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

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1. Introduction

DNA repair pathways have been extensively studied in solid tumors [1]. Two important enzymes that facilitate DNA damage repair are poly (ADP-ribose) polymerase 1 (PARP-1) and 2 (PARP-2) [2]. PARP-1 is a cell cycle regulated protein. The transition of the cell cycle from G1 to S phase leads to transcription of PARP-1 [3]. PARP-1 is overexpressed in many cancers such as testicular and other germ cell tumors [4], neuroblastoma [5], malignant lymphoma [6], Ewing’s sarcoma [7], breast cancer [8], and colon cancer [9]. PARP-1 also contributes to progression of endometrial cancer [10], BRCA-mutated serous ovarian cancer [11], and BRCA-mutated serous ovarian cancer [12].

When single-strand DNA breaks (SSBs) occur, PARP-1 binds to the damaged DNA sites and initiates the formation of a poly-ADP scaffold that recruits other members of the base excision repair (BER) pathway, such as XRCC1 [13]. Blocking PARP-1 inhibits BER, leading to the accumulation of SSBs and double-strand breaks (DSBs), which in turn
activates homologous recombination (HR) repair [14,15]. The most critical proteins are BRCA1 [16,17] and BRCA2 [18] in HR, however, these two genes are often mutated in tumors leading to defects in HR [19,20]. Without effective HR repair, cells use non-conservative forms of DNA repair such as non-homologous end joining (NHEJ), which may generate large-scale genomic rearrangements leading to the lethality of tumor cells [21]. In 2005, Farmer H and Bryant HE found that BRCA-1/2 deficient tumors were sensitive to PARP inhibitors (PARPis) [22,23]. FDA has approved PARPis Olaparib (2014) [24] and Rucaparib (2016) [25] monotherapy in patients with BRCA-mutated advanced ovarian cancer. To date, there are a great deal of research on the use of PARP inhibitors in cancers with DNA repair such as non-homologous end joining (NHEJ), which may generate large-scale genomic rearrangements leading to the lethality of tumor cells [21]. In 2005, Farmer H and Bryant HE found that BRCA-1/2 deficient tumors were sensitive to PARP inhibitors (PARPis) [22,23]. FDA has approved PARPis Olaparib (2014) [24] and Rucaparib (2016) [25] monotherapy in patients with BRCA-mutated advanced ovarian cancer. To date, there are a great deal of research on the use of PARP inhibitors in cancers with BRCA mutations in clinical trials [26–28].

Acute myeloid leukemia (AML) is a highly heterogeneous disease with poor clinical prognosis. DNA damage response (DDR) in hematological malignancies has been extensively studied but not fully understood [29,30]. It has been reported BRCA1 expression level was reduced in AML samples [31]. When AML was treated with DNA-damaging agents, the loss of BRCA1 function leads to the accumulation of DDR proteins [32]. AML cells with low expression of key members of the DDR pathway such as Rad51, ATM, BRCA1, and BRCA2, displayed extremely high sensitivity to PARPi Furthermore, they showed that combined PARPi with GSK3 inhibitor treatment was an effective therapeutic strategy for PARPi-resistant AML.

Currently, the studies combining PARPi with other inhibitors, particularly those that enhance DNA damage, have been successfully applied in both pre-clinical and clinical trials. Gojo et al. demonstrated that a combination therapy of veliparib, a PARPi, plus the DNA-alkylating agent temozolomide was efficacious against advanced AML using doses that were well-tolerated [33]. In another study, combining PARPis with DNA demethylating agents showed synergy in treating AML [34].

There is an urgent need for new therapeutic strategies to improve survival in patients with acute myeloid leukemia (AML). PARP inhibitors (PARPis) have shown significant benefits in a variety of malignancies patients and are considered as potential target drugs.

We found that AML patients had elevated PARP-1 expression and patients with higher expression had poor prognosis. Therefore, we speculate that the use of PARPis in AML, especially relapse/refractory patients, may improve clinical outcomes. In this study, we found that PARPi BMN673 (Talazoparib) combined with novel SAHA-bendamustine NL101 had a synergistic inhibitory effect on AML cell lines and clinical patient specimens. Our experiments provide theoretical basis for the clinical treatment of PARPis.

Our study suggested that PARP-1 is an independent prognostic biomarker for AML. In addition, our findings provide a powerful rationale for the clinical investigation of the BMN673 and NL101 combination therapy.

Evidence before this study

PARP inhibitors (PARPis) have shown significant benefits in a variety of malignancies and are considered as a potential treatment for AML. In our study, we showed that high PARP-1 expression correlates with poor clinical outcome in AML. In particular, we explored the combination treatment of PARPi BMN673 with a novel SAHA-bendamustine hybrid NL101 in AML.

2.1. Materials & methods

Clinical data were collected from the medical records of AML patients at Zhejiang Institute of Hematology, China. From July 2010 to April 2016, 339 patients were included in this study with detailed diagnostic and treatment information. Cytogenetically normal acute myeloid leukemia (CN-AML) was defined as AML with the karyotype 46 XY [20] or 46 XX [20] in all 20 metaphase cells analyzed. Gene mutations of NPM1, FLT3-ITD, CEBPA, DNMT3a, IDH1 and IDH2 were analyzed by whole-genome sequencing. Patients with secondary AML or acute promyelocytic leukemia were excluded. Patient characteristics were summarized using descriptive statistics, which include frequency counts, median, and range. This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China). Informed consent was obtained from all patients according to institutional guidelines.

2.2. Antibodies and reagents

The antibodies GAPDH (#5174), Caspase3 (#9662), PARP (#9532), CyclinB1 (#4135), CDC2 (#9116), p-CDC2 (#9111), CDC25A (#3652), DAN damage kit (#9947) including p-ATM (Ser1981), p-CHK1 (ser345), p-CHK2 (Thr81), phospho-Histone H2AX (Ser139), and 488 Conjugate secondary antibody (#4412) were purchased from CST (Danvers, MA). CHK1 (10362–1-AP), CHK2 (13954–1-AP) and ATM (27156–1-AP) antibodies were purchase from ProteinTech (Rosemont, USA). Anti-Poly (ADP-Ribose) Polymer antibody (ab14459) and Anti-human CD45-FITC (ab134199) antibody was purchased from Abcam (Cambridge, MA). BMN673 was obtained from MedChemExpress (Monmouth Junction, NJ). NL101 was gifted by Hangzhou Minsheng Institute of Pharmaceutical Research (Hangzhou, China).

2.3. RNA expression by real time reverse transcription PCR (qRT-PCR)

Mononuclear cells (MNC) were separated from the bone marrow (BM) of AML patients at the time of initial diagnosis by Ficoll-Hypaque (TBD Science, Tianjin,China) density gradient centrifugation. RNA was extracted using the TRIzol reagent (Takara, Japan) and was reverse transcribed with PrimerScript RT agent Kit (Takara, Japan). Quantitative assessments of cDNA amplification for PARP-1, BRCA1 and GAPDH were performed in triplicate using SYBR-Green PCR Master Mix kit (Takara, Japan) on an IQ5 real time PCR instrument (Bio-Rad, Hercules, CA). The primers sequences were as follows: PARP-1 5′-TCT GAT CCT CCG TGG GAT GA-3′ (forward) and 5′-TGG CCA TAC TCT GCT GCA AAG-3′ (reverse); BRCA1 5′-GAA ACC GTG CCA AAA GAC TTC-3′ (forward) and 5′-CCA AGG TTA GAG AGT TGG ACA C-3′ (reverse); GAPDH 5′-ACC
ACC CTG TTG CTG TAG CCA A-3′ (forward) and 5′-GTC TCC TCT GAC TTC AAC AGC G-3′ (reverse).

2.4. Cell lines and primary patient cells

The AML cell lines MV4-11 and MOLM-13 were kindly gifted by Professor Ravi Bhatia (City of Hope National Medical Center, Duarte, CA). HL-60 and Kasumi-1 were obtained from Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). MV4-11-luciferase was gifted by Professor Xu Rongzhen (The Second Affiliated Hospital of Zhejiang University). These cell lines were authenticated by DNA short-tandem repeat analysis by Shanghai Biwing Applied Biotechnology (Shanghai, China). MV4-11, MOLM-13 cell lines and primary AML cells were cultured in IMDM medium (Corning) supplemented with 10% fetal bovine serum (Gibco) at 37 °C in a humidified incubator containing 5% CO2. HL-60 and Kasumi-1 cell lines were cultured in RPMI1640 medium supplemented with 10% FBS. Primary AML cells were isolated by Ficoll-Hypaque density gradient centrifugation from the bone marrow after obtaining written informed consent.

2.5. Cell viability assay

Cells were seeded in 96-well plates at 1–2 × 10^4 (AML cell lines) or 1 × 10^5 (primary AML cells) per well. At the end of the drugs treatment, 20 μl MTS solution (Promega, Madison, WI) was added to each well and the cells were incubated for an additional 4 h at 37 °C. The plates were read at a wavelength of 490 nm. Experiments using AML cell lines were done in three independent replicates.

2.6. Flow cytometric analysis

To analyze cell cycle distribution, cells were treated with drugs for 24 h and fixed with 75% ethanol at 4 °C overnight. The next day, the cells were resuspended in buffer with 50 μg/ml propidium iodide (PI) and 100 μg/ml RNase A for 30 min at room temperature. For apoptosis assessment, cells were treated with drugs for 48 h and then co-stained with 10 μl Annexin V-Fluorescein Isothiocyanate (FITC) and 5 μl PI using an apoptosis detection kit (Mulisciences, Hangzhou, China). The engrafted MV4-11 cells were analyzed using anti-human FITC-labeled CD45 antibody (abcam, USA). The DNA content, apoptic cells and hCD45+ cells were analyzed by FACScan flow cytometer (Becton Dickinson, San Diego, CA).

2.7. Western blot analysis

Cells were lysed in RIPA buffer (Thermo Fisher Scientific, Waltham, MA) on ice for 30 min, and centrifuged at 12,000 × g for 15 min at 4 °C to pellet cell debris. The protein concentration in the supernatant was determined using BCA reagent (BBI life science, Shanghai, China). Protein samples were separated by SDS-PAGE gel (Thermo Fisher Scientific, Waltham, MA) and transferred to PVDF membranes (Millipore, Burlington, MA). Membranes were blocked using Tris-buffered saline (TBS) containing 5% non-fat milk for 1 h and incubated with primary antibodies overnight at 4 °C. After washing with TBS buffer containing 1% Tween-20 three times, membranes were incubated with secondary antibodies (CST, Danvers, MA) for 1 h. The target proteins were visualized using an ECL kit (Thermo Fisher Scientific, Waltham, MA) and imaged using the ChemiDoc MP Imaging System (Bio-rad, Hercules, CA).

2.8. Immunofluorescence microscopy

Cells were cytospun onto a glass slide at 400 ×g for 5 min and then fixed for 30 min in blocking solution containing 5% BSA and 0.3% Triton X-100 at room temperature. Anti-human γ-H2AX (ser139) (CST, Danvers, MA) was diluted in PBS containing 1% BSA and incubated overnight at 4 °C. Slides were then washed three times with PBS and incubated in 488 Conjugate secondary antibody (CST, Danvers, MA) for 1–2 h at room temperature in the dark. After washed three times
injected into the tail vein of mice. Cell engraftment was assessed by Ara-C/aclarubicin (HAA)-based treatment group. Ara-C (DA)-based treatment group, idarubicin/Ara-C (IA)-based, and homoharringtonine/Multivariable analysis for OS and EFS in CN-AML patients. Table 2 Characteristics of high and low PARP-1 expressing AML patients. Table 1 with PBS, nuclei were counterstained with DAPI (Sigma-Aldrich, St. Louis, MO). Cells were counted in at least three fields, with >100 cells per condition. The frequency of cells with >5 foci per well was scored [41,42].

2.9. Mice models

For AML xenografts, female B-NSG mice (6–8 weeks old) (Biocytogen, China) were used. MV4-11-luc cells (1 in 10^6 cells) were injected into the tail vein of mice. Cell engraftment was assessed by intraperitoneal injection of luciferin (100 mg/kg) followed by imaging using IVIS Lumina LT system (PerkinElmer, CA, USA). Mice were randomly sorted into four groups before treatment. Mice were observed and weighed daily, and leukemic burden was assessed by bioluminescence imaging every 7 days. Mice were treated with either 0.3 mg/kg BMN673, 12 mg/kg NL101, in combination at indicated concentrations, or vehicle. NL101 was diluted in PBS to 12 mg/mL and stored at −20 °C. BMN673 was prepared in 0.01% carboxymethyl cellulose (CMC) and stored at −20 °C in the dark. BMN673 was administered orally, 5 days per week for 3 weeks. NL101 was administered by intravenous injection twice (day 11–12) post-transplant. Animals experiments were approved by the Ethics Committee for Laboratory Animals of the First Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China) and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.10. Statistical analysis

Kaplan-Meier survival curves were compared using the log-rank test. The multivariate Cox proportional hazards model was used to identify independent outcome predictors after adjustment for confounders. Statistical analyses for biological assays were performed using the two-tailed unpaired t-test or multiple-group ANOVA. The combination index (CI) was calculated using CalcuSyn software (Biosoft, Cambridge, UK). For in vivo experiments, we used GraphPad Prism software (San Diego, CA) to calculate statistical significance using ANOVA or Mann-Whitney tests.

3. Results

3.1. PARP-1 expression positively correlates with clinical characteristics

We compared PARP-1 expression in normal bone marrow (BM) cells, AML cell lines and cytogenetically normal AML (CN-AML) patient samples by real-time PCR. PARP-1 expression was significantly increased in CN-AML patients (P < .05, t-test) and AML cell lines (P < .01, t-test) compared to normal BM cells (Fig. 1a). PARP-1 protein levels had a strong correlation with mRNA levels (Fig. S1). The expression of PARP-1 in two normal control specimens (N#1 and N#2) was relatively low (Fig. S1b). The median PARP-1 expression value of AML patients was 1.16 (Range: 0.14–5.58). Patients were classified into high expression group (113, 33.3%) and low expression group (226, 66.7%) according to the mRNA expression level of PARP-1. Patients with high PARP-1 expression had more BM blasts (72.00 [Range: 51.50–85.00] vs 63.00 [Range: 37.00–78.38], P = .003, t-test), elevated peripheral blood WBC levels (17.00 × 10^9/L [Range: 3.60 × 10^9–87.20 × 10^9] vs 9.65 × 10^9/L [Range: 2.42 × 10^9–38.60 × 10^9], P = .008, t-test), and a higher incidence of FLT3-ITD mutation (28.2% vs 17.3%, P = .031, Pearson Chi-Square) than those with low expression (Table 1). There was no statistically significant correlation between PARP-1 expression and other variables such as gender, age, hemoglobin levels, platelet counts, FAB classifications, bone marrow transplantation condition, remission

### Table 1

| Variables | Low expression | High expression | P value |
|-----------|----------------|-----------------|---------|
| Number    | 226            | 113             | 0.727   |
| Gender = 1 (%) | 133 (58.8) | 64 (56.6) |          |
| Age (median [IQR], years) | 54.50 [40.00, 64.00] | 57.00 [39.00, 64.00] | 0.801 |
| Blast (median [IQR]) % | 63.00 [37.00, 78.38] | 72.00 [51.50, 85.00] | 0.003 |
| WBC (median [IQR]) | 9.65 [2.42, 8.80] | 17.00 [3.60, 8.70] | 0.008 |
| Hb (median [IQR]) | 84.00 [66.25, 104.00] | 88.00 [70.00, 102.00] | 0.600 |
| PLT (median [IQR]) | 49.50 [26.00, 92.00] | 44.00 [25.00, 80.00] | 0.393 |

### Table 2

| Variables | Overall survival | Event free survival |
|-----------|-----------------|---------------------|
| PARP-1 expression (High vs Low) | 1.949 (1.384,2.745) | -0.001 | 1.822 (1.323,2.509) | -0.001 |
| Age (>60) | 2.526 (1.774,3.596) | 0.001 | 2.29 (1.65,3.182) | <0.001 |
| WBC (>10) | 1.193 (0.988,1.963) | 0.058 | 1.218 (0.883,1.679) | 0.229 |
| FLT3-ITD | 2.592 (1.744,3.852) | <0.001 | 2.108 (1.448,3.07) | <0.001 |
| NPM1 | 0.701 (0.462,1.062) | 0.094 | 0.85 (0.578,1.251) | 0.41 |
| DNMT3a | 2.029 (1.307,1.49) | 0.002 | 1.827 (1.22,2.781) | 0.005 |
| BMN | 0.493 (0.231,1.043) | 0.011 | 0.794 (0.428,1.472) | 0.464 |
| HAA treatment | 0.499 (0.343,0.726) | <0.001 | 0.574 (0.4,0.824) | 0.003 |
| IA treatment | -0.001 | -0.001 | -0.001 | -0.001 |
condition, treatment regimen, and gene mutations in \textit{NPM1}, \textit{CEBPA}, \textit{DNMT3a}, \textit{IDH1}, and \textit{IDH2}.

3.2. Overexpression of PARP-1 is associated with poor clinical outcome in CN-AML patients

AML patients with high PARP-1 expression ($n = 113$) had a relatively short overall survival (OS) ($P = .005$, log-rank test) and event free survival (EFS) ($P = .004$, log-rank test) compared to patients in low expression group ($n = 226$) (Fig. 1b and c). To identify the potential confounders and interactions, we conducted interactive analyses. In the multivariable analysis for OS and EFS, high PARP-1 expression is associated with poor survival after adjusting for age, WBC, FLT3-ITD mutation, \textit{NPM1} and \textit{DMNT3a} mutations, BMT, and treatment protocols regardless of OS [HR (95% CI), 1.949 (Range: 1.384–2.745); $P < .001$, cox proportional hazards regression model; Table 2] or EFS [HR (95% CI), 1.822 (Range: 1.323–2.509); $P < .001$, cox proportional hazards regression model; Table 2]. In addition, we examined the expression of BRCA1, another gene that plays an important role in DNA damage repair. However, there was no difference in OS among different BRCA1 expression groups (Fig. S2).

3.3. PARPi BMN673 and a novel SAHA-bendamustine hybrid, NL101, synergistically inhibited growth of AML cells

Given the poor prognosis of patients with high PARP-1 expression, we first tested if PARPi BMN673 in combination with HDACi SAHA or bendamustine could improve the outcome of AML patients. We first found minimal growth inhibition of AML cell lines by BMN673 in combination with SAHA after treatment for 48 h (Fig. S3a–d, up panel). We found that BMN673 combined with SAHA had a synergistic inhibitory effect only in HL-60 but not in MV4-11, MOLM-13 and Kasumi-1 (Fig. S3a–d, down panel, and Table S1). However, BMN673 combined with bendamustine displayed synergistic inhibition in all AML cell lines (Fig. S3e–h, Table S1). Moreover, we studied the combination of BMN673 with cytarabine (Ara-C) and daunorubicin (DNR) in MV4-11 and HL-60 cells (Fig. S3i–l), and found no significant synergistic effects. Then, AML cell lines were treated with BMN673, NL101, or in combination for 48 h. Compare to SAHA or bendamustine, NL101 at lower doses in combination with BMN673 was more efficacious at inhibiting the growth of all AML cell lines (Fig. 2a–d, up panel). The combination also effectively inhibited primary AML cells growth compared to single agent treatment (Fig. 2e–g, up panel). In addition, FLT3-ITD mutation in MV4-11, MOLM-13 cell lines and one of the patient samples did not affect cell sensitivity to the treatment. The characteristics of the patient samples are presented in Table S2. The dose-effect curves were determined by CalcuSyn analyses (Fig. 2a–g, and Fig. S3a–l, down panel). The CI values were presented in Table S1. We demonstrated that BMN673 combined with NL101 had a strong synergistic effect (CI < 1.0) on AML cell lines and primary AML cells in vitro.

3.4. Combination treatment of BMN673 and NL101 induce G2/M cell cycle arrest and trigger apoptosis

To explore the mechanism of synergistic effect, we studied the effects of BMN673 and NL101 combination on cell cycle after exposure to drugs for 24 h. Significant G2/M accumulation and downregulation
of the G0/G1 peak were observed after treatment with the drug combination in MV4-11 and HL-60 cells compared to untreated cells and single agent treatment cells ($P < .05$, One-way ANOVA) (Fig. 3a). Western blot analysis showed an increase in cell cycle regulator P21cip/waf1, G2/M regulatory molecules, cyclin B1, CDC2, p-CDC2 (Tyr-15) and CDC25A. GAPDH served as a loading control. (c) Apoptosis induced by various treatments at 48 h ($** P < .01$, One-way ANOVA, combination treatments versus control and single treatments). (d) Western blot of cleaved Caspase-3 and cleaved PARP-1 in AML cells. GAPDH served as a loading control.

3.5. The combination of BMN673 and NL101 causes an increase in DNA-damage response and inhibition of poly (ADP)ribosylation

Inhibition of DNA damage repair is the major mechanism of PARPi. Considering that NL101 can cause DNA damage [39], we hypothesized that co-treatment of BMN673 and NL101 would yield higher levels of DNA damage. Indeed, we observed significantly higher levels of γ-H2AX foci in MV4-11 and HL-60 cells treated with both BMN673 and NL101, compared to single drug and control (Fig. 4a-c). Likewise, combination treatment increased levels of DNA damage markers p-ATM (Ser1981), p-CHK1 (Ser317), p-CHK2 (Thr68) and γ-H2AX (Fig. 4d). Moreover, we checked the inhibition of poly(ADPribosyl)ation (PAR) in AML cells at different time point. Both BMN673 and the combination treatment could significantly inhibit PAR activity at early and late stages (Fig. 4e).

3.6. The combination of BMN673 and NL101 improves survival of AML xenograft models

To clarify in vivo efficacy of the combination of BMN673 and NL101, we used an intravenous MV4-11-luc xenograft mouse model. Drugs were administered 9 days after injection of cells and bioluminescence imaging was performed on days 9, 16, and 23 post transplantation. Mice were observed daily and using hind limb paralysis as an endpoint [43]. Combination treatment of BMN673 (0.3 mg/kg) and NL101 (12 mg/kg) showed the largest reduction in tumor burden on days 16 and 23 (Fig. 5a and b). Treatment using NL101 alone showed a modest reduction, but mice treated with BMN673 (0.3 mg/kg) alone had no significant reduction in tumor burden (Fig. 5a and b). hCD45-positive blast cells were significantly diminished in the bone marrow of mice treated with the drug combination (Fig. 5a and b). Treatment using NL101 alone showed a modest reduction, but mice treated with BMN673 (0.3 mg/kg) alone had no significant reduction in tumor burden (Fig. 5a and b). Tumor inffltration was reduced in the spleen (Fig. S4). All treatment groups prolonged survival, with the combination group providing the best survival (Fig. 5d). Drug doses were well tolerated, and there is no obvious effect on body weight throughout the duration of treatment in all groups (Fig. 5e). Therefore, our data demonstrated that the combination of NL101 and BMN673 could be a novel treatment regimen for AML.

4. Discussion

Chemotherapy is the standard treatment for AML despite several decades of clinical efforts to improve outcomes of this disease. However, long-term survival of AML patients remains poor for refractory/relapse cases [44–46]. We hope to explore new treatments that induce DNA
damage and perturb cellular DDR in AML cells by understanding the role of PARP-1 in AML cells.

PARP-1 plays a pivotal role in DNA repair, particularly in response to DNA-damaging agents [47,48]. We found that PARP-1 was highly expressed in CN-AML patients and AML cell lines compared to normal BM donors. Furthermore, survival of CN-AML patients with higher PARP-1 expression was poor (Fig. 1). We hypothesized that high PARP-1 expression may be related to the insensitivity to chemotherapy, particularly DNA-damaging agents.

To date, two studies have shown lower BRCA1 expression in haematologic malignancies such as AML [31,49]. Our study found that PARP-1 was highly expressed in CN-AML patients and AML cell lines compared to normal BM donors. Furthermore, survival of CN-AML patients with higher PARP-1 expression was poor (Fig. 1). We hypothesized that high PARP-1 expression may be related to the insensitivity to chemotherapy, particularly DNA-damaging agents.

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so we believe that there is sufficient evidence to use the combination of NL101 and BMN673 as a new therapy for AML.

BMN673 combined with NL101 in AML cell lines inhibited cell survival, impaired cell cycle progression, and induced apoptosis (Figs. 2 and 3). In agreement with the mechanisms of BMN673 and NL101, we observed an increase of DNA damage in AML cells (Fig. 4). In vivo experiments supported the in vitro results and demonstrated that the effective dose had minimal side effects (Fig. 5). The cyclin inhibitor p21 is normally induced by p53 and other p53-independent pathways leading to arrest cell cycle. In our study, we found that the combination treatment of NL101 and BMN673 resulted in G2/M phase arrest and significant upregulation of p21 and G2/M regulatory molecules cyclin B1 and p-CDC2 (Tyr-15).

The findings from our present study indicate that PARP-1 expression negatively impacts the prognosis of AML patients. PARPi BMN673 combined with SAHA-Bendamustine Hybrid NL101 showed strong
synergistic inhibitory effects on AML in vitro and in vivo. Our work paves the way for the potential use of BMN673 and NL101 in AML therapy.

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Authors’ contributions

LCY, LX, and JJR performed most experiments, analyzed the data, wrote the manuscript; MZX, WJH and HX helped to correct the manuscript. JJ and YYP designed the overall study and managed patients, provided facilities support; HJS, LFL and PJJ helped to correct the manuscript. JJ and YYP designed the overall study and supervised the experiments. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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