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

Hetty E. Carraway[1], Joy Nakitandwe[2], Alexandru Cacovean[3], Yan Ma[3], Brian Munneke[3], Ganesh Waghmare[3], Clarissa Mandap[3], Uzma Ahmed[3], Nicole Kowalczyk[3], Thomas Butler[3], Stephan W. Morris[3]

[1]Department of Hematology and Medical Oncology, Taussig Cancer Center Institute, Cleveland Clinic, Cleveland, OH, USA

[2]Department of Pathology and Laboratory Medicine, Diagnostics Institute, Cleveland Clinic, Cleveland OH, USA

[3]Biomea Fusion, Inc., Redwood City, CA, USA

Running title: NUP98 AML CR with covalent menin inhibitor BMF-219 **(icovamenib)**

Corresponding author: Hetty E. Carraway, MD, carrawh@ccf.org, 216-445-5899 (phone), 216-444-9464 (fax)

Authors' Contribution: *H.E.C. and J.N. performed the study, collected and analyzed data, and wrote the manuscript; A.C., Y.M., B.M., G.W., C.M., U.A., N.K., T.B., and S.W.M. designed and performed the study, collected and analyzed data, and wrote the manuscript; all authors read and approved the manuscript.*

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Acute myeloid leukemia (AML) containing nucleoporin 98 (*NUP98*) fusions is the most common genotype among children with *relapsed/refractory (R/R)* disease, representing a poor prognosis group.[1] *NUP98* fusions occur in ~5% 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 6 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% vs. 36% for NUP98-NSD1 fusion-negative cases (p = .038).*[3]

NUP98 chimeric proteins fuse the N-terminus with various C-terminal partners, including epigenetic modifiers and transcription factors like HOX proteins.[2] Over 30 NUP98 fusions occur in malignancies including *de novo* and therapy-related AML, MDS, CML, T-ALL, and MPAL.[2,4] *It is important to note that many NUP98 fusions are cryptic on conventional cytogenetic analysis; accordingly, only molecular methods such next-generation sequencing (NGS) technologies 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 lab-developed tests may or may not identify these fusions. Clinicians should consult with commercial vendors and/or their molecular diagnostic laboratory to determine if a specific assay can reliably identify the various NUP98 fusions*.

Preclinical models demonstrate *NUP98* fusions such as *NUP98-HOXA9*, *NUP98-HOXD13*, *NUP98-JARID1A*, and *NUP98-NSD1* as 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 (*KMT2A*r) and *NPM1*-mutant (*NPM1*c) leukemias.[9,10] Disruption of this interaction using small-molecule inhibitors is a promising therapy under clinical evaluation for these leukemias, early results demonstrating complete remissions (CRs) 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*r, *KMT2A*r, and *NPM1*c AML, suggests that *NUP98*r 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 (PDX)* models expressing NUP98-HOXA9, NUP98-HOXD13, NUP98-JARID1A, and NUP98-NSD1. Rasouli *et al*.[13] examined menin-KMT2A inhibition in primary patient AML cells harboring NUP98 fusions, demonstrating the non-covalent reversible inhibitor revumenib to elicit differentiation and suppress proliferation but not apoptotic death in *NUP98-NSD1* AML samples. Rasouli and co-workers also showed *NUP98*r leukemia containing other fusion partners such as *TOP1* and *DDX10* to be menin inhibitor-sensitive.

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

The patient, a 39-year-old Caucasian male with a history that included melanoma *in-situ* post multiple excisions, was diagnosed with primary NUP98-NSD1+ AML with leukocytosis (WBC 317.6 x 10^9/L), anemia (Hgb 7.1 g/dL) and thrombocytopenia (74 x 10^9/L) along with significant lymphadenopathy and splenomegaly. Bone marrow (BM) 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% *(variant of uncertain significance, VUS).* Skin fibroblast germline testing was positive for CHEK2. Induction with cytarabine and idarubicin (7+3) yielded a Day 14 BM showing persistent AML (10% blasts in hypocellular marrow) and second induction with 7+3 resulted in CR1. One cycle of High-dose Ara-C consolidation then preceded curative intent hematopoietic stem cell transplant (HSCT). Conditioning therapy with busulfan and cyclophosphamide was followed by a matched unrelated donor (MUD) allogeneic-HSCT. Post-transplant, the patient received tacrolimus, methotrexate and mycophenolate mofetil.

Five months post-transplant, the patient became cytopenic and transfusion dependent. BM aspirate revealed 13% blasts and 46XY, del(5)(q22q35[5]//46XX[15] karyotype. BM NGS showed NUP98-NSD1, CEBPA 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 1 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 IRB 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 that regulate multiple transcriptional and cell-cycle regulation pathways. Preclinical data with BMF-219 show potent cytotoxicity on various menin-dependent AML cells, as well as cell lines and patient samples representing other hematologic malignancies.[14]

BMF-219 500 mg PO QD was initiated in continuous 28-day cycles. On C1D15, the patient presented afebrile but with a dry cough. Chest CT showed peribronchial ground glass opacities and the patient was hospitalized for suspected Grade 3 differentiation syndrome, receiving 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 0.42 x 10^9/L on C1D1 to 0.88 x 10^9/L on C1D15, and an increase in the absolute monocyte count from 0.10 x 10^9/L on C1D1 to 0.24 x 10^9/L on C1D15.* The patient was discharged after a two-day hospitalization 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 (**Fig. 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.

First BM assessment at C2D1 revealed a PR (ELN2017), with decreased blasts from 13% at baseline to 6% (**Fig. 1**). Marrow karyotype was normal with 46,XX [20], and WBC, Hgb, and platelets were 1.99 x 10^9/L (65% neutrophils), 7.6 g/dL, and 140 x 10^9/L , respectively. BM at C3D1 demonstrated a CR with 0% blasts, no circulating blasts, and normal

female donor karyotype along with recovering normal hematopoiesis (WBC 2.68 x 10^9/L (65% neutrophils), Hgb 10.3 g/dL, platelets 165 x 10^9/L). Per flow cytometry (sensitivity $\geq 10^{-5}$) on C3D1, the patient was MRD+ (~0.2% of WBCs). Gene expression changes consistent with the anti-leukemic response were observed (**Fig. 2**). C4D1 assessments demonstrated continued cytogenetic CR with 1% marrow blasts but with MRD-negativity, BM NGS without detectable mutations, and WBC 4.16 x 10^9/L (80% neutrophils), Hgb 11.5 g/dL, and platelets 143 x 10^9/L.

At C5D1 and C6D1, the CR continued with 3% marrow blasts and WBC 5.21 x 10^9/L (76% neutrophils), Hgb 13.9 g/dL, platelets 128 x 10^9/L but MRD-positivity was noted (~0.7% of WBCs). NGS revealed NUP98-NSD1 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 had been cleared, allowing the patient's dose to be increased to 650 mg QD 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 Cycle 7 after 8 days. BM 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*r AML. For example, Heikamp *et al.*[12] showed responsiveness of a *NUP98-NSD1* PDX model that was also *WT1* mutant, while another *NUP98-NSD1* AML PDX containing mutations in *WT1*, *ASXL1*, *IDH1*, *BCORL1* and *FLT3*-ITD did not respond. Of note, Rasouli and colleagues[13] showed menin inhibitor monotherapy sufficient to suppress *NUP98*r AML with co-occurring *FLT3*-ITD and *WT1* mutations; however, menin and FLT3 inhibitor co-administration 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 NUP98r leukemia) or FLT3 strongly enhanced the anti-leukemic activity of menin inhibition alone in NUP98r patient samples and PDX models. A broader clinical assessment of menin inhibitor monotherapy, as well as combinations with other active agents, in *NUP98*r AML is warranted.

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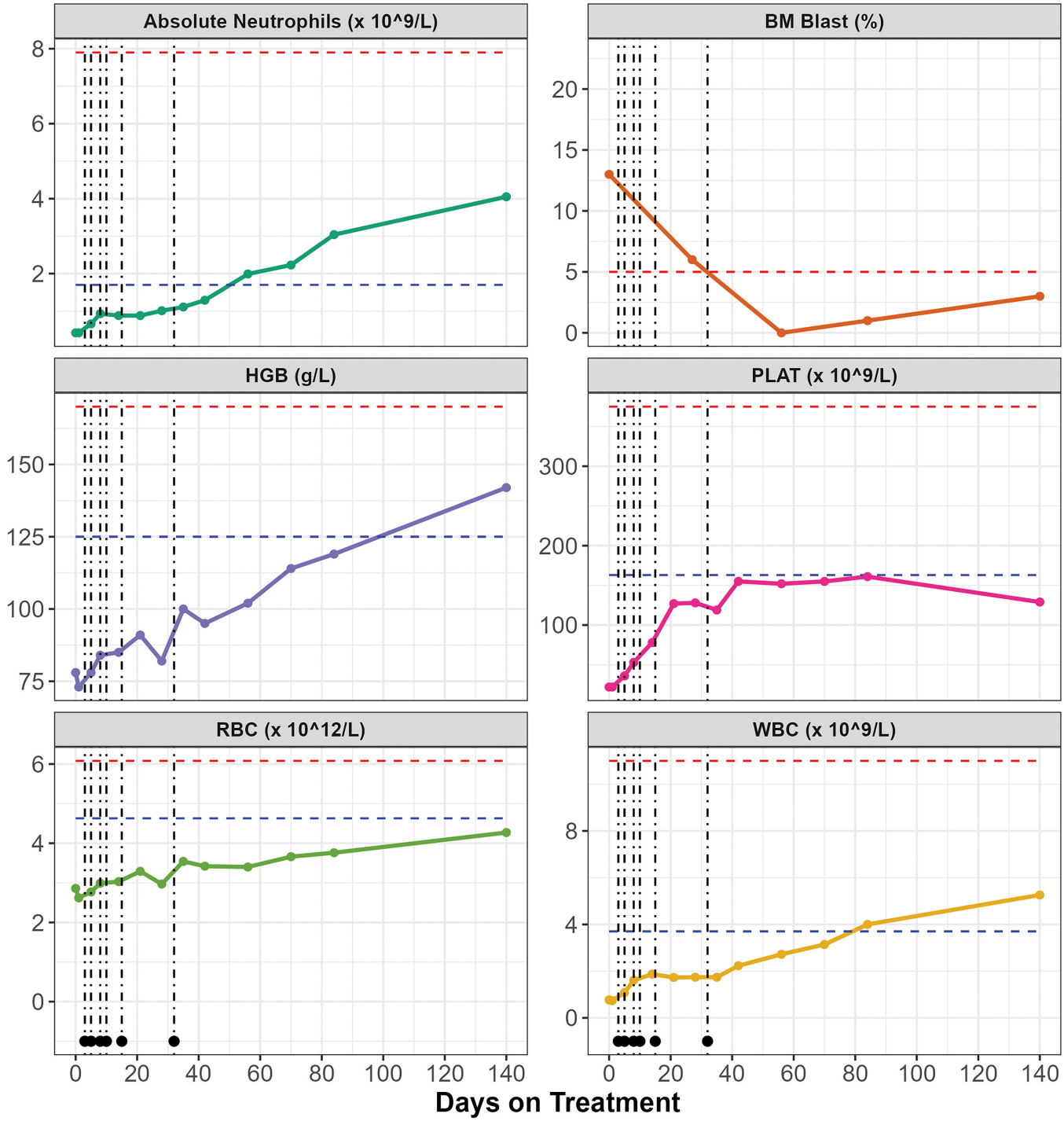
	At AML Diagnosis	Post-Allogenic HSCT	*Screening*	*C1D1*	UNS	UNS	*C1D4*	UNS	*C1D8*	UNS	UNS	UNS	UNS	*C1D15*	*C1D22*	*C2D1*	*C2D8*	*C2D15*	*C3D1*	*C3D15*	*C4D1*	*C6D1*	*EOT (C7D13)*
	D-314	D-31	D-1	D1	D3	D4	D5	D6	D8	D9	D10	D12	D13	D14	D21	D28	D35	D42	D56	D70	D84	D140	D180
NGS	NUP98-NSD1 t(5;11)(q35.3;p15.4) 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%	NUP98-NSD1 t(5;11)(q35.3;p15.4) CEBPA p.G99Vfs*63, c.295_296ins5 VAF 7% RUNX1 VUS pG365S, c.1093G>A ,VAF 9.1%	NSD1-NUP98 fusion																		No detectable mutations	NUP98-NSD1	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%
Cytogenetics	(46, XY [20])	46XY, del(5)(q22q35[5]//46XX[15]														(46, XX [20])			(46, XX [20])			46, XY, t(2;10)(p11.2;q24), del (5)(q22.q35), del (13)(q12q22)[2]//46,XX[19]	
Skin fibroblast germline	CHEK2 (+)																						
BM Blasts (%)	10	13	13													6			0, MRD +		1, MRD -	3, MRD +	87 (collected C7D7)
WBC (x10^9/L) RR: 3.70-11.0	317.58	1.18	0.77	0.74	0.9	1.41	1.08	NA	1.61	1.37	1.4	1.99	2.16	1.87	1.73	1.74	1.74	2.23	2.72	3.14	4.00	5.26	0.72
HGB (g/dL) RR: 12.5-17.0	7.1	6.3	7.8	7.3	7.4	8	7.8	NA	8.4	8.2	7.4	9	8.6	8.5	9.8	8.2	10	9.5	10.2	11.4	11.9	14.2	7.7
PLAT (x10^9/L) RR: 163-375	74	51	22	22	28	36	36	NA	53	50	51	65	72	78	127	128	119	155	152	155	161	129	15
ANC (10^9/L)	NA	873	0.42	0.42	0.54	0.79	0.66	NA	0.93	0.82	0.66	1.13	1.04	0.88	0.88	1.01	1.11	1.29	1.99	2.23	3.04	4.05	0.18

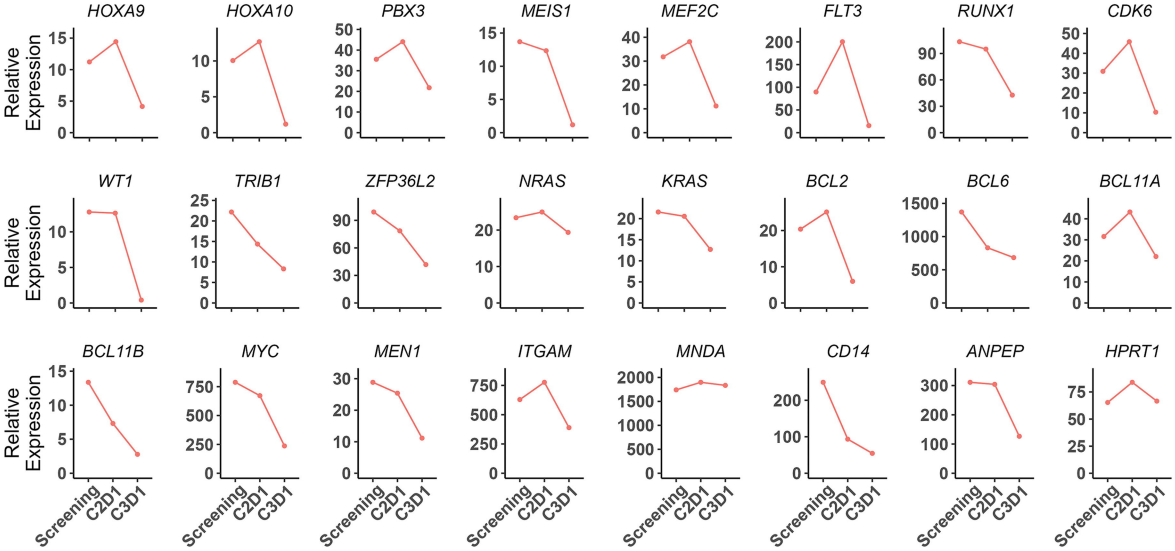
Table 1: Select Laboratory Parameters During Patient's Course RR= Reference Range; ANC= Absolute Neutrophil Count; NGS= Next Generation Sequencing; NA = Not Available; UNS= Unscheduled Visit; CxDy= Cycle X Day Y; HSCT= Hematopoietic stem cell transplant; EOT= End of Treatment; D= Day on Study; D-y= Days preceding C1D1; PLAT= Platelets; ANC= Absolute neutrophil count. RRs are per central lab. Central lab parameters reported where available. Local lab parameters reported only if central lab values are not available.

Figure Legends

Figure 1. Anti-leukemic response and normal hematopoietic cell recovery in a NUP98-NSD1-positive AML patient treated with the covalent menin inhibitor BMF-219 (icovamenib). *Horizontal dashed blue and red lines in each graph represent the lower limit of normal (LLN) and upper limit of normal (ULN), respectively, for each parameter; the vertical interrupted black lines in each graph indicate the timing of administration of packed red blood cell (PRBC) transfusions. BM= Bone marrow; PLAT= Platelets.*

Figure 2. Gene expression profiling in a patient with AML containing the NUP98-NSD1 fusion under treatment with covalent menin inhibitor BMF-219 (icovamenib). *RNA-seq analysis of bone marrow aspirates revealed differentially expressed genes before and after treatment. Gene expression levels are presented as transcripts per million (TPM). Note at Cycle 3 Day 1 (C3D1), coincident with attainment of CR, 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 (MNDA). Housekeeping gene HPRT1 maintained essentially constant expression across the screening, Cycle 2 Day 1 (C2D1), and C3D1 timepoints*


Absolute Neutrophils (x 10^9/L)
BM Blast (%)
HGB (g/L)
PLAT (x 10^9/L)
RBC (x 10^12/L)
WBC (x 10^9/L)
Days on Treatment
Limit
Lower limit of normal (LLN)
Upper limit of normal (ULN)
Transfusion
PRBC


HOXA9
HOXA10
PBX3
MEIS1
MEF2C
FLT3
RUNX1
CDK6
WT1
TRIB1
ZFP36L2
NRAS
KRAS
BCL2
BCL6
BCL11A
BCL11B
MYC
MEN1
ITGAM
MNDA
CD14
ANPEP
HPRT1
Relative Expression
Screening
C2D1
C3D1