

Homoharringtonine is Synergistically Lethal with BCL-2 inhibitor APG-2575 in Acute Myeloid Leukemia

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Abstract

Background: Despite advances in targeted agents development, effective treatment of acute myeloid leukemia (AML) remains a major clinical challenge. The BCL-2 inhibitor exhibited well clinical activity in AML treatment, and APG-2575, a novel BCL-2 selective inhibitor, demonstrated anti-tumor activity in hematologic malignancies.

Methods: The synergistic effects of APG-2575 and HHT were studied in AML cell lines and primary samples. MTS was used to measure the cell viability. Annexin V/propidium iodide staining was used to measure the apoptosis rate by flow cytometry. We established AML xenograft tumor model to evaluate the anti-leukemic effect of the combination of BCL-2 inhibitor and HHT *in vivo*. Western blot was used to examine the expression of related proteins level.

Results: In this study, we demonstrated that APG-2575, showed comparable anti-leukemic effect to the FDA-approved BCL-2 inhibitor ABT-199, in AML cell lines or primary AML patient samples. However, similar to ABT-199, APG-2575 treated AML cells showed increased MCL-1, which lead to the resistance of AML cells to the BCL-2 inhibitor. Our previous studies found homoharringtonine (HHT) induced AML cells apoptosis by promoting MCL-1 degradation through the PI3K/AKT/GSK3 β signaling pathway. Herein, we show that combined treatment of HHT with APG-2575 synergistically inhibited AML cell growth and expedited apoptosis by suppressing MCL-1. In addition, APG-2575 combined with HHT had a strong synergistic anti-tumor effect in AML xenograft mouse model.

Conclusion: Overall, our results provide an effective AML treatment strategy through a reasonable combination of APG-2575 and HHT, which is worthy of further clinical research.

1. Background

Acute myeloid leukemia (AML) is a clonal aggressive hematological malignant disease with clinical and genetic heterogeneity [1, 2]. Although treatment of AML has made a significant achievement, the majority of patients experience a relapse of primary AML and develop resistance to chemotherapy [3]. In particular, elderly patients with AML are ineligible for intensive chemotherapy and their 5-year overall survival rate is only 5–15% [4]. It remains a major challenge in AML management due to refractoriness, relapse and drug resistance.

The BCL-2 is dysregulated in AML and its overexpression mediates therapeutic resistance and poor clinical prognosis, which makes BCL-2 an encouraging therapeutic target [5–7]. ABT-199 (venetoclax/GDC-0199), selectively targeting BCL-2, is a novel BCL-2 homology domains 3 (BH3) mimetic agent. *In vitro* studies had confirmed the remarkable anti-leukemic effect of ABT-199 in either chemotherapy-sensitive or chemotherapy-resistant AML cells, and more importantly, it was reported to eliminate leukemia stem cells [8, 9]. The combination of ABT-199 with hypomethylating agents (decitabine or azacitidine) achieved complete remission (CR) or CR with incomplete blood count recovery (CRI) in 67% of elderly patients with AML [10]. 54% of elderly AML adults who received combined regimen

of ABT-199 and low-dose cytarabine (LDAC) achieved CR/CRi [11]. In addition, clinical trials of ABT-199 in combination with FLT3 inhibitors, IDH1/2 inhibitors and other agents on AML have been launched [12].

APG-2575 (lisaftoclax) is a BCL-2 selective inhibitor independently developed by Suzhou Ascentage Pharma, several clinical trials have been initiated in hematologic malignancies [13]. The results of the phase I trial (NCT03537482) showed that APG-2575 had anti-tumor activity and tolerable safety in patients with relapsed/refractory chronic lymphocytic leukemia (R/R CLL), small lymphocytic lymphoma (SLL) and other hematologic tumors [14]. APG-2575 combined with rituximab/ibrutinib or HHT/azacytidine is currently being tested clinically for SLL/CLL or R/R AML (NCT04494503, NCT04501120). APG-2575 in combination with Bruton's tyrosine kinase (BTK) inhibitor (ibrutinib) or MDM2-p53 inhibitor (APG-115) showed a synergistic anti-tumor effect in diffuse large B-cell lymphoma (DLBCL) [15]. In addition, combination therapy of APG-2575 and tyrosine kinase inhibitor (TKI) (olverembatinib/HQP1351) showed synergistic anti-leukemic effects in FLT3-ITD mutant AML [13]. MCL-1 was highly expressed in multiple hematological malignancies [16], and was shown to mediate the development of hematological malignancies, including AML [17, 18]. Moreover, up-regulation of MCL-1 expression was observed during treatment with ABT-199, leading to the development of drug resistance [16, 19].

Homoharringtonine (HHT) is an alkaloid obtained from the *Cephalotaxus hainanensis*. It is successfully applied in the treatment of hematological diseases due to its effective anti-tumor activity [20, 21]. In our previous study, we found that HHT down-regulated the BCL-2 and MCL-1 expression [22], making us hypothesize combination of HHT with APG-2575 may enhance the response and reverse the APG-2575 resistance in AML cells.

In the present study, we evaluated the synergistic anti-AML effect of combined treatment of APG-2575 and HHT in AML. Our results revealed that the addition of HHT potentiated the anti-leukemic effect of APG-2575 in AML cell lines and primary AML cells *in vitro* and *in vivo*. Mechanistically, HHT inhibited the PI3K/AKT/GSK3 β signaling pathway, leading to MCL-1 dual phosphorylated and degraded, which overcame the resistance of APG-2575 mediated by overexpressed MCL-1. In conclusion, our results support further exploring the effect of the combination regimen of HHT and APG-2575 in AML treatment.

2. Materials And Methods

2.1 Materials

APG-2575 was provided by Ascentage Pharma Group Co., Ltd. (Suzhou, China), and ABT-199 was purchased from Selleck Chemicals (Houston, TX, USA). HHT was purchased from Sigma-Aldrich (St. Louis, MO, USA). The antibodies GAPDH, β -actin, PARP, Caspase3, Caspase7, Cleaved-Caspase3 (Cleaved-C3), Cleaved-Caspase7 (Cleaved-C7), PI3K (p85 α), AKT, AKT (Ser473), GSK3 β , GSK3 β (Ser9), MCL-1, MCL-1 (Thr163) and MCL-1 (Thr163/Ser159) were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2 Cell lines and primary cells

MV4-11 cell line was kindly endowed by Professor R. Bhatia (City of Hope National Medical Center, Duarte, CA, USA). THP-1, HL-60, U937 and OCI-AML3 cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. Kasumi-1 cell line was gifted by Professor Chen Saijuan (Shanghai Institute of Hematology, Shanghai, China). MV4-11 cells were maintained in Iscove's modified Dulbecco's medium (IMDM, Gibco, Billings, MT, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), and THP-1, HL-60, U937 and OCI-AML3 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640, Gibco) medium supplemented with 10% FBS at 37°C in a humidified incubator containing 5% CO₂.

Bone marrow samples were obtained from AML patients following written informed consent. Mononuclear cells were isolated by Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO, USA) density gradient centrifugation. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University, China.

2.3 Cell viability assay

20,000 AML cells (cell density of 2.0×10^5 cells/ml) or 100,000 primary AML cells (cell density of 1.0×10^6 cells/ml) were seeded in a 96-well plate and treated with designated drugs for the 24 or 48 h. Then 10 μ l MTS solution (Promega CellTitre 96, Promega Corporation, Madison, WI, USA (5mg/ml) was added by incubation for additional 3–4 h at 37°C. The plates were measured at an absorbance of 490 nm. IC50 values and combination index (CI) were calculated by CalcuSyn Software (Biosoft, Cambridge, UK).

2.4 Flow-cytometric analysis of apoptosis

The extent of apoptosis was assessed through an apoptosis detection kit (Beyotime Institute of Biotechnology, Hangzhou, China). Cells were treated with drugs for 24 h, and then harvested and washed with phosphate buffered saline (PBS). According to the manufacturer's instruction, after resuspending in binding buffer and staining with Annexin V-FITC (Annexin-V) and propidium iodide (PI) in dark at room temperature for 15 min, cells were subjected to FACScan™ flow cytometer (Becton Dickinson, San Diego, CA, USA). Data were presented as percentages of Annexin V + cells.

2.5 Western blot analysis

Cells were harvested and washed in PBS, and then lysed in radioimmunoprecipitation (RIPA) buffer in the presence of protease and phosphatase inhibitor (Cell Signaling Technology (CST), Beverly, MA, USA) on ice for 30 min. Cells were centrifuged at 12,000 g for 15 min at 4°C to pellet cell debris. The protein concentration was determined using BCA reagent. Equal protein was electrophoresed in 10% SDS-PAGE (Life Technologies, Carlsbad, CA, USA) and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The transblotted membranes were blocked with 5% non-fat milk in TBS-T for 1 h and then incubated with primary antibodies overnight at 4 °C. Then the membranes were washed thrice with TBS-T buffer, 15min each time, followed by secondary HRP-conjugated antibody (CST, Beverly, MA, USA) for 1 h at room temperature. The target proteins were visualized using an ECL detection kit (Amersham, Little Chalfont, UK) and analyzed using IMAGE LAB™ software (Bio-Rad Laboratories, Hercules, CA, USA).

2.6 AML xenograft model

Thirty-five BALB/c Nude mice and twenty NOD SCID mice (female, 6–8 weeks of age, weight 17–24 g) were purchased from Shanghai Lingchang Biotechnology Co., Ltd. (Shanghai, China) and Beijing Vital River Laboratory Animal Technology Co., Ltd, respectively. MV4-11 cells were resuspended in PBS and Matrigel (1×10^6 cells/mouse), and then subcutaneously injected into the right back of BALB/c Nude mice to establish a subcutaneous xenograft tumor model. When the tumor volume reached to 100–200 mm³, the mice were randomly divided into 6 groups (vehicle control, 50 mg/kg APG-2575, 50 mg/kg ABT-199, 1 mg/kg HHT, 50 mg/kg APG-2575 + 1 mg/kg HHT, 50 mg/kg ABT-199 + 1 mg/kg HHT). The administration was started on the day of grouping (ie D1; APG-2575/1BT-199, PO, QDx21D; HHT, IP, QDx14D). The mouse body weight and tumor size were measured twice a week during the experiment. After the administration, all mice were killed by cervical dislocation. In addition, OCI-AML3 cells were injected subcutaneously on the right back of NOD SCID mice, and the other operations referred to the above.

Tumor volume (TV) was calculated as: $TV = L \cdot W^2 / 2$, Where L and W represent the length and width of the tumor, respectively. The relative tumor volume (RTV) was: $RTV = V_t / V_1$. V_1 is the TV on the first day of administration (Day1) and V_t is the TV at the time of measurement (Day t). The evaluation index of anti-leukemic effect was the relative tumor proliferation rate: $(T/C (\%)) = (T_{RTV} / C_{RTV}) \cdot 100\%$ [T_{RTV} : the RTV of the treatment group, C_{RTV} : the RTV of the vehicle control group]. Synergy analysis was measured by using the formula [23]: expected value = $(A/C) \times (B/C)$ [actual value = $(AB)/C$; A: RTV of A drug; B: RTV of B drug; C = The RTV of the vehicle control group; AB = the RTV of the combined administration group]. The synergy index = expected value/actual value; ratio > 1, indicating that the two drugs have a synergistic effect; ratio = 1, indicating that the two drugs have an additive effect; ratio < 1, indicating that the two drugs have a weaker than additive effect. Change of body weight (%) = $(\text{measured weight} - \text{weight at grouping}) / \text{weight at grouping} \cdot 100\%$. In addition, complete regression (CR), partial regression (PR) and stable disease (SD) among the number of animals in each group represented the remission rate. $T/C (\%) \leq 42\%$ and statistical analysis $P < 0.05$ is effective. If the weight of the mouse drops by more than 20% or the number of drug-related death exceeds 20%, the drug dose is considered to be severely toxic.

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Suzhou GenePharma (Approval No: IACUC-2021003).

2.7 Statistical analyses

Experiments were presented as the means \pm standard deviation (SD) in three independent experiments. Student's t-test and the one-way ANOVA test were used to assess statistical significance ($P < 0.05$ was considered statistically significant). Statistical analyses were conducted using Prism software v8.3.0 (GraphPad Software, La Jolla, CA, USA). The combination index (CI) was calculated using CalcuSyn software (Biosoft, Cambridge, UK).

3. Results

3.1 APG-2575 shows comparable anti-leukemic effect to ABT-199

MV4-11 and OCI-AML3 cells were treated with APG-2575 or ABT-199 (0.1–0.8 μM) for 24 h. The results showed that APG-2575 or ABT-199 treatment inhibited AML cells viability in a dose-dependent manner. MV4-11 cells showed equal response to APG-2575 and ABT-199. However, OCI-AML3 cells were more sensitive to APG-2575 than ABT-199 (Fig. 1A). The IC₅₀ values of six cell lines (HL-60, Kasumi-1, MV4-11, THP-1, U937 and OCI-AML3) were determined after treatment with APG-2575 or ABT-199 for 24 h. The anti-leukemic activity of APG-2575 in AML cells was equal or even better than that of ABT-199 (Fig. 1B, Table 1). Additionally, towards better integrating clinical practice, primary AML samples were used to evaluate the anti-leukemic effect of APG-2575 and ABT-199. Five patient samples were treated with APG-2575 or ABT-199 (0.1–0.8 μM) for 48 h (Fig. 1C). We also observed the similar efficacy of APG-2575 and ABT-199. The clinical characteristics of the patient samples were presented in Table 3.

Table 1
IC₅₀ values of APG-2575 and ABT-199 against AML cell lines

FAB type	Cell Line	APG-2575 IC ₅₀		ABT-199 IC ₅₀	
		(μM) [Mean (SD)]			
M2	HL-60	0.05	0.01	0.06	0.01
M2	Kasumi-1	0.10	0.04	0.09	0.02
M5	MV4-11	0.26	0.01	0.30	0.04
M5	THP-1	6.19	0.07	5.92	0.13
M4	U937	15.35	0.53	16.46	1.79
M4	OCI-AML3	27.77	6.55	36.73	5.39

3.2 HHT and APG-2575 synergistically inhibit AML cells growth

To test the synergistic anti-leukemic activity between HHT and APG-2575, we treated several AML cells lines with HHT, APG-2575 and their combination at various concentrations and monitored cell viability. Remarkably, HHT in combination with APG-2575 augmented the inhibitory effect to a greater extent than either of the two agents alone in HL-60, Kasumi-1, MV4-11 and OCI-AML3 cell lines (Fig. 2A). And the CI of each combination indicated synergy between HHT and APG-2575 in the four AML cell lines. Significantly, OCI-AML3 cells were resistant to APG-2575 and a strong synergistic effect (CI < 0.3) was observed (Fig. 2A). MV4-11 cells which were moderately sensitive to APG-2575, and OCI-AML3 cells which were

inherently resistant to APG-2575 for the high levels of Mcl-1 (Table 1, Fig. 2C) were selected for the subsequent phenotype and mechanistic studies.

Considering that APG-2575 is a BCL-2 inhibitor and exerts an anti-leukemic effect through the apoptotic pathway, we tested whether the combination treatment could synergistically trigger apoptosis in AML cells. MV4-11 and OCI-AML3 cell lines were exposed to APG-2575 and HHT alone or in combination for 24 h. The Annexin-V/PI staining results indicated that APG-2575 induced AML cells apoptosis, and combination treatment resulted in superior induction of apoptosis compared with either single agent (Fig. 2B). Moreover, we analyzed the level of proteins involving apoptosis signaling pathway by western blot. Cleaved PARP, cleaved caspase-3 and cleaved caspase-7 were more significantly increased in the combined treatment group compared with the single-agent group (Fig. 2D), indicating HHT potentiates APG-2575 induced apoptosis in AML cells.

3.3 HHT and APG-2575 combination exerts synergistically anti-leukemic effect in primary AML samples

We further validated the synergistic effect of HHT and APG-2575 combination in primary AML patient samples. The results revealed that APG-2575 or HHT can inhibit the viability of primary AML cells, and indeed, synergies ($CI < 1.0$) were also observed in AML specimens (Fig. 3A, Table 2). The characteristics of the AML patients were presented in supplemental Table 1. Furthermore, inhibition rate was presented for every dose combination of HHT and APG-2575 using the Synergy Finder application (<https://synergyfinder.fimm.fi>). Synergy score was calculated based on the Loewe model. Dose-dependent inhibition of proliferation was observed upon HHT or APG-2575 treatment for 48 h, according to the overall drug combination dose-response matrix pattern (Fig. 3D). The average synergy score was 16.18 and the most synergistic area score was 20.41 in AML patient 08. These results indicated HHT in combined with APG-2575 exerted a synergistically anti-leukemic effect in primary AML samples.

Table 2
The combination index values of AML primary patients

CI	ED50	ED75	ED90
patient 01	0.22006	0.38335	0.92697
patient 02	1.79E-05	0.00131	0.09882
patient 03	0.54117	0.82208	1.34239
patient 05	0.30568	0.0932	0.04407

3.4 APG-2575 synergized with HHT suppresses AML xenograft tumor growth

To evaluate the anti-leukemic effect of the combination of BCL-2 inhibitor and HHT *in vivo*, we established AML xenograft mouse model. The APG-2575 and HHT monotherapy group showed anti-leukemic effect in MV4-11 xenograft mouse model, with T/C values of 62.76% and 32.43% respectively, however, the APG-2575/HHT co-treatment group presented a significant synergistic anti-leukemic effect, with a T/C value of 3.31%. The synergy index was 6.14 and tumor remission rate was 100% (6/6 PR). In parallel comparison, another FDA-approved BCL-2 inhibitor ABT-199 combined with HHT also showed an obvious synergistic anti-AML effect, with a T/C value of 2.59%. The synergy index was 11.07 and the tumor remission rate was 100% (6/6 PR) ((Fig. 4A, supplemental Table 2). These results suggested that APG-2575 combined with HHT had a significant synergistic anti-AML effect, and the synergistic effect was comparable to that of ABT-199 combined with HHT. No significant weight loss was observed in the animals in each administration group (Fig. 4B), indicating that the animals were well tolerated the compounds.

OCI-AML3 cells were used to establish a mouse subcutaneous xenograft tumor model as well. OCI-AML3 cell was characterized by a rapidly growing xenograft tumor. On the 15th day after administration, the animals in the vehicle control group were euthanized because the tumor volume exceeded the allowable range of animal welfare. Therefore, the experiment was terminated on the 15th day. APG-2575 and HHT single agent did not show significant antitumor activity in this model, with a T/C value of 61.36% and 66.77% respectively (Fig. 4C). Notably, the co-treatment group had significant synergistic anti-leukemic effect with T/C value of 33.02% (compared with the vehicle control group, $P < 0.05$). The synergy index was 1.24 (supplemental Table 3). No significant weight loss was observed in the animals in each group (Fig. 4D).

3.5 HHT reverses the resistance of APG-2575 via inhibiting the PI3K/AKT/GSK3 β pathway

As MCL-1 is a vital player in the resistance to BCL-2 inhibitor in AML cells. We examined the expression of MCL-1 protein after exposure to APG-2575 or HHT alone or combined regimen. Western blot analysis revealed that, while exposure to APG-2575 lead to increase MCL-1 expression, treatment with HHT alone or the combination results in a significant reduction in the protein level of MCL-1 in MV4-11, OCI-AML3 cells. It was reported that GSK3 β was involved in MCL-1 degradation [26–29]. We next examined whether the combination of APG-2575 and HHT affects the PI3K/AKT/GSK3 β signaling pathway. As shown in Fig. 5A, HHT in the presence or absence of APG-2575 led to a reduction of PI3K, p-AKT (Ser473), and p-GSK3 β (Ser9) expression, but has no obvious effect on the total protein level of AKT and GSK3 β . Of note, our study indicated that APG-2575 showed no effects on PI3K/AKT/GSK3 β signaling pathway. In addition, monophosphorylated and dual phosphorylated MCL-1 were detected by western blot. The results suggested that HHT could reduce p-MCL-1 (Thr163) and up-regulate p-MCL-1 (Thr163/Ser159), and notably, this effect of HHT was enhanced in the combination treated cells. Treatment with APG-2575 increased MCL-1 level and HHT promoted the phosphorylation and degradation of MCL-1 via inhibiting the PI3K/AKT/GSK3 β signaling pathway, thereby reversing the resistance of APG-2575 (Fig. 5B).

4. Discussion

AML is a genetically heterogeneous disorder characterized by a poor survival rate. Regardless of whether regimens combine conventional therapies with multiple novel target drugs or hematopoietic stem cell transplantation (HSCT), AML remains an incurable malignancy of the bone marrow. New regimens with better tolerance and efficacy, less toxicity and fewer side effects are urgently needed. On June 7, 2021, Ascentage Pharma Ascent Pharmaceuticals presented the latest clinical progress of the new generation of Bcl-2 inhibitor APG-2575 at the ASCO meeting. Among 15 patients with R/R CLL/SLL, 12 cases achieved partial remission (PR) and the overall remission rate (ORR) was as high as 80.0%. No dose-limiting toxicity (DLT) was observed at 1200 mg and no tumor lysis syndrome occurred [15]. It means that there are great potentials for clinical development of single drug and combination therapy of APG-2575, which will bring better options for patients. MCL-1 plays a vital role in the development of hematological malignancies and high repression of MCL-1 is associated with BCL-2 inhibitor resistance. Our previous studies identified that HHT could reduce MCL-1 and exert significant anti-leukemic activity [24, 25]. Therefore, we propose the combined application of APG-2575 and HHT for the treatment of AML in order to reverse the resistance of APG-2575.

In this study, we first compared the anti-leukemic effect of APG-2575 and ABT-199 and the results suggested that the two agents were comparable in inhibiting AML cells growth. Next, we found HHT in combination with APG-2575 displayed an excellent anti-leukemic effect. APG-2575 showed dose-dependent growth inhibition and accelerate apoptosis when administrated as a monotherapy, and the combined regimen exhibited more obvious synergistic activity, as evidenced by the activation of PARP and caspase family members. Studies had shown that tumor cells with high expression of anti-apoptotic protein MCL-1 have primary resistance to the BCL-2 selective inhibitor [26]. Interestingly, the two agents exerted more significant growth inhibition and apoptosis in OCI-AML3 cells (inherent resistance to APG-2575). These results demonstrated that HHT enhanced the anti-leukemic effect of APG-2575, suggesting HHT as a potential drug to overcome the resistance of APG-2575.

According to the cell experiments *in vitro*, MV4-11 cells are moderately sensitive to APG-2575. Therefore, we first used human MV4-11 cells to establish a mouse xenograft tumor model to evaluate the anti-tumor effect of the combination of APG-2575 and HHT. The results suggested that APG-2575 and ABT-199 have equally anti-leukemic effects *in vivo*. The data proved that APG-2575 combined with HHT had a synergistic effect *in vivo* through tumor volume, synergy factor, T/C value. And we further confirmed co-administration of APG-2575/HHT also exerting synergistic anti-leukemic effect in the OCI-AML3 xenograft model. Together, these above results verified that the combination of APG-2575 and HHT has a significant synergistic anti-AML effect *in vitro* and *in vivo*, indicating that this combined regimen could bring clinical benefits to AML patients.

GSK3 β has been reported to be related to p-MCL-1 (Thr163/Ser159), and the double-phosphorylated MCL-1 (Thr163/Ser159) is degraded through the ubiquitinase pathway. Phosphorylation at serine 9 weakens GSK3 β constitutive activity [27, 28]. Our results suggested that single-agent APG-2575 could not down-

regulate p-GSK3 β (S9), thereby preventing the conversion of p-MCL-1 (Thr163) to p-MCL-1(Thr163/Ser159). The accumulated p-MCL-1 (Thr163) could stabilize the structure of MCL-1 and made MCL-1 being more difficult to be degraded. APG-2575 combined with HHT can inhibit the PI3K/AKT/GSK3 β signaling pathway. Because p-GSK3 β (Ser9) is down-regulated, it was beneficial to the conversion of mono-phosphorylated p-MCL-1(Thr163) to double-phosphorylated p-MCL-1(Thr163/Ser159), and further degradation through ubiquitinase. The inhibition of the signaling pathway leads to the decrease of MCL-1 content and reverses the resistance of APG-2575.

Conclusion

Some studies have reported that high expression of MCL-1 was related to non-remission following ABT-199 treatment, and the original resistance in AML cells could reverse by co-administration of MCL-1 inhibitor. In this study, HHT combined with APG-2575 treatment could reduce the level of MCL-1. Overall, HHT combined with APG-2575 could overcome the resistance to APG-2575 in AML, and these preclinical results provided strong evidence for the treatment of AML patients with APG-2575 combined with HHT.

Declarations

Ethics approval and consent to participate

Human samples have been performed in accordance with the Declaration of Helsinki and have been approved by Clinical Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are not publicly available due confidentiality for another study but are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing financial interests.

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Author contributions

Contribution: W.W., J.J., and J.S. designed experiments; W.W., S.H., Q.L., S.M., Y.Q., Q.Y., F.L., J.P., X.L., and X.H. performed experiments and/or collected data; W.W., J.J., and J.S. analyzed and interpreted data; W.W. wrote the manuscript, which was revised and approved by J.J., and J.S.

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Table

Table 3 is not available with this version.

Figures

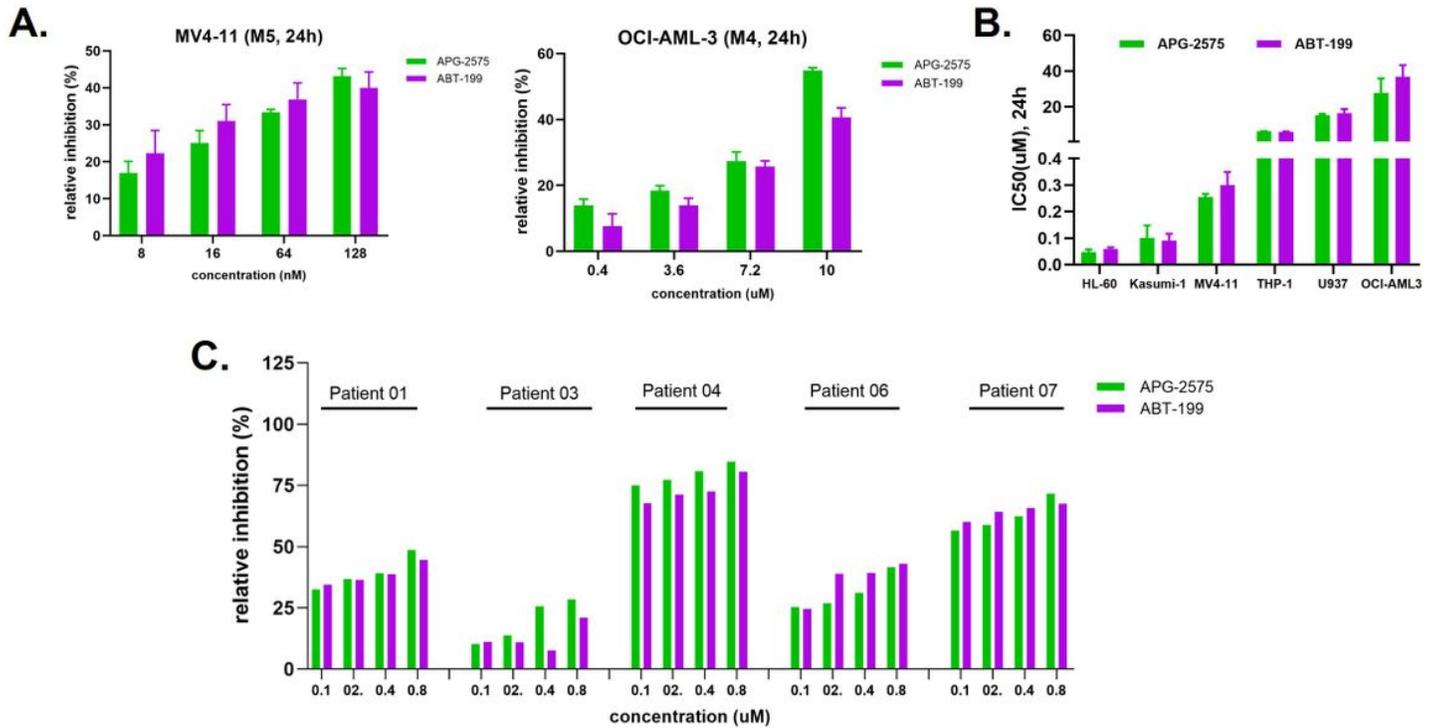
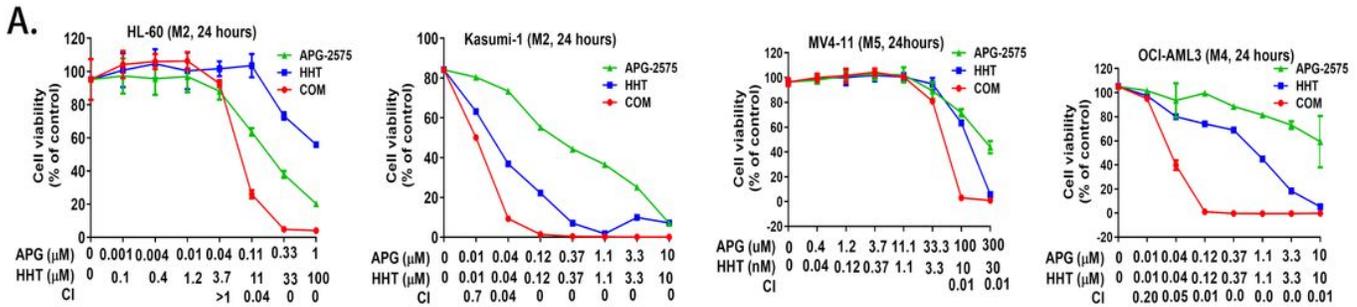


Figure 1

APG-2575 showed comparable anti-leukemic effect to ABT-199. (A) The inhibition ratio of different concentrations of APG-2575 or ABT-199 in MV4-11, OCI-AML3 cells for 24 h. (B) The IC₅₀ values of APG-2575 or ABT-199 in HL-60, Kasumi-1, MV4-11, THP-1, U937 and OCI-AML3 for 24 h. (C) The inhibition ratio of APG-2575 and ABT-199 in primary AML at different concentrations for 48 h.



B.

Control APG-2575 HHT COM MV4-11 OCI-AML-3

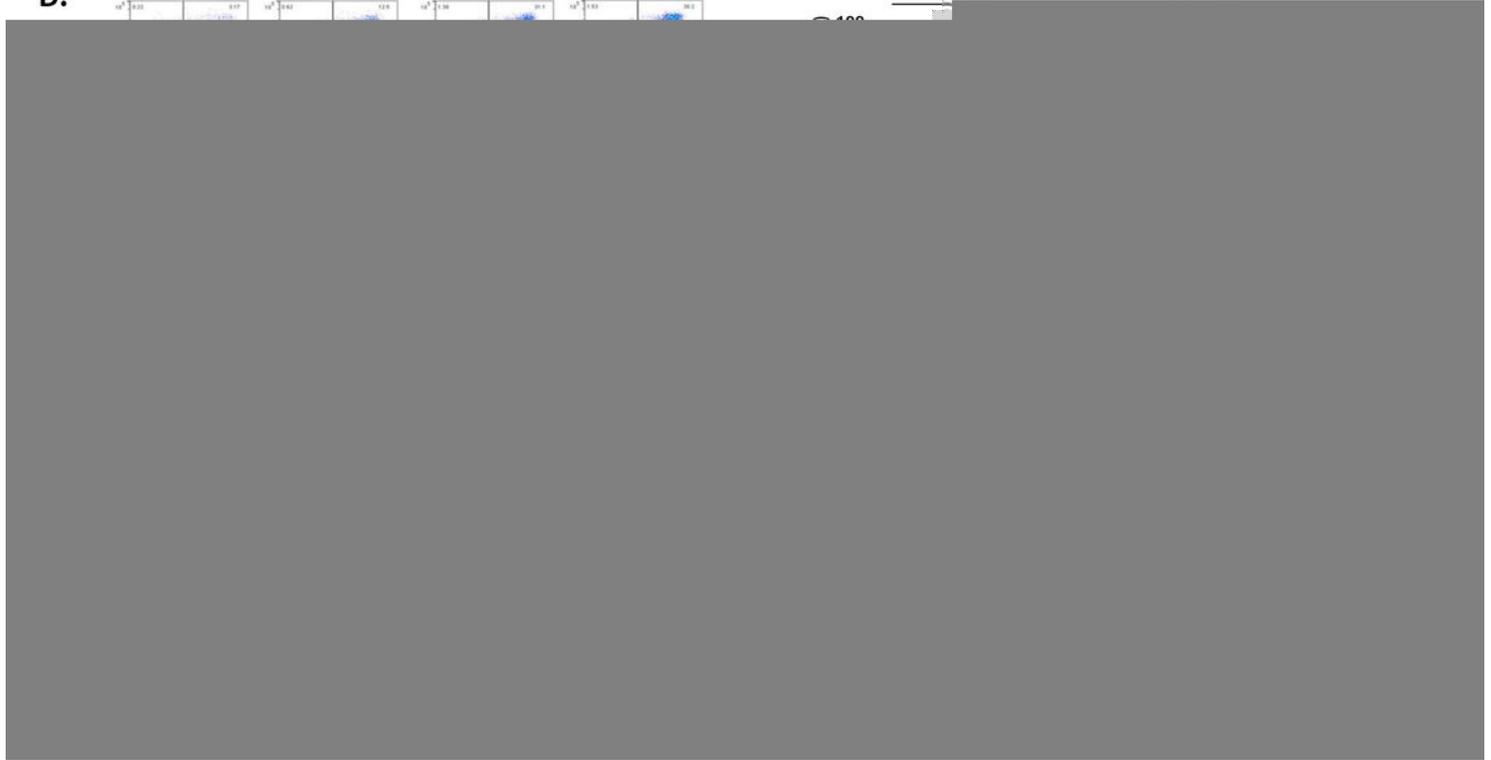


Figure 2

HHT and APG-2575 synergistically inhibit AML cells growth. (A) Cell viability of AML cell lines (HL-60, Kasumi-1, MV4-11 and OCI-AML3) treated with APG-2575 and HHT single agent or their combination for 24 h. The CI indexes were also presented. (CI: <0.1, very strong; 0.1~0.3, strong; 0.3~0.7, medium; 0.7~0.85, moderate; 0.85~0.90, slight). (B) The apoptosis of MV4-11 and OCI-AML3 cells treated with APG-2575 and HHT single agent or their combination for 24 h. (C) The expression of MCL-1 in AML cell lines. (D) The levels of apoptosis related proteins were determined after drug exposure for 24 h.

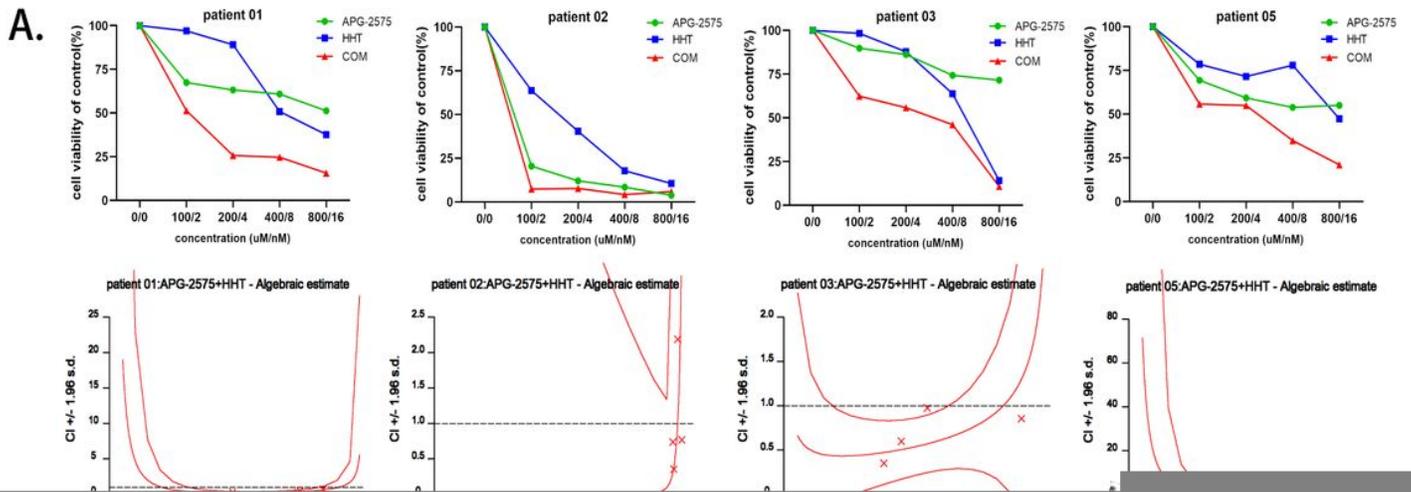


Figure 3

HHT and APG-2575 combination exerts synergistically anti-leukemic effect in primary AML samples. (A) Cell viability of AML patients treated with APG-2575 or HHT single agent or their combination for 48 h. The CI indexes (down panel) were calculated by CalcuSyn software. **(B)** The apoptosis of primary AML samples treated with APG-2575 and HHT single agent or their combination for 24 h. **(C)** Cell viability was calculated for every dose combination of APG-2575 and HHT using the SynergyFinder Web application. synergy score: <-10, antagonistic; from -10 to 10, additive; >10, synergistic.

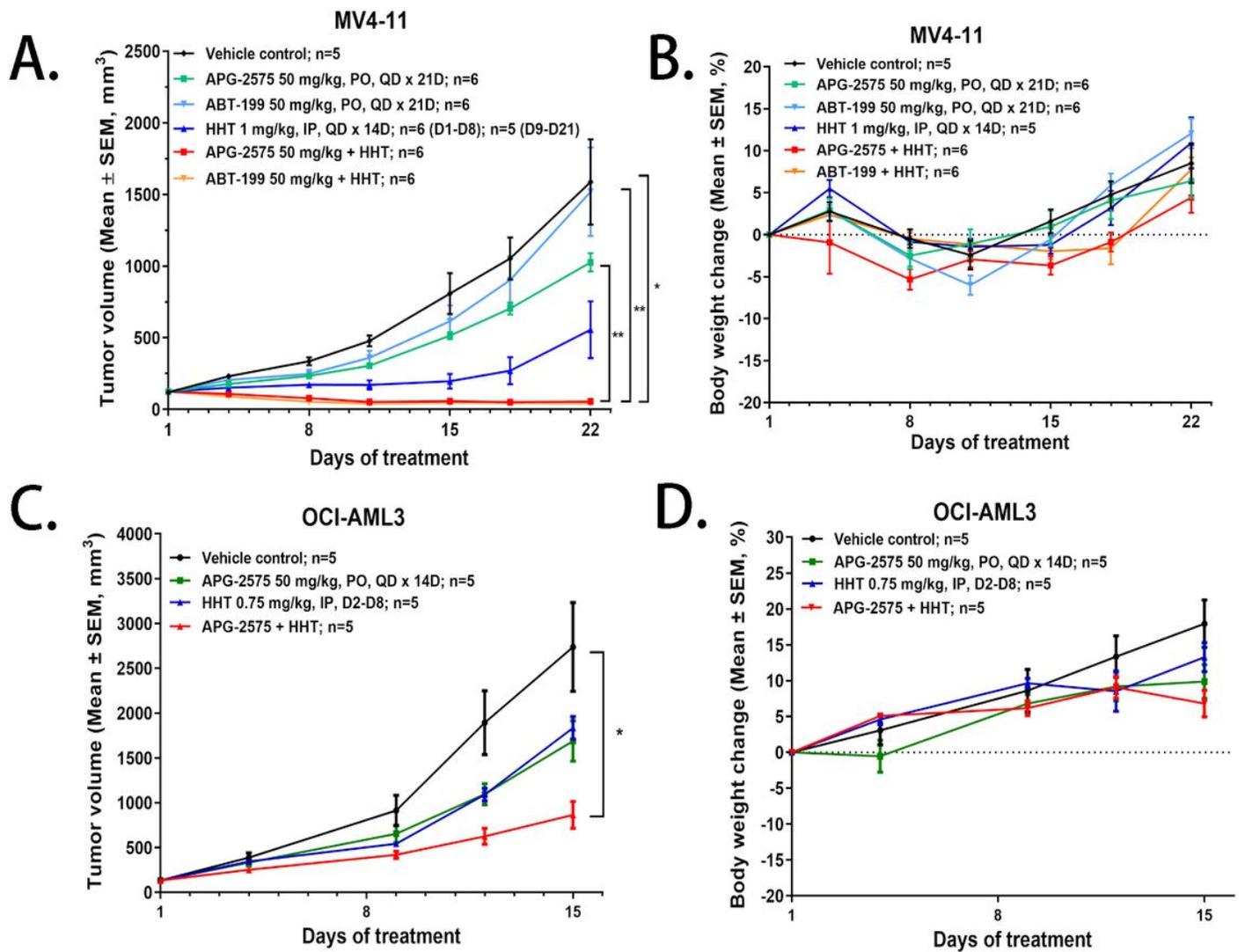


Figure 4

APG-2575 synergized with HHT suppresses AML xenograft tumor growth. (A and B) The average tumor volume cure and the tumor weight curve in MV4-11 xenograft mouse model for each group. (C and D) The average tumor volume cure and the tumor weight curve of OCI-AML3 xenograft mouse model. (* P < 0.05; ** P < 0.01; *** P < 0.001).

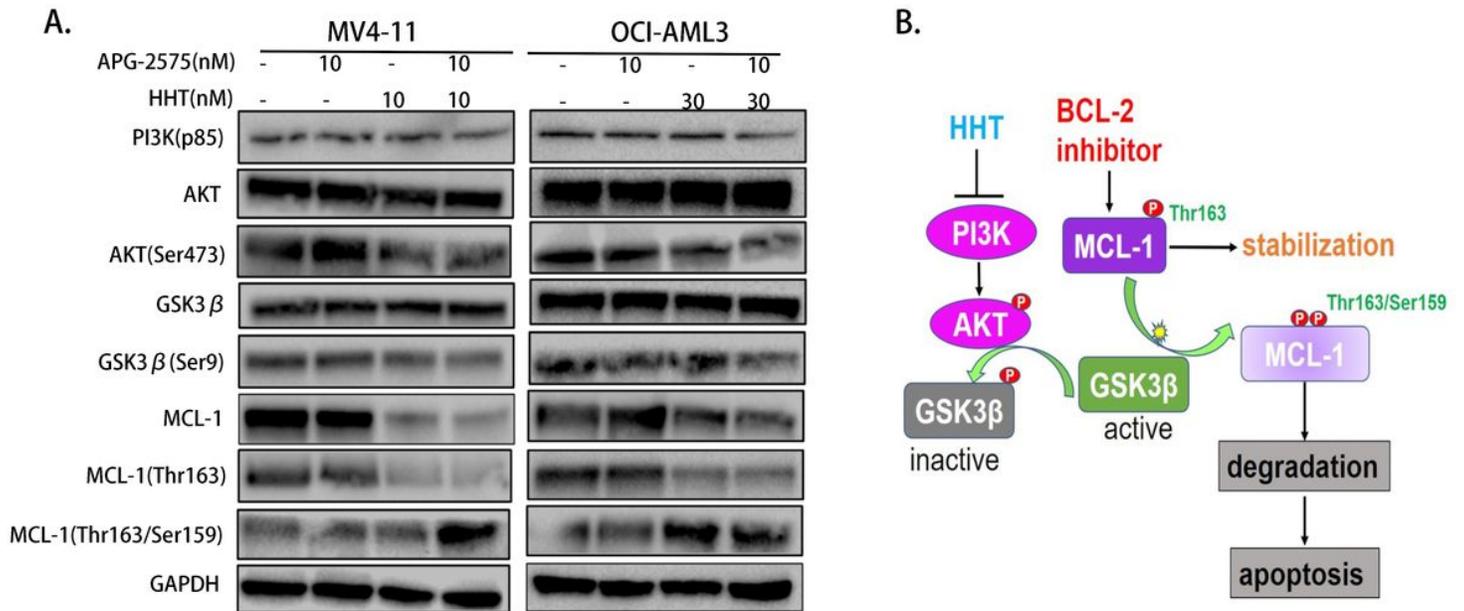


Figure 5

HHT reverses the resistance of APG-2575 via inhibiting the PI3K/AKT/GSK3 β pathway. (A) The level of proteins in PI3K/AKT/GSK3 β signaling pathway and the MCL-1(Thr163, Thr163/Ser159) were measured after treatment with APG-2575 and HHT for 4 h. And the MCL-1 for 6 h. (B) Schematic of APG-2575 and HHT in AML. HHT inhibits the PI3K/AKT/GSK3 β pathway, which interfere with MCL-1 stability, thereby accelerating MCL-1 degradation.

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