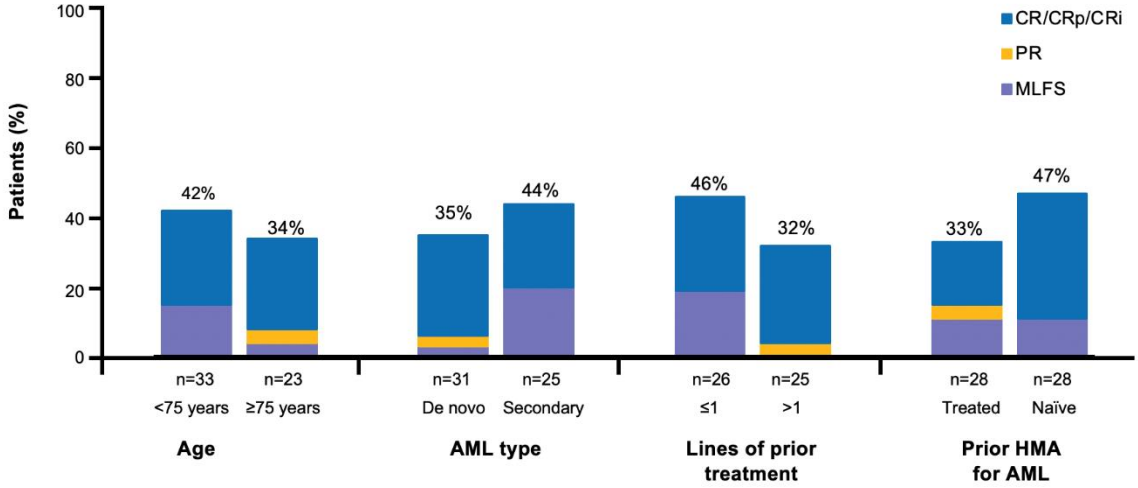
idasa, idasanutlin; ven, venetoclax.

Supplemental Figure 3. Swimplots of individual patients with MLFS or better showing dose interruptions/modifications and responses.



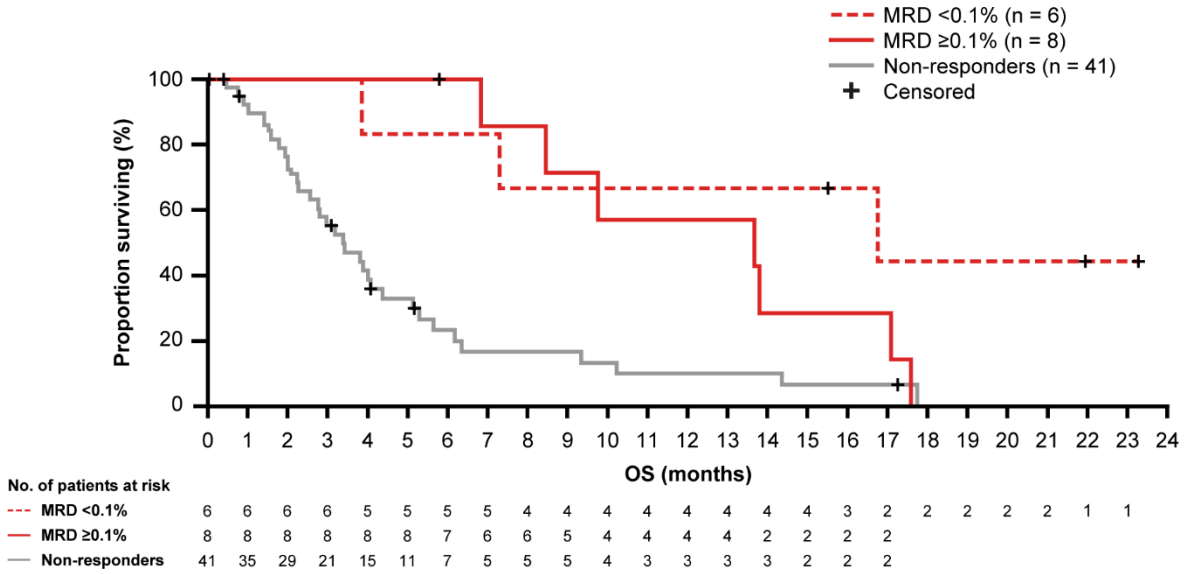
CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; idasa, idasanutlin; MLFS, morphologic leukemia-free state; PR, partial response; ven, venetoclax.

Supplemental Figure 4. Response rates to ven-idasa in patient subgroups based on baseline characteristics.



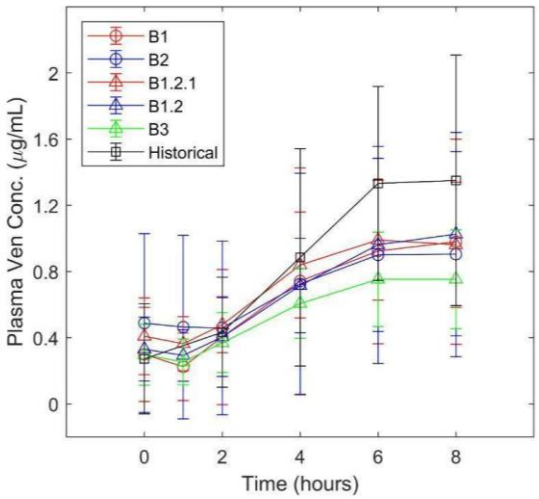
AML, acute myeloid leukemia; CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; HMA, hypomethylating agents; idasa, idasanutlin; Int, intermediate; MLFS, morphologic leukemia-free state; PR, partial response; ven, venetoclax.

Supplemental Figure 5. Kaplan-Meier curves of OS in the ven-idasa arm by MRD status for responders (CR/CRi/CRp) and non-responders.



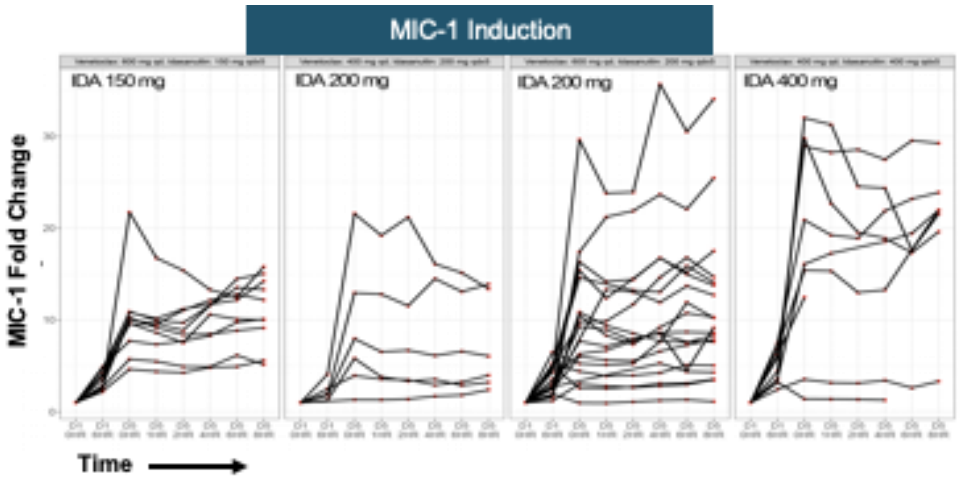
CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; idasa, idasanutlin; MRD, minimal residual disease; OS, overall survival; ven, venetoclax.

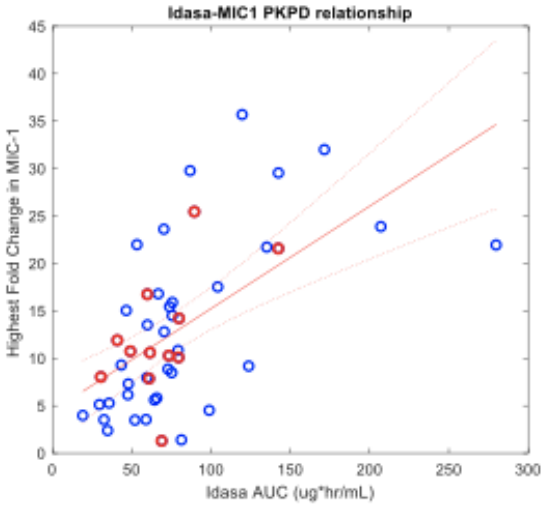
Supplemental Figure 6. Pharmacokinetics of ven. Dose normalized (400 mg) plasma ven concentration-time profile (pharmacokinetic profile) in patients treated with ven-idasa in comparison to historical plasma ven concentrations after 400 mg oral ven in patients with AML.



AML, acute myeloid leukemia; Conc., concentration; idasa, idasanutlin; ven, venetoclax.

Supplemental Figure 7. MIC-1 induction by idasa dose and idasa exposure. Top figure displays change in MIC-1 protein plotted over time. Each graph represents a different dose of idasa. Bottom figure represents best fold change induction of MIC-1 plotted against idasa exposure. A highly significant correlation is observed (P = 7.464 x 10^{-6}).





Linear regression model:
MIC_fold ~ 1 + Idasa_AUC

Estimated Coefficients:

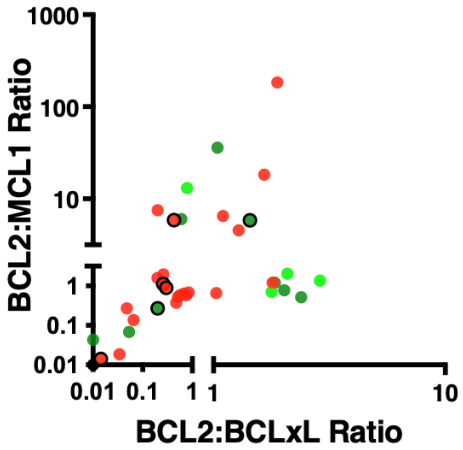
	Estimate	SE	tStat	pValue
(Intercept)	4.4451	1.9692	2.2574	0.028776
Idasa_AUC	0.10791	0.021374	5.0487	7.464e-06

Number of observations: 48, Error degrees of freedom: 46
Root Mean Squared Error: 6.98
R-squared: 0.357, Adjusted R-Squared: 0.343
F-statistic vs. constant model: 25.5, p-value = 7.46e-06

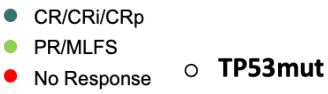
Red circles represent patients with CR/CRi/CRp, suggesting that MIC-1 may not relate to clinical response.
AUC, area under the curve (area under the plasma concentration versus time curve within a dosing interval of 24 hours); CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; IDA/idasa, idasanutlin; PKPD, pharmacokinetic/pharmacodynamic; MIC-1, macrophage inhibitory cytokine-1; SE, standard error.

Supplemental Figure 8. Scatter plot of BCL-2:BCL-xL and BCL-2:MCL-1 status for responders and non-responders BCL-2 family scatter plot. BCL-2, BCL-xL and MCL-1 protein were assessed by flow cytometry in blood at baseline. Ratios of percent positive cells are depicted. Using a cut-off of 1.5, patients who are low for both BCL-2:MCL-1 and BCL-2:BCL-xL are enriched for non-response, whereas a high ratio for either enriches for responders. Black circles - patients with a *TP53* mutation >1% VAF at baseline. While interpretability is limited due to the small number of patients and missing flow cytometry data, *TP53* mutation status did not appear to be associated with either high or low BCL-2 family ratios.

ven-idasa; *P* = 0.09669*

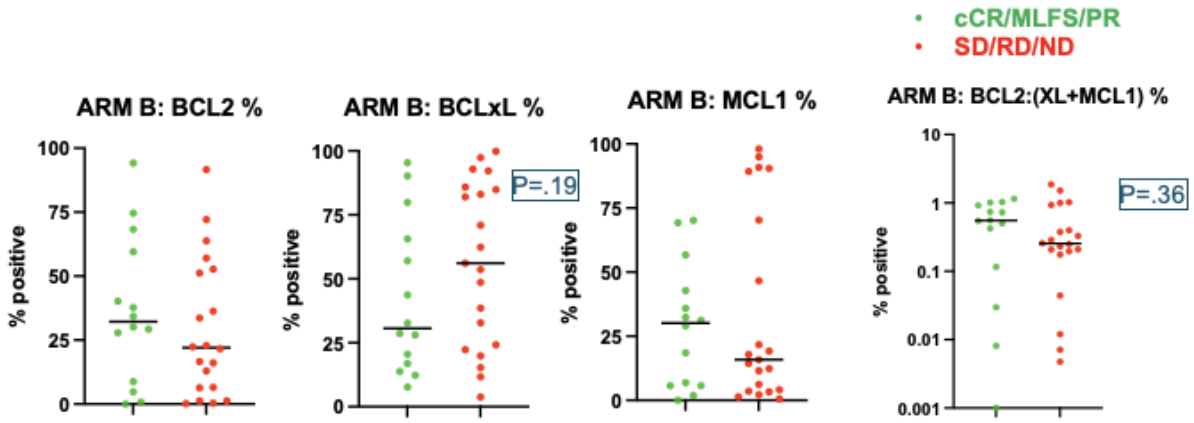


Arm B	Responder (PR or better)	Non-Responder (SD, RD, ND)
High (n=19)	10 (53)	9 (47)
Low (n=17)	4 (24)	13 (76)



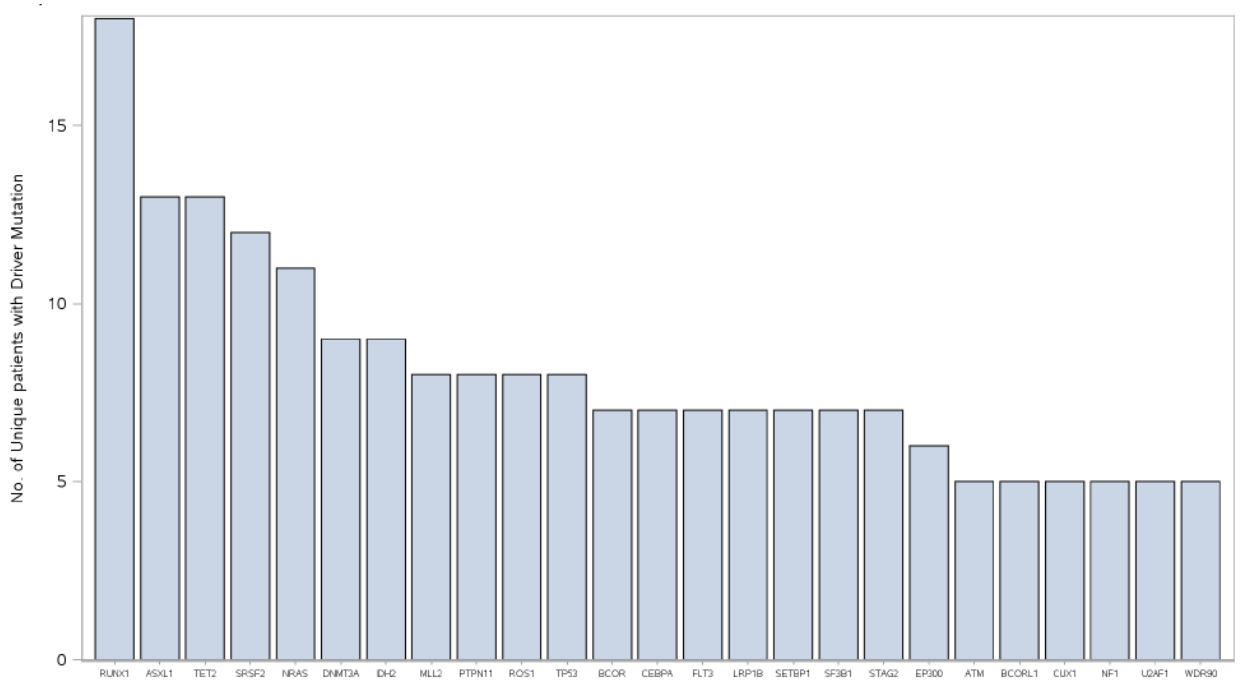
*Fisher's exact test.
BCL, B-cell lymphoma; CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, complete remission with incomplete platelet recovery; idasa, idasanutlin; MCL-1, myeloid leukemia 1; MLFS, morphologic leukemia-free state; ND, not done; PR, partial response; RD, residual disease, SD, stable disease; ven, venetoclax; xL, extra large.

Supplemental Figure 9. BCL-2 family flow. BCL-2 family assessed by flow cytometry in blood at baseline in responders and non-responders treated with ven-idasa. Percent positive BCL-2, BCL-xL and MCL-1 cells are plotted. In addition, the ratio of BCL-2 to (BCL-xL + MCL-1) was calculated (right). For ven-idasa, non-responders had high BCL-xL and somewhat lower BCL-2.

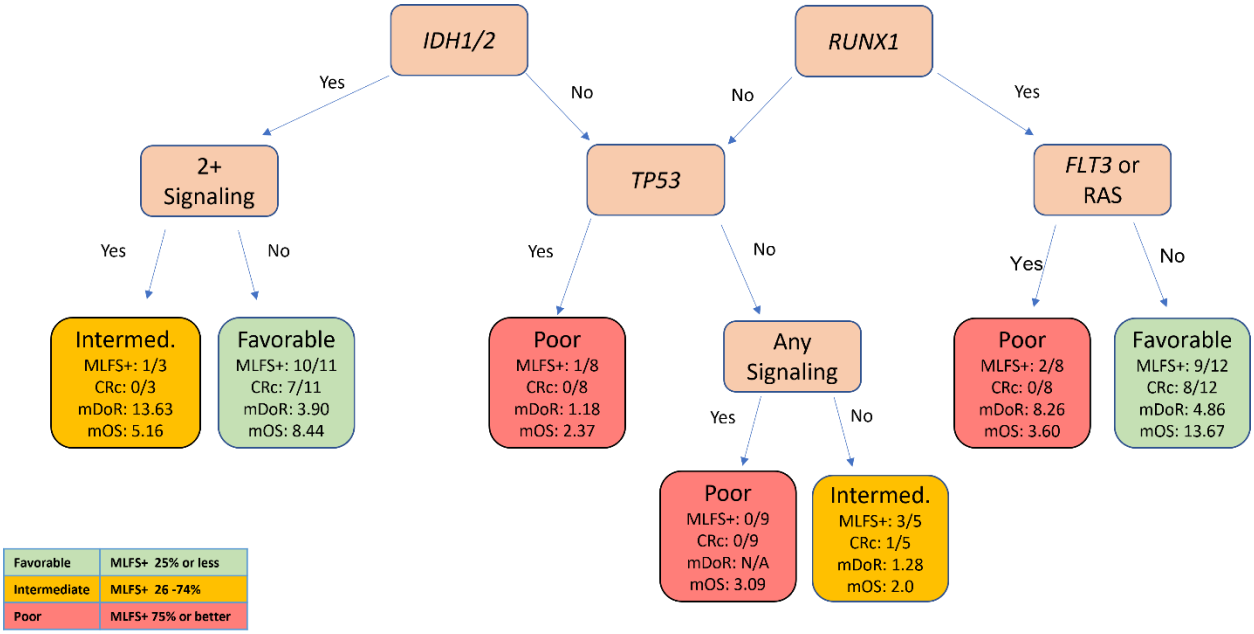


BCL, B-cell lymphoma; cCR, composite complete remission; idasa, idasanutlin; MCL-1, myeloid leukemia 1; MLFS, morphologic leukemia-free state; ND, not done; PR, partial response; RD, residual disease; SD, stable disease; ven, venetoclax; xL, extra large.

Supplemental Figure 10. Frequently mutated genes. Most commonly mutated genes in the ven-idasa arm.



Supplementary Figure 11. Proposed hierarchy of co-mutations and associated response. *IDH1/2* and *RUNX1* were favorable prognostic mutations unless they co-occurred with RAS signaling mutations, 2 or more for *IDH1/2* and *FLT3* or *N/KRAS* for *RUNX1*. In the absence of *IDH1/2* or *RUNX1* mutations, *TP53* was unfavorable. In the absence of *IDH1/2*, *RUNX1*, or *TP53* mutations, any RAS signaling mutations were poor prognostic mutations. Antileukemic response rate (MLFS+), CRc rate, mDOR and mOS are shown for each group.



CR, complete remission; CRc, composite complete remission; mDOR, median duration of response; MLFS, morphologic leukemia-free state; mOS, median overall survival.

Supplemental Figure 12. Clonal dynamics on ven-idasa. ScDNA-seq using the Tapestry platform (Mission Bio) reveals outgrowth of clones harboring mutations in *TP53* and *RAS* pathway genes (*NRAS* and *NF1*).

Subclones identified

Variant	WT	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
***EPHA3*:chr3:89498433:C/G**	WT	WT	WT	Het	Het	WT
***FGFR2*:chr10:123263332:C/A**	WT	Het	WT	Het	Het	Het
***FOXO3*:chr6:108985398:C/G**	WT	WT	WT	Het	Het	Het
***NOTCH4*:chr6:32171570:C/G**	WT	WT	WT	Het	WT	Het
***TET2*:chr4:106158055:G/T**	WT	Het	WT	Het	Het	Het
***TET2*:chr4:106197258:T/A**	WT	Het	WT	Het	Het	Het
***TP53*:chr17:7577509:C/T**	WT	WT	WT	Hom	Hom	Hom
***TP53*:chr17:7578403:C/T**	WT	WT	Het	WT	WT	WT
Proportion of each clone						
Screen	13.70%	86.16%	0.14%	0.00%	0.00%	0.00%
C1D22 (MLFS)	27.65%	0.61%	69.93%	1.41%	0.40%	0.00%
C6D1 (relapse)	1.74%	0.00%	3.14%	69.34%	12.54%	13.24%

Subclones identified

Variant	WT	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
***IDH2*:chr15:90631934:C/T**	WT	WT	WT	Het	Het	Het
***NRAS*:chr1:115258748:C/T**	WT	Het	Het	WT	Het	WT
***SETBP1*:chr18:42531907:G/A**	WT	Het	Het	WT	WT	WT
***TP53*:chr17:7577535:C/G**	WT	WT	WT	WT	WT	Hom
***TP53*:chr17:7578260:C/G**	WT	WT	Het	WT	WT	WT
Proportion of each clone						
Screen	11.94%	82.12%	0.00%	5.87%	0.06%	0.00%
C10D1 (SD)	53.75%	13.22%	18.03%	1.63%	7.92%	5.45%

Subclones identified

Variant	WT	Clone 1	Clone 2
MIB1:chr18:19371473:A/T	WT	Het	Het
NF1:chr17:29657521:G/C	WT	WT	Hom
SF3B1:chr2:198266834:T/C	WT	Het	Het
Proportion of each clone			
C1D28 (MLFS)	53.72%	45.57%	0.71%
C1D42 (relapse)	6.65%	8.67%	84.68%

Subclones identified

Variant	WT	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6
DNMT3A:chr2:25463483:G/A	WT	Het	WT	Het	Hom	Het	Het
DNMT3A:chr2:25469085:CG/C *R458Gfs*	WT	WT	Hom	WT	WT	Het	WT
DNMT3A:chr2:25469502:C/T *L422=*	WT	Het	WT	Het	Hom	Het	Het
KRAS:chr12:25398255:G/T *Q22K*	WT	WT	WT	Het	WT	WT	WT
TP53:chr17:7578190:T/C *Y181C*	WT	WT	WT	WT	WT	WT	Het
Proportion of each clone							
Screen	0.79%	16.85%	80.83%	0.00%	0.33%	1.19%	0.00%
C1D42 (CRp)	4.55%	83.04%	0.08%	5.36%	4.87%	1.22%	0.89%

CRp, complete remission with incomplete platelet recovery; Het, heterozygous; Hom, homozygous; MLFS, morphologic leukemia-free state; idasa, idasanutlin; scDNA-seq, single cell DNA sequencing; SD, stable disease; ven, venetoclax; WT, wild-type.