

Homoharringtonine Combined with the Heat Shock Protein 90 Inhibitor IPI504 in the Treatment of *FLT3*-ITD Acute Myeloid Leukemia



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Abstract

As a heterogeneous group of clonal disorders, acute myeloid leukemia with internal tandem duplication of fms-like tyrosine kinase 3 (*FLT3*-ITD) mutation usually shows an inferior prognosis. In the present study, we found that homoharringtonine (HHT), a protein translation inhibitor of plant alkaloid in China, exhibited potent cytotoxic effect against *FLT3*-ITD (+) cell lines and primary leukemia cells, and a remarkable synergistic anti-leukemia action was demonstrated in vitro and in vivo in xenograft mouse models when co-treated with the heat shock protein 90 inhibitor IPI504. Mechanistically, HHT combined with IPI504 synergistically inhibited the growth of leukemia cells by inducing apoptosis and G1 phase arrest. This synergistic action resulted in a prominent reduction of total and phosphorylated *FLT3* (p-*FLT3*) as well as inhibition of its downstream signaling molecules such as STAT5, AKT, ERK and 4E-BP1. Furthermore, co-treatment of HHT and IPI504 led to a synergistic or additive effect on 55.56% (10/18) of acute myeloid leukemia cases tested, including three relapsed/refractory patients. In conclusion, our findings indicate that the combination of HHT and HSP90 inhibitor provides an alternative way for the treatment of *FLT3*-ITD positive acute myeloid leukemia, especially for relapsed/refractory AML.

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Background

As a genetically heterogeneous group of diseases, acute myeloid leukemia (AML) shows distinct features in the fields of clinicopathology, cytogenetics and genetics [1,2]. However, the common pathogenesis of AML are block in cellular differentiation and/or aberrant proliferation in myeloblasts [3]. The *FLT3* (FMS-like tyrosine kinase 3) gene with a mutation of internal tandem duplications (ITD) in the juxtamembrane domain occurs approximately 30% in AML with normal karyotype [4]. And accumulating evidence reveals that AML accompanied with *FLT3*-ITD mutation has poor prognosis and a higher disease relapse rate, which reminds us that *FLT3*-ITD is a desirable therapeutic target in personalized

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medicine of AML [5–7]. Despite improvement of complete remission (CR) induced by conventional chemotherapy, consolidation and/or allogeneic hematopoietic stem cell transplantation (HSCT) in AML, disease relapse and drug resistance are still two key obstacles in long-term remission and overall survival (OS) of patients [8]. Therefore, it is urgent to find novel available strategies or regimens in the treatment of AML patients with *FLT3*-ITD.

Homoharringtonine (HHT), also known as omacetaxine mepe-succinate, is a classical anti-leukemia drug which has been used for nearly 40 years in China. Although HHT has been studied for many years, the precise targets are still unclear [9,10]. Our previous study demonstrated that HHT can inhibit the proliferation of leukemia cells by down-regulating the expression of the phospho-eIF4E (p-eIF4E), an indispensable protein for survival and growth in normal cells or malignant cells [11–13]. Recently, our studies and others' have shown that HHT is more sensitive to *FLT3*-ITD (+) AML cell lines and primary samples as compared with AML with wild-type *FLT3*. However, to date, it exhibits limited anti-leukemic activity with monotherapy.

Previous studies have shown that the heat-shock protein 90 (*Hsp90*) was a molecular chaperone of *FLT3* and highly expressed in *FLT3*-ITD (+) AML and other tumors [14–16]. And as a molecular target in leukemic therapy, several small-molecule drugs that target the molecular chaperone *Hsp90* have been developed as potential anticancer agents over the past decade [17,18]. Here, we find out that a novel inhibitor of *Hsp90* named as IPI-504, which derives from the well-studied *Hsp90* inhibitor 17-allylamino-17-demethoxy-geldanamycin (17-AAG) [19,20], can effectively induce apoptosis on *FLT3*-ITD (+) cell lines and patient samples.

Therefore, in the present study, we examined whether HHT would strengthen the anti-leukemic effect when combined with the *Hsp90* inhibitor IPI504 in vitro and vivo. Simultaneously, we also explored the potential mechanisms for the synergistic effect of both two drugs.

Materials and Methods

Cell culture

The human AML cell lines THP-1, Kasumi-1 and MV4-11 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MOLM-13 was kindly provided by Prof. Jie Jin (Department of Hematology, The First Affiliated Hospital of Zhejiang University, Hangzhou, China). The cells were cultivated in RPMI 1640 medium (GIBCO, Bethesda, MD, USA) (THP-1, Kasumi-1) and IMDM (GIBCO, Bethesda, MD, USA) (MV4-11, MOLM-13) containing 10% fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 units/mL penicillin. All cells were maintained at 37°C with 5% CO₂.

Patient Samples and Ethics Statement

The human primary AML cells were isolated from patients with their informed consent according to the Declaration of Helsinki. All experiments were approved by the ethics committee of Second Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China).

Cell Viability and Apoptosis Assay

The assessment of cell viability and apoptosis were performed as before [12]. HHT was purchased from Nanjing Spring & Autumn Biological Engineering Co., Ltd, China. IPI504 was purchased from MCE (MedChemExpress, USA).

Cell Cycle Assay

Different treated cells were harvested and fixed with 75% ethanol overnight at -20°C, then washed with PBS and incubated with propidium iodide (PI) staining solution for 30 min. Cells were detected by flow cytometry and analyzed by ModFit LT5.0 (Verity Software House, USA).

Western Blotting

The immunoblotting was conducted as before [12]. The primary antibodies used were presented as follows, *FLT3*, p-AKT (Ser473), AKT, p-ERK (T202/Y204), Caspase-3, PARP, 4E-BP1 and p4E-BP1 (S65), which were all purchased from Cell Signaling Technology (Beverly, MA). ERK1/2, STAT5, p-STAT5 (Y694) and β-actin were obtained from Huabio (Hangzhou, China). p-*FLT3* (Y591), MCL-1, BCL-XL, BCL-2, CDK-2, CDK-4 and CDK-6 were purchased from Abcam (Cambridge, MA).

Colony Formation Assays

MV4-11 and MOLM-13 cells (600 cells per plate) were seeded in low-melting-point agarose, in the presence of 2.5 nM HHT and/or 5 nM IPI504, then reconstituted with 2×IMDM medium supplemented with 20% FBS. After two weeks, the colonies were visualized by staining with 0.1% crystal violet and then counted under light microscope (cells > 50 will be counted as a colony). The drug-treated colony formation efficiency was calculated through dividing it by control colony numbers.

Xenotransplantation and In Vivo Drug Treatment

All animal procedures were approved by the Institution's Ethics Committee. NSG (NOD/SCID/IL2Rγ^{-/-}) Mice at 6–7 weeks of age (Beijing Biocytogen Co., Ltd., China) were injected through tail veins with MOLM-13 (2 × 10⁵ cells) stably transduced with a luciferase reporter gene. Fourteen days after injection, the engrafted mice were treated with vehicle (1%DMSO in 200 µL PBS intraperitoneal), HHT (in 200 µL PBS per mouse, 0.5 mg/kg/d, intraperitoneal), and/or IPI504 (in 200 µL PBS, 50 mg/kg, every other day, intraperitoneal). And engraftment was assessed by intraperitoneal injection of luciferin (2 mg/mouse) (Promega, Madison, WI) after anesthesia (fentanyl/fluniasone and midazolam) and bioimaging of mice was performed at scheduled time points by using an in vivo IVIS 100 bioluminescence/optical imaging system (Xenogen, Alameda, CA).

Statistical Analysis

The statistical analyses were performed with IBM SPSS Statistics 20. The combination index (CI) value was calculated by CalcuSyn software (Biosoft, Cambridge, UK). The IC₅₀ was determined by GraphPad software. Survival analysis was performed by using the Kaplan-Meier method and differences in survival were analyzed by Log-rank test. Other differences were evaluated by t-test analysis of variance and *P* values <.05 were considered statistically significant. All results are shown as means ± SEM. **P* < .05; ***P* < .01; ****P* < .001.

Results

The Cytotoxic Effect of HHT and IPI504 on the AML Cells

In this study, we used the MTT assay to examine the viability of AML cells treated by HHT and IPI504, alone or combined. Briefly, AML cells were treated with increasing concentrations of HHT (1, 2, 4, 8, 16 and 32 nM) or IPI504 (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 µM) for 24 h and 48 h. DMSO was used as the control. We observed that

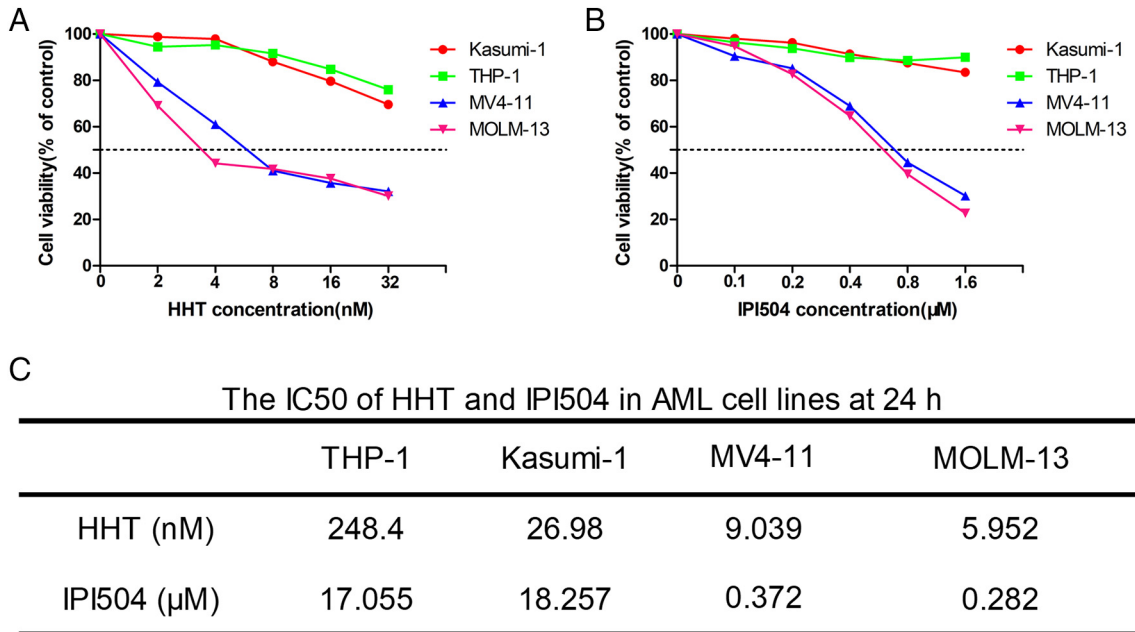


Figure 1. HHT and IPI-504 inhibit the growth of AML cell lines. (A and B) The cell viability analyzed by MTT assays in AML cell lines treated with increasing concentrations of HHT and IPI504 at 24 h. (C) The IC50 of HHT and IPI504 in AML cell lines at 24 h. The data are presented as mean ± SEM from at least three independent experiments.

FLT3-ITD (+) cell lines MV4-11 and MOLM-13 were significantly more susceptible to HHT or IPI504 than *FLT3*-ITD negative cell lines Kasumi-1 and THP-1 (Figure 1, A and B). Moreover, both HHT and IPI504 showed higher cytotoxicity in MOLM-13 than

that in MV4-11 cells, in which 24 h IC50 values of HHT and IPI504 for MV4-11 and MOLM-13 cells were 9.039 nM and 5.952 nM, 0.372 µM and 0.282 µM, respectively (Figure 1C). HHT or IPI504 treatment inhibited the cell proliferation in a dose and time

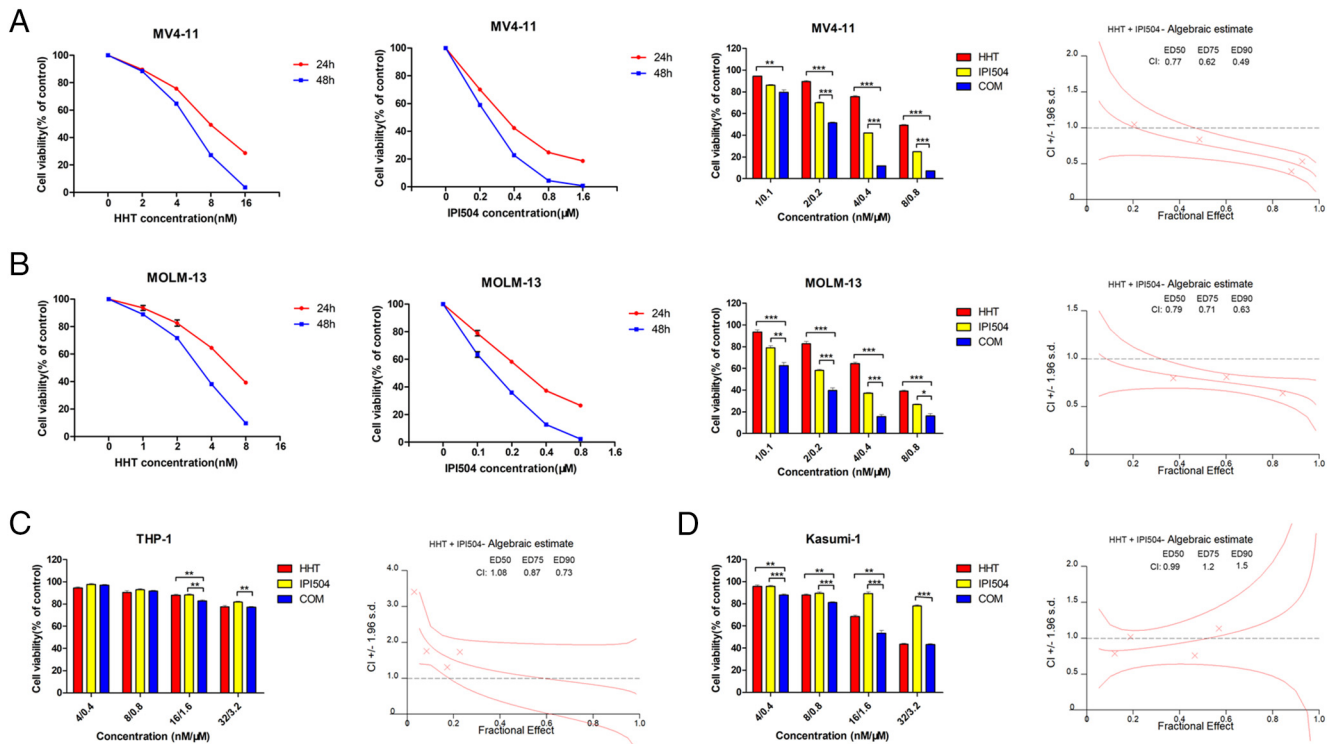


Figure 2. HHT and IPI-504 synergistically inhibit the growth of AML cell lines. The cell viability induced by HHT, IPI504 and HHT + IPI504 in a fixed ratio (1:100) in (A) MV4-11 cells, (B) MOLM-13 cells, (C) THP-1 cells and (D) Kasumi-1 cells at 24 h or 48 h, and the CI values at ED50, ED75 and ED90 were presented. (mean ± SEM, n=3, *P < .05, **P < .01, ***P < .001).

dependent manner. Notably, co-treatment with HHT and IPI504 exhibited a significant synergistic effect on *FLT3*-ITD positive cell lines (Figure 2, A and B) and primary AML cells (Figure 3, A and B), but no significant synergism was found in *FLT3*-wild type cell lines (Figures 2, C and D; 3, C and D). The IC₅₀ values of HHT and IPI504 for the primary samples were shown in Supplementary Table 1 and the clinical characteristics of primary samples were presented in Supplementary Table 3. The dose-effect curves were determined by calculusyn analyses. The primary samples combination index (CI) values were calculated according to the median effect method of Chou and Talala [21], which was presented in Supplementary Table 2. After 18 primary samples co-treated by HHT and IPI504 for 48 h, the CI values between the *FLT3*-ITD positive and the wild type were compared in Figure 3E. The CI value less than 1.0 meant a synergistic effect. All results were performed by three independent experiments. Taken together, these results suggested that HHT and the *Hsp90* inhibitor IPI504 exhibit potent cytotoxic effect and significant synergism when combined with both drugs in AML cell lines and primary samples.

HHT and IPI504 Induce Apoptosis in AML Cell Lines Harboring *FLT3*-ITD

The *FLT3*-ITD (+) cell lines MV4-11 and MOLM-13 were treated by HHT and/or IPI504 for 24 h and 48 h and then apoptosis was analyzed. We observed that combination of HHT and IPI504

resulted in a prominent augmentation in apoptosis of MV4-11 and MOLM-13 cells compared with the control (DMSO) or both drugs alone ($P < .05$) (Figure 4, A and B). Next, we performed western blot to analyze the relevant signaling molecules of apoptotic pathway. As shown in Figure 4C, the expression levels of cleaved PARP and cleaved caspase-3 were greatly increased both in MV4-11 and MOLM-13 cells after co-treated by HHT and IPI504 for 24 h. And the expression of pro-survival protein MCL-1 and BCL-XL were remarkably attenuated after cells exposed by HHT and IPI504 for 24 h. However, there was no obvious change in the expression level of BCL-2 (Figure 6C).

Effects of HHT Combined with IPI504 on Cell Cycle and Colony Formation

Next, we explored the effects of HHT, IPI504 or the combination of both on the cell cycle and colony formation in *FLT3*-ITD (+) cells. MV4-11 and MOLM-13 cells were treated with the indicated concentrations of HHT and/or IPI504 for 48 hours. As displayed in Figure 5, A and B, treatment of cells by low dose of HHT at 2 nM or IPI504 at 0.2 μ M, and it did not affect G₀/G₁ and S phase cells and apoptosis obviously. However, co-treatment with HHT at 2 nM and IPI504 at 0.2 μ M caused a prominent G₀/G₁ phase arrest, S phase decreasing and apoptosis increasing. We observed that in MV4-11 and MOLM-13, G₀/G₁ phase cells increased by 14.92% and 17.44%, respectively, S phase cells decreased by 15.23% and 13.09%,

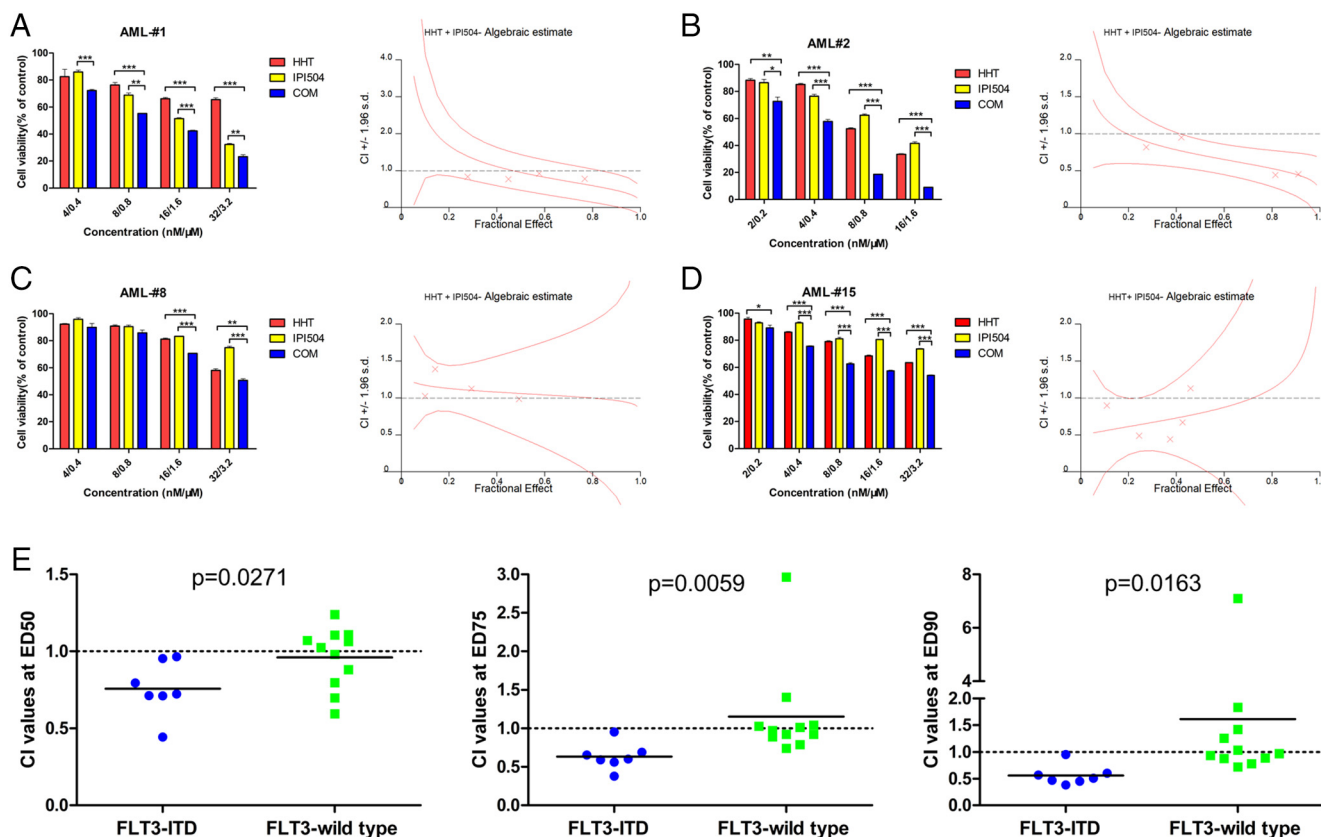


Figure 3. HHT and IPI504 inhibit the growth of primary AML cells. (A and B) The *FLT3*-ITD (+) primary AML cells and (C and D) *FLT3*-wild type primary AML cells were treated with HHT, IPI504 and HHT+IPI504 for 48 h. The rate of cell viability was measured by MTT assay. (E) The combination index (CI) values of 18 patient samples at the ED₅₀, ED₇₅ and ED₉₀ were showed (FLT3-ITD (+) n= 7; FLT3-wt n=11, solid bars represent the mean CI values for each group). The CI values were calculated by Calculusyn according to the median effect method of Chou and Talalay [21]. (mean \pm SEM, n=3, * $P < .05$, ** $P < .01$, *** $P < .001$)

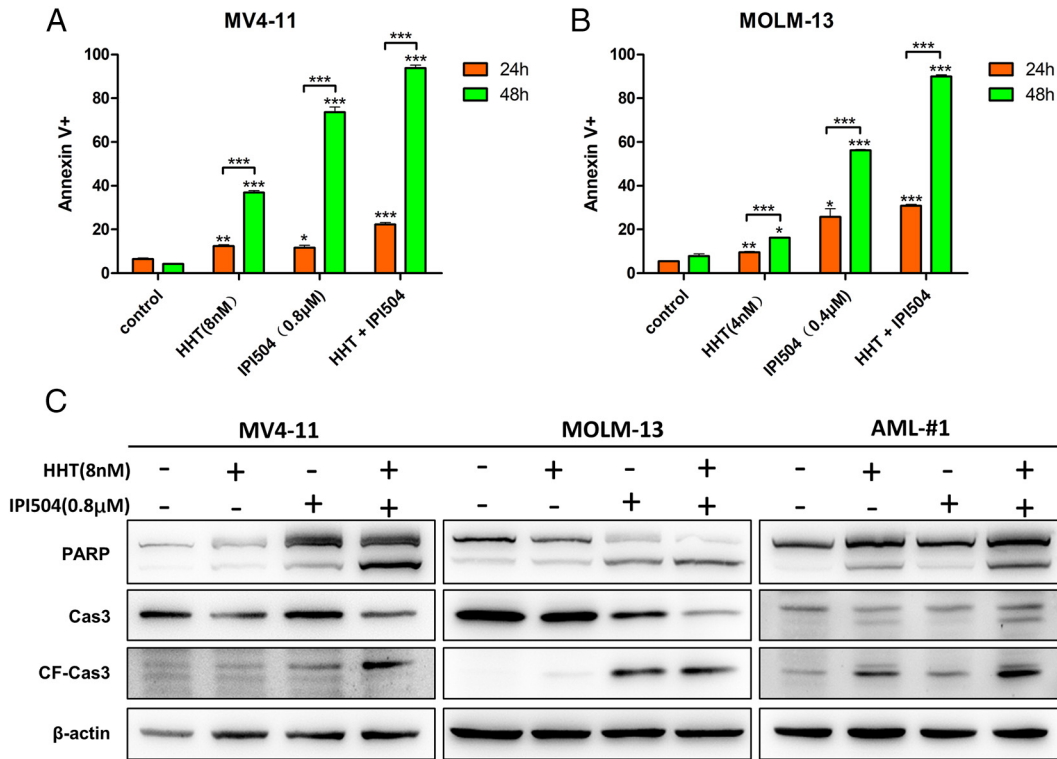


Figure 4. HHT and IPI504 synergistically induced apoptosis in AML cell lines harboring *FLT3*-ITD. (A and B) The *FLT3*-ITD mutant cell lines MV4-11 and MOLM-13 were exposed by HHT (8 nM or 4 nM) and/or IPI504 (0.8 μ M or 0.4 μ M) for 24 h and 48 h. Cells were harvested and co-stained with Annexin V and PI before apoptosis was analyzed by flow cytometry (mean \pm SEM, n=3, **P* < .05, ***P* < .01, ****P* < .001). (C) After treated with HHT (8 nM) and/or IPI504 (0.8 μ M) for 24 h, MV4-11, MOLM-13 and primary cell lysates were subjected to western blot analysis using the PARP, caspase3 and cleaved caspase3 antibodies, β -actin was displayed as a loading control.

respectively. Consistent with above observations, this co-treatment also increased apoptotic cells by 28.72% and 36.93% in MV4-11 and MOLM-13 cells, respectively. We also performed colony formation assay to evaluate the long-term effects of HHT and IPI504. As shown in Figure 5, C and D, low dose of HHT (2.5 nM) or IPI504 (5 nM) alone exhibited 44.00% or 49.76% and 53.00% or 41.20% of colony formation inhibition, respectively, for MV4-11 or MOLM-13 cells, whereas combination of HHT and IPI504 at the same dose caused 99.2% or 73.87% of colony formation inhibition, suggesting that the combination of HHT and IPI504 at low dose significantly enhance cell killing ability.

In western blotting assay, MV4-11 and MOLM-13 cells treated with the indicated concentration of HHT and IPI504 for 24 hours displayed an apparent down-regulation in cyclin dependent kinase 2, 4 and 6 (CDK-2, CDK-4, CDK-6) proteins, which represented the G1 phase arrest of cell cycle (Figure 6D). Taking all the results into consideration, it further confirmed the synergistic anti-proliferative effects of HHT combined with IPI504 in *FLT3*-ITD (+) AML.

HHT and IPI504 Down-Regulated the *FLT3*-ITD Mutant and Its Oncogenic Signaling Pathways

It is well known that the mutation of *FLT3*-ITD causes aberrant activation of downstream kinases such as *STAT5*, *AKT* and *MAPK/ERK* [22–24]. To determine whether the combination of HHT and IPI504 would synergistically affect *FLT3*-ITD mutant protein, leukemia cells were treated with HHT at 8 nM or IPI504 at 0.8 μ M for 24 hours and then collected for analysis of *FLT3*-ITD using western blot. We found the combination of HHT and IPI504 synergistically

reduced the protein levels of both total *FLT3* and phosphorylated *FLT3* (p-*FLT3*) in MV4-11, MOLM-13 and primary sample cells (Figure 6A). To determine whether the combination of HHT and IPI504 would affect *FLT3*-ITD downstream molecules such as *STAT5*, *AKT* and *MAPK/ERK*, MV4-11 and MOLM-13 cell lines were treated with HHT (8 nM) plus IPI504 (0.8 μ M) for 6 h and then collected for western blotting analysis. We observed that the combination of HHT and IPI504 greatly reduced phosphorylated protein levels of *STAT5* (p-*STAT5*), *AKT* (p-*AKT*) and *ERK1/2* (p-*ERK1/2*), but it did not affect their total protein levels. In line with this, similar results were also observed in primary AML samples (Figure 6B). Because 4E-binding protein 1 (*4E-BP1*) played a key role in protein synthesis, whose phosphorylation loosened its inhibition on eukaryotic translation initiation factor 4E (*eIF4E*), which facilitated protein translation initiation [25], we next evaluated the effect of this combination on p4E-BP1 and found out that phosphorylated 4E-BP1 (p4E-BP1) was obviously attenuated in MV4-11 and MOLM-13 cells treated by HHT (8 nM) combined with IPI504 (0.8 μ M) for 24 h (Figure 6A). Therefore, we have reasons to believe that HHT and IPI504 synergistically abrogate proliferation and induce apoptosis through regulating the major molecular target *FLT3* and then lead to an inhibition in its downstream signaling cascades on *FLT3*-ITD mutant acute myeloid leukemia.

Combination of HHT and IPI504 Enhances Anti-Leukemia In Vivo

To validate the synergic anti-leukemia effect of HHT and IPI504 in vivo, we engrafted NSG mice with MOLM-13 cell line expressing

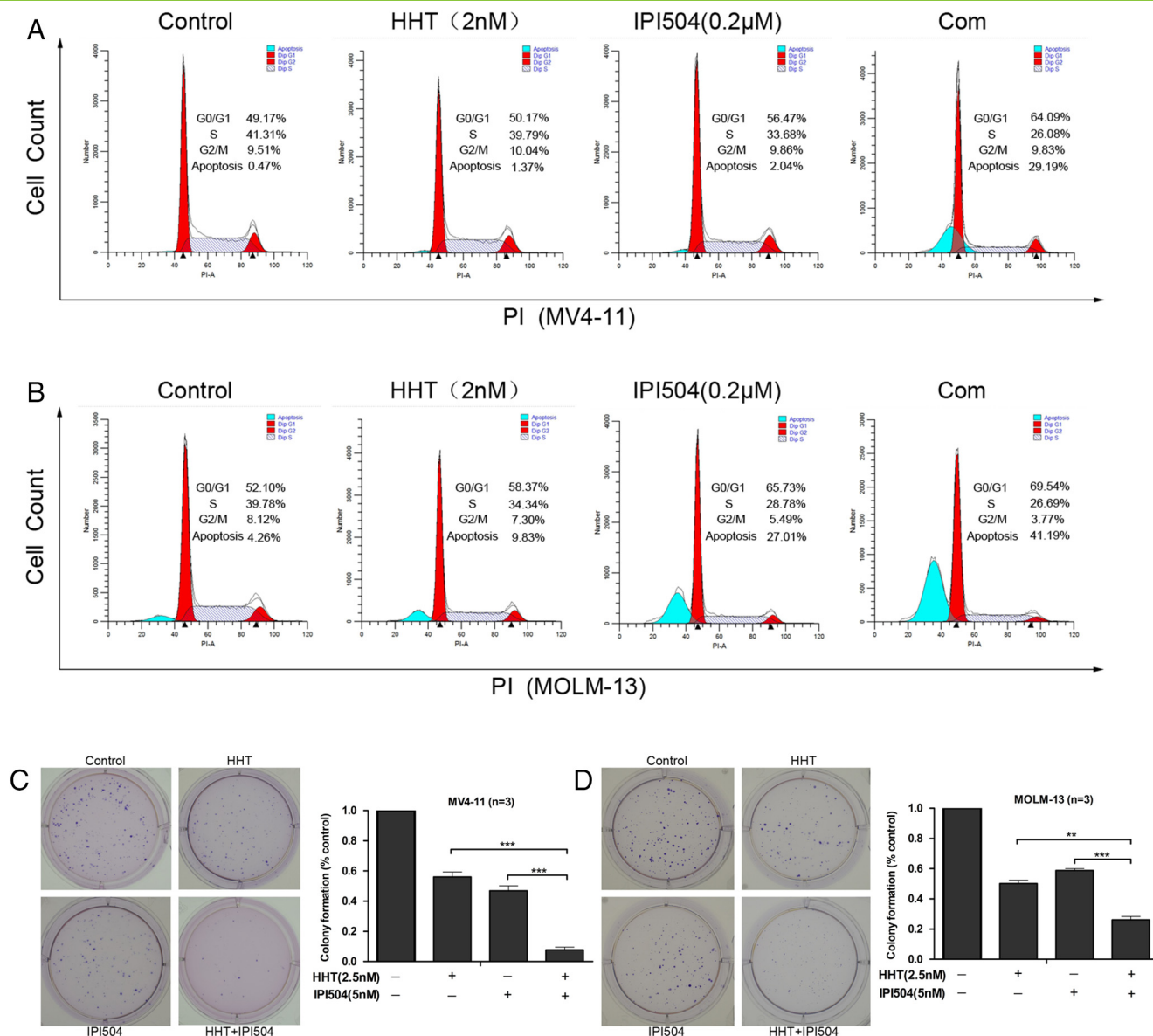


Figure 5. Effects of HHT, IPI504, HHT+ IPI504 on cell cycle and colony formation. (A and B) The representative flow cytometry analysis for PI staining in MV4-11 and MOLM-13 cell lines treated with HHT, IPI504 or HHT+IPI504 at the indicated concentrations for 48 h are depicted. (C and D) Representative images of colonies formation for MV4-11 and MOLM-13 cells under HHT (2.5 nM), IPI504 (5 nM) and HHT+IPI504 treatment about 14 days. Quantification of colony counts (% of control) with different group treatment as indicated. (mean \pm SEM, n=3, * P < .05, ** P < .01, *** P < .001)

the luciferase reporter gene. After 14 days, leukemic mice were intraperitoneally injected daily with vehicle, HHT (0.5 mg/kg per day), IPI504 (50 mg/kg, every other day), or the combination. Compared with those receiving vehicle, HHT or IPI504 monotherapy, mice treated with HHT and IPI504 showed less engraftment of MOLM-13 and a slower increase of leukemic signal up to 35 days (Figure 7, A and B), which was consistent with the results in vitro. Meanwhile, the survival time of mice treated with HHT+IPI504 was longer than those receiving vehicle, HHT or IPI504 monotherapy. And we also analyzed the statistical significances between each survival curve and vehicle by Log-rank test which P value were 0.004 (HHT compared to vehicle), 0.017 (IPI504 compared to vehicle) and 0.001 (HHT+IPI504 compared to vehicle), respectively (Figure 7D). Notably, only the group of mice treated with the combination of

HHT and IPI504 showed an increasing body weight up to 35 days (Figure 7C), suggesting that the combination of HHT and IPI504 might improve the quality of life for AML. As described above, our data further proves the synergistic anti-leukemia effect of HHT plus the *Hsp90* inhibitor IPI504 in vivo, which provides a firm base in future clinical therapy for *FLT3*-ITD (+) acute myeloid leukemia.

Discussion

In this report, we demonstrated that the combination of HHT and the *Hsp90* inhibitor IPI504 exhibited a significant anti-leukemic action on *FLT3*-ITD (+) AML in vitro and in vivo. Mechanistically, the combination of HHT and IPI504 synergistically inhibited *FLT3* protein and its downstream *STAT5*, *AKT*, *ERK* and *4E-BP1*, leading to apoptosis and cell arresting at G1.

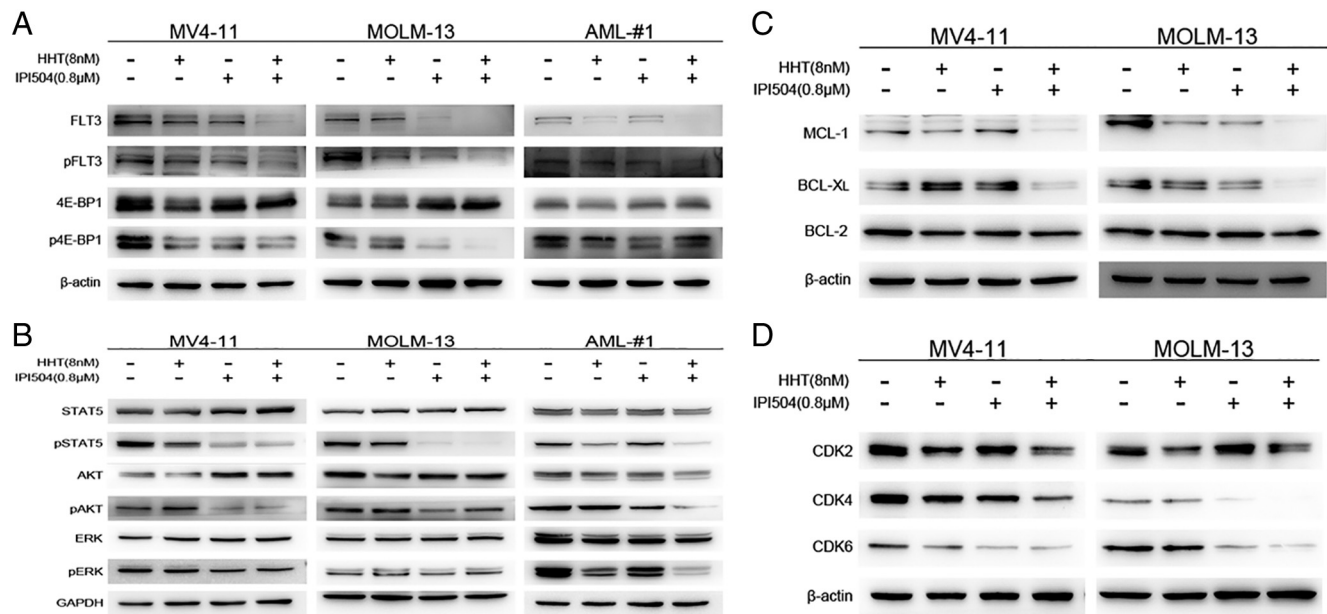


Figure 6. HHT + IPI-504 synergistically inhibit major targets and oncogenic signaling pathways. (A-D) Western blots exhibiting expression levels of total FLT3, phosphorylated FLT3 (pFLT3) and its downstream signaling proteins or targets in MV4-11, MOLM-13 or primary sample treated with vehicle, HHT (8 nM) and/or IPI504 (0.8 μ M) for (A, C and D) 24 hours and (B) 6 hours, with GAPDH or β -actin being the loading control.

Acute myeloid leukemia with *FLT3*-ITD mutations are general associated with decreased disease-free survival (DFS) and poor overall survival (OS) [26]. Although *FLT3* tyrosine kinase inhibitors (TKI) have been developed to treat *FLT3*-mutant AML patients, limited efficacy was observed because of relapse and rapid drug resistance [27]. Recently, we and others observed that HHT behaved a sensitive cytotoxic effect on *FLT3*-ITD (+) AML cells [28,29]. As many works highlighted, *Hsp90* is a molecular chaperone of mutant *FLT3* which is involved in holding conformation, stabilization and function of oncoproteins. The *Hsp90* inhibitors have been developed to selectively kill *FLT3*-mutant cells in AML [14,16,30–32]. We therefore speculated that whether anti-leukemic effect might be enhanced when combining HHT with the *Hsp90* inhibitor. As expected, HHT and the *Hsp90* inhibitor IPI504 synergistically inhibited the proliferation and clonogenic growth of human *FLT3*-ITD (+) AML cell lines. And we also evaluated the effects on primary AML patient cells. We observed that 39%(7/18) *FLT3*-ITD mutant patient samples presented a significant synergistic effect after performed by HHT + IPI504 for 48 h. In addition, the combination of HHT with IPI504 also exhibited great synergistic action for several *FLT3*-wild type AML cells, implying other underlying mechanisms may be involved in the therapy of both two drugs which need further study (Supplementary Figure 2). More importantly, similar results were also observed in three relapsed/refractory AML samples (Supplementary Figure 1, B, D and E). Consistent with in vitro observations, the combination of HHT with IPI504 not only significantly reduced tumor burden but also prolonged tumor-bearing mouse survival time as compared to the vehicle or monotherapy in *FLT3*-ITD (+) AML xenograft mouse models.

Evasion of apoptosis, an important hallmark of cancer, is caused by activation of anti-apoptotic molecules of the BCL-2 protein family [33–35]. And aberrant activation of BCL-2 members such as MCL-1, BCL-XL and BCL-2 were involved in anti-apoptosis and drug

resistance in *FLT3*-ITD mutant AML [36–38]. Our studies reveal that the combination of HHT with IPI504 potently inhibits the expressions of anti-apoptotic molecules MCL-1 and BCL-XL, which may explain why the combination of HHT with IPI504 efficiently kill relapsed/refractory AML cells. It is as well know that *FLT3*-ITD mutations lead to loss of autoinhibitory function for *FLT3* kinase, which then results in constitutive activation of its downstream signaling pathways, including the *JAK/STAT5*, *PI3K/AKT* and *RAS/MAPK* [23,26,39]. In the study, we demonstrate that HHT combined with IPI504 synergistically decrease the expression of total and phosphorylated forms of FLT3 as well as its downstream members such as p-STAT5, p-AKT and p-ERK in human *FLT3*-ITD (+) AML cell lines and patient samples.

Since *JAK/STAT5* signaling is significantly activated in leukemic stem cells of high-risk AML patients [40], the *STAT5* activation induced by *FLT3*-ITD confers drug resistance by protecting the *mTOR/4EBP1/MCL-1* pathway [41]. Interestingly, our results show that the combination of HHT with IPI504 could disrupt the *mTOR/4EBP1/MCL-1* pathway with inhibition of p-4EBP1, MCL-1 and p-STAT5, suggesting that the combination of HHT with the *Hsp90* inhibitor IPI504 may affect leukemic stem cells.

Conclusions

Taken together, in this study, we mainly investigated the synergistic anti-leukemic effect between HHT and the *Hsp90* inhibitor IPI504 on *FLT3*-ITD mutant cells and then further confirmed by primary AML samples as well as orthotopic xenograft models. All of the results provide a solid base for future personal therapy with novel therapeutic regimen HHT + *Hsp90* inhibitors in de novo or relapsed/refractory AML patients.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tranon.2019.02.016>.

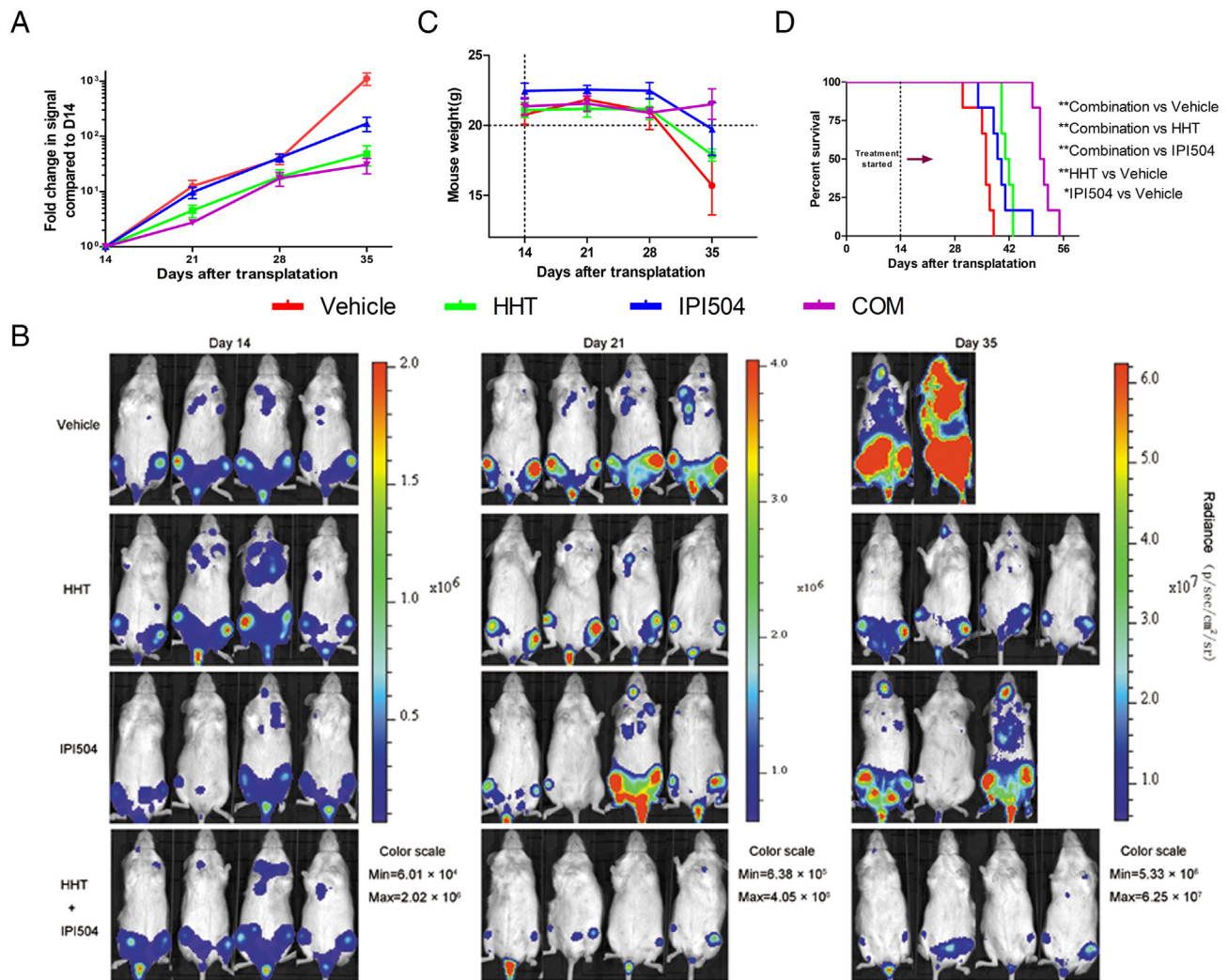


Figure 7. In vivo effect of HHT and IPI504 combination. (A, B) The trend of bioluminescence intensities and xenogen images of NSG mice transplanted with luciferase-labeled MOLM-13 cells before (day 14) and after treatment with vehicle ($n = 6$), HHT ($n = 6$), IPI504 ($n = 6$), and their combination ($n = 6$). (C) The change of body weight of mice treated with vehicle, HHT, IPI504 and combination of both. (D) Kaplan-Meier survival curves were analyzed for the mice and Log-rank test was used to calculate the statistical significances with HHT compared to vehicle ($P = .004$), IPI504 compared to vehicle ($P = .017$) and HHT+IPI504 compared to vehicle ($P = .001$), respectively.

Declarations

Ethics Approval and Consent to Participate

The human primary AML cells were isolated from patients with their informed consent according to the Declaration of Helsinki. This study was approved by the ethics committee of Second Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China).

Consent for Publication

All the patients involved in the study have agreed to publish their individual data. Authors have reviewed and approved the manuscript for submission.

Conflict of Interest

The authors have no conflicts to declare.

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Authors' Contributions

R.Z.X and J.P.S conceived of the study, initiated, designed, and supervised the experiments and wrote the manuscript. Z.X.W, H.F.Z and Q.F.Y designed and performed experiments and wrote the manuscript. X.Z.Z, X.D.J, X.Y.L, Y.X, L.L.Y, B.W.W, A.M, L.Z, X. B.X, Y.L and R.L.G performed experiments.

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