OMTN, Volume *29*

Supplemental information

Double-stranded RNA induction as a potential dynamic biomarker for DNA-demethylating agents

Minjeong Kang, Raisa Kharbash, Ja Min Byun, Jaemin Jeon, Ahsan Ausaf Ali, Doyeong Ku, Jimin Yoon, Yongsuk Ku, Jooyeon Sohn, Seung-Jae V. Lee, Dong-Yeop Shin, Youngil Koh, Sung-Soo Yoon, Junshik Hong, and Yoosik Kim

SUPPLEMENTAL FIGURES

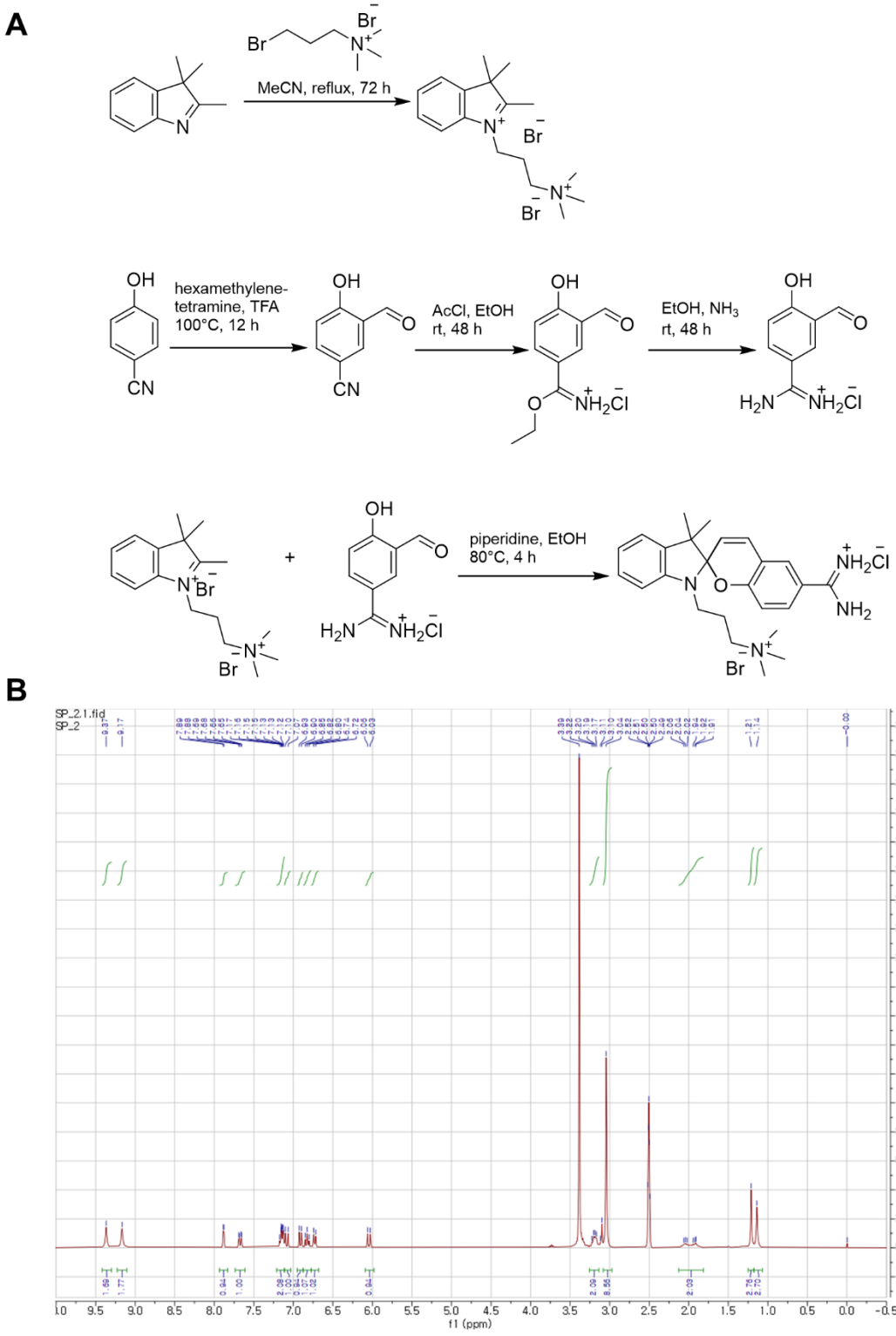

Figure S1. Synthesis and characterization of Am-SP. (A, B) Synthesis steps (A) and ^{1}H-NMR spectrum (DMSO-d_6) (B) of the products.

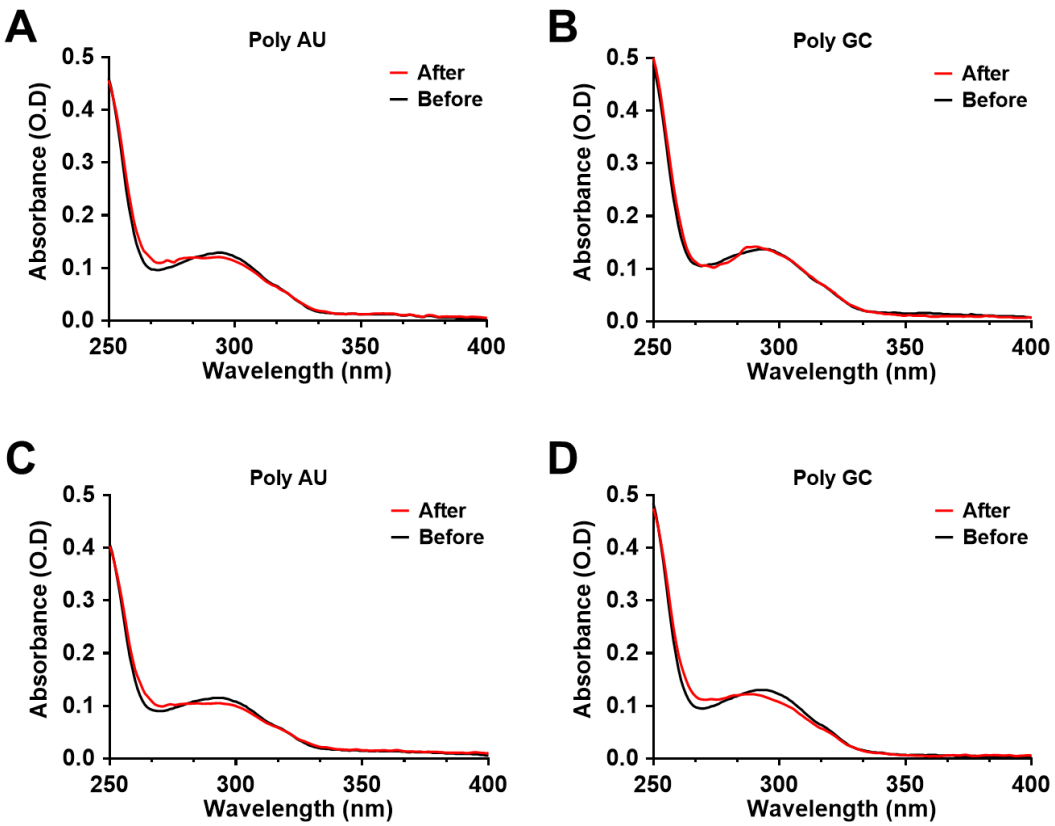

Figure S2. Interaction between Am-SP and synthetic dsRNAs. (A, B) The absorbance spectra of Am-SP after incubating it with 100 μM 20 bp long poly AU (A) or poly GC (B) dsRNAs in 9 mM Na^+ and 1 mM cacodylate buffer at pH 7. (C, D) The absorbance spectra of Am-SP after incubating it with 100 μM 20 bp long poly AU (A) or poly GC (B) dsRNAs in TDW at pH 7.

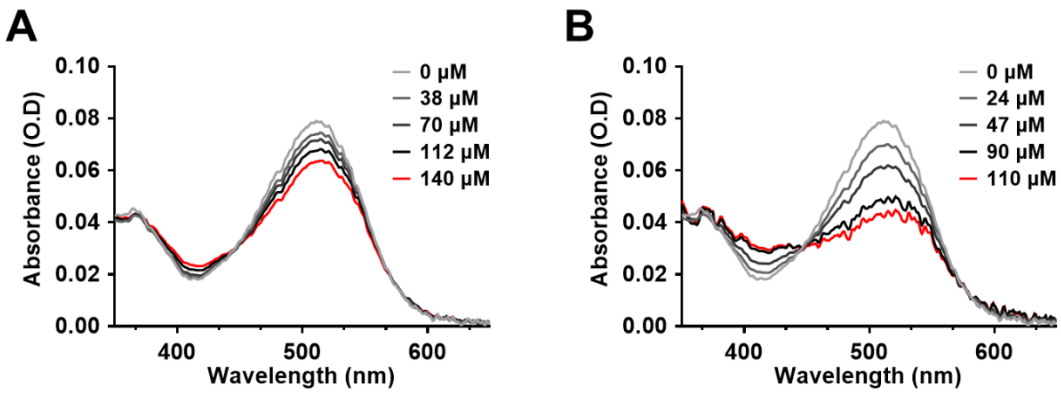

Figure S3. The absorbance changes of the Am-MC by dsRNAs. (A, B) The absorbance spectra of Am-MC when indicated concentration of 20 bp poly AU (A) or poly GC (B) dsRNAs were added in 9 mM Na^+ and 1 mM cacodylate buffer at pH 7.

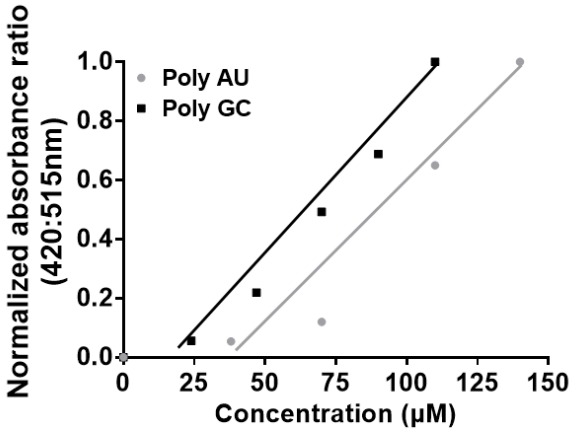

Figure S4. The absorbance ratio of the Am-MC by dsRNAs. The absorbance ratio (420:515 nm) for increasing concentration of 20 bp poly AU or poly GC dsRNAs in TDW at pH 7.

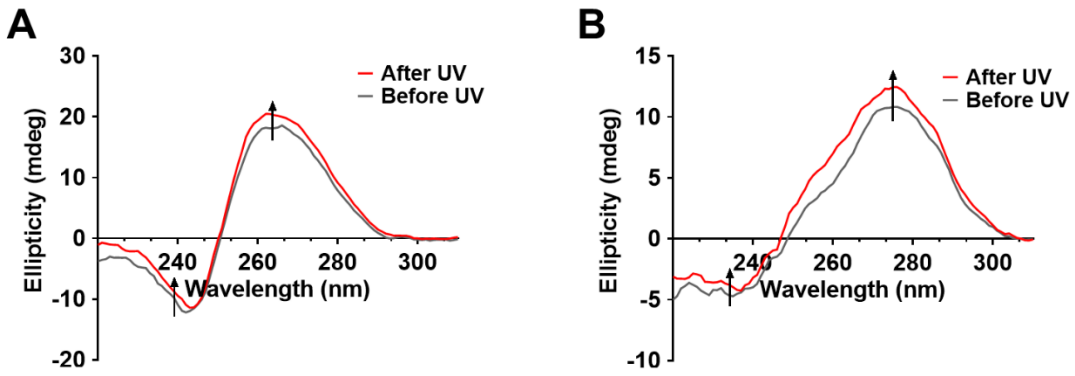

Figure S5. Circular dichroism analysis of Am-MC and dsRNA interaction. (A, B) The circular dichroism spectra of Am-MC with 100 μM of 20 bp poly AU (A) or poly GC (B) dsRNAs in TDW at pH 7.

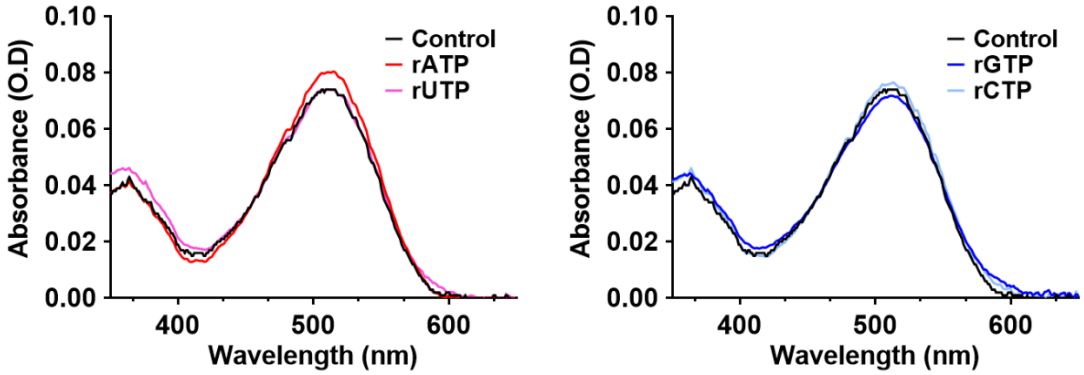

Figure S6. Interaction of Am-MC with rNTPs. The absorbance spectra of Am-MC after incubating it with 250 μM rNTPs in TDW at pH 7.

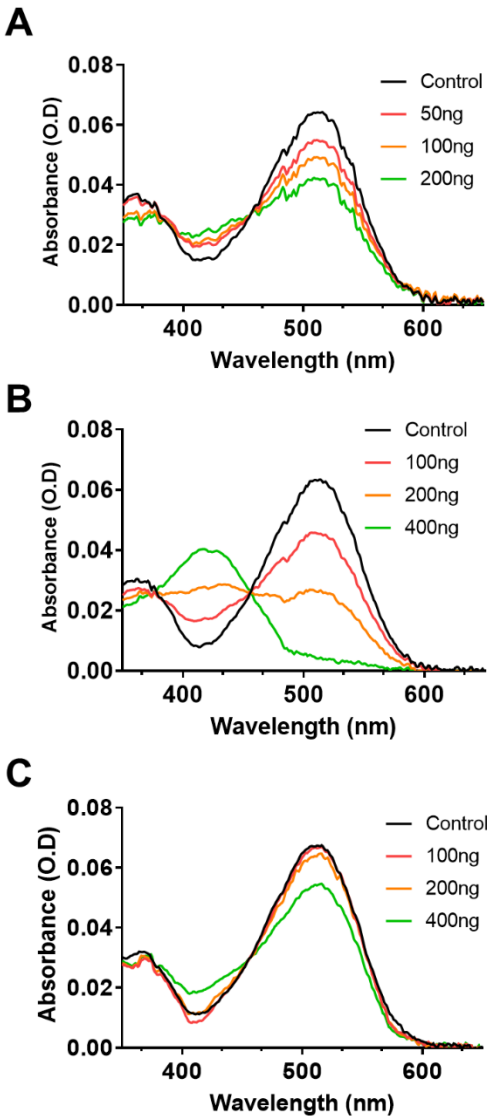

Figure S7. Interaction of Am-MC with ssRNA, dsRNA, and dsDNA. (A-C) The absorbance spectra of Am-MC after incubating it with indicated amount of 1,929 nucleotide long Luciferase mRNA (A), 717 bp long EGFP dsRNA (B), and 717 bp long EGFP dsDNA (C) in TDW at pH 7.

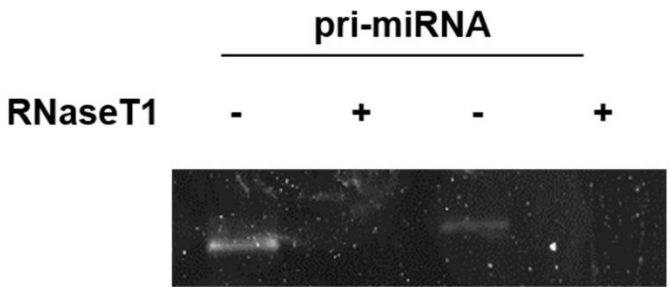

Figure S8. Effect of RNase T1 on small hairpin RNAs. Gel electrophoresis analysis of pri-miRNA hairpin RNAs with or without RNase T1 treatment. Two biological replicates are shown.

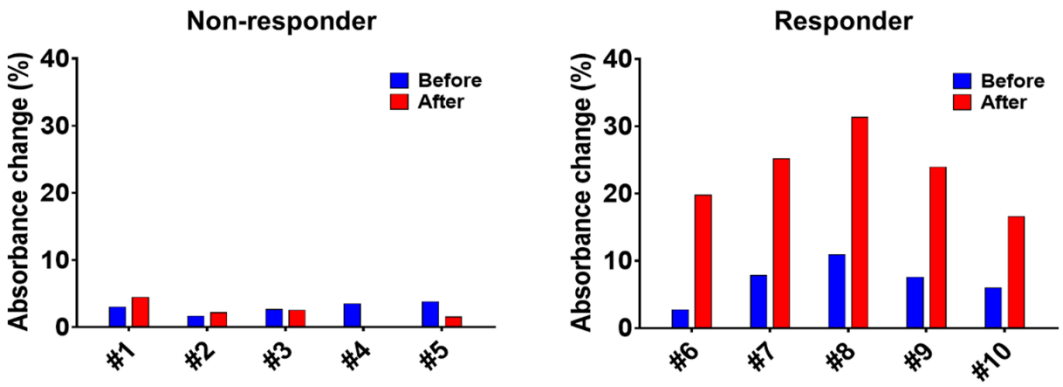

Figure S9. Quantified Am-MC absorbance for patient samples. Quantified Am-MC absorbance change at 515 nm for individual patient before and after the HMA therapy.

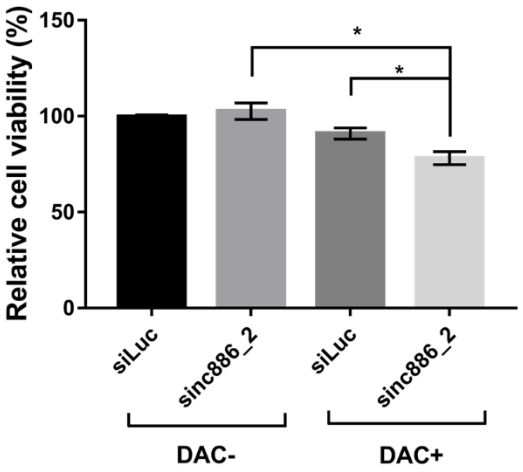

Figure S10. Effect of nc886 knockdown on cell viability. The effect of knockdown of nc886 on cell viability with or without DAC treatment. sinc886_2 was used to downregulate nc886 expression in MCF-7 cells. MCF-7 cells four days after DMSO (DAC-) or DAC treatment were used. The average of three biological replicates is shown with error bars indicating S.D. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$.

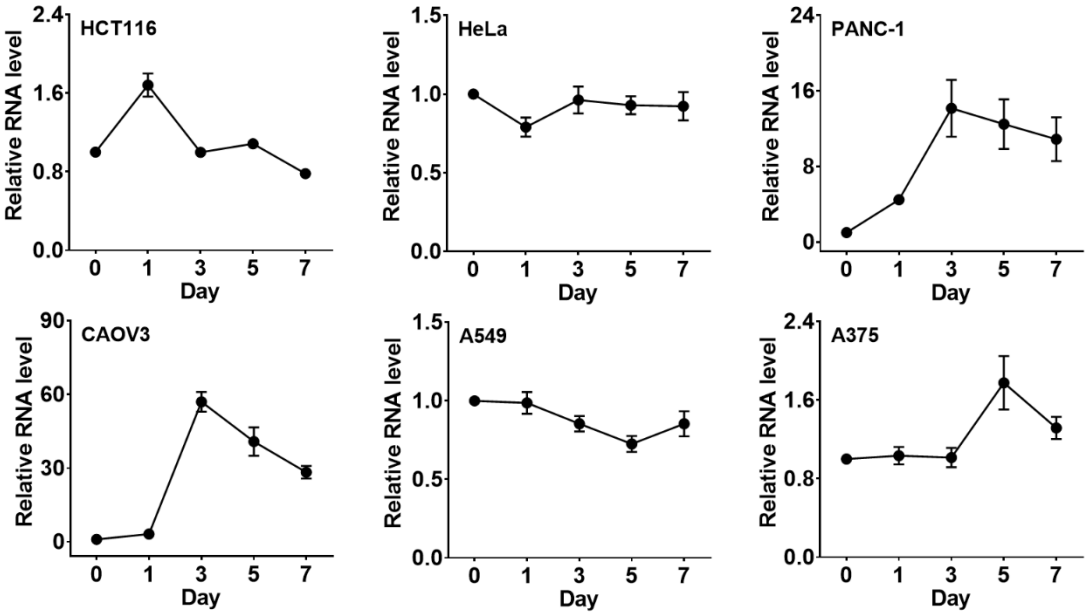

Figure S11. nc886 expression in cancer cell lines treated with DAC. nc886 RNA levels for the indicated cell lines measured by RT-qPCR. RNA expression was normalized to that of vtRNA1-1 RNA. The x-axis denotes the days after DAC treatment. The average of three biological replicates is shown with error bars indicating S.D.

SUPPLEMENTAL TABLES

Table S1. Demographics and clinical characteristics of analyzed patients with myelodysplastic syndrome or acute myeloid leukemia of the elderly.

Patient	#1	#2	#3	#4	#5
Sex	F	M	M	F	M
Age	50	65	74	68	45
Disease	MDS	AML	AML	AML	MDS
IPSS-R	High	NA	NA	NA	Low
Bone marrow blast (%)	4	56	44.4	61	0.2
Hb (g/dL)	7.5	7.5	7.5	8.5	5.9
ANC (/μL)	1,660	7027	1,350	370	4,441
Platelet (/μL)	99,000	79,000	34,000	42,000	155,000
G-banding	47,XX,+14[4]/46,XX[16]	45,X,-Y,t(8;16;21)(q22;q12;q22)[20]	46,XY,inv(11)(p15q22)[20]	46,XX,der(7)t(1;7)(q21;q32),inv(16)(p13.1q22)[3]/46,sl,del(6)(q13q23)[2]/46,sl,del(9)(q13q22)[2]/46,XX[13]	46,XY[20]
Therapy	decitabine	decitabine	decitabine	decitabine	Azacitidine
Cycles number at the time of response evaluation	3	4	3	4	9
Response [a)]	No (stable disease)	No (stable disease)	No (progressive disease)	No (progressive disease)	No (progressive disease)

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; IPSS-R, Revised International Prognostic Scoring System; ANC, Absolute neutrophil count

[a)] according to the 2006 International Working Group criteria for MDS[1] and 2017 European LeukemiaNet criteria for AML[2]

Patient	#6	#7	#8	#9	#10
Sex	M	F	M	M	M
Age	59	72	61	57	55
Disease	MDS	AML	AML	MDS	MDS
IPSS-R	Very high	NA	NA	Very high	Intermediate
Bone marrow blast (%)	7.4	24.2	59.4	16.4	9.4
Hb (g/dL)	9.1	8.3	10.6	7.6	7.5
ANC (/μL)	720	221	313	78	1,139
Platelet (/μL)	18,000	3,000	41,000	15,000	435,000
G-banding	46,XY,+1,der(1;7)(q10;p10)[6]/46,XY[14]	46,XX[9]	46,XY[20]	46,XY[20]	46,XY[20]
Therapy	azacitidine	azacitidine	decitabine	decitabine	Azacitidine
Cycles number at the time of response evaluation	4	3	2	4	3
Response [a)]	Yes (complete remission)	Yes (complete remission)	Yes (complete remission)	Yes (marrow complete remission)	Yes (marrow complete remission)

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; IPSS-R, Revised International Prognostic Scoring System; ANC, Absolute neutrophil count

[a)] according to the 2006 International Working Group criteria for MDS[1] and 2017 European LeukemiaNet criteria for AML[2]

Table S2. List of primer sequences used for the RT-qPCR.

Gene name	Forward (5'→3')	Reverse (5'→3')
GAPDH	CTC CTC CAC CTT TGA CGC TG	TCC TCT TGT GCT CTT GCT GG
MER57B1	CCT CCT GAG CCA GAG TAG GT	ACC AGT CTG GCT GTT TCT GT
MER4D	CCC TAA AGA GGC AGG ACA CC	TCA AGC AAT CGT CAA CCA GA
MER21C	GGA GCT TCC TGA TTG GCA GA	ATG TAG GGT GGC AAG CAC TG
ERVL	ATA TCC TGC CTG GAT GGG GT	GAG CTT CTT AGT CCT CCT GTG T
ERV9-1	TCT TGG AGT CCT CAC TCA AAC TC	ACT GCT GCA ACT ACC CTT AAA CA
MLTA10	TCT CAC AAT CCT GGA GGC TG	GAC CAA GAA GCA AGC CCT CA
MLT1C49	TAT TGC CGT ACT GTG GGC TG	TGG AAC AGA GCC CTT CCT TG
MLT1B	TGC CTG TCT CCA AAC ACA GT	TAC GGG CTG AGC TTG AGT TG
vtRNA 1-1	GGC TGG CTT TAG CTC AGC GG	AAA AGG ACT GGA GAG CGC CCG
nc886	CGG GTC GGA GTT AGC TCA AGC GG	AAG GGT CAG TAA GCA CCC GCG

Table S3. The sequences of siRNAs used in this study.

Gene name	Sense (5'→3')	Antisense (5'→3')
siLuc	CUU ACG CUG AGU ACU UCG A	UCG AAG UAC UCA GCG UAA G
sinc886	ACC UCC UCA UGC CGG ACU U	AAA GUC CGG CAU GAG GAG G
sinc886_2	UUU CUA UCU GUC CAU CUC UG	CAG AGA UGG ACA GAU AGA AA

SUPPLEMENTAL MATERIALS AND METHODS

Synthesis procedures

1-(3′-Trimethylammonio-N-propyl)-2,3,3-trimethylindolenium bromide

2,3,3-Trimethylindolenine (3.2 g, 0.020 mol) and (3-Bromopropyl)-trimethylammonium bromide (5.7 g, 0.021 mol) were dissolved in dry acetonitrile (40 mL) under nitrogen, and the mixture was stirred under reflux for 72 h. After cooling to room temperature, the solvent was removed in a vacuum, and the residue was crystallized from the mixture of methanol/acetone (1/5 volume ratio). The product was filtered and dried. Yield: 4.5 g (53%). 1H NMR (DMSO-d_6): δ 8.18 (d, 1H), 7.87 (d, 1H), 7.64 (m, 2H), 4.54 (t, 2H), 3.73 (m, 2H), 3.16 (s, 9H), 2.98 (s, 3H), 2.39 (m, 2H), 1.57 (s, 6H) ppm.

3-Formyl-4-hydroxybenzamidine hydrochloride

Step 1. To a solution of 4-cyanophenol (5 g, 0.0419 mol) in trifluoroacetic acid (25 ml) hexamethylenetetramine (11.7 g, 0.0839 mol) was added under nitrogen. The mixture was stirred at 100 °C for 12 h. After cooling to 0 °C, water (30 mL) was added, and the product was extracted with dichloromethane (20 ml × 3). The organic phase was washed with water and brine and dried over $MgSO_4$. The solvent was removed in a vacuum, and the residue was purified by a column (eluant: hexane/ethyl acetate 85/15). Yield: 1.4 g (23%).

Step 2. To a stirred suspension of 3-formyl-4-hydroxybenzonitrile (1.8 g, 0.0122 mol), in ethyl alcohol (30 ml), acetyl chloride (11.3 ml, 0.1590 mol) was added dropwise. The

stirring was continued at room temperature for 48 h. The volatiles were removed under reduced pressure to give 2.8 g (100%) of the product.

Step 3. Ethyl 3-formyl-4-hydroxybenzimidate hydrochloride (2.8 g, 0.0121 mol) was dissolved in ethyl alcohol (170 mL). The solution was saturated with ammonia gas for 8 h. Saturated solution was stirred at room temperature for 48 h. The solvent was removed in a vacuum, and the residue was dissolved in the mixture of 1 M HCl (60 mL) and dichloromethane (60 mL) and stirred at room temperature for 2 h. The aqueous layer was separated, and water was removed in a vacuum. The residue was crystallized from water and dried. Yield: 1.5 g (62%). 1H NMR (DMSO-d_6): δ 12.05 (s, 1H), 10.35 (s, 1H), 9.33 (s, 2H), 9.06 (s, 2H), 8.16 (d, 1 H), 7.98 (d, 1H), 7.30 (d, 1H) ppm.

Synthesis of Am-SP

1-(3′-Trimethylammonio-N-propyl)-2,3,3-trimethylindolenium bromide (1 g, 0.0025 mol) and 3-formyl-4-hydroxybenzamidine hydrochloride (0.5 g, 0.0025 mol) were dissolved in ethyl alcohol (75 mL), piperidine was added (0.24 ml, 0.0025 mol), and the reaction was stirred at 80 °C for 4 h. Solvent was removed in a vacuum, and the residue was purified by a column (eluat: dichloromethane/methyl alcohol 9/1). The obtained purple oil was crystallized by stirring in tetrahydrofuran. Yield: 0.55 g (42%). 1H NMR (DMSO-d_6): δ 9.37 (s, 2H), 9.17 (s, 2H), 7.89 (d, 1 H), 7.68 (dd, 1H), 7.15 (m, 2H), 7.09 (d, 1H), 6.92 (d, 1H), 6.83 (t, 1H), 6.74 (d, 1H), 6.05 (d, 1H), 3.35 (m, 2H), 3.19 (m, 2H), 3.05 (s, 9H), 1.99 (m, 2H), 1.22 (s, 3H), 1.15 (s, 3H) ppm.

SUPPLEMENTAL REFERENCES

1. Cheson, BD, Greenberg, PL, Bennett, JM, Lowenberg, B, Wijermans, PW, Nimer, SD, Pinto, A, Beran, M, de Witte, TM, Stone, RM*, et al.* (2006). Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood* **108**: 419-425.
2. Dohner, H, Estey, E, Grimwade, D, Amadori, S, Appelbaum, FR, Buchner, T, Dombret, H, Ebert, BL, Fenaux, P, Larson, RA*, et al.* (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* **129**: 424-447.