LOR-253, a small molecule anticancer agent that acts through induction of the tumor suppressor Krüppel-like factor 4 (KLF4), has demonstrated antitumor activity as a single agent in a Phase 1 study in patients with advanced or metastatic solid tumors. Recently, the vast majority of patients with acute myeloid leukemia (AML) were shown to inappropriately express the embryonic CDX2 gene in bone marrow stem and progenitor cells, resulting in down-regulation of KLF4 expression, speculated to be the leukemogenic event. Consequently, we examined the antitumor activity and mechanism of action for LOR-253 in AML cells and cells representing other hematological malignancies. Indeed, LOR-253 was found to inhibit proliferation of various human leukemia and lymphoma cell lines in vitro with low IC50 values. Furthermore, LOR-253 induced high levels of KLF4 mRNA expression in AML cells and a resultant significant increase in expression of p21, a cell cycle-dependent kinase inhibitor that is transcriptionally regulated by KLF4. Consistent with these findings, in AML cells we found that LOR-253 induced G1/S cell cycle arrest and apoptosis, based on positive Annexin V staining, activated caspase-3, and increased BAX mRNA expression. Studies are underway to further characterize the pathway that mediates KLF4 induction by LOR-253, to characterize the effects of LOR-253 in combination with approved chemotherapies for AML, and to assess the efficacy of LOR-253 in animal models of AML. Induction of CDX2 represents a novel approach to the treatment of AML and other hematologic malignancies, and LOR-253 is the only clinical stage agent to act through this mechanism of action.

**Abstract**

**Introduction**

LOR-253 is a novel small molecule being developed by Lorus Therapeutics as an anticancer agent for treatment of hematologic cancers. In preclinical studies, LOR-253 has shown significant antitumor activity in a range of tumor types, including non-small cell lung cancer (NSCLC) and colon cancer, with minimal toxicity at efficacious doses. A Phase 1 study with LOR-253 was recently completed in patients with advanced or metastatic tumors, demonstrating evidence of antitumor activity with minimal toxicity.

Mechanism of action and efficacy studies have revealed that the antitumor activity of LOR-253 is associated with induction of expression of KLF4, a tumor suppressor that is downregulated in several cancers including colon and NSCLC. Several reports have shown that KLF4 expression is regulated by the homeobox gene CDX2 in colon cancer and leukemia. Notably, the vast majority of patients with acute myeloid leukemia (AML) were shown to aberrantly express CDX2 in bone marrow stem and progenitor cells, resulting in downregulation of KLF4 expression as the leukemogenic event (Faber et al., 2013). LOR-253 induces KLF4 expression in AML, leading to cell death by apoptosis.

**LOR-253 induces G1 arrest and apoptosis in AML**

- Approximately 90% of patients with AML aberrantly express CDX2/KLF4, leading to cell death by apoptosis.
- LOR-253 is a novel anticancer small molecule and the only clinical-stage therapy that specifically induces expression of the tumor suppressor KLF4 in cancer.
- LOR-253 showed potent antiproliferative activity in a panel of AML and other leukemia cell lines, with IC50 values ranging from 0.007 - 0.3 μM.
- Treatment of AML cells with LOR-253 resulted in high level induction of CDX2/KLF4 and p21, time-dependent manner.
- Anticancer activity of LOR-253 in AML is mediated through cell cycle arrest in G1 and apoptosis.
- LOR-253 has strong anticancer synergy when used in combination with conventional chemotherapy, daunorubicin, azacitidine, decitabine or cytarabine.
- Collectively, these preclinical data strongly support further development of LOR-253 as an AML therapy.

**References**


**Induction of KLF4 by LOR-253 as an innovative therapeutic approach to induce apoptosis in acute myeloid leukemia.**

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**Anticancer activity of LOR-253 in vitro**

LOR-253 is a novel anticancer agent for treatment of hematologic cancers. In preclinical studies, LOR-253 has shown significant antitumor activity in a range of tumor types, including non-small cell lung cancer (NSCLC) and colon cancer, with minimal toxicity at efficacious doses. A Phase 1 study with LOR-253 was recently completed in patients with advanced or metastatic tumors, demonstrating evidence of antitumor activity with minimal toxicity.

**Figure 1.** CDX2 is a homeodomain transcription factor that functions in embryonic organogenesis and early hematopoietic development in vertebrates. CDX2 is not normally expressed in adult normal and human normal hematopoietic cells, but is highly expressed in leukemia and lymphoma cell lines. CDX2 expression is regulated by the homeobox gene CDX2 in colon cancer and leukemia. Notably, the vast majority of patients with acute myeloid leukemia (AML) were shown to aberrantly express CDX2 in bone marrow stem and progenitor cells, resulting in downregulation of KLF4 expression as the leukemogenic event (Faber et al., 2013). LOR-253 induces CDX2 and KLF4 expression in AML, leading to cell death by apoptosis.

**Figure 2.** Antiproliferative activity of LOR-253 in solid tumor, leukemia and lymphoma cell lines. Leukemia and lymphoma cell lines (1 × 10⁶/well) in 96-well microplates were seeded in 96-well cell culture plates and incubated overnight at 37 °C. Following overnight incubation, 50 μL of growth medium were seeded in 96-well plates and treated with increasing concentrations of LOR-253 for 16 h and stained with propidium iodide (PI) followed by cell cycle analysis with flow cytometry. Each data point showed cell cycle arrest at G1 phase with LOR-253 treatment compared to DMSO control. (A) LOR-253 induces KLF4 and p21 in AML. THP1 and HL-60 cells were treated with 0.5 μM LOR-253 or DMSO for 16 h and expression of KLF4 and p21 were quantitated with qRT-PCR. (B) LOR-253 induces KLF4 and p21 in AML. THP1 and HL-60 cells were treated with 0.5 – 1 μM LOR-253 for 40 h. KLF4 expression peaked at 16 – 24 h in both cell lines. By contrast, p21 levels increased steadily in THP1 up to 40 h and tracked closely with KLF4 expression in HL60. For all studies KLF4 and p21 expression levels were normalized to β-actin.

**Figure 3.** LOR-253 induces KLF4 and p21 expression in AML. (A) AML cell lines THP1 and HL60 were treated with 0.5 μM LOR-253 or DMSO for 16 h and expression of KLF4 mRNA was measured by qRT-PCR. The table shows mean increase of KLF4 expression in THP1 and HL60 in response to LOR-253 treatment. Cells were treated with DMSO or 0.5 μM LOR-253 and stained for KLF4 and p21 expression over a time course up to 40 h. KLF4 expression peaked at 16 – 24 h in both cell lines. By contrast, p21 levels increased steadily in THP1 up to 40 h and tracked closely with KLF4 expression in HL60. For all studies KLF4 and p21 expression levels were normalized to β-actin.

**Figure 4.** (A) Effect of LOR-253 on cell cycle in AML. THP1 and HL-60 cells were treated with increasing concentrations of LOR-253 for 16 h and stained with propidium iodide (PI) followed by cell cycle analysis with flow cytometry. Each data point showed cell cycle arrest at G1 phase with LOR-253 treatment compared to DMSO control. (B) LOR-253 induces apoptosis in AML. THP1 cells treated with 0.5 μM LOR-253 for 48 h showed upregulation of pro-apoptotic gene BAX and decreased expression of anti-apoptotic BCL2 by qRT-PCR. (C) Treatment of THP1 and HL-60 cells with 0.5 μM LOR-253 for 48 h resulted in translocation of caspase 3 activity compared to DMSO control. (D) THP1 cells were treated with increasing concentrations of LOR-253 for 48 h, followed by double staining with Annexin V-PI and Fli. LOR-253 treated cells showed increased levels of apoptotic cells (Annexin V+ /PI-; Annexin V+ /PI+).

**Figure 5.** LOR-253 synergizes with AML chemotherapy drugs. (A) For concurrent treatment, HL-60 cells (k × 10⁶/well) were seeded in 96-well plates and incubated overnight at 37 °C followed by cell cycle analysis with flow cytometry. Each data point showed cell cycle arrest at G1 phase with LOR-253 treatment compared to DMSO control. (B) Sequential treatment: LOR-253 and chemotherapy with AML. HL-60 cells were treated with LOR-253 at 0.5X IC50 (0.02 μM) for 48 h followed by drug combination therapy. Each data point showed cell cycle arrest at G1 phase with LOR-253 treatment compared to DMSO control. (C) LOR-253 induces apoptosis in AML. THP1 cells treated with 0.5 μM LOR-253 for 48 h showed increased expression of pro-apoptotic gene BAX and decreased expression of anti-apoptotic BCL2 by qRT-PCR. (D) Treatment of THP1 and HL-60 cells with 0.5 μM LOR-253 for 48 h resulted in translocation of caspase 3 activity compared to DMSO control. (E) THP1 cells were treated with increasing concentrations of LOR-253 for 48 h, followed by double staining with Annexin V-FTIC and PI. LOR-253 treated cells showed increased levels of apoptotic cells (Annexin V+ /PI-; Annexin V+ /PI+).

**Figure 6.** LOR-253 induces G1 arrest and apoptosis in AML. (A) AML cell lines THP1 and HL-60 were treated with 0.5 μM LOR-253 or DMSO for 16 h and expression of KLF4 mRNA was measured by qRT-PCR. Cell cycle analysis was performed by flow cytometry as described in Fig. 2. The dashed line (----) indicates the predicted percentage of the tumor suppressor KLF4 expression in HL60. For all studies KLF4 and p21 expression levels were normalized to β-actin.