Introduction
Acute leukemia (AL) occurs when primitive or immature white blood cells grow uncontrollably, disturbing normal hematopoiesis. According to the pathogenic cellular origin, AL can be divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). ALL is the most common pediatric cancer type[1], whereas AML is more prevalent among adults[2, 3]. AL accounts for nearly 27 000 diagnoses and 12 000 deaths yearly in the US[4]. It progresses quickly and needs immediate treatment. With the progress and availability of combination chemotherapy and hematopoietic cell transplantation, the complete response (CR) rate and overall cure rate of AL patients have improved markedly[5]. However, ~20% of ALL patients still experience relapse, and only a minority of them will survive[6]; more than 60% of AML patients cannot be cured[7]. Consequently, there is still an urgent need to seek more effective therapy.

Cyclin-dependent kinases (CDKs) are a family of serine/threonine protein kinases whose activity depends on binding to specific cyclin partners[8]. Some CDKs (CDK1, CDK2, CDK4 and CDK6) have a role in regulating cell cycle transition[9]. Another family of CDKs that regulates transcription was also identified and includes CDK7 and CDK9[8, 10]. CDK7...
is an integral part of transcription factor II H (TFIIH), which phosphorylates the serine 5 (ser5) residue of RNA polymerase II (RNA pol II) to facilitate transcription initiation. CDK9, as the catalytic part of elongation factor P-TEFb, phosphorylates the serine 2 (ser2) residue of RNA pol II, which is required for efficient transcription elongation. Owing to the central role of CDKs in the control of cell division, it is not surprising that cancers exhibit some features that derange the normal controls over the cell cycle. Additionally, over the past 20 years, numerous drugs that target cell cycle-related CDKs have emerged and have been tested in clinical trials. Recent studies have indicated that the inhibition of transcription-related CDK9 might contribute predominantly to the anti-tumor activity of most clinical CDK inhibitors, such as flavopiridol and dinaciclib. CDK9 is essential for the efficient transcription of numerous genes, especially those encoding short-lived anti-apoptotic proteins, such as Mcl-1, XIAP and Bcl-2. Additionally, CDK9 inhibition through siRNA or inhibitors induces potent cell apoptosis in many tumor cell lines.

LS-007, also known as CDKI-73, is one of the most potent CDK9 inhibitors identified to date. Previous studies have shown that LS-007 had little toxicity on normal T- and B-cells while exhibiting potent efficiency alone or in combination with fludarabine against chronic lymphocytic leukemia (CLL) primary cells. In addition, LS-007 exerted a potent anti-tumor effect in ovarian cancer A2780 cell lines through simultaneously targeting CDK9 and its downstream Mnk-eIF4E pathways. These results provided convincing evidence that LS-007 may be a promising anti-tumor candidate deserving further development. CDK9 is often abnormally activated in lymphoid or myeloid leukemia, especially in those with rearranged mixed lineage leukemias. Therefore, in this study, we evaluated the effects of LS-007 in AL cell lines and primary AL blasts as both a single agent and in combination with the BH3 mimetic ABT-199. Our data demonstrated that LS-007 exhibited activity as a single agent and exhibited a remarkable degree of synergy with ABT-199 in AL.

Materials and methods

Compounds
LS-007 was kindly provided by Prof Shu-dong WANG (University of South Australia, Australia). Flavopiridol HCl (S2679) and ABT-199 (S8048) were purchased from Selleck (Houston, TX, USA). The compounds were dissolved in DMSO at 10 mmol/L and stored at -80°C.

In vitro kinase assay
The inhibition of CDKs was measured using mobility shift assays by Shanghai ChemPartner (Shanghai, China). The inhibition of other kinases was measured by WuXi AppTec (Shanghai, China) or by enzyme immunosorbent assays.

Cell culture
The human acute leukemia cell lines CCRF-CEM, U937, and Molt-4 were purchased from the American Type Culture Collection (Manassas, VA, USA). The HL-60 and THP-1 cell lines were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Jurkat cells were kindly gifted by Prof Jian-ping ZUO (SIMM, Shanghai, China). All of the cells were cultured in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 4.5 g/L glucose, 0.11 g/L sodium pyruvate, and 10% FBS (Gibco, Grand Island, NY, USA) and maintained at 37°C and in 5% CO₂.

Proliferation assays
Cells were seeded at the appropriate density in 96-well plates and cultured for 2 h before exposure to increasing doses of LS-007 or flavopiridol. After 72 h of incubation, 10% 1.5 mg/mL resazurin (Sigma, St Louis, MO, USA) was added, and the cells were incubated for another 2–4 h. Fluorescence intensity was detected at an excitation wavelength range of 540±35 nm and an emission wavelength range of 590±35 nm using a Synergy 2 Multi-Mode Microplate Reader (BioTek, Burlington, VT, USA). The dosages corresponding to the half-maximal inhibition (IC₅₀) were calculated using SoftMax Pro-based non-linear 4-parameter regression analysis. IC₅₀ (mean±SD) values are presented as histograms.

Apoptosis assays
An Annexin V-FITC/PI double staining apoptosis detection kit (KeyGEN Biotech, Nanjing, China) was used to quantitatively measure the percentage of cells in apoptosis. Cells (5×10⁶ per well in 6-well plates) were incubated in media alone or with various concentrations of LS-007 (or flavopiridol) for 24 h or were incubated with 0.2 μmol/L of the test compounds for 12, 24, or 48 h. The samples were collected, stained, and detected using a FACS Calibur instrument (BD Biosciences, Franklin Lake, NJ, USA). The results were analyzed using BD CellQuest Pro software.

Western blotting
Cells were cultured for 2 or 24 h with various concentrations of LS-007 (or flavopiridol), harvested, washed and lysed with RIPA lysis buffer (strong) (Beyotime, HaiMen, China). The protein concentration of lysates was unified, mixed with gel electrophoresis loading buffer, and boiled for 5–10 min. Subsequently, the cell lysates were resolved using SDS–PAGE, were transferred to nitrocellulose membranes and then were probed using the following primary antibodies: RNA pol II, p-RNA pol II ser2, p-RNA pol II ser5 (Abcam, Cambridge, Cambridgeshire, UK); RB, p-RB ser780, p-RB ser807/811, PP1α, p-PP1α thr320, β-tubulin, PARP, XIAP, Mcl-1, Bcl-2, cleaved-caspase3 (Cell Signaling, Boston, MA, USA); p-RB thr821 (Gene Tex, San Antonio, TX, USA). Next, the membranes were incubated with anti-mouse or anti-rabbit secondary antibodies conjugated to horseradish peroxidase, and the proteins were detected using an enhanced chemiluminescence detection reagent (Thermo Fisher Scientific, Rockford, IL, USA) and ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, Buckinghamshire, UK).
Real-time PCR
Cells were left untreated or were exposed to LS-007 (or flavopiridol) for 6 h. Total cellular RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into complementary DNA using a PrimeScript™ RT reagent kit (Takara, Otsu, Shiga, Japan). Real-time PCR was then performed using Applied Biosystems Viia™7 (Waltham, MA, USA) and SYBR-Green Master mix (Takara, Otsu, Shiga, Japan) using the following primers (Table 1).

The reaction parameters were as follows: 95 °C for 5 min followed by 41 cycles of 95°C for 5 s and 60 °C for 34 s. All samples, including the controls, were tested in duplicate.

Table 1. Primers sequences (5′–3′).

| Gene      | Forward primer sequence | Reverse primer sequence |
|-----------|-------------------------|-------------------------|
| Mcl-1     | AAGGCAAGTGGCAAGAGGA     | TTAATGAATTCGGCGGGTAA    |
| XIAP      | TGGCATTTCCAGATGGGGGC    | TAGGACTTGCCACCTTTTCGC   |
| GAPDH     | GAAGGGTGAAGGTCGGAGT     | GAAGGTGATGGATGGAGT     |

Primary lymphocyte isolation
Bone marrow samples from ALL and AML pediatric patients were obtained according to the provision of informed consent using the guidelines approved by the Committee on the Use of Human Subjects in Research at Shanghai Children’s Hospital and the Children’s Hospital of Fudan University. Mononuclear cells were separated from freshly collected bone marrow using Ficoll-Paque Plus (GE Healthcare, Little Chalfont, Buckinghamshire, UK) density gradient centrifugation following the manufacturer’s instructions. Subsequently, enriched cells were maintained in RPMI-1640 medium supplemented with 10% FBS and were immediately seeded in 96-well plates for viability tests after 72 h of treatment with LS-007/flavopiridol. Additionally, the cells were seeded in 12-well plates and exposed to LS-007/flavopiridol for 48 h (for apoptosis detection) or 12 h (for protein expression detection).

Statistical analysis
Combination index (CI) values were calculated using the IC_{50} ratio (50% of cells that underwent apoptosis at that dose) obtained with LS-007 alone to that obtained with LS-007 plus ABT-199 as previously described\[24\]. Student’s t test was applied for statistical comparison using GraphPad Prism 6. Unless otherwise indicated, the results are expressed as the mean±SD from at least three independent experiments. Differences were considered to have statistically significant at \(P<0.05\), \(P<0.01\) vs CON; \(P<0.01\) vs ABT-199 and \(P<0.01\) vs LS-007.

Results
LS-007 potently inhibits the proliferation of AL cell lines and patient-derived AL blasts
The activity of LS-007 against human acute leukemia was evaluated in a panel of established AL cell lines, including ALL and AML cell lines. As shown in Figure 1A, LS-007 exhibited potent cytotoxicity against these cell lines, with IC_{50} values ranging from 100–200 nmol/L, and the mean IC_{50} for all of the tested leukemia cell lines was 163±48 nmol/L, which was similar to that of flavopiridol (147±25 nmol/L).

Next, bone marrow samples from 21 ALL and 15 AML pediatric patients were also tested to obtain results that are more clinically relevant. Lymphocytes were separated using Ficoll-Paque PLUS for corresponding experiments\[25\]. Similarly to the effects on cell lines, LS-007 greatly affected the survival of all of these patients-derived lymphocytes, with a mean LD_{50} value of 67 nmol/L against ALL (Figure 1B) and 102 nmol/L against AML (Figure 1C) cells, values that were slightly lower than those for flavopiridol (LD_{50} of 100 nmol/L, 112 nmol/L, respectively) (Figure 1B, 1C). It was noteworthy that LS-007 also exhibited cytotoxicity in one of two relapsed ALL samples.
with an LD₅₀ value of 5 nmol/L, although this result needs to be confirmed.

**LS-007 is a potent and selective inhibitor of CDK**

Inhibition of the kinase activity of various cyclin/CDKs was examined using a mobility shift assay at the molecular level. Similar to previously reported data[19], we also demonstrated that LS-007 was a potent CDK9 inhibitor, with an IC₅₀ value of 5.78 nmol/L. Additionally, LS-007 inhibited CDK2, CDK1 and CDK4 with IC₅₀ values of 3.27, 8.17 and 8.18 nmol/L, respectively. Compared to flavopiridol, LS-007 was an equally potent inhibitor of CDK9 but exhibited 1.8-, 2.6- and 17-fold stronger inhibition of CDK1, CDK4 and CDK2, respectively. LS-007 also inhibited CDK6 and CDK7 (IC₅₀: 37.68 and 134.26 nmol/L, respectively) but was much weaker than the other tested CDKs (Table 2). The efficacy against CDK8 was also measured; only a 28% inhibitory rate was found at 100 nmol/L LS-007 (Table 3).

**LS-007 inhibits cellular CDK activity**

We next investigated the effect of LS-007 on cellular CDK activity in two AL cell lines: HL-60 and CCRF-CEM. As illustrated in Figure 2, ser2 phosphorylation of the carboxy-terminal domain (CTD) of RNA pol II was attenuated upon exposure to 0.1 μmol/L of LS-007 for 2 h and almost completely disappeared at 0.2 μmol/L, indicating remarkable cellular CDK9 inhibition. Moreover, compared with control-treated cells, LS-007 also attenuated the phosphorylation of PP1α (thr320) and RNA pol II CTD (ser5) in a concentration-dependent manner (Figure 2A). There was slightly greater inhibition of ser2 phosphorylation than that of ser5 and PP1α, consistent with a lower IC₅₀ of CDK9. However, we also observed that this compound elicited no effect on the phosphorylation of RB at concentrations within the 0.1-0.4 μmol/L range, indicating that LS-007 negligibly impairs CDK2/CDK4/CDK6 activity at concentrations less than 0.4 μmol/L at the cellular level (Figure 2). Significant suppression of phosphorylation of RNA pol II (ser2) was also found in primary cells treated with LS-007, indicating that LS-007 potently attenuated cellular CDK9 activity in primary cells (Figure 3F).

**LS-007 induces dose- and time-dependent apoptosis in established AL cell lines and patient-derived AL blasts**

The mechanism of LS-007-induced cell killing was confirmed to be through apoptosis (Figure 3A–3C), which was mediated

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**Table 2.** CDK inhibitory activity for LS-007.

| Kinase | IC₅₀ (nmol/L) | LS-007 | Flavopiridol |
|-------|---------------|--------|-------------|
| CDK1  | 8.17±0.07     | 14.97±0.74 |            |
| CDK2  | 3.27±0.15     | 52.02±3.81 |            |
| CDK4  | 8.18±0.58     | 20.66±1.14 |            |
| CDK6  | 37.68±0.81    | 170.40±4.80 |           |
| CDK7  | 134.26±1.29   | 199.17±4.58 |           |
| CDK9  | 5.78±0.05     | 5.34±0.20  |            |

**Table 3.** Inhibitory activity on non-CDK kinases for LS-007.

| Kinase name | 10 μmol/L inhibitory rate (%) | 100 nmol/L inhibitory rate (%) |
|-------------|-------------------------------|-------------------------------|
| ABL1        | 84                            | 2                             |
| AKT1        | 17                            | 0                             |
| AKT2        | 36                            | -1                            |
| AμRA        | 100                           | 46                            |
| AMMKB       | 98                            | 13                            |
| AXL         | 64                            | -2                            |
| BMX         | 9                             | -1                            |
| BTK         | 69                            | -20                           |
| CAMK1D      | 45                            | 2                             |
| CDK8        | 98                            | 28                            |
| CHEK1       | 28                            | 2                             |
| CHEK2       | 71                            | 2                             |
| CLK1        | 103                           | 41                            |
| c-KiT       | 33                            | 17                            |
| EGFR        | 17                            | 17.7                          |
| EGFR/T790M/L858R | 7                  |                               |
| EPHA2       | 58                            | 8.1                           |
| ErbB2       | 0                             |                               |
| ErbB4       | 66                            | 2.3                           |
| ERK2        | 21                            | 0                             |
| Fli-1       | 77                            | 29.6                          |
| GRK2        | 5                             | 0                             |
| GSK3β       | 107                           | 55                            |
| IGF1R       | 54                            | 17.7                          |
| JAK1        | 98                            | 40                            |
| JAK2        | 96                            | 16                            |
| JAK3        | 97                            | 25                            |
| JNK1        | 65                            | 2                             |
| Lck         | 44                            | 0                             |
| MST4        | 62                            | -3                            |
| p38α        | 6                             | -1                            |
| PAK2        | 17                            | 0                             |
| PDK1        | 19                            | 3                             |
| PDGFR-α     | 3                             |                               |
| PDGFR-β     | 10                            |                               |
| PI3Kβ       | 45                            | 1                             |
| PKA         | 46                            | -3                            |
| PLK1        | 31                            | 2                             |
| RET         | 47                            |                               |
| RON         | 37                            |                               |
| TAK1        | 95                            | 32                            |
| TEC         | 20                            | 5                             |
in a concentration- and time-dependent manner, as indicated by flow cytometric analysis using Annexin V/PI double staining. After treatment with 0.1 μmol/L LS-007 for 24 h, approximately 20% of HL-60 cells underwent apoptosis. Additionally, 80% of cells with well-characterized apoptosis were observed at the concentration of 0.4 μmol/L (Figure 3A). Treatment with 0.2 μmol/L LS-007 significantly increased apoptotic cells compared with control cells within 12 h, and this population continued to increase over time (Figure 3B). The apoptotic cleavage of poly (ADP-ribose) polymerase (PARP) and caspase 3 were also increased in a dose-dependent manner by LS-007 (Figure 3D, 3F). In addition, increased numbers of apoptotic cells were observed in 5 tested primary leukemia samples that were exposed to 0.2 or 0.4 μmol/L LS-007 for 48 h (Figure 3C). Collectively, these findings suggest that LS-007 induces apoptosis in AL cells.

LS-007 down-regulates both the transcriptional and protein levels of XIAP and Mcl-1

Inhibition of transcriptional CDKs may induce cell death through the down-regulation of several short-lived proteins, including the anti-apoptotic proteins Mcl-1 and XIAP.[24] We therefore examined the change in Mcl-1, XIAP and Bcl-2. Indeed, the expression of Mcl-1 and XIAP was dose-dependently decreased after treatment with LS-007 (Figure 3D, 3F), and this effect was mediated by significant inhibition at the transcription level (Figure 3E). However, no reduction in the Bcl-2 protein level was observed following exposure to LS-007. The above results re-emphasized that LS-007 inhibits CDK9 activity and is efficient in both cultured acute leukemia cell lines and primary leukemia lymphocytes.

The combination of LS-007 and ABT-199 synergistically induces AL cell apoptosis

ABT-199 (venetoclax) is currently being evaluated in clinical trials for the treatment of leukemia. It is a highly potent, orally bioavailable Bcl-2-selective inhibitor and shows promise for the treatment of Bcl-2-dependent hematological malignancies.[27–31] Therefore, we explored whether there is a synergistic interaction between LS-007 and ABT-199 against AL cells. Three human AL cell lines, HL-60, CCRF-CEM and Molt-4, were treated with increasing doses of LS-007 alone or concurrently with ABT-199. The concentration of ABT-199 was determined from preliminary experiments. At the indicated concentration of ABT-199 alone, compared with the control, a 10%–20% inhibitory rate was achieved in HL-60, Molt-4 and CCRF-CEM cells. The combination of LS-007 and ABT-199 produced marked synergistic apoptosis induction against these cell lines, demonstrated by an obvious left shift of the alive-dose curves. We also determined the combination therapy effect by calculating the combination index (CI). CI values were calculated by the ratio of concentrations inducing 50% cellular apoptosis obtained with LS-007 combined with ABT-199 to the ratio obtained with LS-007 alone as described previously.[24] The mean CI values from three independent experiments were 0.44±0.03, 0.61±0.17 and 0.54±0.13 for HL-60, Molt-4 and CCRF-CEM cells, respectively (Figure 4A–4C).

The Annexin V/PI assay further revealed that LS-007 or ABT-199 alone weakly increased the accumulation of apoptosis in vitro. However, combined therapy resulted in a dramatic increase in apoptotic cells (Figure 4D). Consistent with this result, increased cleaved PARP and caspase 3 were also observed (Figure 4E). Next, the effects of combination therapy on apoptosis signaling were assayed. As expected, ABT-199 or LS-007 alone modestly reduced XIAP expression, whereas the maximal suppression of XIAP was observed in dual-treated cells. Up-regulation of Mcl-1 may mediate resistance to Bcl-2 inhibitors in many tumors.[33] In the present study, ABT-199 alone slightly elevated the level of Mcl-1, and LS-007 reversed
the up-regulation in dual-treated cells. However, no alteration in Bcl-2 was observed using the two drugs alone or combined.

**Discussion**

Given the key role of CDKs in both cell cycle regulation and cellular transcription processes, which are frequently altered in malignant cells, their potential as molecular targets for anti-cancer therapies has become increasingly recognized\[12\]. With the development of the pan-CDK inhibitor flavopiridol, more specific CDK inhibitors have been developed with encouraging results\[12\]. In this study, we present our findings on the CDK inhibitor LS-007 in acute leukemia both alone and in combination with ABT-199. LS-007 has been reported to be one of the most efficient CDK9 inhibitors and is effective in both CLL and ovarian tumors\[19, 20\]. We evaluated the inhibitory activity of LS-007 against various CDKs and 41 types of non-CDK kinases at the molecular level. Consistent with previous reports\[19\], LS-007 effectively inhibits the activity of CDK1, 2, and 9. LS-007 also obviously attenuates CDK4 activity while displaying a lower potency against CDK6/7 and most non-CDK kinases in biochemical kinase assays. Compared to flavopiridol, LS-007 appears to be an equally potent inhibitor of CDK9 and more selective for the CDK family. By contrast, flavopiridol can affect the activity of EGFR and some other non-CDK kinases, as well as CDKs\[26\]. Furthermore, we investigated the activities downstream of CDK inhibition-related phosphorylation of RNA pol II, PP1α and RB. We showed a concentration-dependent de-phosphorylation of

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**Figure 3.** LS-007 induces apoptosis in a dose- and time-dependent manner. CCRF-CEM and HL-60 cells were treated with increasing concentrations of LS-007 for 24 h (A) or with 0.2 mol/L LS-007 for 12, 24, or 48 h (B), and 5 patient-derived lymphocytes were treated with LS-007 for 48 h (C). Apoptotic cells were detected by Annexin \( V \) /PI staining and analyzed by flow cytometry. Cells (D, E) or patient-derived lymphocytes (F) were treated with LS-007 or flavopiridol and then were collected for Western blotting (D, F) and qPCR for Mcl-1 or XIAP (E). *P*<0.05, **P*<0.01 vs CON.
CDK9 downstream involving the RNA pol II CTD at ser2 residues. Moreover, the dose-dependent de-phosphorylation of PP1α at thr320 was also displayed using the same concentrations of LS-007, indicating CDK1 inhibition at the cellular level. In contrast to the biochemical kinase assay results, LS-007 hardly altered the RB phosphorylation status, which is an indicator of CDK2/4/6 activities, in CCRF-CEM and HL-60 cells.

Hematologic malignancies, such as AL, often have a high demand for the transcription and translation of anti-apoptotic proteins to resist apoptosis[34]. Many of these proteins have short half-lives at both the mRNA and protein levels[35]. Additionally, the inhibition of transcriptional CDKs might induce cell death through the down-regulation of these short-lived anti-apoptotic proteins, including Mcl-1 and XIAP[36]. In our study, LS-007 resulted in in vitro cell killing, in AL cell lines and in samples derived from 21 ALL and 15 AML pediatric patients accompanied by the induction of apoptosis. As expected, LS-007 and flavopiridol clearly induced Mcl-1 and XIAP down-regulation via transcriptional repression caused by CDK9-related RNA pol II dephosphorylation. However, the expression of Bcl-2 remained unchanged. These findings are consistent with previous studies of flavopiridol and SNS-032[31,14].

CDK inhibitors always show tremendous preclinical activity, but their clinical application was limited by modest efficacy along with various side effects[12]. Nevertheless, it seems encouraging that the combination of CDK inhibitors with other chemotherapeutic drugs will enhance the efficacy and reduce unfavorable toxicity of CDK inhibitors, which has been confirmed in many preclinical and clinical settings aimed at lowering the cancer burden[37,38]. On the other side, the over-expression of the anti-apoptotic Bcl-2 family proteins Bcl-2, Bcl-xL, and Mcl-1 is frequently associated with acute leukemia progression and resistance to conventional chemotherapies[39–41]. Bcl-2 and Mcl-1 usually compensate each other’s function in tumor cells[42]. Our previous data demonstrated that LS-007 had little effect on the Bcl-2 levels, although it could significantly reduce Mcl-1 expression. Thus, we hypothesized that Bcl-2 inhibitors may have the potential to be effective in combination with LS-007 toward a broad spectrum of hematological malignancies. ABT-199 is a highly selective Bcl-2 inhibitor and was granted breakthrough designation by the FDA for relapsed or refractory CLL with a 17p deletion[28,40]. In addition, ABT-199 showed potent efficacy in treating AL, especially AML[27] and ALL[29], and a phase II trial in subjects with AML has already been completed[49]. Therefore, here, we used this compound for the investigation of the synergistic effect. As expected, apoptosis induced by co-treatment with LS-007 and ABT-199 was remarkable without reaching the dosages inducing obvious apoptosis by LS-007 or ABT-199 alone, indicating stronger efficacy with less toxicity.
at the same time. In contrast to a previous study, in which the CDK9 inhibitor wogonin potentiated the anti-cancer activity of the Bcl-2 family inhibitor ABT-263 mainly through down-regulating the Mcl-1 levels\(^{[30]}\), maximal suppression of XIAP, other than through Mcl-1, was observed when LS-007 was combined with ABT-199. As an anti-apoptotic protein, the synergistic inhibition of XIAP may contribute to the greatly increased cells in apoptosis. Moreover, LS-007 inhibited the phosphorylation of RNA pol II, induced apoptosis in leukemia associated with the down-regulation of Mcl-1, whose up-regulation was thought to confer drug resistance when exposed to Bcl-2 inhibitors, and might delay drug resistance caused by Bcl-2 inhibitors. However, the actual mechanism of such a combination needs to be further explored.

Altogether, our study demonstrated that LS-007, a novel CDK inhibitor, showed potent anti-tumor activity against AL cell lines and primary AL blasts and provides a rationale for including AML and ALL patients in clinical trials for LS-007 in combination with ABT-199.

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Author contribution

Shao XIE, Yi CHEN and Jian DING designed the experiments; Shu-dong WANG provided the LS-007 compound; Shao XIE, Hui JIANG, Xiao-wen ZHAI, and Fan WEI performed the research; Shao XIE and Yi CHEN analyzed the data and wrote the paper, which was revised by Jian DING and Yi CHEN.

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