Complete remission of *NUP98* fusion-positive acute myeloid leukemia with the covalent menin inhibitor BMF-219, icovamenib

Acute myeloid leukemia (AML) containing nucleoporin 98 (*NUP98*) fusions is the most common genotype among children with relapsed/refractory disease, representing a poor prognosis group.[1] *NUP98* fusions occur in ~5% of cases of childhood AML, and are especially frequent in acute erythroid and megakaryocytic leukemias.[1,2] *NUP98* translocations also occur in adults; in *FLT3*-ITD AML – a setting with frequent co-occurring *NUP98* fusions – one of the more common, *NUP98-NSD1*, was reported in six of 97 (6.2%) cases.[3] Adults with AML harboring *NUP98-NSD1* fusions have a poor response to therapy, with induction failure rates following intensive anthracycline/cytarabine-containing chemotherapy of 83% *versus* 36% for *NUP98-NSD1* fusion-negative cases (P=0.038).[3]

NUP98 chimeric proteins fuse the N-terminus with various C-terminal partners, including epigenetic modifiers and transcription factors, such as HOX proteins.[2] Over 30 NUP98 fusions occur in malignancies including *de novo* and therapy-related AML, myelodysplastic syndromes, chronic myeloid leukemia, T-cell acute lymphoblastic leukemia, and mixed phenotype acute leukemia.[2,4] It is important to note that many *NUP98* fusions are cryptic on conventional cytogenetic analysis; accordingly, only molecular methods such as next-generation sequencing (NGS) can reliably identify the variety of *NUP98* fusions.[2] Although many commercially available NGS diagnostic assays capture *NUP98* fusions, this is not invariably the case; likewise, 'homebrew" NGS assays offered as laboratory-developed tests may or may not identify these fusions. Clinicians should consult with commercial vendors and/or their molecular diagnostic laboratory to determine whether a specific assay can reliably identify the various *NUP98* fusions.

Preclinical models demonstrate that *NUP98* fusions such as *NUP98-HOXA9, NUP98-HOXD13, NUP98-JARID1A,* and *NUP98-NSD1*, are potent drivers of leukemogenesis.[5,6] *WT1* or *FLT3*-ITD mutations that co-occur with *NUP98* fusions worsen prognosis.[1,2] NUP98 fusions elicit leukemogenesis through interaction with histone-modifying chromatin complexes.[6,7] For example, NUP98-NSD1 recruits WDR82–Set1A/COMPASS complexes, resulting in histone H3 lysine 4 tri-methylation at the *HOXA* genes and *MEIS1* promoter.[7] NUP98 fusions also interact with mixed lineage leukemia-1 (MLL1; aka KMT2A) chromatin complexes. For instance, NUP98-HOXA9 colocalizes with KMT2A at the *HOXA/B* genes, *KMT2A* deletion delays leukemogenesis in a *NUP98-HOXA9* mouse model, and KMT2A is required for the gene expression signature observed in *NUP98* fusion-positive AML.[8]

Interaction between *KMT2A* and the scaffold protein menin is essential for *KMT2A*-rearranged and *NPM1*-mutant leukemias.[9,10] Disruption of this interaction using small-molecule inhibitors is a promising therapy under clinical evaluation for these leukemias and early results have demonstrated complete remissions in some patients.[9,11]

The association of NUP98 fusions with KMT2A complexes on promoters, together with the observation that similar leukemia-associated gene expression signatures occur in *NUP98*-rearranged, *KMT2A*-rearranged, and NPM1-mutant AML, suggests that *NUP98*-rearranged AML may depend on the menin-KMT2A interaction. Indeed, Heikamp *et al.*[12] showed that interruption of this interaction halts leukemic progression in cell lines and patient-derived xenograft models expressing *NUP98-HOXA9, NUP98-HOXD13, NUP98-JARID1A*, and *NUP98-NSD1*. Rasouli *et al.*[13] examined menin-KMT2A inhibition in primary patients' AML cells harboring *NUP98* fusions, demonstrating that the non-covalent reversible inhibitor revumenib elicits differentiation and suppresses proliferation but not apoptotic death in *NUP98-NSD1* AML samples. Rasouli and co-workers also showed that *NUP98*-rearranged leukemias containing other fusion partners, such as *TOP1* and *DDX10*, are sensitive to menin inhibition.

These preclinical results support menin inhibition as a potential therapy for *NUP98*-rearranged AML but clinical proof has not been described so far, to our knowledge. Here we report a patient with relapsed *NUP98-NSD1* AML who achieved a long-lasting complete remission with the covalent menin inhibitor BMF-219 (icovamenib).

The patient, a 39-year-old Caucasian male with a history that included melanoma *in-situ* after multiple excisions, was diagnosed with primary *NUP98-NSD1*-positive AML with leukocytosis (white blood cell count [WBC]=317.6x10^9/L), anemia (hemoglobin [Hb]=7.1 g/dL) and thrombocytopenia (platelet count=74x10^9/L) along with significant lymphadenopathy and splenomegaly. Bone marrow biopsy showed AML with normal cytogenetics (46, XY [20]); NGS revealed *NUP98-NSD1*, *CEBPA* p.G99Vfs*63, c.295_296ins5 variant allelic frequency (VAF, 32.3%), *NRAS* p.Q61K, c.181C.A (VAF, 1.9%), *NRAS* p.G13D, c.38G>A (VAF, 38.4%), *WT1* p.P132Rfs26, c395delC (VAF, 35.2%), and *RUNX1* p.G365S, c.1093G>A (VAF, 49.6%), a variant of uncertain significance (VUS). Skin fibroblast germline testing was

positive for *CHEK2*. Bone marrow studies on day 14 after induction with cytarabine and idarubicin ("7+3" regimen) showed persistent AML (10% blasts in hypocellular marrow), while a second induction course with the "7+3" regimen resulted in a first complete remission. One cycle of high-dose Ara-C consolidation then preceded hematopoietic stem cell transplantation with curative intent. Conditioning therapy with busulfan and cyclophosphamide was followed by a matched unrelated donor allogeneic hematopoietic stem cell transplant. Following the transplant, the patient was given tacrolimus, methotrexate and mycophenolate mofetil.

Five months after the transplant, the patient became cytopenic and transfusion-dependent. A bone marrow aspirate revealed 13% blasts and 46XY, del(5)(q22q35[5]//46XX[15] karyotype. Bone marrow NGS showed *NUP98-NSD1, CEB-*

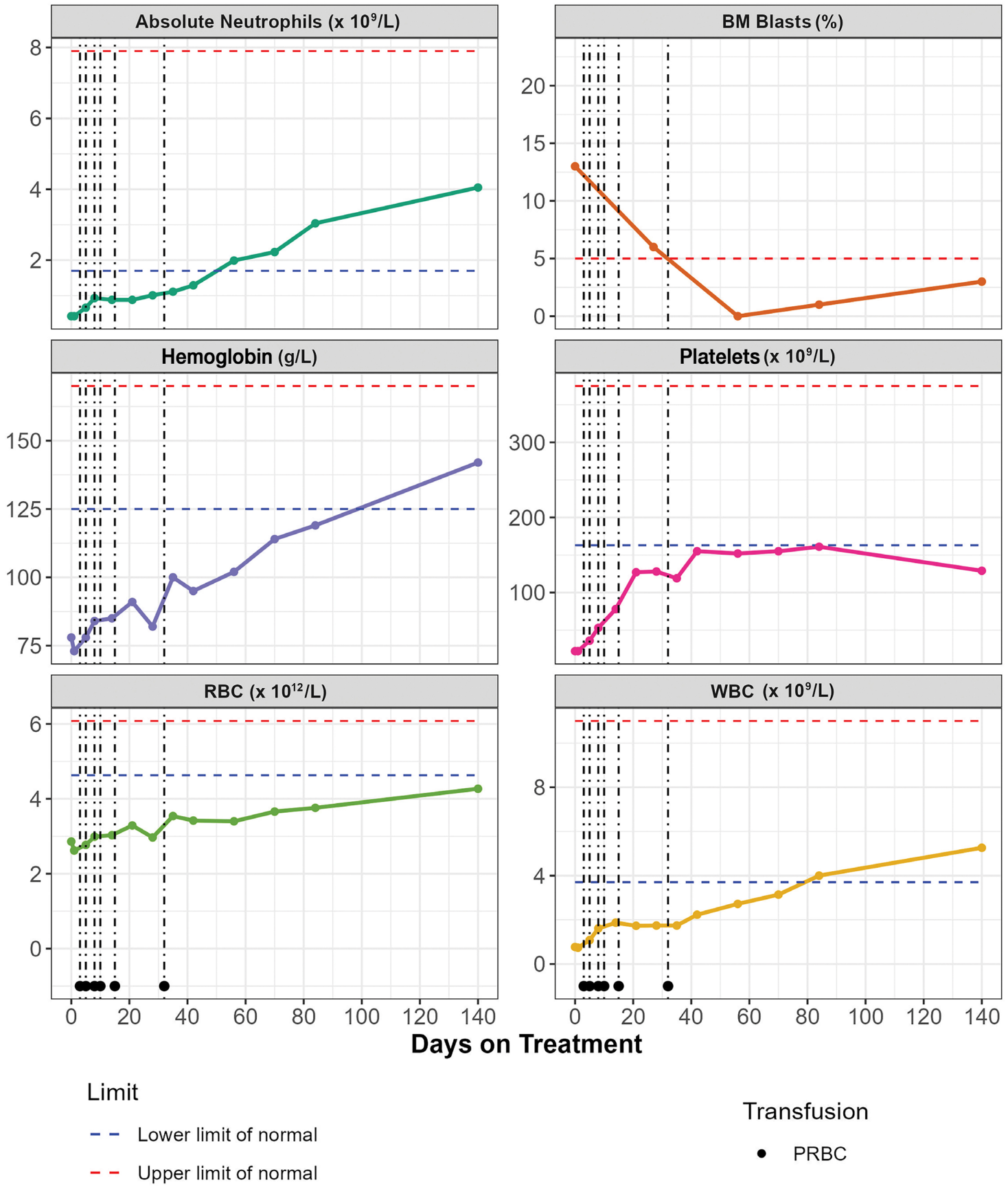

Figure 1. Antileukemic response and normal hematopoietic cell recovery in a patient with *NUP98-NSD1*-positive acute myeloid leukemia treated with the covalent menin inhibitor BMF-219 (icovamenib). Horizontal dashed blue and red lines in each graph represent the lower limit of normal and upper limit of normal, respectively, for each parameter; the vertical interrupted black lines in each graph indicate the timing of administration of packed red blood cell transfusions. BM: bone marrow; RBC: red blood cells; WBC: white blood cells; PRBC: packed red blood cells.

Table 1. Select laboratory parameters during the patient's course.

Time	Day	Test							
		NGS	Cytogenetics	Skin fibroblast germline	BM blasts (%)	WBC[a] x10⁹/L	Hb[b] g/dL	Plts[c] x10⁹/L	ANC x10⁹/L
At AML diagnosis	D-314	*NUP98-NSD1* t(5;11) (q35.3;p15.4) *CEBPA* p.G99Vfs*63, c.295_296ins5 VAF 32.3% *NRAS* p.Q61K, c.181C.A VAF 1.9% *NRAS* p.G13D, c.38G>A VAF 38.4% *WT1* p. P132Rfs26, c395delC VAF 35.2%, VUS for *RUNX1* p.G365S, c.1093G>A VAF 49.6%	46, XY [20]	CHEK2 (+)	10	317.58	7.1	74	NA
After allogeneic HSCT	D-31	*NUP98-NSD1* t(5;11) (q35.3;p15.4) *CEBPA* p.G99Vfs*63, c.295_296ins5 VAF 7% *RUNX1* VUS pG365S, c.1093 G>A, VAF 9.1%	46XY, del(5) (q22q35[5]//46XX[15]	-	13	1.18	6.3	51	873
Screening	D-1	*NSD1-NUP98*	-	-	13	0.77	7.8	22	0.42
C1D1	D1	-	-	-	-	0.74	7.3	22	0.42
UNS	D3	-	-	-	-	0.9	7.4	28	0.54
UNS	D4	-	-	-	-	1.41	8	36	0.79
C1D4	D5	-	-	-	-	1.08	7.8	36	0.66
UNS	D6	-	-	-	-	NA	NA	NA	NA
C1D8	D8	-	-	-	-	1.61	8.4	53	0.93
UNS	D9	-	-	-	-	1.37	8.2	50	0.82
UNS	D10	-	-	-	-	1.4	7.4	51	0.66
UNS	D12	-	-	-	-	1.99	9	65	1.13
UNS	D13	-	-	-	-	2.16	8.6	72	1.04
C1D15	D14	-	-	-	-	1.87	8.5	78	0.88
C1D22	D21	-	-	-	-	1.73	9.8	127	0.88
C2D1	D28	-	46, XX [20]	-	6	1.74	8.2	128	1.01
C2D8	D35	-	-	-	-	1.74	10	119	1.11
C2D15	D42	-	-	-	-	2.23	9.5	155	1.29
C3D1	D56	-	46, XX [20]	-	0 MRD +	2.72	10.2	152	1.99
C3D15	D70	-	-	-	-	3.14	11.4	155	2.23
C4D1	D84	No detectable mutations	-	-	1 MRD -	4.00	11.9	161	3.04

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Time	Day	Test							
		NGS	Cytogenetics	Skin fibroblast germline	BM blasts (%)	WBC[a] x10^9/L	Hb[b] g/dL	Plts[c] x10^9/L	ANC x10^9/L
C6D1	D140	*NUP98-NSD1*	46, XY, t(2;10)(p11.2;q24), del(5)(q22.q35), del(13)(q12q22) [2]//46, XX[19]	-	3 MRD +	5.26	14.2	129	4.05
EOT (C7D13)	D180	*NUP98-NSD1* *CEBPa* p.G99Vfs*63, c.295_296ins5 VAF 30% *KRAS* p.G13D, c.38G>A VAF 34.1% *NRAS* p.G13D, c.38G>A VAF 6.2% *WT1* p.P132Rfs*26, c.395delC VAF 45.4% VUS for *RUNX1* p.G365S, VUS VAF 46.7%	-	-	87 (collected C7D7)	0.72	7.7	155	0.18

[a]Reference range: 3.70-11.0 (x10^9/L). [b]Reference range: 12.5-17.0 (g/dL). [c]Reference range: 163-375 (x10^9/L). Reference ranges are for the central laboratory. Central laboratory parameters reported when available. Local laboratory parameters reported only if central laboratory values were not available. NGS: next-generation sequencing; BM: bone marrow; WBC: white blood cells; Hb: hemoglobin; Plts: platelets; ANC: absolute neutrophil count; AML: acute myeloid leukemia; VAF: variant allele frequency; VUS: variant of unknown significance; NA: not available; HSCT: hematopoietic stem cell transplant; CxDy: cycle x day y of treatment; UNS: unscheduled visit; EOT: end of treatment.

PA p.G99Vfs*63, c.295_296ins5 (VAF, 7%), and *RUNX1* VUS pG365S, c.1093 G>A (VAF, 9.1%).

The patient was enrolled in the COVALENT-101 study, ‘‘A phase I first-in-human dose-escalation and dose-expansion study of BMF-219, an oral covalent menin inhibitor, in adult patients with acute leukemia (AL), diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), and chronic lymphocytic leukemia (CLL/SLL)” (NCT05153330), approximately 10 months following his AML diagnosis. The study had been approved by the Institutional Review Board at the site and informed consent was obtained from the patient. BMF-219 (icovamenib) is a small molecule designed to disrupt interactions of menin with various proteins, such as KMT2A and JunD, which regulate multiple transcriptional and cell-cycle regulation pathways. Preclinical data on BMF-219 showed potent cytotoxicity on various menin-dependent AML cells, as well as cell lines and patients’ samples representing other hematologic malignancies.[14]

BMF-219 500 mg PO once daily was initiated in continuous 28-day cycles. On day 15 of cycle 1 (C1D15) of treatment, the patient was afebrile but with a dry cough. Chest computed tomography showed peribronchial ground glass opacities and the patient was hospitalized for suspected grade 3 differentiation syndrome, and given prednisone, allopurinol, acyclovir, piperacillin-tazobactam, and azithromycin. Although the patient’s baseline cytopenias persisted at C1D15, the peripheral blood counts revealed an emerging differentiation response to BMF-219 as evident by an increase in the absolute neutrophil count from 0.42x10^9/L on C1D1 to 0.88x10^9/L on C1D15, and an increase in the absolute monocyte count from 0.10x10^9/L on C1D1 to 0.24x 10^9/L on C1D15. The patient was discharged after 2 days in hospital and received prednisone as an outpatient until C1D20, with complete resolution of his symptoms.

Select laboratory parameters at diagnosis and throughout the patient’s course are shown in Table 1. The patient’s hematologic parameters showed steady improvement with BMF-219 (Figure 1). At study entry, the patient was receiving blood products 3-4 times weekly. This frequency decreased to weekly by the end of cycle 1, and 5 weeks after treatment initiation the patient became transfusion-independent and remained so for the duration of BMF-219 therapy.

The first bone marrow assessment at C2D1 revealed a partial response (ELN2017), with a decrease in blasts from 13% at baseline to 6% (Figure 1). The marrow karyotype was normal with 46,XX [20]; the WBC count, Hb concentration and platelet count were 1.99x10^9/L (65% neutrophils), 7.6 g/dL, and 140x10^9/L, respectively. Bone marrow analysis at C3D1 demonstrated a complete remission with 0% blasts, no circulating blasts, and normal female donor karyotype along with recovering normal hematopoiesis

(WBC count=2.68x10^9/L [65% neutrophils], Hb=10.3 g/dL, platelet count=165x10^9/L). Tested by flow cytometry (sensitivity ≥10^{-5}) on C3D1, the patient had measurable residual disease (~0.2% of WBC). Gene expression changes consistent with the anti-leukemic response were observed (Figure 2). C4D1 assessments demonstrated continued cytogenetic complete response with 1% marrow blasts but with minimal residual disease-negativity, bone marrow NGS without detectable mutations, a WBC count of 4.16x10^9/L (80% neutrophils), Hb of 11.5 g/dL, and platelet count of 143x10^9/L.

At C5D1 and C6D1, the complete remission continued with 3% marrow blasts, a WBC count of 5.21x10^9/L (76% neutrophils), Hb of 13.9 g/dL, and platelet count of 128 x10^9/L but positive minimal residual disease was noted (~0.7% of WBC). NGS revealed the *NUP98-NSD1* fusion and cytogenetics showed 46XY, t(2;10)(p11.2;q24), del(5)(q22.q35), del(13)(q12q22)[2]//46,XX[19]. The next dose level in the ongoing COVALENT-101 dose-escalation trial had been cleared, allowing the patient's dose to be increased to 650 mg once daily towards the end of cycle 6. The higher dose level was well tolerated but, unfortunately, the patient's leukemia progressed, and treatment was discontinued in C7D8. Bone marrow NGS at study discontinuation showed *NUP98-NSD1* and mutations in *CEBPA* (VAF, 30%), *KRAS* G13D (VAF, 34.1%), *NRAS* G13D (VAF, 6.2%), *WT1* (VAF, 45.4%), and *RUNX1* VUS (VAF, 46.7%).

Co-occurring mutations may affect the efficacy of menin inhibition in *NUP98*-rearranged AML. For example, Heikamp *et al.*[12] showed responsiveness of a *NUP98-NSD1* patient-derived xenograft model that was also *WT1* mutant, while another *NUP98-NSD1* AML patient-derived xenograft model containing mutations in *WT1, ASXL1, IDH1, BCORL1* and *FLT3*-ITD did not respond. Of note, Rasouli and colleagues[13] showed that menin inhibitor monotherapy was sufficient to suppress *NUP98*-rearranged AML with co-occurring *FLT3*-ITD and *WT1* mutations; however, co-administration of a menin inhibitor with an FLT3 inhibitor enhanced the antileukemic activity of either alone. Similarly, Miao *et al.*[15] found that combinations of a menin inhibitor with kinase inhibitors targeting either CDK6 (overexpressed in *NUP98*-rearranged leukemia) or FLT3 strongly enhanced the antileukemic activity of menin inhibition alone in samples from patients with *NUP98*-rearranged AML and patient-derived xenograft models. A broader clinical assessment of menin inhibitor monotherapy, as well as combinations with other active agents, in *NUP98*-rearranged AML is warranted.

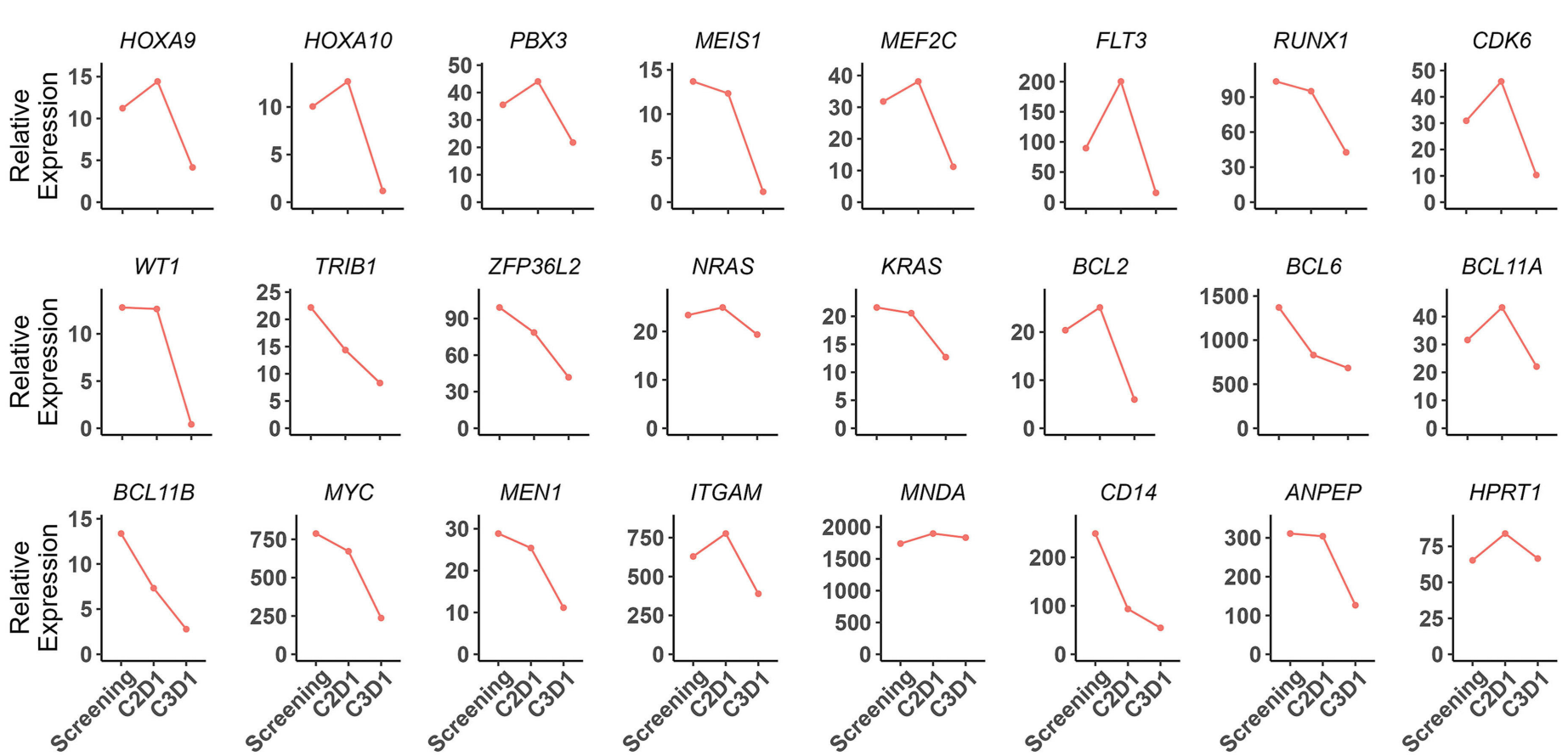

Figure 2. Gene expression profiling in a patient with acute myeloid leukemia containing the *NUP98-NSD1* fusion under treatment with covalent menin inhibitor BMF-219 (icovamenib). RNA-sequencing analysis of bone marrow aspirates revealed differentially expressed genes before and after treatment. Gene expression levels are presented as transcripts per million. Note at cycle 3 day 1 of treatment, coincident with attainment of complete remission, the pro-leukemogenic gene expression program in the bone marrow was downregulated by more than two-fold compared to the pre-treatment screening sample. These changes included suppression of key hematopoietic transcription factors (*HOXA9, HOXA10, MEIS1, MEF2C*), other relevant transcription factors (*WT1, TRIB1, ZFP36L2, BCL6, BCL11B, cMYC),* kinases (*FLT3, CDK6*), and the *MEN1* gene, which encodes menin. Notably, there was no noticeable upregulation of markers of differentiation as observed with non-covalent menin inhibitors. Instead, BMF-219 led to their downregulation (*CD14, ANPEP, ITGAM*) or maintenance of gene expression level (M*NDA*). Expression of the housekeeping gene *HPRT1* remained essentially constant across the screening, cycle 2, and cycle 3 timepoints. C2D1: cycle 2 day 1; C3D1: cycle 3 day 1.

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Disclosures

AC, YM, BM, GW, CM, UA, NK, TB and SWM are current or former employees of Biomea Fusion, Inc. HEC and JN have no conflicts of interest to disclose.

Contributions

HEC and JN performed the study, collected and analyzed data, and wrote the manuscript. AC, YM, BM, GW, CM, UA, NK, TB and SWM designed and performed the study, collected and analyzed data, and wrote the manuscript. All authors read and approved the manuscript.

Data-sharing statement

Original data are available upon request to the corresponding author.

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