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Anti-leukemic Activity of AIU2008 in FLT3-ITD-positive Acute Myeloid Leukemia

HYO JEONG KIM[1], HWANI RYU[1], HYUN-KYUNG CHOI[2], JIE-YOUNG SONG[1], SANG-GU HWANG[1] and JIYEON AHN[1]

[1]*Division of Radiation Biomedical Research, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea;*
[2]*Department of Medicinal Chemistry, Jungwon University, Goesan, Republic of Korea*

Abstract. *Background/Aim: FMS-like tyrosine kinase 3 (FLT3) is a class III receptor tyrosine kinase involved in signal transduction underlying survival, proliferation, and differentiation of hematopoietic cells. An internal tandem duplication (ITD) in FLT3 in the juxtamembrane domain is a common mutation causing human acute myeloid leukemia (AML) and activates constitutive signaling. Materials and Methods: We evaluated the novel FLT3 inhibitor 5-(4-fluorophenyl)-N-(naphthalen-1-yl)oxazol-2-amine (AIU2008) for the treatment of AML. Results: AIU2008 was designed by modifying FLT3 inhibitor 7c, and showed improved anti-leukemic efficacy in FLT3-ITD–positive AML cells. Specifically, AIU2008 inhibited cell growth and apoptotic death. In addition, AIU2008 down-regulated DNA repair genes involved in homologous recombination and non-homologous end joining. It contributed to the synergistic inhibition of AML cell growth in combination treatment with PARP inhibitors. Conclusion: AIU2008 is a promising FLT3 targeting agent, and may be used in combination with PARP inhibitors for the treatment of AML.*

Mutation in FMS-like tyrosine kinase 3 (FLT3), a type III receptor tyrosine kinase (RTK), is the most common genetic alteration in acute myeloid leukemia (AML) (1, 2). Internal tandem duplicates (ITD) in the juxtamembrane domain and a point mutation at D835 in the tyrosine kinase domain (TKD) are common *FLT3* mutations (3). *FLT3* mutations cause spontaneous induction of kinase activity with or without ligands.

Correspondence to: Jiyeon Ahn, Division of Radiation Biomedical Research, Korea Institute of Radiological & Medical Sciences, 75 Nowon-ro, Nowon-gu, Seoul 01812, Republic of Korea. Tel: +82 29701311, e-mail: ahnjy@kirams.re.kr

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Signal transducer and activator of transcription 5 (STAT5) is a well-known downstream mediator of RTKs, particularly in hematopoietic cancers. *FLT3* harboring an ITD or TKD mutation is constitutively activated even in the absence of the ligand, resulting in aberrant STAT5 activation (4, 5). Aberrant signaling associated with FLT3 and STAT5 causes abnormal hematopoietic cell survival and proliferation, leading to leukemia.

Aberrant STAT5 signaling and Rac family small GTPase 1 (RAC1) activation caused by *FLT3* mutation contribute to the generation of reactive oxygen species (ROS) (6). High levels of endogenous ROS increase DNA damage, such as double strand breaks (DSBs) and DNA oxidation (7, 8). Since AML is characterized by high levels of ROS, DNA damage is constantly being induced (6). Due to increased genomic instability, FLT3-ITD–positive AML cells show increased DNA repair activity, including homologous recombination (HR), non-homologous end joining (NHEJ), and nucleoside excision repair (NER) (9). Although this allows for cell survival, error-prone DNA repair mechanisms are also increased (10, 11). Abnormally low DNA repair activity can result in disease pathogenesis, whereas elevated DNA repair activity causes mismatch repair and inhibits apoptosis, causing abnormal cell survival (12). According to previous studies, FLT3 inhibitors down-regulate DNA repair genes associated with HR and NHEJ and show synergistic anti-leukemia activity with PARP inhibitors in combination therapy (13). Thus, decreasing DNA repair activity contributes to the mitigation of malignancy of AMLs. According to reports by Sallmyr *et al.* and our previous studies, FLT3 inhibition results in suppression of HR and NHEJ (6, 14, 15).

In our previous study, we designed and synthesized several FLT3 inhibitors and screened them by conducting *in vitro* FLT3 kinase inhibition tests and AML growth inhibition. We then suggested a novel FLT3 inhibitor, 7c (5-(4-fluorophenyl)-N-phenyloxazol-2-amine) that inhibits the FLT3-STAT5 axis and has a synergistic effect in combination treatment with a PARP inhibitor (15). In this study, we

Table I. *IC_{50} values (nM) of AIU2008.*

Compound	IC_{50} (nM) (*in vitro* kinase)			IC_{50} of Cell growth (nM)		
	FLT3	FLT3 (D835Y)	FLT3 (ITD)	MV4-11	Molm-13	HL-60
AIU2008	103.1±1.4	60.4±2.7	6.4±0.5	41.6±3.1	45.6±2.7	>10,000

Table II. *In vitro kinase inhibitory activities of AIU2008 (1 μM) against off-target kinases.*

Kinase	Inhibition (%)	Kinase	Inhibition (%)	Kinase	Inhibition (%)
Abl	20±6	cSRC	25±2	MEK1	3±4
ARK5	59±1	DDR2	0±10	p70S6K	0±5
CDK1	10±10	EGFR	0±6	PDGFRα	72±1
CDK5/p35	64±3	FAK	2±3	PDK1	0±9
CK1γ1	0±6	FGFR1	17±5	Pim-1	7±1
cKit	94±1	FLT1	79±5	PKCα	0±9
cKit(D816H)	75±5	FLT3	91±1	TrkA	57±4
cKit(V560G)	94±1	FLT4	90±1	WEE	1±3
c-RAF	0±5	Fms	91±1	PI3 Kinase	0±9

developed 5-(4-fluorophenyl)-N-(naphthalen-1-yl)oxazol-2-amine (AIU2008) by modifying compound 7c, which showed improved AML growth inhibition. Therefore, we aimed to identify the anti-leukemic activities of AIU2008 and the combination effect of AIU2008 and PARP-1 inhibitors in FLT3-ITD–positive AML.

Materials and Methods

Synthesis of chemicals. All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Sodium azide and 4-fluorophenacyl bromide in DMSO were stirred at room temperature for 2 h to obtain 2-azido-1-(4-fluorophenyl)ethan-1-one. The synthesized 2-azido-1-(4-fluorophenyl) ethan-1-one was dissolved in 1,4-dioxane, triphenyl phosphine and 1-naphthyl isothiocyanate were added at room temperature, and the mixture was refluxed at 100˚C for 4 h. The final compound 5-(4-fluorophenyl)-N-(naphthalene-2yl)oxazol-2-amine was obtained. Proton nuclear magnetic resonance (NMR) spectra were recorded as described previously (15).

5-(4-fluorophenyl)-N-(naphthalen-1-yl)oxazol-2-amine (AIU2008). Yellow solid, mp=165-167˚C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.29-8.37 (m, 1H), 8.12 (d, *J*=7.32 Hz, 1H), 7.81-7.99 (m, 1H), 7.43-7.671 (m, 7H), 7.33-7.27 (t, *J*=8.99 Hz, 2H); MS (FAB) m/z 305 (MH^+).

Cell culture. Human AML MV4-11 and HL-60 cells were obtained from ATCC (Manassas, VA, USA). Molm-13 cells were kindly provided by Dr. In-Ki Kim (Asan Medical Center, Seoul, Korea). The cells were cultured in RPMI 1640 (Molm-13; Welgene, Korea) and IMDM (MV4-11, HL-60; Gibco, USA) media with 10% FBS (Welgene) and 1% penicillin (Gibco). Cells were incubated at 37˚C, 5% CO_2, and 95% humidity.

Cell viability assay. Cells were seeded in a 96-well plate. After 24 h, they were treated with AIU2008 at a concentration of 3.9-1000 nM, using vehicle (DMSO) as a control. Vehicle-and compound-treated cells were incubated at 37˚C, 5% CO_2, and 95% humidity for 5 days. Cell Counting Kit-8 (CCK-8; Dojindo, Japan) solution was added to each well. The 450 nm absorbance was measured by using a 96-well plate reader (Multiskan EX; ThermoLabsystems, Waltham, MA, USA)

Western blotting. Protein preparation, separation, and detection were conducted as described previously (15). Primary antibodies used in this study included the following: anti-cleaved caspase 3 (Cell Signaling Technology (CST #9661; Danvers, MA, USA), anti-phospho-FLT3 (CST #3461), anti-FLT3 (CST #3462), anti-phospho-STAT5a/b (Santa Cruz Biotechnology (sc) #81524; Dallas, TX, USA), anti-STAT5 (sc #271542), anti-BRCA1 (sc #6954), anti-BRCA2 (sc #293185), anti-BARD1 (sc #74559), anti-RAD51 (Abcam (ab) #63801; Cambridge, UK), anti-XRCC4 (sc #8285), anti-Ku80 (CST #2180), anti-Ku70 (sc #5309), anti-phospho-histone H2AX, and anti-β-actin (Sigma-Aldrich #A5316).

Quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was prepared using TRIzol® (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was synthesized using CycleScript reverse transcriptase (Bioneer, Daejeon, Republic of Korea). cDNA was mixed with target sequence primers and Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Scientific). The designed primer sequences of housekeeping genes and target genes have been described previously (15). Thermal cycling conditions were as follows: initial duration at 95˚C for 10 min, followed by 50

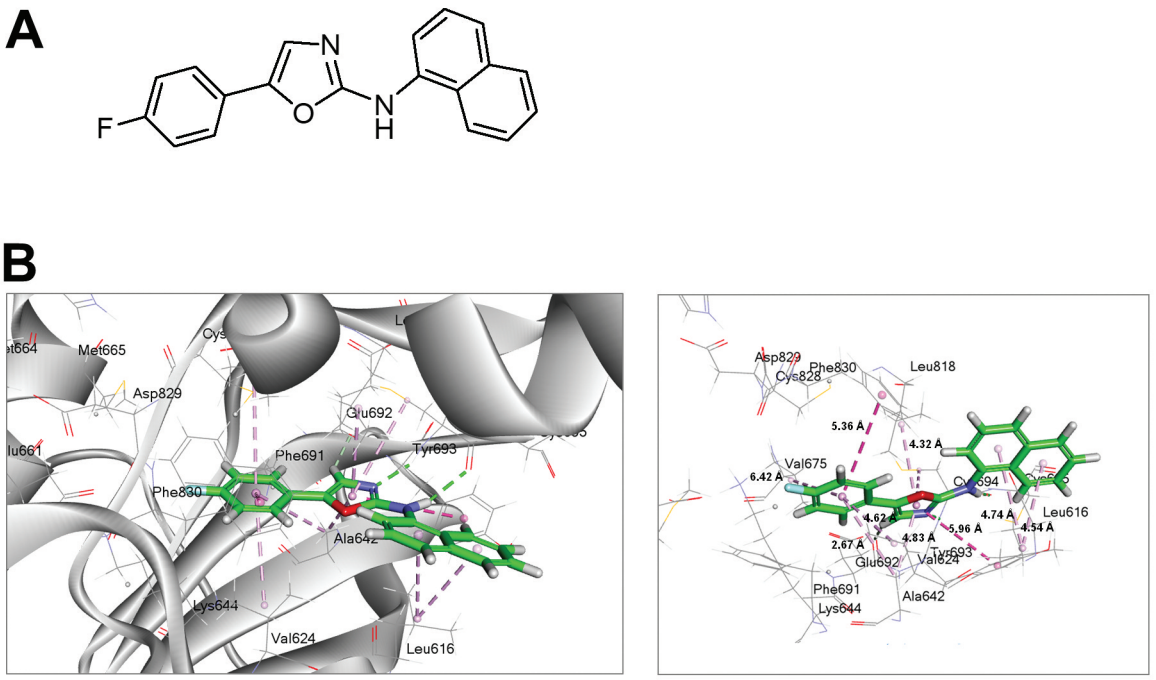

Figure 1. *Chemical structure and target binding mode of AIU2008. (A) Chemical structure of AIU2008. (B) 3D binding mode of AIU2008 with FLT3. Green, AIU2008; pink dotted line, pi–alkyl bond; dark pink dotted line, pi-pi stacked bond. Relevant distances are expressed in Å.*

cycles at 95˚C for 15 s, 60˚C for 30 s, and 72˚C for 30 s. Data were obtained and analyzed using the LightCycler 96 system and software (Roche, Germany).

In vitro kinase assay. In vitro kinase profiling of AIU2008 was performed by Reaction Biology Corp. (Malvern, PA, USA).

Molecular docking study. The FLT3 protein structure in PDB format was provided by Zorn *et al*. (16) and downloaded from the RCSB website. Docking analysis of AIU2008 with the FLT3 protein (PDB: 4XUF) was performed as described previously (15).

Statistical analysis. Results are represented as mean±standard deviations. Student's *t*-test was performed using Microsoft Excel (Microsoft Co., Redmon, WA, USA) software program to indicate significant differences between vehicle- and compound-treated groups.

Results

AIU2008 inhibits FLT3 and FLT3 mutant kinases in vitro. To improve the efficacy of compound 7c reported previously (15), we designed and synthesized a novel compound, AIU2008, in which a phenyl group is substituted with a naphthyl group of 7c. Kinase inhibitory activities were determined by an *in vitro* kinase assay. As shown in Table I, kinase inhibition of wild-type FLT3 and D835Y and ITD mutants was calculated as half maximal inhibitory concentration (IC_{50}), and was 50%, 72%, and 80% lower than compound 7c, respectively (15). In addition, AIU2008 mainly inhibited FLT members and class III RTK family members including cKIT and PDGFR (Table II). To investigate the physical mechanism of FLT3 inhibition by AIU2008, we explored the binding mode of AIU2008 and the crystal structure of FLT3 (PDB code: 4XUF) by *in silico* docking. AIU2008 was bound to the FLT3 structure by a pi-alkyl bond (Figure 1). The aromatic ring of AIU2008 and pi-alkyl interactions were observed between Val^{624}, Val^{675}, Leu^{818}, Ala^{642}, and Leu^{616}, and pi-pi stacked between Phe^{830} and Tyr^{693}. It showed hydrogen bonding between Cys^{694} and the nitrogen of the amine group of the oxazole ring. However, the fluorine interactions were not visible.

AIU2008 induced cell death of FLT3 mutated AML cells. After verifying the *in vitro* FLT3 inhibitory activity, we investigated the cancer cell-based toxicity effect by CCK-8 proliferation assay and immunoblotting of apoptotic targets. Human AML cell lines, Molm-13, MV4-11, and HL-60 were treated with vehicle or AIU2008 for 5 days, and then CCK-8 solution was added. As shown in Figure 2A, the survival rate of Molm-13 and MV4-11 decreased in proportion to the concentration of AIU2008. The IC_{50} of cell viability was 45.6 nM and 41.6 nM in Molm-13 and MV4-11, respectively. However, AIU2008-induced growth inhibition did not appear in the HL-60, FLT3-

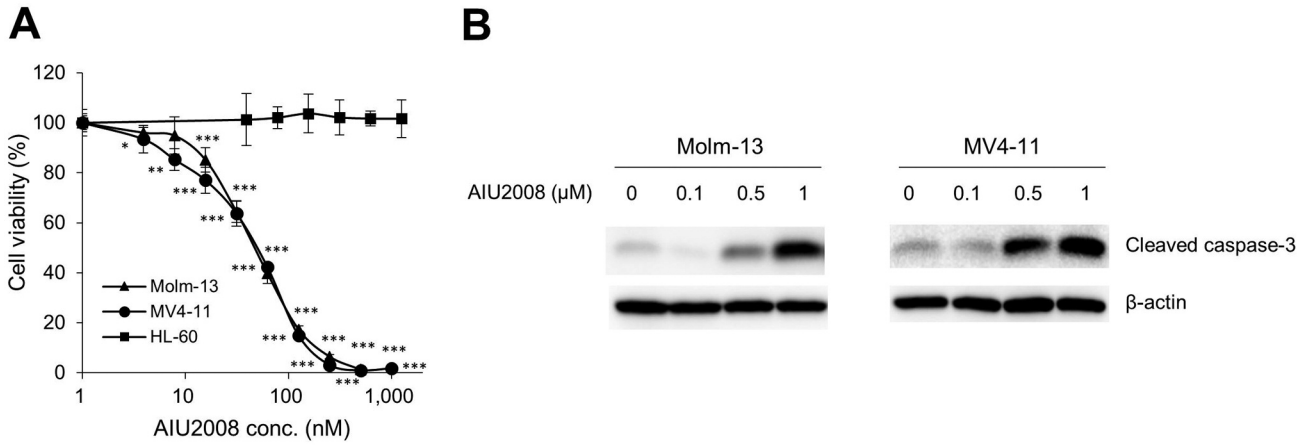

Figure 2. *AIU2008 induced growth inhibition and apoptotic cell death in AML cell lines. (A) Human FLT3-ITD–positive AML cell lines, Molm-13 and MV4-11, and human FLT3-null HL-60 were treated with AIU2008 or DMSO (vehicle). After 5 days, CCK-8 solution was added and fluorescence at 450 nm measured. Cell viability was expressed as percentage compared to the control. Student's t-test was used to identify significant differences between the control and AIU2008 treatment groups. *p<0.05; **p<0.01; ***p<0.001. (B) Molm-13 and MV4-11 cells were treated with AIU2008 and DMSO in a dose-dependent manner for 48 h. The apoptotic signaling marker cleaved caspase3 was detected by immunoblotting. β-actin was used as loading control.*

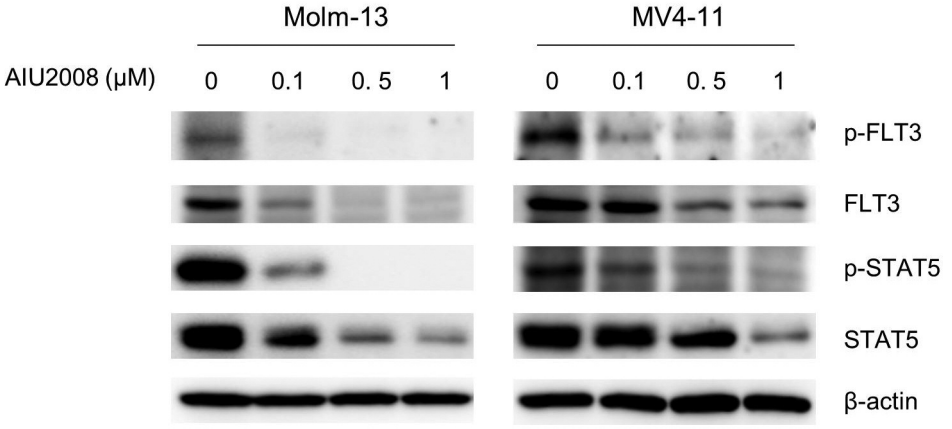

Figure 3. *AIU2008 inhibited FLT3 signaling in FLT3-ITD–positive AML cells. Molm-13 and MV4-11 were treated with AIU2008 at the indicated concentration for 48 h. Whole cell lysates were extracted and subjected to immunoblotting to detect FLT3 and STAT5. β-actin was used as loading control.*

null cells. Only FLT3-ITD–positive cell lines, Molm-13 and MV4-11, showed inhibited cell growth. In addition, AIU2008-treated FLT3-ITD–positive cells had increased levels of cleaved-caspase 3, an apoptotic cell death marker (Figure 2B). Thus, AIU2008 increased apoptotic cell death of FLT3-ITD–positive AML cells.

AIU2008 reduced STAT5 signaling. In FLT3-ITD–positive AMLs, the FLT3 downstream mediator STAT5 is persistent and ligand-independently hyperactivated. High levels of p-STAT5 are a negative prognostic marker in myeloid leukemia and implicated in RTK inhibitor resistance (17). As a secondary confirmation of the inhibitory activity of AIU2008, we determined p-FLT3 and p-STAT5 levels in treated Molm-13 and MV4-11 cells by western blotting. Both p-FLT3 and p-STAT5 were dose-dependently reduced by AIU2008 (Figure 3). Reduction in aberrant STAT5 activation may contribute to alleviating malignant tumors in AML.

AIU2008 suppressed DNA damage repair. To investigate whether AML cell death induced by AIU2008 inhibited DNA repair, we examined the gene expression and protein levels of DNA damage repair genes and proteins in Molm-13 and MV4-11. As a result, HR-related genes including *BRCA1*, *BRCA2*,

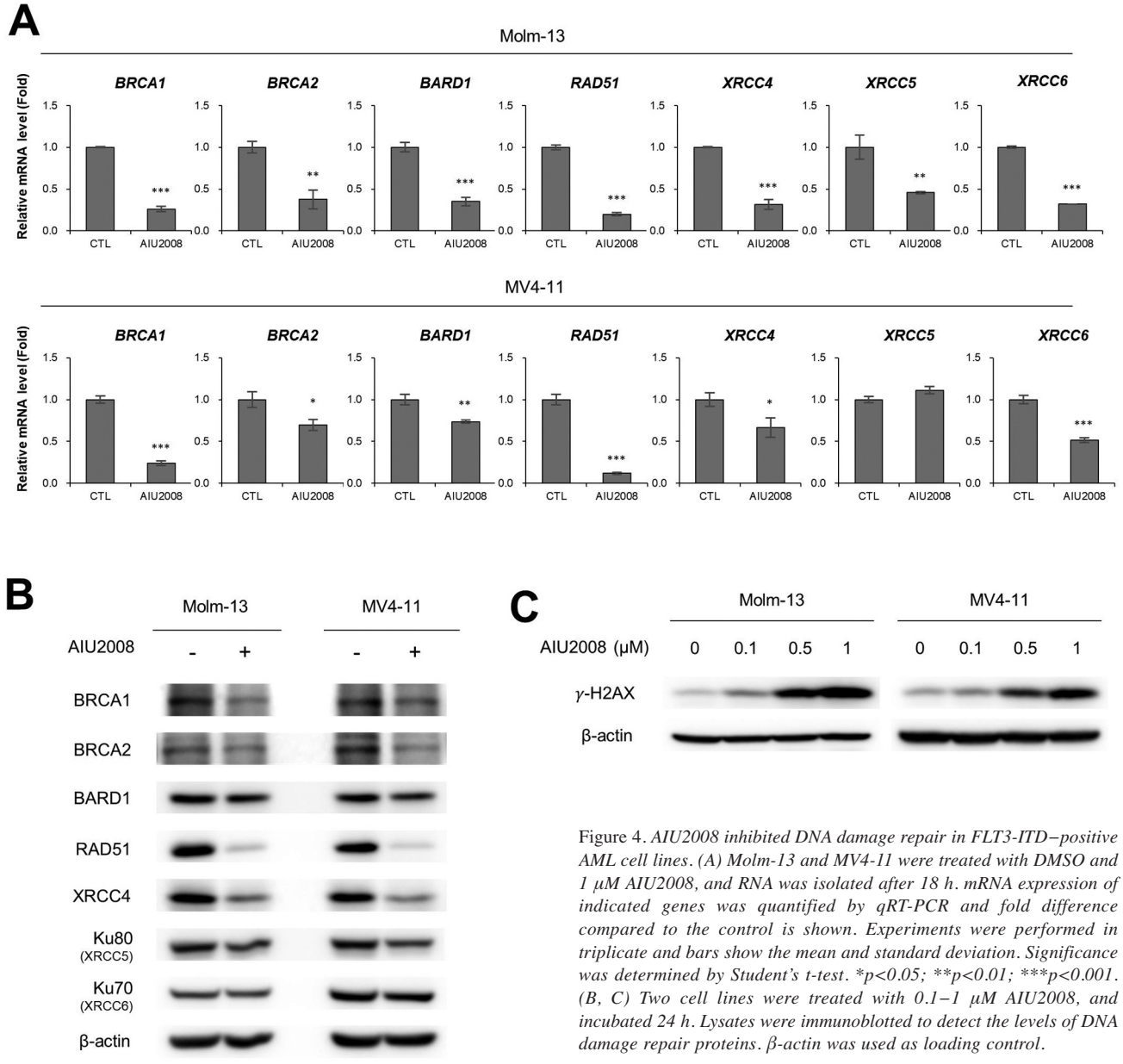

Figure 4. *AIU2008 inhibited DNA damage repair in FLT3-ITD–positive AML cell lines. (A) Molm-13 and MV4-11 were treated with DMSO and 1 μM AIU2008, and RNA was isolated after 18 h. mRNA expression of indicated genes was quantified by qRT-PCR and fold difference compared to the control is shown. Experiments were performed in triplicate and bars show the mean and standard deviation. Significance was determined by Student's t-test. *p<0.05; **p<0.01; ***p<0.001. (B, C) Two cell lines were treated with 0.1–1 μM AIU2008, and incubated 24 h. Lysates were immunoblotted to detect the levels of DNA damage repair proteins. β-actin was used as loading control.*

BARD1, and *RAD51*, and NHEJ-related genes *XRCC4*, *XRCC5*, and *XRCC6*, were significantly down-regulated in both FLT3-ITD–positive cell lines (Figure 4A and B). Consequently, DNA damage accumulated in AIU2008-treated Molm-13 and MV4-11 cells. We also found increased levels of the DSB recognition marker histone variant H2AX (γ-H2AX) (Figure 4C).

Anti-leukemic activities of AIU2008 in combination with a PARP inhibitor. We attempted a combination treatment with a PARP inhibitor, olaparib, to improve lethality. Molm-13 and MV4-11 cells were treated with AIU2008 and olaparib for 5 days, and viability was determined by CCK-8 assay. The combination treatment of AIU2008 and olaparib exerted a synergistic effect, contributing to AML growth inhibition (Figure 5). Therefore, combination treatment with AIU2008 and a PARP inhibitor might be a more effective anti-leukemic therapy.

Discussion

In this study, we devised a novel FTL3 inhibitor AIU2008, which showed improved cell growth inhibition compared to the oxazol-2-amine derivative 7c observed in our previous studies

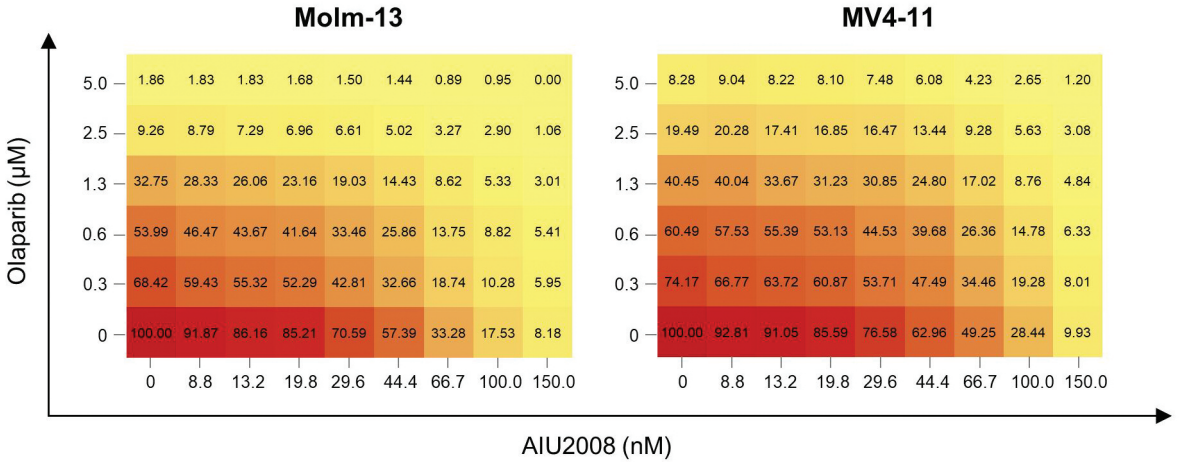

Figure 5. *AIU2008 has a synergistic effect on anti-leukemic activity when combined with PARP inhibitor. Molm-13 and MV4-11 were treated with AIU2008 and olaparib simultaneously, and DMSO was used as control. After 5 days, cell growth inhibition was determined by CCK-8 assay. Representative data of four independent experiments are shown for each combination at the indicated concentrations.*

(13). AIU2008 is also an oxazol-2-amine-based small-molecule compound that contains fluorophenyl and naphthyl groups (Figure 1A). To investigate the efficacy of AIU2008, we examined the *in vitro* kinase profiling and cell viability in Molm-13 and MV4-11. The results showed that AIU2008 was five times more effective than 7c in inhibiting FLT3-ITD kinase *in vitro* and almost twice as effective as 7c in inhibiting AML cell growth, especially in MV4-11. The improved efficacy of AIU2008 is expected to be caused by chemical properties such as drug dissolution, absorption, and cellular stability (18). Therefore, with further study on the physicochemical properties and pharmacokinetics, AIU2008 could become a promising treatment agent for FLT3-ITD–positive AML.

Due to the high ROS levels caused by STAT5 and RAC1, genomic instability is increased in FLT3-ITD–positive AML. To survive ROS-induced genomic damage, AML cells acquire an enhanced DNA damage repair system. Indeed, human AML cells, including Molm-13 and MV4-11, highly express PARP1, an early DNA damage sensor, and RAD51, a key player in HR (12, 19). An overly-active DNA repair system might induce error-prone DNA repair, leading to mutagenesis and malignancy in leukemia. Kinase inhibitors targeting PARP1 effectively treat cancers with impaired HR and NER due to *BRCA* and *DNA-PK* mutations (20, 21). Since AIU2008 can inhibit the DNA repair of HR and NHEJ, we evaluated the effect of the combination treatment with the PARP1 inhibitor olaparib. As shown in our results, decreased DNA repair activity by AIU2008 sensitized Molm-13 and MV4-11 to a PARP inhibitor. Therefore, AIU2008 could be a novel drug targeting FLT3-AML–positive cells and a therapeutic agent for combination treatment with PARP inhibitors. Further investigation into the pharmacokinetics and metabolism of AIU2008 should be conducted.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

AJ designed the study. KHJ and RH performed the experiments. AJ, KHJ, and RH performed the data analysis. CHK designed and synthesized the chemical compound and performed the docking analysis. AJ and KHJ wrote the manuscript. SJY and HSG revised the draft of the manuscript.

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References

1 Birg F, Courcoul M, Rosnet O, Bardin F, Pebusque MJ, Marchetto S, Tabilio A, Mannoni P and Birnbaum D: Expression of the fms/kit-like gene flt3 in human acute leukemias of the myeloid and lymphoid lineages. Blood *80(10)*: 2584-2593, 1992. PMID: 1384791.

2 Kihara R, Nagata Y, Kiyoi H, Kato T, Yamamoto E, Suzuki K, Chen F, Asou N, Ohtake S, Miyawaki S, Miyazaki Y, Sakura T, Ozawa Y, Usui N, Kanamori H, Kiguchi T, Imai K, Uike N, Kimura F, Kitamura K, Nakaseko C, Onizuka M, Takeshita A, Ishida F, Suzushima H, Kato Y, Miwa H, Shiraishi Y, Chiba K, Tanaka H, Miyano S, Ogawa S and Naoe T: Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients. Leukemia *28(8)*: 1586-1595, 2014. PMID: 24487413. DOI: 10.1038/leu.2014.55

3 Kiyoi H, Naoe T, Yokota S, Nakao M, Minami S, Kuriyama K, Takeshita A, Saito K, Hasegawa S, Shimodaira S, Tamura J, Shimazaki C, Matsue K, Kobayashi H, Arima N, Suzuki R, Morishita H, Saito H, Ueda R and Ohno R: Internal tandem duplication of flt3 associated with leukocytosis in acute promyelocytic leukemia. Leukemia study group of the ministry of health and welfare (kohseisho). Leukemia *11(9)*: 1447-1452, 1997. PMID: 9305596. DOI: 10.1038/sj.leu.2400756

4 Gilliland DG and Griffin JD: The roles of flt3 in hematopoiesis and leukemia. Blood *100(5)*: 1532-1542, 2002. PMID: 12176867. DOI: 10.1182/blood-2002-02-0492

5 Vainchenker W and Constantinescu SN: Jak/stat signaling in hematological malignancies. Oncogene *32(21)*: 2601-2613, 2013. PMID: 22869151. DOI: 10.1038/onc.2012.347

6 Sallmyr A, Fan J, Datta K, Kim KT, Grosu D, Shapiro P, Small D and Rassool F: Internal tandem duplication of flt3 (flt3/itd) induces increased ros production, DNA damage, and misrepair: Implications for poor prognosis in aml. Blood *111(6)*: 3173-3182, 2008. PMID: 18192505. DOI: 10.1182/blood-2007-05-092510

7 Rebechi MT and Pratz KW: Genomic instability is a principle pathologic feature of flt3 itd kinase activity in acute myeloid leukemia leading to clonal evolution and disease progression. Leuk Lymphoma *58(9)*: 1-11, 2017. PMID: 28278729. DOI: 10.1080/10428194.2017.1283031

8 Skorski T: Chronic myeloid leukemia cells refractory/resistant to tyrosine kinase inhibitors are genetically unstable and may cause relapse and malignant progression to the terminal disease state. Leuk Lymphoma *52(Suppl 1)*: 23-29, 2011. PMID: 21299457. DOI: 10.3109/10428194.2010.546912

9 Skorski T: Genomic instability: The cause and effect of bcr/abl tyrosine kinase. Curr Hematol Malig Rep *2(2)*: 69-74, 2007. PMID: 20425353. DOI: 10.1007/s11899-007-0010-6

10 Slupianek A, Nowicki MO, Koptyra M and Skorski T: Bcr/abl modifies the kinetics and fidelity of DNA double-strand breaks repair in hematopoietic cells. DNA Repair (Amst) *5(2)*: 243-250, 2006. PMID: 16297667. DOI: 10.1016/j.dnarep.2005.10.005

11 Nowicki MO, Falinski R, Koptyra M, Slupianek A, Stoklosa T, Gloc E, Nieborowska-Skorska M, Blasiak J and Skorski T: Bcr/abl oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. Blood *104(12)*: 3746-3753, 2004. PMID: 15304390. DOI: 10.1182/blood-2004-05-1941

12 Seedhouse CH, Hunter HM, Lloyd-Lewis B, Massip AM, Pallis M, Carter GI, Grundy M, Shang S and Russell NH: DNA repair contributes to the drug-resistant phenotype of primary acute myeloid leukaemia cells with flt3 internal tandem duplications and is reversed by the flt3 inhibitor pkc412. Leukemia *20(12)*: 2130-2136, 2006. PMID: 17066094. DOI: 10.1038/sj.leu.2404439

13 Maifrede S, Nieborowska-Skorska M, Sullivan-Reed K, Dasgupta Y, Podszywalow-Bartnicka P, Le BV, Solecka M, Lian Z, Belyaeva EA, Nersesyan A, Machnicki MM, Toma M, Chatain N, Rydzanicz M, Zhao H, Jelinek J, Piwocka K, Sliwinski T, Stoklosa T, Ploski R, Fischer T, Sykes SM, Koschmieder S, Bullinger L, Valent P, Wasik MA, Huang J and Skorski T: Tyrosine kinase inhibitor-induced defects in DNA repair sensitize flt3(itd)-positive leukemia cells to parp1 inhibitors. Blood *132(1)*: 67-77, 2018. PMID: 29784639. DOI: 10.1182/blood-2018-02-834895

14 Ryu H, Choi HK, Kim HJ, Kim AY, Song JY, Hwang SG, Kim JS, Kim DU, Kim EH, Kim J and Ahn J: Antitumor activity of a novel tyrosine kinase inhibitor aiu2001 due to abrogation of the DNA damage repair in non-small cell lung cancer cells. Int J Mol Sci *20(19)*, 2019. PMID: 31554189. DOI: 10.3390/ijms20194728

15 Kim HJ, Ryu H, Song JY, Hwang SG, Jalde SS, Choi HK and Ahn J: Discovery of oxazol-2-amine derivatives as potent novel flt3 inhibitors. Molecules *25(21)*, 2020. PMID: 33167505. DOI: 10.3390/molecules25215154

16 Zorn JA, Wang Q, Fujimura E, Barros T and Kuriyan J: Crystal structure of the flt3 kinase domain bound to the inhibitor quizartinib (ac220). PLoS One *10(4)*: e0121177, 2015. PMID: 25837374. DOI: 10.1371/journal.pone.0121177

17 Warsch W, Kollmann K, Eckelhart E, Fajmann S, Cerny-Reiterer S, Holbl A, Gleixner KV, Dworzak M, Mayerhofer M, Hoermann G, Herrmann H, Sillaber C, Egger G, Valent P, Moriggl R and Sexl V: High stat5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia. Blood *117(12)*: 3409-3420, 2011. PMID: 21220747. DOI: 10.1182/blood-2009-10-248211

18 Chan A, Orme RP, Fricker RA and Roach P: Remote and local control of stimuli responsive materials for therapeutic applications. Adv Drug Deliv Rev *65(4)*: 497-514, 2013. PMID: 22820529. DOI: 10.1016/j.addr.2012.07.007

19 Li X, Li C, Jin J, Wang J, Huang J, Ma Z, Huang X, He X, Zhou Y, Xu Y, Yu M, Huang S, Yan X, Li F, Pan J, Wang Y, Yu Y and Jin J: High parp-1 expression predicts poor survival in acute myeloid leukemia and parp-1 inhibitor and saha-bendamustine hybrid inhibitor combination treatment synergistically enhances anti-tumor effects. EBioMedicine *38:* 47-56, 2018. PMID: 30472087. DOI: 10.1016/j.ebiom.2018.11.025

20 Dellomo AJ, Baer MR and Rassool FV: Partnering with parp inhibitors in acute myeloid leukemia with flt3-itd. Cancer Lett *454:* 171-178, 2019. PMID: 30953707. DOI: 10.1016/j.canlet.2019.03.048

21 Dedes KJ, Wilkerson PM, Wetterskog D, Weigelt B, Ashworth A and Reis-Filho JS: Synthetic lethality of parp inhibition in cancers lacking brca1 and brca2 mutations. Cell Cycle *10(8)*: 1192-1199, 2011. PMID: 21487248. DOI: 10.4161/cc.10.8.15273

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