RESEARCH ARTICLE

A Phase Ib/II Study of Ivosidenib with Venetoclax ± Azacitidine in *IDH1*-Mutated Myeloid Malignancies

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ABSTRACT The safety and efficacy of combining the isocitrate dehydrogenase-1 (IDH1) inhibitor ivosidenib (IVO) with the BCL2 inhibitor venetoclax (VEN; IVO + VEN) ± azacitidine (AZA; IVO + VEN + AZA) were evaluated in four cohorts of patients with *IDH1*-mutated myeloid malignancies ($n = 31$). Most (91%) adverse events were grade 1 or 2. The maximal tolerated dose was not reached. Composite complete remission with IVO + VEN + AZA versus IVO + VEN was 90% versus 83%. Among measurable residual disease (MRD)–evaluable patients ($N = 16$), 63% attained MRD-negative remissions; *IDH1* mutation clearance occurred in 64% of patients receiving ≥5 treatment cycles ($N = 14$). Median event-free survival and overall survival were 36 [94% CI, 23–not reached (NR)] and 42 (95% CI, 42-NR) months. Patients with signaling gene mutations appeared to particularly benefit from the triplet regimen. Longitudinal single-cell proteogenomic analyses linked cooccurring mutations, antiapoptotic protein expression, and cell maturation to therapeutic sensitivity of *IDH1*-mutated clones. No IDH isoform switching or second-site *IDH1* mutations were observed, indicating combination therapy may overcome established resistance pathways to single-agent IVO.

SIGNIFICANCE: IVO + VEN + AZA is safe and active in patients with *IDH1*-mutated myeloid malignancies. Combination therapy appears to overcome resistance mechanisms observed with single-agent IDH-inhibitor use, with high MRD-negative remission rates. Single-cell DNA ± protein and time-of-flight mass-cytometry analysis revealed complex resistance mechanisms at relapse, highlighting key pathways for future therapeutic intervention.

INTRODUCTION

Acute myeloid leukemia (AML) represents a malignant clonal disorder of precursor hematopoietic cells containing a diversity of recurrent cytogenetic alterations and gene mutations (1, 2). Many mutations are prognostic and/or predictive; a minority have FDA-approved therapies available (3, 4). Molecularly tailored therapy enables individualization of treatment and can improve survival; however, relapse remains the primary cause of treatment failure (4, 5).

Mutations within exon 4 of the isocitrate dehydrogenase-1 gene (*IDH1*) are identified in approximately 7% to 8% of patients with AML (5, 6). The resultant amino acid substitution at R132 enables neomorphic IDH1 activity, catalyzing a reverse redox reaction resulting in the accumulation of 2-hydroxyglutarate (2-HG; refs. 7, 8). 2-HG inhibition of downstream alpha-ketoglutarate-dependent enzymes results in impaired epigenetic regulation, DNA hypermethylation, and arrested myeloid differentiation (7–9).

The small-molecule IDH1 inhibitor ivosidenib (IVO; AG-120) demonstrated efficacy as monotherapy in patients with newly diagnosed (ND) and relapsed/refractory *IDH1*-mutated AML, resulting in complete response (CR) and CR with partial hematologic recovery (CRh) rates of 42% and 30% (10, 11), and was associated with reduced plasma 2-HG concentrations and promoted myeloblast differentiation (4, 11). Relapse following IVO is characterized by mechanisms intrinsic to the IDH enzyme [i.e., development of an *IDH2* mutation (isoform switching), second-site *IDH1* mutations] and/or extrinsic causes including outgrowth of leukemic clones with mutations in genes other than *IDH1* (12, 13).

Additionally, *IDH*-mutated AML has marked dependence on the antiapoptotic protein B-cell lymphoma 2 (BCL2) for survival (14). This vulnerability underscores the favorable results described with the BCL-2 inhibitor venetoclax (VEN) in *IDH*-mutated AML (15, 16). Within this context, however, a differential treatment effect based on *IDH* isoforms may exist; patients with *IDH2* compared with *IDH1*-mutated AML have particularly favorable survival when treated with VEN in combination with azacitidine (AZA) or low-dose cytarabine (LDAC; refs. 17, 18).

A randomized phase III trial evaluating IVO in combination with AZA, versus AZA monotherapy in patients with ND *IDH1*-mutated AML resulted in CR/CRh in 53% versus 18% of patients and a median overall survival (OS) of 24 months versus 7.9 months (19). The survival benefit observed with IVO + AZA resulted in the approval of this molecularly targeted combination, increasing treatment options for ND patients with *IDH1*-mutated AML (19).

Although both VEN and IVO-based therapies in combination with AZA are efficacious in *IDH1*-mutated malignancies,

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Table 1. Patient demographics.

	Cohort				
Characteristic	Overall (*N* = 31)	DL1 (*N* = 6)	DL2 (*N* = 6)	DL3 (*N* = 13)	DL4 (*N* = 6)
Age, median (range)	67 (44–84)	69 (66–84)	68 (44–80)	65 (56–76)	66 (56–76)
Gender, *n*/*N* (%)					
Female	11/31 (35%)	3/6 (50%)	3/6 (50%)	4/13 (31%)	1/6 (17%)
Male	20/31 (65%)	3/6 (50%)	3/6 (50%)	9/13 (69%)	5/6 (83%)
Bone marrow blast %, median (range)	23 (1–80)	31 (1–64)	24 (10–61)	35 (7–80)	14 (6–32)
ECOG, *n*/*N* (%)					
0	8/30 (27%)	1/6 (17%)	0/6 (0%)	4/13 (31%)	3/5 (60%)
1	18/30 (60%)	4/6 (67%)	4/6 (67%)	8/13 (62%)	2/5 (40%)
2	4/30 (13%)	1/6 (17%)	2/6 (33%)	1/13 (7.7%)	0/5 (0%)
Disease, *n*/*N* (%)					
MDS/MPN	9/31 (29%)	1/6 (17%)	1/6 (17%)	2/13 (15%)	5/6 (83%)
t-MDS	1/31 (3.2%)	0/6 (0%)	0/6 (0%)	0/13 (0%)	1/6 (17%)
R/R-MDS	1/31 (3.2%)	1/6 (17%)	0/6 (0%)	0/6 (0%)	0/6 (0%)
ND-AML	14/31 (45%)	1/6 (17%)	3/6 (50%)	9/13 (69%)	1/6 (17%)
De novo AML	9/31 (29%)	1/6 (17%)	2/6 (33%)	5/13 (38%)	1/6 (17%)
ts-AML	5/31 (16%)	0/6 (0%)	1/6 (17%)	4/13 (31%)	0/6 (0%)
R/R-AML	8/31 (26%)	4/6 (67%)	2/6 (33%)	2/13 (15%)	0/6 (0%)
Prior treatment, *n*/*N* (%)	15/31 (48%)	5/6 (83%)	3/6 (50%)	6/13 (46%)	1/6 (17%)
Lines of therapy *n*/*N* (%)					
1	9/31 (29%)	2/6 (33%)	2/6 (33%)	4/13 (31%)	1/6 (17%)
2	1/31 (3.2%)	1/6 (17%)	0/6 (0%)	0/13 (0%)	0/6 (0%)
3	3/31 (9.7%)	1/6 (17%)	0/6 (0%)	2/13 (15%)	0/6 (0%)
4	2/31 (6.5%)	1/6 (17%)	1/6 (17%)	0/13 (0%)	0/6 (0%)
Prior IDH inhibitor, *n*/*N* (%)	3/31 (9.7%)	2/6 (33%)	1/6 (17%)	0/13 (0%)	0/6 (0%)
2022 ELN risk group, *n*/*N* (%)	*N* = 22	*N* = 5	*N* = 5	*N* = 11	*N* = 1
Favorable	8/22 (36%)	3/5 (60%)	2/5 (40%)	2/11 (18%)	1/1 (100%)
Intermediate	3/22 (14%)	1/5 (20%)	1/5 (20%)	1/11 (9.1%)	0/1 (0%)
Adverse	11/22 (50%)	1/5 (20%)	2/5 (40%)	8/11 (73%)	0/1 (0%)

Abbreviations: MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; ELN, European LeukemiaNet; R/R, relapsed/refractory; ND, newly diagnosed; ts-AML, treated-secondary AML; ECOG, Eastern Cooperative Oncology Group; t-MDS: therapy-related MDS.

preclinical data suggest synergism between IDH inhibitors and VEN in differentiating leukemic blasts, via augmentation of proapoptotic activator proteins (16, 19, 20). Thus, therapy with IVO + VEN or IVO + VEN + AZA may additionally be active in *IDH1*-mutated AML.

To the best of our knowledge, the safety and efficacy of IVO + VEN as an oral doublet, or IVO + VEN + AZA as a triplet combination has not previously been evaluated. This analysis reports the safety and efficacy data from the completed phase Ib (P1b) study of the combination of IVO + VEN ± AZA, in patients with IDH1-mutated myeloid malignancies (NCT03471260).

RESULTS

Between March 20, 2018 and May 28, 2021, 31 patients initiated study treatment [dose level (DL)1: *N* = 6, DL2: *N* = 6, DL3: *N* = 13, one patient was not evaluable due to early transition to hematopoietic cell transplantation (HCT) prior to completion of the dose-limiting toxicity (DLT) evaluation period and was replaced, another experienced a DLT requiring enrollment of an additional 6 patients, DL4: *N* = 6]. The median age at treatment initiation was 67 years (range, 44–84; Table 1). Enrolled patients identified as Caucasian [80%; *N* = 25), African American (16%; *N* = 5), or other/undisclosed (3%; *N* = 1).

Patients with AML comprised most of the study population [newly diagnosed (ND-AML); including treatment-naïve and secondary AML (sAML)/treated-secondary AML (ts-AML): 45% (*N* = 14); relapsed/refractory (R/R-AML): 26% (*N* = 8); myelodysplastic syndrome or myeloproliferative neoplasm (MDS or MPN): 29% (*N* = 9)]. DL1 enrolled more patients with R/R-AML (67%; *N* = 4), although DL4 enrolled more patients with MDS or MPN (83%; *N* = 5). Fifteen (48%) patients (R/R-AML: 8, ts-AML: 5, MDS or MPN: 2) received a median of 1 prior therapy (range, 1–4), including three patients treated with prior investigational IDH1 inhibitors [FT-2102 (olutasidenib): *N* = 2, BAY1436032: *N* = 1]. ELN 2022 risk in patients with AML (*N* = 22) was favorable in 36% (*N* = 8), intermediate in 14% (*N* = 3), and adverse in 50% (*N* = 11; ref. 21).

Baseline molecular, cytogenetic, and IDH1 variant data are displayed in Supplementary Fig. S1A–SE. A diploid karyotype

was most common (45%, $N = 14$), followed by adverse/complex cytogenetics (32%, $N = 10$). Grouped analysis across biological pathways identified frequent additional gene mutations in signaling (58%, $N = 18$), methylation (52%, $N = 16$), and cohesin–chromatin pathways (52%, $N = 16$), with no significant difference between patients with ND-AML, R/R-AML, or MDS and MPN. Variant allele frequency (VAF) analysis of bulk NGS sequencing at baseline indicated *IDH1* mutations occurred early, with VAFs similar to mutated methylation or splicing genes. Median mutation burden (including *IDH1*) per patient was 5 (range, 1–9) and did not significantly differ across disease groups (MDS or MPN: 3; ND-AML: 5, R/R-AML: 4.5).

IDH1 variants at baseline included R132C (48%, $N = 15$), R132H (29%; $N = 9$), R132G (13%, $N = 4$), R132S (7%, $N = 2$), and R132 L (3%, $N = 1$; Supplementary Fig. S1). Median *IDH1* VAF was 23% (range, 5%–48%), with no significant difference in VAF observed between variants. Additionally, no significant difference in *IDH1* variant or *IDH1* VAF was observed between patients with MDS or MPN; ND-AML, or R/R-AML.

Pharmacokinetics

Peripheral blood samples were obtained 0, 1, 2, 4, 6, 8, and 24 hours following administration of VEN ± AZA on cycle 1 day 14 (C1D14), and administration of IVO + VEN ± AZA on C2D14 to assess the effects of IVO + VEN combination therapy on plasma VEN concentrations. Median time to peak VEN plasma concentrations was similar across cohorts without [DL1: 4 (0–8), DL2: 8 (1–8), DL3: 6 (2–8), DL4: 6 (6–8) hours] or with (DL1: 4 (0–6), DL2: 8 (2–8), DL3: 6 (4–8), DL4: 5 (4–8) hours] concurrent IVO.

Due to known CYP-inducing effects of IVO, the 24-hour area under the curve (AUC_{0-24}) and maximum concentration (C_{max}) of VEN were evaluated at steady state both with and without concurrent IVO. The AUC and C_{max} of VEN were reduced by approximately 55% and 45%, respectively, in the setting of concurrent IVO (i.e., VEN 800 mg resulted in AUC_{0-24} and C_{max} levels on C2D14 similar to those observed with VEN 400 mg on C1D14 without IVO; Supplementary Table S1 and Supplementary Fig. S2A and S2B). Serum 2-HG analysis during C2 demonstrated serum 2-HG levels in responding patients were suppressed to levels observed in healthy individuals, as previously published (8).

Treatment Characteristics

Median time on study was 5.3 months (range, 1.4–44.9 months); patients received a median of 4 (range, 1–49) cycles of therapy [DL1: 8 (2–49), DL2: 6 (3–41), DL3: 4 (1–25), DL4: 4 (2–11); Supplementary Table S2]. Patients transitioning to HCT ($N = 12$) received a median of 3 (range, 1–5) cycles; patients not receiving consolidative HCT ($N = 19$) received a median of 11 cycles (range, 2–49).

Common causes for discontinuing treatment were transitioning to HCT (39%, $N = 12$) after a median of 3.4 months (range, 1.4–7.3 months), disease progression (26%, $N = 8$), no response ($N = 1$), and death ($N = 1$). Nine patients remain on study treatment as of March 15, 2022 (Supplementary Fig. S3). No patients discontinued therapy due to intolerance.

Adherence across DLs during the DLT evaluation period was >90% for all study agents. Adherence during the first 12 months of therapy is displayed in Supplementary Table S3. 14-Day courses of VEN were effective and well tolerated in both the doublet and triplet DLs; average VEN exposure during the first four cycles of therapy (the median number of cycles received in the triplet cohorts) was 102% (SD: 11%; due to inadvertent continuation of VEN beyond 14 days) and 87% (SD: 29%), for doublet and triplet cohorts, respectively. No significant difference between cohorts was observed with respect to IVO.

Dose Adjustments for Myelosuppression

IVO + VEN and IVO + VEN + AZA resulted in a marked reduction of bone marrow blasts following 1 cycle of therapy (median reduction with IVO + VEN: 75% vs. IVO + VEN + AZA: 91%; Supplementary Fig. S4A). Cycle length (i.e., time from C1D1 until C2D1, etc.) was longer in patients treated with IVO + VEN + AZA versus IVO + VEN for C1 (median 38 vs. 28 days, $P < 0.001$) and C2 (median 35 vs. 28 days, $P = 0.055$) and was consistent with median published cycle lengths of AZA + VEN (Supplementary Fig. S4B). Minimal myelosuppression was observed with IVO + VEN.

Cytopenias in responding patients treated with IVO + VEN + AZA were similar to that observed with AZA + VEN (16). Though not formally classified as protocol-defined DLTs, two previously treated patients with underlying MDS (ts-AML in DL3 and therapy-related MDS in DL4, respectively) treated with IVO + VEN + AZA had C1 durations of 105 and 79 days, related to myelosuppression.

Dose adjustments were more common with IVO + VEN + AZA (47%, $n = 9$) as compared with IVO + VEN (17%, $n = 2$), and mostly following C2. 73% ($N = 8/11$) consisted of reductions in VEN duration to 7 or 10 days in the absence of marrow disease (considered an appropriate and recommended modification for patients in remission with persistent neutropenia). Dose reductions for prolonged cytopenias occurred in 42% ($N = 5/12$) versus 32% ($N = 6/19$) of patients receiving VEN 800 mg versus VEN 400 mg combinations, respectively ($P = 0.71$; Supplementary Table S4).

Two patients (one in DL3 with ND-AML and one in DL4 with therapy-related MDS) discontinued AZA + VEN to continue with IVO monotherapy after C2 and C1, respectively, in the setting of CRc [CR + CRh + complete response with incomplete hematologic recovery (CRi)]. Overall, 94% ($N = 29/31$) of patients remained on all assigned study agents for the duration of time on therapy and no patients discontinued IVO during the study period.

Nonhematologic Adverse Events

Both IVO + VEN and IVO + VEN + AZA were well tolerated. Most (91%) of AEs were grade 1 or 2. Adverse events (AE) occurring in ≥10% of study participants separated by grade, receipt of the doublet or triplet, and DL are displayed in Supplementary Fig. S5A–S5C.

Grade 3–5 AEs per patient are displayed in Table 2. Febrile neutropenia (29%, $N = 9$) and lung infections (19%, $N = 6$) were the most common infectious events, though no significant difference was observed between IVO + VEN and IVO + VEN + AZA cohorts (Table 2). One episode of grade 5 febrile neutropenia occurred in a patient with R/R-AML and active disease receiving IVO + VEN 400 mg. Only one episode of grade 2 QTc prolongation occurred on study.

Table 2. Grade 3–5 nonhematologic AEs occurring during the study period.

	AE by grade			
AE	Overall ($N = 31$)[a]	Grade 3	Grade 4	Grade 5
Febrile neutropenia	9/31 (29%)	8/31 (26%)	0/31 (0%)	1/31 (3%)
Lung infection	6/31 (19%)	6/31 (19%)	0/31 (0%)	0/31 (0%)
Abdominal pain	3/31 (10%)	3/31 (10%)	0/31 (0%)	0/31 (0%)
Differentiation syndrome	3/31 (10%)	3/31 (10%)	0/31 (0%)	0/31 (0%)
Musculoskeletal pain	3/31 (10%)	3/31 (10%)	0/31 (0%)	0/31 (0%)
Otitis media	2/31 (6.5%)	2/31 (6.5%)	0/31 (0%)	0/31 (0%)
Tumor lysis syndrome	2/31 (6.5%)	2/31 (6.5%)	0/31 (0%)	0/31 (0%)
Acute kidney injury	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Conjunctivitis	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Disseminated intravascular coagulation	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Dyspnea	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Eye pain	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Fatigue	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Hypertension	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Hypokalemia	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Hypophosphatemia	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Hypotension	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Hypoxia	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Infections and infestations	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Leukocytosis	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Musculoskeletal and connective tissue disorder	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Sepsis	1/31 (3%)	0/31 (0%)	1/31 (3%)	0/31 (0%)
Sleep apnea	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Thrombocytopenia	1/31 (3%)	0/31 (0%)	1/31 (3%)	0/31 (0%)
Upper respiratory tract infection	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)
Urinary tract infection	1/31 (3%)	1/31 (3%)	0/31 (0%)	0/31 (0%)

Grade 3–5 infectious complications	IVO + AZA ($N = 12$)	IVO + VEN + AZA ($N = 19$)
Febrile neutropenia	4/12 (33%)	5/19 (26%)
Lung infection	3/12 (25%)	3/19 (16%)

[a]n/N (%).

Four patients (DL1: $N = 2$, DL2: $N = 1$, DL3: $N = 1$) developed IDH differentiation syndrome (IDH-DS; grade 2: $N = 1$; grade 3: $N = 3$) after a median of 39 days (range, 17–95 days). Three of the four patients developing IDH-DS received treatment and were successfully managed using hydroxyurea ($N = 1$), corticosteroids ($N = 1$), or both ($N = 1$).

Two patients developed tumor lysis syndrome (TLS; both grade 3, DL1: $N = 1$, DL3: $N = 1$), after 1 and 27 days, respectively; both patients also experienced IDH-DS. The episode of documented TLS on C1D1 occurred in a DL3 patient with ND-AML and a peripheral WBC of 8.9×10^9 cells/μL with 86% circulating blasts. VEN was held for 8 days (thus meeting DLT criteria as the interruption was ≥72 hours) and resumed in subsequent cycles. Both episodes of TLS were successfully managed by temporary cessation of study medications (IVO or VEN) in addition to judicious volume management and diuretic therapy; both patients recovered without use of renal replacement therapy.

The maximum tolerated dose (MTD) was not reached. Based upon observed efficacy including initial responses as well as durability of response, tolerability, and toxicity profiles across dose levels, IVO + VEN 400 mg + AZA (DL3) was selected as the recommended phase II dose (RP2D).

Response Outcomes

The overall response rate [ORR: CR + CRh + CRi + partial response (PR) + morphologic leukemia-free state (MLFS)] for the study population was 94% ($N = 29/31$; 95% CI: 80%–99%)

overall and 67% (N = 4/6, 95% CI: 27%–94%) for DL1, 100% (N = 6/6; 95% CI, 59%–100%) for DL2, 100% (N = 13/13 95% CI: 77%–100%) for DL3, and 100% (N = 6/6; 95% CI, 59%–100%) for DL4. Two patients with R/R-AML treated within DL1 (i.e., VEN 400 mg) did not respond to treatment.

CRc was attained in 87% (N = 27/31; 95% CI, 71%–96%) overall and was 67% (N = 4/6; 95% CI, 27%–94%) in DL1, 100% (N = 6/6; 95% CI, 59%–100%) in DL2, 85% (N = 11/13; 95% CI: 57%–97%) in DL3, and 100% (N = 6/6; 95% CI, 59%–100%) in DL4 (Fig. 1A). CRc was attained in 83% (N = 10/12) versus 90% (N = 17/19) of patients treated with IVO + VEN and IVO + VEN + AZA, respectively (P = 0.63). Median time to best response was 37 days (range, 23–231 days) overall and was 55 (~2 cycles) versus 37 days (~1 cycle) for patients treated with IVO + VEN versus IVO + VEN + AZA, respectively.

CRc rates were 100% (N = 9/9; 95% CI, 68%–100%) in patients with MDS or MPN, 93% (N = 13/14; 95% CI, 69%–100%) in patients with ND-AML, and 63% (N = 5/8; 95% CI, 29%–89%) in patients with R/R-AML, respectively. In ND-AML patients (N = 14), CRc was attained in 100% (N = 9/9; 95% CI, 68%–100%) and 80% (N = 4/5; 95% CI, 34%–99%) of patients with treatment-naïve AML versus sAML/ts-AML, respectively.

Measurable residual disease (MRD)–negative CRc assessed via multiparameter flow cytometry (MFC) was achieved in 63% (N = 10/16; 95% CI, 37%–83%) of MRD-evaluable patients including 67% (N = 2/3) in DL1, 40% (N = 2/5) in DL2, 67% (N = 4/6) in DL3, and 100% (N = 2/2) in DL4. Although not statistically significant, increased MRD-negative rates were observed in patients receiving IVO + VEN + AZA (N = 6/8, 75%; 95% CI, 36%–95%) versus IVO + VEN (N = 4/8, 50%; 95% CI, 19%–81%; P value: 0.60). Of the patients who attained MRD-MFC–negative CRc, 90% (N = 9/10) achieved this within 5 cycles of therapy (Fig. 1B).

In patients with ND-AML evaluable for MRD assessment (N = 10/16), MRD-negative CRc was attained in 60% (N = 6/10; 95% CI, 24%–85%) and was increased with IVO + VEN + AZA (N = 5/6, 83%; 95% CI, 41%–99%) versus IVO + VEN (N = 1/4, 25%; 95% CI, 13%–75%; P = 0.19).

IDH1 mutation clearance assessed using digital-droplet polymerase chain reaction (ddPCR) in responding patients was observed in 37% (N = 10/27). Median baseline and remission *IDH1* VAF in responding patients with detectable *IDH1* mutations by ddPCR and available paired NGS samples (N = 7/17) was 28% (range, 5%–45%) and 6% (range, 1%–36%), respectively. Median *IDH1* VAF at diagnosis did not significantly differ between patients with detectable (28%; range, 5%–46%) versus undetectable (22%; 6%–42%; P = 0.80) *IDH1* mutations by ddPCR in remission.

As depth of remission can evolve with ongoing treatment and *IDH1* clearance occurred after a median of 4 treatment cycles in patients receiving IVO + AZA (19), we evaluated *IDH1* mutation clearance by ddPCR after ≥5 cycles compared with patients receiving 1 to 4 cycles. *IDH1* clearance was observed in 64% (N = 9/14; 95% CI, 37%–85%) of patients receiving ≥5 cycles compared with 8% (N = 1/13; 95% CI, 1%–34%) in those receiving 1 to 4 cycles (P = 0.004; Fig. 1C). *IDH1* mutation clearance in patients receiving ≥5 cycles of therapy was numerically increased with IVO + VEN + AZA (N = 6/7, 86%; 95% CI, 45%–99%) versus IVO + VEN versus (N = 3/7, 43%; 95% CI, 13%–78%; P = 0.26).

In patients receiving ≥5 cycles of therapy, *IDH1* mutation clearance was observed in 71% (N = 5/7, including 100% of patients with treatment-naïve AML), 50% (N = 2/4), and 67% (N = 2/3) of patients with ND-AML, R/R-AML, and MDS or MPN, respectively.

The correlation of morphologic response, MRD-MFC, and *IDH1* mutation clearance measured via ddPCR for MRD-MFC evaluable patients is displayed in Supplementary Figs. S6–S8. In MRD-evaluable patients attaining CRc with available *IDH1* ddPCR following ≥5 cycles of therapy (N = 11), concordant MRD-MFC and *IDH1* results were observed in 55% (N = 6/11). Three patients with no detectable MRD-MFC but with detectable *IDH1* mutations (median ddPCR VAF: 3.7%; range, 0.19%–5.5%) remain in MRD-MFC–negative remissions (one proceeded to HCT and two remain on protocol therapy) after a median follow-up of 37 months. Conversely, two patients who remained MRD-MFC–positive despite no detectable *IDH1* mutation relapsed after a median of 6.5 months (range, 6–23 months) with *IDH1*-negative disease (Supplementary Fig. S9).

Survival Outcomes

After a median follow-up of 24 months, median duration of response (DOR) was 34.6 months (95% CI, 22–not reached (NR)]. Median DOR was 24 months (95% CI, 1.2–NR), 6.7 months (95% CI, 3.9–NR), and NR (DL3: 12-month DOR: 77%; SE: 12%) across DL1–DL3 [DL4 not estimated (NE)], respectively. Median event-free survival (EFS) and OS were 36 (95% CI, 23–NR) and 42 (95% CI, 42–NR) months for the study population, respectively.

Median EFS was 8 (95% CI, 0–NR) and 9 months (95% CI, 7–NR) for DL1 and DL2, and NR for DL3 (12–month EFS: 77%; SE: 12%) or DL4 (12-month EFS: NE). A trend toward improved EFS was observed with IVO + VEN + AZA vs. IVO + VEN (NR; 95% CI, 3.9–NR; vs. 11 months (22.9–NR), P = 0.058). 12-month EFS was 84% (SE: 8%) with IVO + VEN + AZA vs. 50% (SE: 14%) with IVO + VEN, respectively. Median EFS was NR in patients with MDS or MPN (estimated 12-month EFS: 89%; SE: 11%), 36 months (95% CI, 23–NR) in ND-AML, and 6 months (95% CI, 2–NR) in R/R-AML.

Median OS was 26 months (95% CI, 4.1–NR) for DL1, and NR for DL2–4 (Fig. 2A); 12-month OS was 50% (SE 20%), 67% (SE: 19%), 85% (SE: 10%), and not estimated for DL1–4, respectively. No statistically significant difference in OS was observed between IVO + VEN vs. IVO + VEN + AZA [42.1 (95% CI, 7.9–NR) months vs. NR (95% CI: NR), P = 0.13]; 24-month OS was 58% versus 75%, respectively.

Median OS was 42 (95% CI, NR) months in patients with MDS or MPN, NR in ND-AML [estimated 12- and 24-month OS: 79% (SE: 11%) and 67% (SE: 14%)], and 9 months (95% CI: 8–NR) in R/R-AML (Fig. 2B). In patients with ND-AML (N = 14), estimated 12-month OS was 50% (SE: 25%) versus 90% (SE: 10%) with IVO + VEN versus IVO + VEN + AZA (P = 0.30).

Landmark survival analyses were performed at baseline, 3, 5, and 7 months in patients with available *IDH1* ddPCR assessment, with no significant difference in OS observed based on *IDH1* mutation status (Supplementary Fig. S10A–S10D). No significant OS difference was observed at the end of cycle 5 (EOC5), when increased rates of *IDH1* clearance

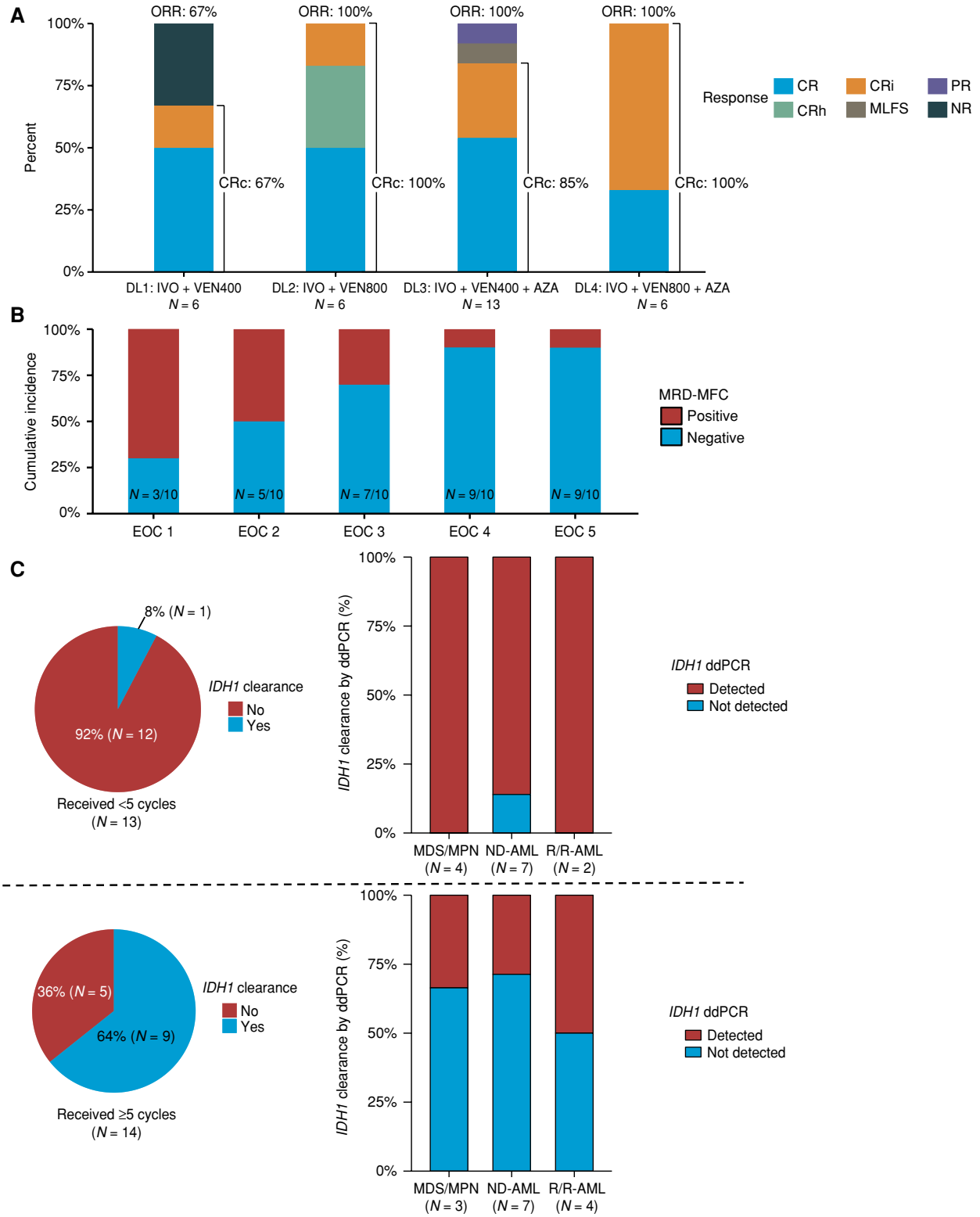

Figure 1. Overall response rate and measurable residual disease (MRD) clearance rates by multiparameter flow cytometry (MRD-MFC) and *IDH1* digital-droplet PCR (ddPCR) in patients treated with IVO + VEN ± AZA. **A,** Overall response and composite complete response (CRc) rates across the study cohort. **B,** Timing of MRD-negative response in 10 patients obtaining MRD-MFC–negative CRc. 90% of patients attained MRD-MFC–negative remissions by 4 cycles of treatment. **C,** Clearance of *IDH1* mutation in remission assessed using ddPCR in patients receiving <5 vs. ≥5 cycles of protocol-directed treatment. *IDH1* mutation clearance was improved with continued cycles of therapy with *IDH1* mutation clearance observed in 64% of patients receiving ≥5 cycles of treatment. *IDH1* mutation clearance was observed across all disease types.

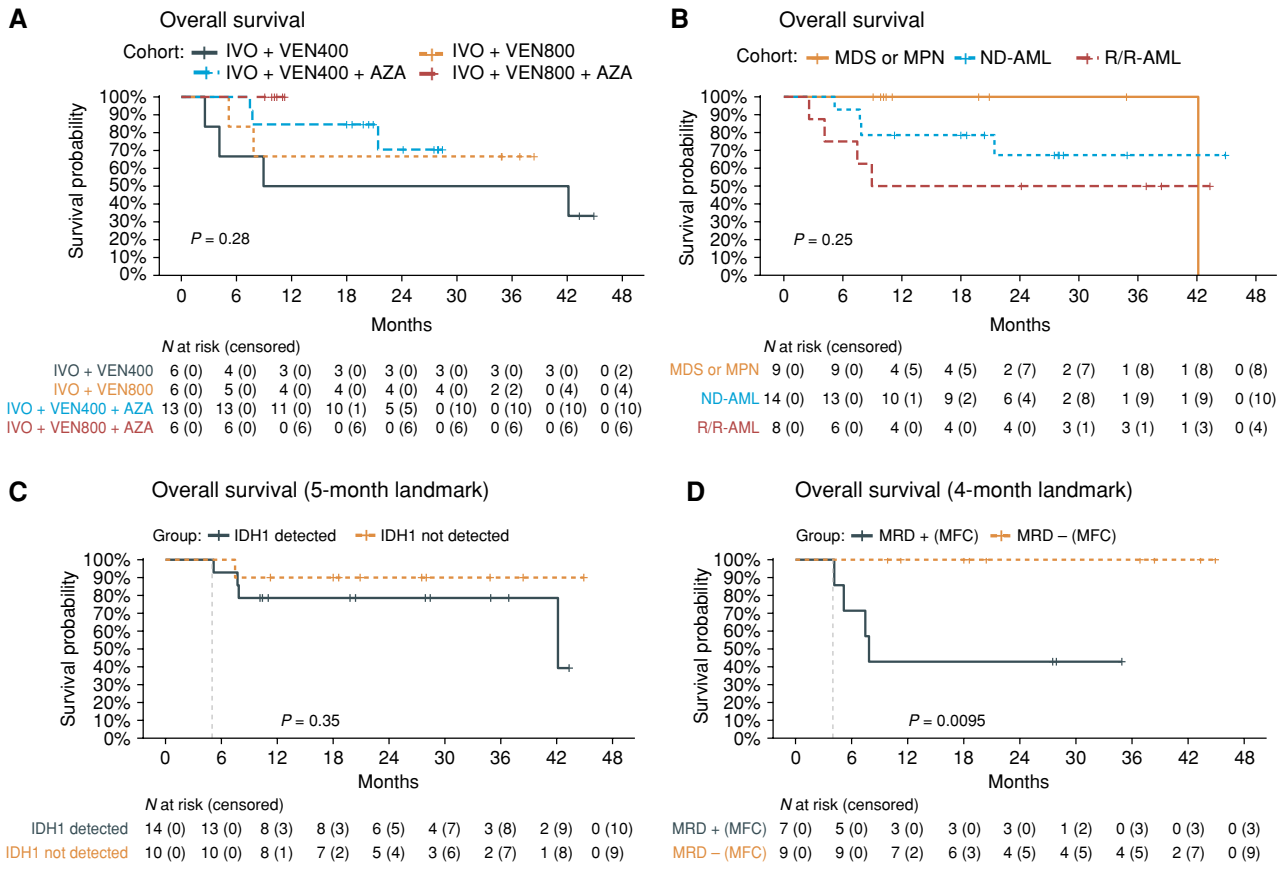

Figure 2. Overall survival in patients treated with IVO + VEN or IVO + VEN + AZA. **A,** Overall survival across study cohorts. **B,** Overall survival by disease type. **C,** Landmark analysis (5-month landmark shown here, remainder in Supplementary Fig. S10) depicting overall survival based upon *IDH1* clearance vs. *IDH1* persistence in patients attaining CRc. **D,** Landmark analysis (4-month landmark) demonstrating overall survival based upon the detection or absence of MRD between C1 and C4 using MFC.

were noted, based on *IDH1* persistence in CRc [12-month OS *IDH1* undetected: 90% (SE: 10%) vs. *IDH1* detected: 79% (SE: 11%), $P = 0.35$; Fig. 2C]. Patients with ND-AML with undetectable *IDH1* in CRc ($N = 6$) demonstrated 12- and 24-month OS of 100% (SE: NA).

Conversely, a landmark analysis including surviving patients at EOC4 with evaluable MRD-MFC ($N = 16$) demonstrated clearance of MRD-MFC between cycles 1 to 4 was associated with a significant improvement in OS compared with patients with detectable MRD-MFC during this period (NR vs. 8 months, $P = 0.0095$); 12- and 24-month OS in patients with negative MRD-MFC CRc ($N = 9$) was 100% (SE: NA; Fig. 2D).

No significant difference in OS (NR vs. 42 months, $P = 0.29$) was observed between patients receiving HCT ($N = 11$) compared with those who did not ($N = 16$) in CRc. The estimated 24-month OS in all patients ($N = 12$) proceeding to HCT was 92% (SE: 8%). One death in remission approximately 4 months after HCT occurred secondary to infection.

Molecular Correlates of Response and Survival

No significant difference in response or survival was observed based upon baseline *IDH1* VAF or variant measured using an institutional next-generation myeloid gene sequencing panel performed on bone marrow mononuclear cells. After adjusting for disease and treatment with IVO + VEN or IVO + VEN + AZA, patients with methylation gene mutations ($N = 16$; i.e., *DNMT3A, TET2, IDH2*) other than *IDH1* had improved survival compared with patients with wild-type status (HR 0.11; 95% CI, 0.17–0.65; $P = 0.0157$; Supplementary Fig. S11A). Patients with mutations in signaling pathway genes ($N = 13$; *FLT3*-TKD, *K/NRAS, NF1, PTPN11, JAK/STAT, KIT*, and *CSF3R*) demonstrated a trend toward inferior survival (HR 7.5; 95% CI, 0.93–61; $P = 0.058$) in the overall population (Supplementary Fig. S11B). This association was stronger when restricted to genes regulating RAS/RTK pathway signaling (*N/KRAS, FLT3*-ITD/TKD, *PTPN11*, and *NF1*) associated with primary and acquired resistance to IDH inhibitor and AZA + VEN combinations (HR: 4.89; 95% CI, 1.02–23.51; $P = 0.048$).

Patients with signaling pathway mutations appeared to benefit from treatment with IVO + VEN + AZA versus IVO + VEN (median OS NR vs. 8.4 months; HR, 0.24; 95% CI, 0.047–1.25; $P = 0.090$), corresponding to 12-month survival of 90% (SE: 9%) versus 38% (SE: 17%; Supplementary Fig. S11C). Similar survival was observed when restricted to patients with AML (median OS: NR vs. 7.9 months; HR, 0.26; 95% CI, 0.051–1.37; $P = 0.11$), albeit these results were not statistically significant (Supplementary Fig. S11D).

Molecular Characterization of Relapse

Overall, nine responding patients (33%; ND-AML: 5/14, R/R: AML: 2/8, MDS or MPN: 2/9) experienced relapse after a median of 6.5 months (range, 2.2–36.4 months). Of these 9 patients, 6 received IVO + VEN (including 3 patients in DL1 with likely insufficient VEN exposure). More patients receiving IVO + VEN either failed to attain an initial response ($N = 2$) or relapsed ($N = 6$) on study therapy compared with IVO + VEN + AZA [$N = 3$; 67% (95% CI, 37%–88%) vs. 16% (95% CI, 5%–39%), $P = 0.007$].

Targeted NGS analysis in 89% ($N = 8$) of the 9 relapsing patients revealed clonal changes in 75% ($N = 6$). Cytogenetic evolution occurred in 57% ($N = 4/7$) of patients with available cytogenetic analysis at relapse. Emergent mutations detected on bulk NGS at relapse included transcription factor mutations in 50% ($N = 4$; *ETV6*, *RUNX1*, and *CEBPA*); and signaling mutations in 25% ($N = 2$; *NRAS*, *NF1*, and *PTPN11*) of patients (Fig. 3A). Notably, emergent and/or persistent transcription factor mutations were present in 80% ($N = 4/5$) of IVO + VEN patients at relapse compared with no patients who progressed while receiving the triplet regimen. One patient initially treated with IVO + VEN + AZA had an identifiable *RUNX1* mutation at relapse after receiving more than a year of IVO maintenance.

Among patients with relapsed disease, 100% ($N = 4$) of patients with undetectable *IDH1* using ddPCR in remission experienced relapse without recurrent *IDH1* mutations; conversely, all ($N = 4$) patients with persistently detectable *IDH1* mutations in remission had detectable *IDH1* mutations at relapse (Fig. 3A). No significant VAF difference was observed between relapsing patients who cleared their *IDH1* mutation versus those who did not.

Single-Cell Sequencing Identifies Genomic Drivers of Relapse

Single-cell DNA sequencing (scDNA-seq) without ($N = 2$; accession #15 and accession #16; Fig. 3B and C) and with analysis of surface protein expression (DAb-seq; $N = 2$; accession #18 and accession #20]; Fig. 4A–H; Supplementary Fig. S12) was performed in four patients treated with triplet therapy including one long-term responder to evaluate clonal dynamics under the therapeutic pressure of targeted therapy. In addition to conserved mechanisms of relapse to IDH-inhibitor and VEN-based therapies, complex clonal dynamics were observed at the single-cell level (22, 23).

For instance, in a patient with R/R-AML (accession #15) treated with 5 cycles of IVO + VEN + AZA, an *IDH1* + *SF3B1* + *TP53*-mutated clone was present in remission, and eliminated. Relapse was driven by selective outgrowth of clones containing *TP53* without *IDH1* (Fig. 3B). In a patient with sAML (accession #16) treated with 3 cycles of IVO + VEN + AZA, an *IDH1* + *NPM1* + *NRAS* G12A-mutated clone was replaced with emergent clones containing *IDH1* + *NPM1* with an alternative *NRAS* isoform G13R, and a clone containing *KRAS* (Fig. 3C). At all three time points assayed, the dominant clone contained only an *IDH1* mutation. Whole-exome sequencing (WES) performed at progression did not demonstrate any evidence of second-site *IDH1* mutations; however, clinical flow cytometry revealed the relapsed blast population was nearly universally comprised of a monocytic phenotype, suggesting the dominant *IDH1*-mutated clone was contained within this more mature blast population previously correlated with VEN resistance (24).

The observed elimination of *TP53* and *IDH1* comutated clones, outgrowth of alternative signaling isoforms, and presence of a monocytic phenotype at relapse in these cases underscore that sensitivity or resistance to treatment contributing to the observed heterogenous response to therapy is influenced by the molecular composition of individual leukemic clones, variant isoforms, and cellular phenotype.

Genotype–phenotype relationships were further explored using DAb-seq analysis in two responding patients, demonstrating IVO + VEN + AZA appears effective at eliminating and suppressing leukemic clones with cooperating mutations including transcription factor gene mutations, a known resistance mechanism to IVO monotherapy (12). In a patient with ND-AML (accession #20) attaining a CRc with IVO + VEN + AZA most *IDH1*-mutated cells displayed a maturing myeloid progenitor phenotype (Fig. 4A–F) and were eliminated after one cycle of treatment, with subsequent expansion of $CD14^+$ monocytic and *IDH1* wild-type cell populations (Fig. 4G). Neither scDNA-seq nor ddPCR detected any *IDH1* mutation in remission, confirming clearance of the *IDH1*-mutated clone. However, persistent mutations in *TET2, ASXL1*, and to a lesser extent *SRSF2* were observed in multiple cell populations including $CD14^+$ monocyte/dendritic, $CD16^+$ monocytic, primitive/maturing myeloid progenitor, and erythroid progenitor cell populations (Fig. 4A–F). After transitioning to IVO maintenance while in remission (EOC1-EOC23) following C2 due to persistent cytopenias, a subsequent decrease in $CD14^+$ monocytic populations was observed between EOC1 (when receiving AZA + VEN + IVO) and EOC7 (when receiving IVO maintenance) sampling (Fig. 4G).

Following relapse, EOC26 sampling demonstrated outgrowth of an immature myeloid population with strong expression of $CD34^+/CD117^+/HLA\text{-}DR^+/CD99^+$ compared with the initiating leukemic clone was identified, consistent with a more primitive myeloid phenotype (Fig. 4F). Clinical MFC also identified this primitive $CD34^+$ cell population, confirming a phenotypic shift occurred at relapse (Supplementary Fig. S13). Simultaneous bulk NGS revealed expansion of a *RUNX1* p.Y414fs*186 containing clone that was not detected with DAb-seq (due to poor coverage secondary to its location within a CG-rich region on the edge of the primer) following transitioning to IVO monotherapy at the EOC2 (Fig. 4H).

Conversely, DAb-seq analysis in a patient with ND-AML (accession #18) with cooccurring *IDH1* and *RUNX1* (p.L204Q) mutations treated with IVO + VEN + AZA demonstrated that treatment eliminated most *RUNX1* and *IDH1* comutated cells, with persistent *IDH1/RUNX1* comutated clones in remission observed within a monocytic cell population with increased CD16 expression (Supplementary Fig. S12A–S12D). Paired ddPCR analysis in remission confirmed persistence of *IDH1*-mutated cells, whereas MFC-MRD was undetectable, suggesting these residual *IDH1*-mutated cells were present within preleukemic clones. In contrast to patient #3 who transitioned to IVO monotherapy, this latter patient remains on IVO + VEN + AZA in a durable remission after 27 months of follow-up.

Cellular Correlates of Resistance

To assess if increased levels of intracellular signaling or alternative antiapoptotic proteins correlated with resistance, time-of-flight mass cytometry (CyTOF) was performed at

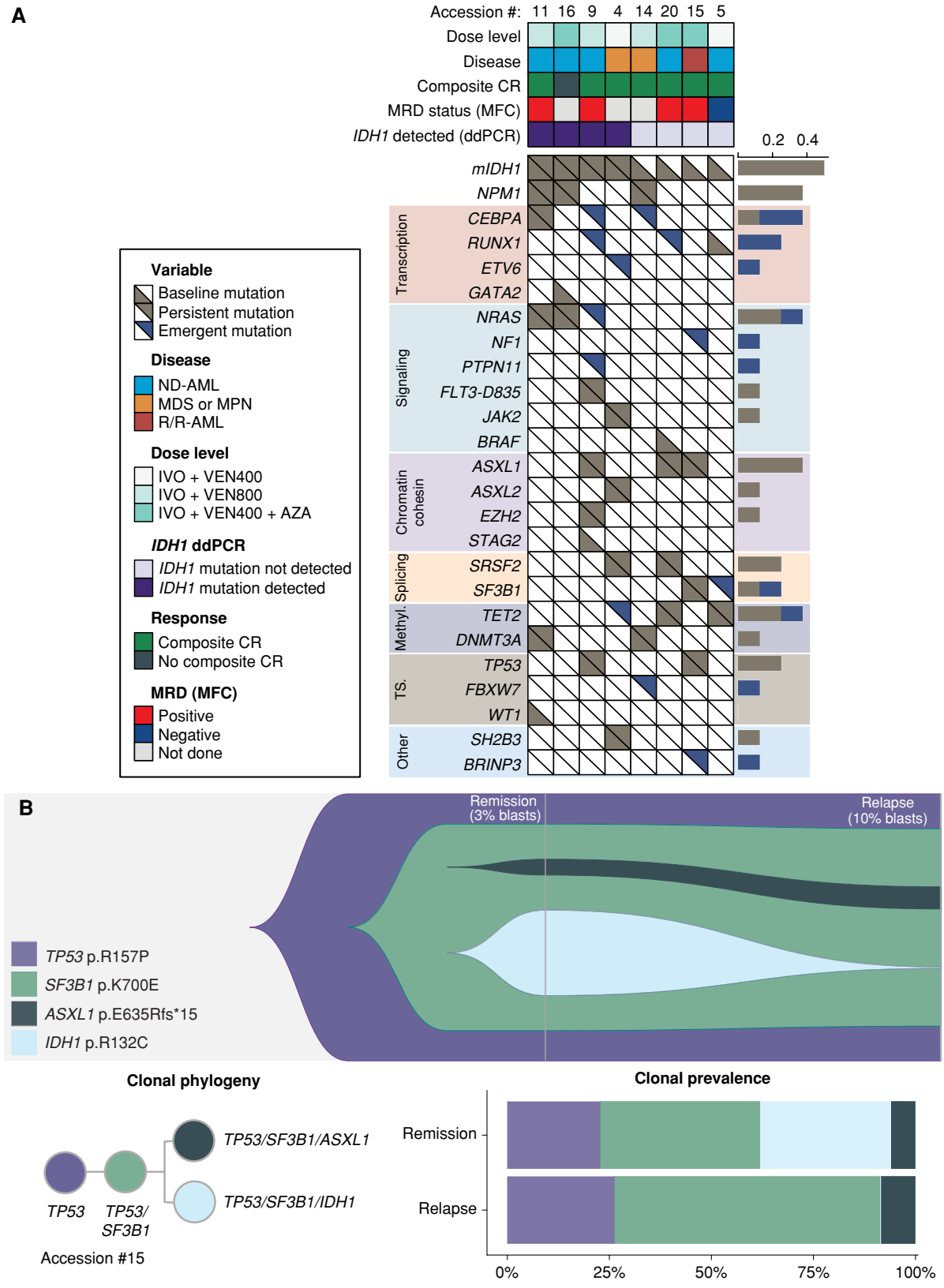

Figure 3. Bulk NGS and single-cell correlates of relapse in patients treated with IVO + VEN ± AZA. **A,** Bulk next-generation myeloid gene panel sequencing at the time of diagnosis and at relapse in responding patients treated with IVO + VEN or IVO + VEN + AZA who ultimately relapsed following treatment. **B,** scDNA-seq in a patient with R/R-AML (Accession #15) at the time of remission and relapse identified expanding leukemic clones contributing to relapse. (*continued on next page*)

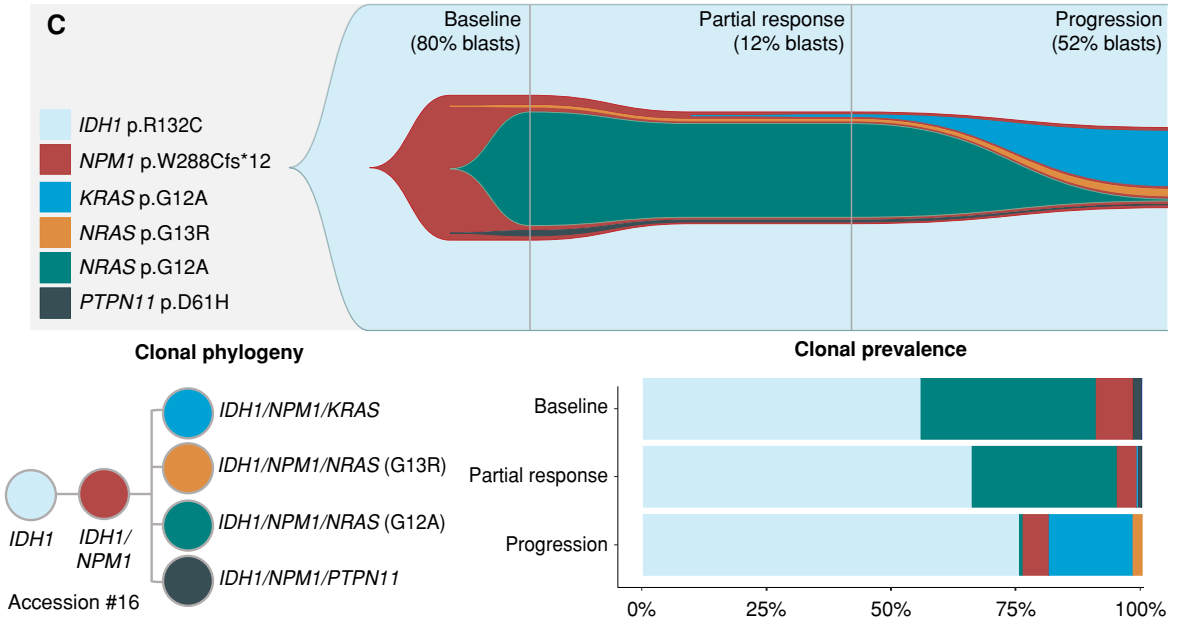

Figure 3. ***(Continued)*** **C,** scDNA-seq in a patient with ND-AML (Accession #16) demonstrating differing clonal architecture with respect to signaling mutations and variants throughout treatment. Methyl., methylation; T.S., tumor suppressor.

baseline and following treatment with IVO + VEN ± AZA. Notably, IVO + VEN ± AZA effectively eliminated BCL-2–dependent blast populations whereas persistent and/or expanding myeloid cell populations were reliant on increased alternative antiapoptotic protein levels (typically MCL-1), and in long-term responders often with a maturing monocytic immunophenotype.

For instance, CyTOF analysis in two patients with ND-AML (accessions #20 and #11) who attained CR/CRi and ultimately relapsed demonstrated increased BCL-xL and/or MCL-1 levels relative to BCL-2 in expanding $CD34^+$ cell populations and relatively abundant CD44 levels (previously correlated with VEN resistance; Fig. 5A and B; Supplementary Fig. S14; ref. 25). Conversely in two patients with ongoing response (accessions #27 and #26), BCL-2 levels were higher relative to other alternative antiapoptotic protein levels in decreasing cell $CD34^+$ cell populations (Fig. 5C and D; Supplementary Fig. S15).

Increased alternative antiapoptotic protein levels were also observed in healthy maturing myeloid progenitor cell populations in a long-term responder with R/R-AML treated with IVO + VEN (accession #10) who attained a CRh, consistent with a known on-target effect of venetoclax therapy. This patient demonstrated an expanding cell population with a maturing monocytic phenotype ($CD33^+$/HLA-DR^+/$CD14^+$/$CD64^+$) at the end of cycle 3 with concurrent upregulation of MCL-1 (Supplementary Fig. S16). The patient remains in remission with a response lasting over 3 years.

DISCUSSION

The combination of IVO + VEN ± AZA is safe, well tolerated, and highly active, resulting in durable responses and prolonged survival in patients with high-risk *IDH1*-mutated myeloid malignancies.

Reassuringly, treatment with IVO + VEN + AZA (one of the first triplet regimens explored to date) was well tolerated. The safety profile of IVO + VEN ± AZA was largely comparable with reported safety of IVO + AZA or AZA + VEN (16, 19). Most AEs were grade 1–2, with no significant difference in grade 3 to 5 AEs observed between cohorts. Common grade 3 to 5 AEs included febrile neutropenia ($N = 9$; 29%) and lung infections ($N = 6$; 19%). All episodes of TLS and IDH-DS were successfully managed and resolved with medical therapy.

Though treatment modifications for myelosuppression (47%; $N = 9$) were required analogous to current HMA + VEN based therapies, adherence to study agents in the triplet cohorts was > 90% for cycles 1 to 4 and >70% during cycles 1 to 12, and no patients discontinued IVO (16). The MTD was not reached. Given prolonged myelosuppression observed in two patients treated within DL4 (IVO + VEN 800 mg + AZA), DL3 (IVO + VEN 400 mg + AZA) was selected as the recommended phase II dose.

The overall CRc rate of 87% [N = 27/31, including 93% (N = 13/14) in patients with ND-AML] with IVO + VEN ± AZA compares favorably with a post hoc subgroup analysis of AZA + VEN (CR/CRi: 66.7% [N = 22/33]), and the randomized prospective analysis of IVO + AZA [CR/CRh: 53% (N = 38/72)] in patients with ND, *IDH1*-mutated AML (17, 19). In contrast to IVO monotherapy or IVO + AZA, responses occurred early (median time to best response of IVO + VEN: 55 days and IVO + VEN + AZA: 37 days) consistent with other VEN-containing regimens (11, 16, 19).

Sixty-three percent of MRD-evaluable patients ($N = 10/16$) attained MRD-negative CRc, and *IDH1* mutation clearance assessed via ddPCR in patients receiving ≥5 cycles of therapy occurred in 64% ($N = 9/14$, including 100% of patients with treatment-naïve AML). Only 50% of MRD-negative patients attained an MRD-negative CRc by the end of cycle 2, whereas 90% of patients attained an MRD-negative CRc by the end of

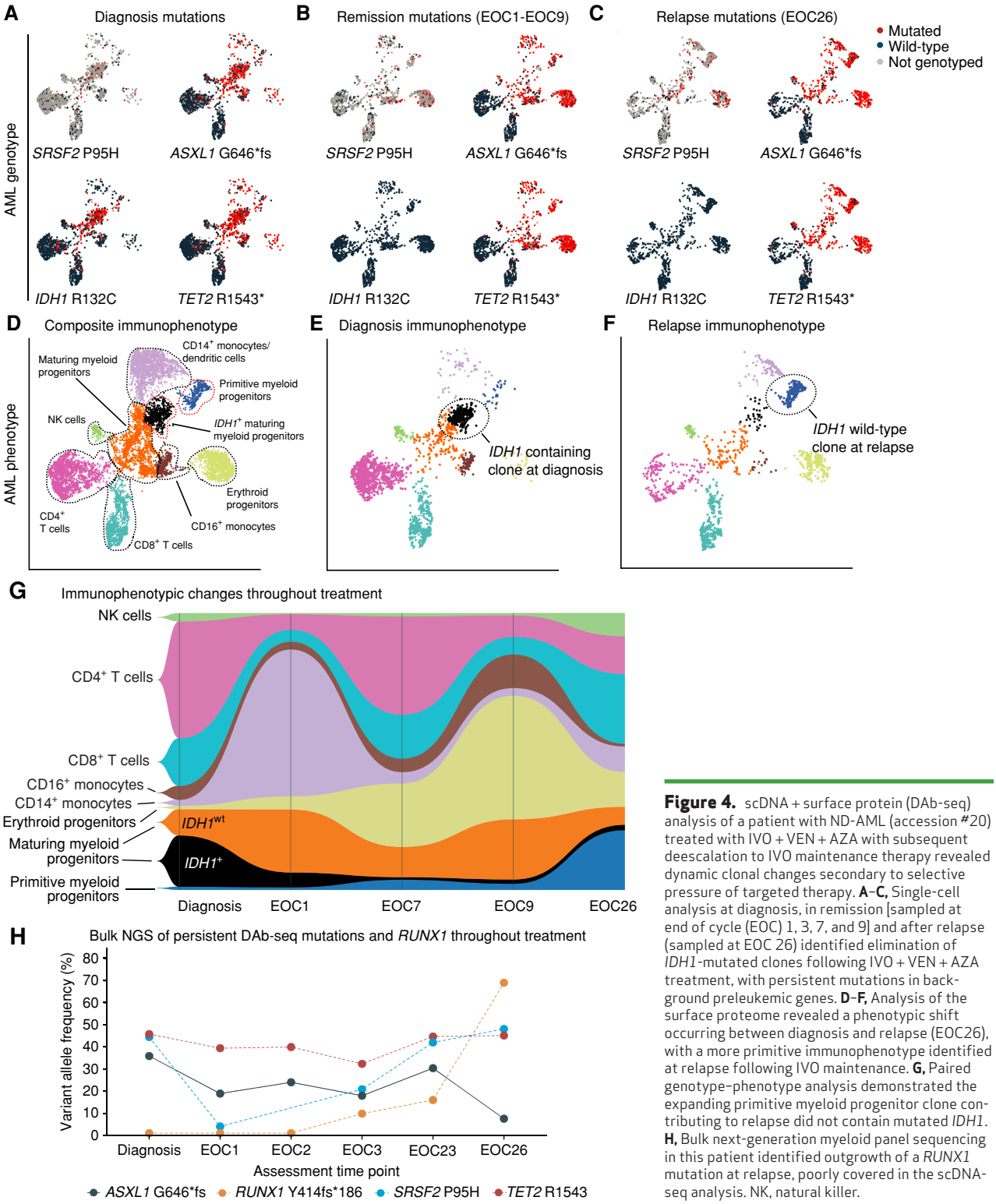

Figure 4. scDNA + surface protein (DAb-seq) analysis of a patient with ND-AML (accession #20) treated with IVO + VEN + AZA with subsequent deescalation to IVO maintenance therapy revealed dynamic clonal changes secondary to selective pressure of targeted therapy. **A–C,** Single-cell analysis at diagnosis, in remission [sampled at end of cycle (EOC) 1, 3, 7, and 9] and after relapse (sampled at EOC 26) identified elimination of *IDH1*-mutated clones following IVO + VEN + AZA treatment, with persistent mutations in background preleukemic genes. **D–F,** Analysis of the surface proteome revealed a phenotypic shift occurring between diagnosis and relapse (EOC26), with a more primitive immunophenotype identified at relapse following IVO maintenance. **G,** Paired genotype–phenotype analysis demonstrated the expanding primitive myeloid progenitor clone contributing to relapse did not contain mutated *IDH1*. **H,** Bulk next-generation myeloid panel sequencing in this patient identified outgrowth of a *RUNX1* mutation at relapse, poorly covered in the scDNA-seq analysis. NK, natural killer.

cycle 4, highlighting MRD status can evolve with subsequent cycles in patients receiving lower-intensity treatment.

Though not statistically significant, MRD-negative CRc rates (75% vs. 50%) and *IDH1* clearance (86% vs. 43%) were numerically improved with IVO + VEN + AZA versus IVO + VEN. These results suggest regimens incorporating combined targeted therapies (in particular IVO + VEN + AZA) effectively eliminate the *IDH1*-mutated clone at rates comparable to IVO + AZA for ND-AML (*IDH1* clearance: 52%; $N = 17/33$) and are encouraging in the context of published MRD-MFC data in *IDH*-mutated patients treated with AZA + VEN (MRD-negative rate: *IDH1*/2: 49%, $N = 21/43$; *IDH1*: 42%,

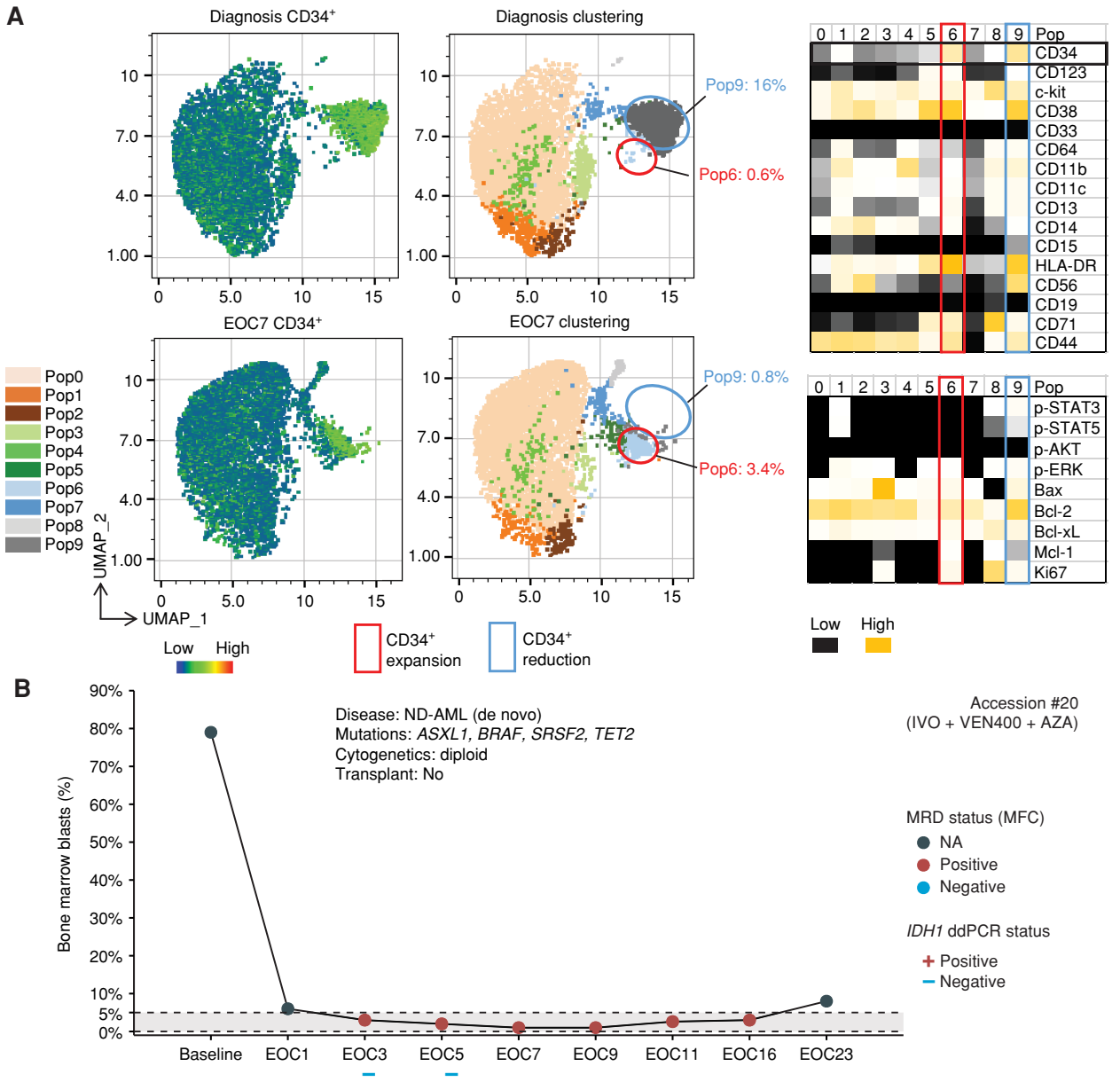

Figure 5. Time-of-flight mass cytometry (CyTOF) identifies cellular pathways associated with divergent CD34+ populations. **A** and **B,** CyTOF of a patient with ND-AML (accession #20) who attained morphologic remission but remained MRD-positive and developed morphologic relapse following cycle 23. PCA clustering of CyTOF performed in remission (end of cycle 7) identified an expanding CD34+ cell population with increased MCL-1 levels. (*continued on following page*)

N = 5/12]; refs. 11, 12, 19, 26). Although compelling, the small numbers of patients included within this molecular subgroup across studies warrant cautious interpretation and generalization of these results while awaiting validation in larger ongoing prospective studies.

Responses to IVO + VEN ± AZA were durable (median DOR was approximately 35 months), correlating with a median EFS of 36 months. Treatment with IVO + VEN + AZA corresponded with a trend toward improved median EFS and was associated with a lower rate of primary or secondary treatment failure compared with IVO + VEN. Thus, triplet therapy with IVO + VEN + AZA compared with IVO + VEN may more effectively prevent disease relapse through obtainment of deeper remission.

IVO + VEN ± AZA resulted in durable survival within a high-risk patient population, with a median OS for the study population of 42 months. Median OS was not reached in patients with ND-AML with an estimated 12- and 24-month OS of 79% and 67%, exceeding currently published survival rates in patients with *IDH1*-mutated AML treated with AZA + VEN (median OS: 15.2 months) or IVO + AZA (median OS: 24 months; refs. 17, 19). These favorable survival outcomes were seen regardless of allogeneic HCT consolidation status.

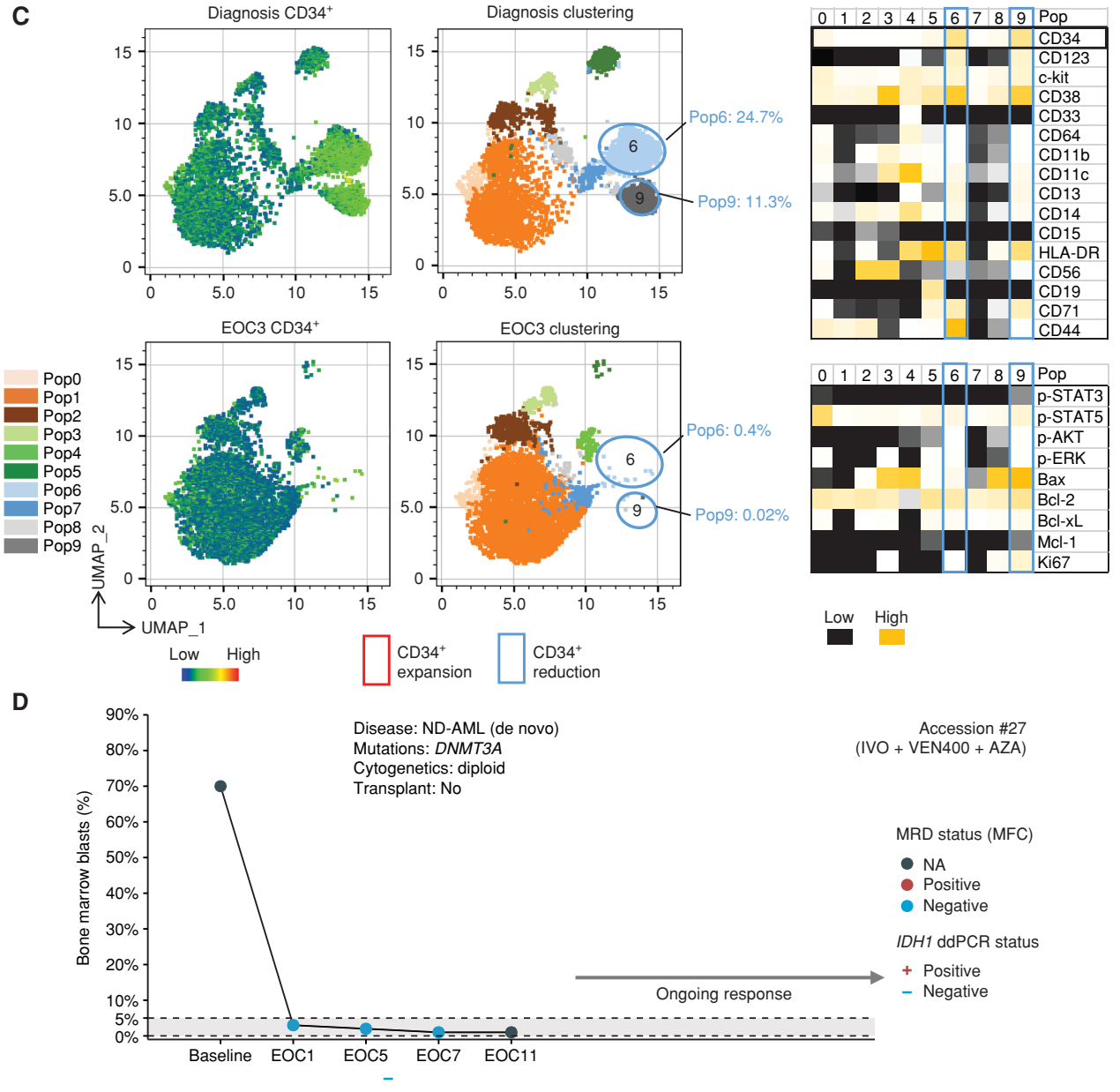

Figure 5. ***(Continued)*** **C** and **D,** CyTOF of a patient with ND-AML (accession #27) with an ongoing response following 18 cycles of treatment. In contrast to the previous patient, PCA clustering of CyTOF performed at the end of cycle 3 identified marked reductions in CD34+ cell populations with increased BCL-2 levels relative to BCL-xL or MCL-1. NA, not applicable.

MRD-MFC is now a standardized assessment in AML and correlates with DOR and OS in patients treated with lower-intensity therapy including AZA + VEN (21, 26). MRD-MFC status in remission following IVO + VEN or IVO + VEN + AZA remained a robust prognostic marker with 24-month OS of 100% in MRD-MFC–negative patients. *IDH1* clearance in remission, though associated with favorable survival, was less prognostic—likely due in part to the molecular heterogeneity observed in myeloid malignancies, with alternative *IDH1*-negative leukemic clones driving relapse (1, 2). Ongoing enrollment in expansion cohorts will further define the prognostic role of MRD-MFC and evaluate the impact of treatment augmentation in MRD-positive patients treated with IVO + VEN + AZA.

Although mutations in RAS/RTK pathway genes remained independently associated with inferior survival, IVO + VEN + AZA appeared to improve survival in patients with signaling mutations compared with IVO + VEN [12-month OS 90% (SE: 9%) vs. 38% (SE: 17%)], albeit the results were not statistically significant.

Longitudinal scDNA-seq sampling demonstrated the elimination of large leukemic clones containing *IDH1* + *NPM1* + *NRAS* G12A mutations with IVO + VEN + AZA. At progression, outgrowth of minor *IDH1* + *NPM1* + *NRAS* G13R and *KRAS* containing clones was observed. Similarly, scDNA-seq revealed the elimination of a *TP53* + *SF3B1* + *IDH1* containing clone in remission with outgrowth *TP53* clones without cooccurring *IDH1* at

progression, a known relapse mechanism to IDH inhibitor and HMA + VEN combinations (12, 13, 18, 27). These results imply clonal composition determines sensitivity to IVO + VEN + AZA and highlight potential inequities regarding the influence of specific signaling mutations (i.e., *KRAS* vs. *NRAS*) and variants (i.e., *NRAS* G12A vs. G13R) confer on relapse.

In contrast to IDH-inhibitor monotherapy, emergent mutations in transcription factor genes rather than RAS/RTK signaling mutations were common at relapse, predominantly in patients treated with IVO + VEN or IVO (including a patient deescalated from IVO + VEN + AZA; refs. 12, 13, 28). DAb-seq analysis of two patients resolved clonal dynamics within phenotypically and genotypically defined AML populations throughout treatment. Paired DAb-seq and bulk NGS analysis in a patient relapsing on IVO maintenance identified an emergent mutation in *RUNX1* with subsequent expansion of a primitive myeloid phenotype at relapse. Alternatively, a patient with a similar mutation profile pattern (though a notably different *RUNX1* variant) at baseline remains in remission now >24 months on IVO + VEN + AZA.

Whether these emergent mutations recapitulate arrested differentiation or simply represent clonal markers of progenitor cell populations with differing stemness and differentiation states expanding under the selective pressure of therapy remains to be defined; however, understanding how genotype–phenotype relationships confer sensitivity and/or resistance to treatment is critical for future targeted therapy development (12, 27–30). No cases of *IDH* isoform switching or second-site *IDH1* mutations were identified, suggesting combined therapy including VEN may effectively suppress these relapse mechanisms (12, 13, 28).

DAb-seq analysis in patients with ND-AML identified $CD14^+$ monocytic expansion following IVO + VEN + AZA treatment. Despite initial expansion, the $CD14^+$ monocytic clone regressed after withdrawal of AZA + VEN and did not appear to drive relapse when the patient transitioned to IVO monotherapy. It is plausible AZA + VEN may select for monocytic populations containing preleukemic mutations in remission with a selective fitness advantage, which ultimately drive relapse after accumulating additional cytogenetic and/or molecular aberrations (24). Such appeared to be the case in the setting of an ND-AML patient who attained a PR followed by progression, with clinical MFC demonstrating a nearly universal monocytic phenotype at relapse.

CyTOF analysis of patients relapsing following treatment with IVO + VEN + AZA revealed increased levels of the alternative antiapoptotic protein MCL-1, further supporting the role of increased alternative antiapoptotic protein levels as a cellular mechanism of resistance to IVO + VEN + AZA. Expanding myeloid populations with monocytic markers and increased MCL-1 were also observed in responding patients. Thus, immunophenotype, even in the absence of clear molecular correlations such as RAS/RTK pathway mutations, appears to correlate with increased MCL-1 levels (24, 30, 31).

In conclusion, we demonstrate the combination of IVO + VEN and especially IVO + VEN + AZA as safe and effective regimens for the treatment of *IDH1*-mutated myeloid malignancies. Enrollment continues in phase II expansion cohorts treated with IVO + VEN + AZA. Translational analyses identifying key resistance mechanisms including mutations in transcription factor, tumor suppressor, and signaling pathway genes in addition to MCL-1 upregulation and monocytic expansion, provide the foundation for future investigations directed at successfully irradicating resistant leukemic clones and further improving survival in patients with *IDH1*-mutated myeloid malignancies.

METHODS

Trial Design, Randomization, and Blinding

The P1b portion of this P1b/PII nonrandomized, open-label, dose-escalation study was designed to determine the MTD and RP2D. A modified toxicity probability interval was utilized to guide dose-escalation decisions (displayed with the full study protocol in the supplementary protocol).

DLT was defined as any grade 3 or 4, clinically significant nonhematologic adverse event or abnormal laboratory value occurring during C1 and C2 not attributable by the investigator to a clearly identifiable cause including disease progression, underlying illness, concurrent illness, or concomitant medication (exceptions to this definition are displayed in the supplementary protocol). Patients removed from study prior to completion of the formal DLT period (i.e., due to proceeding with allogeneic HCT) were replaced. Phase Ib accrual was designed to continue until 30 patients enrolled or the trial was halted for toxicity.

Inclusion/Exclusion Criteria

Eligibility included men and women ≥18 years with an adequate performance status (ECOG 0–2) and an advanced myeloid malignancy (*de novo* AML, sAML, ts-AML, and R/R-AML, or high-risk MDS, MPN, or chronic myelomonocytic leukemia (CMML; ≥10% bone marrow blasts or intermediate/high-risk scores assessed by IPSS, R-IPSS, or D-IPSS, hereafter collectively referred to as "MDS or MPN") with a confirmed *IDH1* R132 mutation. Prior receipt of VEN or IVO was exclusionary; prior treatment with an alternative IDH inhibitor was permitted. Prior hydroxyurea and/or cytarabine was permitted to attain a WBC $<25 \times 10^9$/L in patients with rapidly proliferative disease prior to initiation of protocol-directed treatment. Additional inclusion and exclusion criteria are available within the supplementary protocol. Written informed consent was obtained from all study participants. All studies were conducted in accordance with recognized ethical guidelines and approved by participating centers local institutional review committees.

Treatment Administration

The P1b portion evaluated four successive combination dose levels, enrolling cohorts of six patients each (DL1: IVO 500 mg + VEN 400 mg; DL2: IVO 500 mg + VEN 800 mg; DL3: IVO 500 mg + VEN 400 mg + AZA 75 mg/m^2; DL4: IVO 500 mg + VEN 800 mg + AZA 75 mg/m^2). Treatment consisted of continuous 28-day (D) cycles (C). VEN 400 mg or 800 mg PO was administered D1–14. IVO 500 mg PO, a known moderate CYP3A inducer, started on C1D15 followed by continuous administration. Initiating IVO on C1D15 allowed for an assessment of steady-state VEN plasma concentrations on C1D14 and C2D14, before and after initiation of IVO. DL #3 and #4 received AZA 75 mg/m^2 (intravenous or subcutaneous) on D1–7 in addition to IVO and VEN. Dose modifications prior to completion of the DLT period in patients attaining a response were considered on an individual basis at the discretion of the treating physician and principal investigator.

All patients were hospitalized for C1 initiation, for at least 24 hours after receiving their target VEN dose. A VEN ramp-up was used with TLS monitoring and prophylaxis as appropriate. Triazole antifungal prophylaxis was prohibited 72 hours prior to treatment initiation and during the first two cycles of therapy in the phase I portion. Following completion of VEN pharmacokinetic studies in C2, patients could receive triazole antifungals and other moderate and strong CYP3A4 inhibitors with concurrent FDA-recommended VEN dose

adjustments in addition to standard antimicrobial prophylaxis as indicated. Serial electrocardiogram monitoring of the QTc interval was performed on D1 and D15 of C1–2 and C3D1.

Cytogenetic, Molecular, and Flow-Cytometric Analysis of MRD

Conventional karyotyping was performed via standard metaphase cytogenetics (32, 33). MRD was assessed via 8-color MFC on bone marrow samples using leukemia-associated immunophenotype (LAIP) or deviation from normal assessment with a minimum sensitivity of 10^{-3} (range, 10^{-3}–10^{-4}; 0.1–0.01%) in patients attaining a CRc (34, 35). Patients with MDS, MPN, or specimens not meeting the criteria for adequate MRD assessment were excluded from MRD analyses. Mutation assessment at prespecified study time points utilized NGS interrogating the entire exonic or hotspot regions of 81 genes frequently mutated in myeloid malignancies (Supplementary Table S5; ref. 36). The analytical sensitivity was established at 1% to 2% mutant reads in a background of wild-type reads. Variants identified using NGS throughout treatment are displayed in Supplementary Table S6.

Certain genes from the 81-gene NGS panel were assessed and grouped according to biological pathway unless otherwise specified, including methylation (*IDH1, IDH2, DNMT3A*, and *TET2*), active signaling (*NRAS, KRAS, PTPN11, JAK2, FLT3*-ITD, *FLT3*-TKD [D835], *KIT, STAT3, STAT5A, STAT5B, BRAF, NF1*, and *CSF3R*), cohesin-chromatin (*ASXL1, EZH2, BCOR, BCORL1, SMC3, STAG2*, and *RAD21*), splicing (*SRSF2, U2AF1, SF3B1*, and *ZRSR2*), transcription factor (*RUNX1, CEBPA*, and *GATA2*), tumor suppressor (*TP53, WT1*, *FXW7*, and *PHF6*), or mutated *NPM1*. Other genes assayed but not listed were not considered as part of a specific biological pathway for the purpose of this analysis. All genes classified by biological pathway were allocated into only one group and did not span multiple biological pathways.

Study Objectives

The primary P1b objective was evaluation of the safety and efficacy of IVO + VEN ± AZA, with identification of the MTD and RP2D. Secondary objectives included markers of efficacy (ORR, CRc), durability (DOR: date of first response until change in therapy, progression, or death), and survival (OS: C1D1 until death or last contact; EFS: C1D1 until morphologic relapse or progression or death; patients not attaining a protocol-defined response were considered as progressing on C1D1; ref. 37).

Exploratory Objectives

Exploratory outcomes included the impact of *IDH1* mutation or MRD-MFC detection in remission on survival. Correlative biomarkers predictive of response or resistance to IVO + VEN ± AZA included ddPCR for specific *IDH1* R132 variants (sensitivity 0.25%–0.1%), scDNA-seq/DAb-seq, and CyTOF. WES was performed on patient samples with persistent *IDH1* mutations at the time of relapse to assess for second-site mutations in the *IDH1* gene. All correlative analyses were preferentially performed using bone marrow samples (peripheral blood was used in the minority of cases where corresponding bone marrow samples were unavailable).

Statistical Considerations

The operating characteristics for the dose-escalation rule are displayed in the supplementary protocol, Table 8.1. Between group comparisons utilized Fisher exact test and the Wilcoxon rank-sum test for categorical and continuous variables as appropriate. The log-rank method with unstratified cox proportional hazard modeling was utilized for the assessment of time-to-event endpoints between DL and disease types. 95% exact confidence intervals were calculated using Blaker's binomial method as implemented in the R package PropCIs (38). Descriptive statistics were utilized to evaluate molecular and cellular correlates in subgroups with insufficient statistical power. Analyses were conducted using R version 2022.12.0.

scDNA-seq

According to the manufacturer's protocol, single-cell library DNA preparation was performed using the Tapestri platform and reagents (MissionBio). Briefly, cryopreserved bone marrow mononuclear cells (BMNC) were thawed, washed with RPMI-1640 supplemented with 40% FBS, 1% BSA, and 5 mmol/L EDTA, and counted using a Countess cell counter (Invitrogen). Cells were normalized to 3,000 to 4,000 cells/μL using a cell buffer. Next, cells were loaded onto the Tapestri instrument for single-cell encapsulation, lysis, and barcoding. The DNA from barcoded cells was amplified via multiplex PCR using a targeted myeloid panel that included 279 amplicons across 37 genes associated with myeloid malignancies (https://designer.missionbio.com/catalogpanels/Myeloid-MDACC). DNA libraries were extracted from the droplets, followed by the purification with Ampure XP beads (Beckman Coulter). The purified DNA libraries were indexed and then sequenced on Illumina's NovaSeq 6000 SP with 250-bp paired-end multiplexed runs.

scDNA-seq with Antibody–Oligonucleotide Conjugates

Samples for combined antibody-Oligonucleotide Conjugates (AOC)-based protein detection were prepared in the same manner described in scDNA-seq with the following modifications. First, BMNCs were normalized to 25,000 cells/μL in 40 μL and incubated with Human TruStain FcX (BioLegend, catalog #422301) blocking buffer in 1x staining buffer for 15 minutes on ice. Next, cells were stained with TotalSeq-D Human Heme Oncology Cocktail (BioLegend, cat. #399906), CD99 (BioLegend, cat. #371325), and CD366 (BioLegend, cat. #345057) AOCs for 30 minutes on ice. Following staining, cells were washed three times in wash buffer and were loaded onto the Tapestri instrument for single-cell encapsulation, lysis, and barcoding with a 2-μmol/L antibody tag forward primer. DNA libraries were prepared and purified as above. Protein PCR products, which exist in the supernatant from the Ampure XP bead purification step, were isolated by a 5-minute incubation with 2-μL 5′ Biotin Oligo for 5 minutes at 96°C, followed by a 5-minute incubation on ice. Isolated proteins were washed using 2 × binding and washing buffer and streptavidin beads. Protein libraries were generated using the washed proteins, library template, and i5/i7 indices via PCR. The protein library PCR product was cleaned again using Ampure XP beads. Protein libraries were quantified, quality checked, and sequenced on Illumina's NovaSeq 6000 S4 with 150-bp paired-end multiplexed runs.

scDNA-seq Data Analysis

FASTQ files generated by the sequencer were processed using the Tapestri Pipeline v2. They included adapter trimming and sequence alignment (Burrows–Wheeler Aligner) to the human genome (GRCh37/hg19), barcode correction, and cell find. Both DNA and protein data were analyzed and visualized using Mission Bio's Python-based Mosaic library (version 1.8). Somatic variants were manually reviewed outside of Mosaic, and only the selected variants were subsequently examined in Mosaic. "filter_variants" was applied with parameters: min_dp = 5, min_gq = 0, min_vaf = 21, min_prct_cells = 0, min_mut_prct_cells = 0, min_std = 0. Protein reads were first normalized by centered log ratio and then underwent dimensionality reduction [PCA with components = 10, and uniform manifold approximation and projection (UMAP) with attribute = "pca," n_neighbors = 20, metric = "cosine," and min_dist = 0]. Lastly, such transformed counts were clustered with the Louvain community detection algorithm (attribute = "umap," method = "graph-community," and k = 150) to be plotted into final figures. Randomness was controlled in all steps.

Cell populations for DAb-seq analysis were defined as follows: $CD4^+$ T cells ($CD3^+$, $CD4^+$), $CD8^+$ T cells ($CD3^+$, $CD8^+$), natural killer cells ($CD45^+$, $CD16^+$), $CD14^+$ monocytes/dendritic cells ($CD11b^+$, $CD11c^+$, $CD14^+$, $CD64^+$, HLA-DR^+), $CD16^+$ monocytes ($CD11b^+$, $CD16^+$), erythroid progenitors ($CD71^+$), primitive myeloid progenitors ($CD34^+$, $CD117^+$). Maturing myeloid progenitors were separated

from primitive myeloid progenitors based on a manual review of differential expression of ($CD34^+$, $CD117^+$, and $HLA\text{-}DR^+$).

CyTOF Assay and Analyses

CyTOF assay was performed as described in previous reports (39, 40). CyTOF data were first normalized by using bead-normalization and analyzed with FlowJo v10 (FlowJo, LLC) with Plugins supported by R v4.1 and GraphPad Prism v9. Two algorithms, UMAP (41) followed by FlowSOM (42), were applied for dimension reduction, clustering, and visualization on high-dimensional CyTOF data. Antibodies used for CyTOF analysis are displayed in Supplementary Table S7.

Data Availability

Clinical data may be made available on an individual basis after discussion with the corresponding author. Single-cell and WES data are available at: http://www.ncbi.nlm.nih.gov/bioproject/957456. Accession number: PRJNA957456.

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Note

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