Utilization of KLF-4 as a pharmacodynamic biomarker for in vivo anticancer activity of a novel small molecule drug LOR-253

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Abstract

LOR-253 is a potent and selective growth inhibitor of several cancer types, including non-small cell lung cancer (NSCLC), colon cancer and leukemia. LOR-253 stimulates Krüppel Like Factor-4 (KLF-4), a tumor suppressor factor which is characteristically deficient in a variety of cancers, and so represents a new approach to cancer therapy. The current Phase I dose-escalation study is being conducted at Memorial Sloan-Kettering Cancer Center in New York and MD Anderson Cancer Center in Houston. In the present preclinical study, the changes in KLF-4 expression levels were evaluated in vivo to examine the utility of KLF-4 as a pharmacodynamic biomarker, aiming at linking the antitumor effects of LOR-253 to the induction of KLF-4. In the human H226 NSCLC xenograft mouse model, LOR-253 demonstrated dose-dependent antitumor activity when administered at 1, 5, and 15 mg/kg and mediated significant tumor growth inhibition of about 55% at the highest dose given by intravenous bolus injection (p=0.05). Tumors were harvested 24h after the last dose following the treatments at 1, 5, and 15 mg/kg and KLF-4 mRNA and protein levels were analyzed by real-time RT-PCR and Western blot, respectively. A dose-dependent induction of KLF-4 mRNA and protein levels was observed. Pharmacodynamic studies to characterize the effect of LOR-253 on intratumoral KLF-4 were also conducted in the H226 NSCLC xenograft mouse model to determine the optimal time point for tumor biopsy sampling, following the treatment schedule in the ongoing Phase 1 clinical trial (2 days dosing, 12 day break, 2 days of dosing, 12 day break (1 cycle)). Tumor samples were taken 24h after the second, fourth and eighth dose of LOR-253 and analyzed for KLF-4 expression. It was shown that tumor sampling after the second and eighth doses was optimal at demonstrating increased intratumoral KLF-4 levels. Furthermore, antitumor efficacy of LOR-253 was also evaluated in nude rats bearing established human H226 NSCLC tumors when LOR-253 was infused intravenously following the current Phase I clinical dosing schedule. A significant efficacy was observed at the end of the first cycle of treatment, demonstrating 44% decrease in mean tumor volume when compared with the control treatment (p=0.025). Inhibition of tumor growth correlated with increased KLF4 protein levels when analyzed by Western blot. Real time PCR of mRNA in NSCLC tumors from rat models is in progress. Taken together, our in vivo data provide strong preclinical support for utilizing KLF-4 as a potential pharmacodynamic biomarker for LOR-253.

Introduction

LOR-253 is a novel small molecule that is being developed by Lorus Therapeutics Inc. as an anticancer agent for treatment of solid tumors. In preclinical studies, LOR-253 has shown significant anticancer activity in a range of tumor types, including NSCLC and colon cancer, with minimal toxicity at efficacious doses. Mechanism of action and efficacy studies have revealed that the anticancer 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.

LOR-253 is currently in a Phase I clinical study in patients with advanced or metastatic solid tumors who are unresponsive to conventional therapy or for which no effective therapy is available. The Phase I trial includes a biomarker study designed to explore the effects of LOR-253 at relevant doses and to evaluate KLF4 as a tumor biomarker in patient biopsies, with a focus on NSCLC and colon cancer.

To support the clinical development of LOR-253, we examined in vivo expression of KLF4 mRNA and protein in animal models of NSCLC. We show that KLF4 expression significantly correlates with anticancer activity of LOR-253 in NSCLC xenografts. Our findings identify optimal time points for analysis of KLF4 following LOR-253 treatment, and provide strong support for the use of KLF4 as a biomarker for LOR-253 in patient biopsies.

In vivo Dose Response Study Results

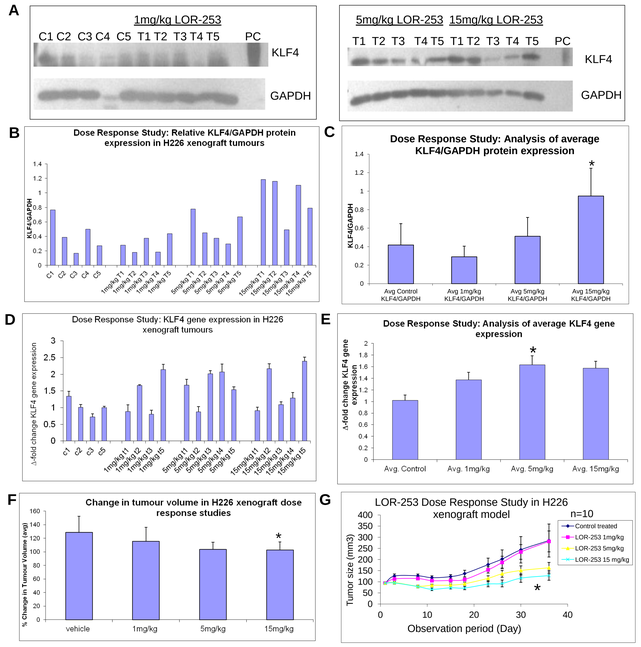

Figure 1. In vivo Dose Response treatment in H226 xenograft CD-1 nude mice with 1, 5 or 15mg/kg LOR-253. LOR-253 was administered intravenously for 5 consecutive days at the indicated doses. Tumors were analyzed 16h after the final dose. **A.** Western Blot (WB) analysis of tumor proteins blotted with KLF4 and GAPDH antibodies. **B and C.** Densitometry analysis of WBs from panel A showing KLF4 levels relative to GAPDH from individual tumors (panel B) and average levels in all tumors (panel C). KLF4 protein levels increase in a dose dependent manner, with 15mg/kg dose demonstrating significance (*P<0.05). **D and E.** Real time PCR analysis of tumor RNA also shows a dose dependent increase in KLF4 mRNA, with a significant increase at 5mg/kg (*P=0.05). **F.** LOR-253 shows dose response antitumor activity in NSCLC that is significant at 15 mg/kg (*P<0.05). **G.** A subsequent dose response study was conducted with 3 rounds of 5 consecutive days of LOR-253 treatment. LOR-253 showed 55% tumor growth inhibition at 15mg/kg (*P=0.05). PC: Positive control KLF4-expressing cell line.

In vivo Biomarker Studies: 2 doses of LOR-253

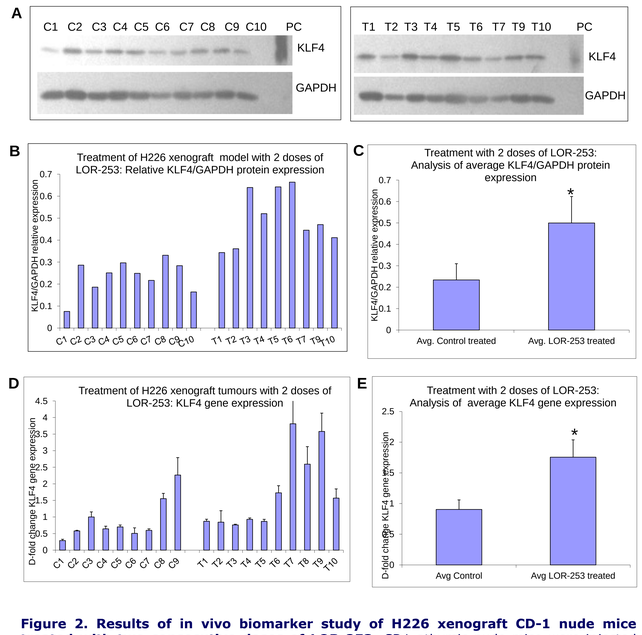

Figure 2. Results of in vivo biomarker study of H226 xenograft CD-1 nude mice treated with two consecutive doses of LOR-253. CD1 athymic nude mice were injected subcutaneously in the right flank region with human H226 NSCLC cells. When tumors reached the desired size (100-120mm^3), LOR-253 or vehicle control was administered intravenously at 15 mg/kg by bolus injection once daily for two days. Tumors were isolated 24 hr after the second dose and analyzed for KLF4 RNA and protein. **A.** WB analysis of tumor protein in control and LOR-253 treated tumors. WBs were, blotted with KLF4 and GAPDH antibodies. **B and C.** Densitometry analysis of WBs from panel A showing KLF4 levels relative to GAPDH from individual tumors (panel B) and average levels in all tumors (panel C). Mean KLF4 protein levels are significantly increased following treatment with 2 doses of LOR-253 (*P<0.001). **D and E.** Real time PCR analysis of tumor RNA in individual tumors (panel D) and average levels (panel E). Mean KLF4 levels are increased after two doses of LOR-253, with a trend towards significance (*P=0.068). PC: Positive control KLF4-expressing cell line.

In vivo Biomarker Studies: 8 doses of LOR-253

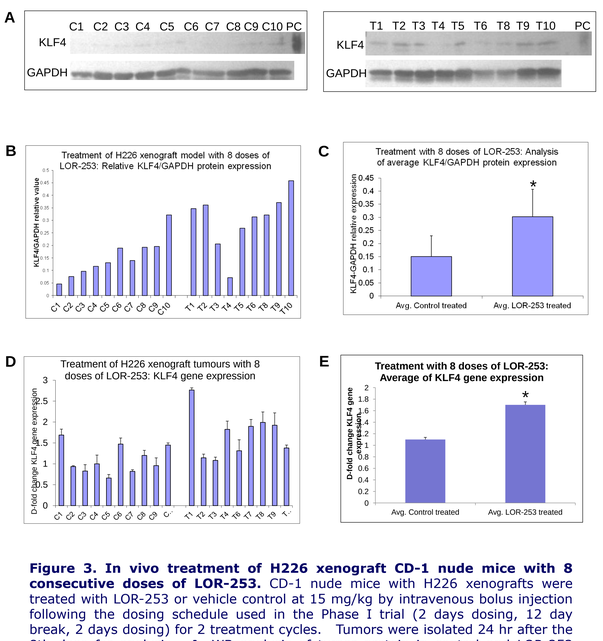

Figure 3. In vivo treatment of H226 xenograft CD-1 nude mice with 8 consecutive doses of LOR-253. CD-1 nude mice with H226 xenografts were treated with LOR-253 or vehicle control at 15 mg/kg by intravenous bolus injection following the dosing schedule used in the Phase I trial (2 days dosing, 12 day break, 2 days dosing) for 2 treatment cycles. Tumors were isolated 24 hr after the 8th dose for analysis. **A.** WB analysis of tumor protein in control and LOR-253 treated tumors. WBs were, blotted with KLF4 and GAPDH antibodies. **B and C.** KLF4 levels relative to GAPDH from individual tumors (panel B) and average levels in all tumors (panel C). Mean KLF4 protein levels are significantly increased following treatment with 8 doses of LOR-253 (*P<0.01). **D and E.** Real time PCR of tumor RNA in individual tumors (panel D) and average levels (panel E). As with KLF4 protein levels, mean KLF4 mRNA levels are significantly increased after 8 doses of LOR-253 (*P<0.01). PC: Positive control KLF4-expressing cell line.

Antitumor activity of LOR-253 in Rat Infusion Model

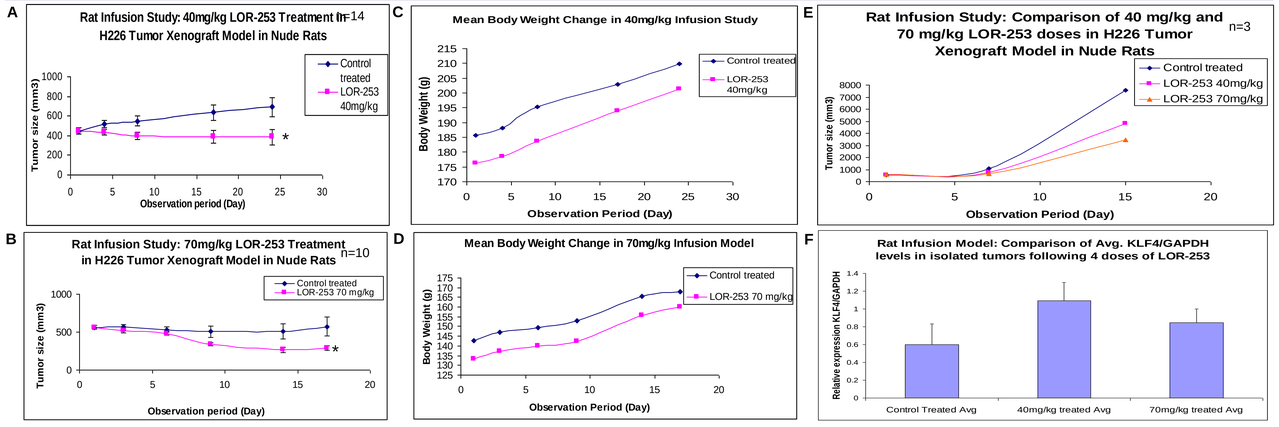

Figure 4. In vivo treatment of H226 xenografts in NIH nude rats with LOR-253. LOR-253 was administered by intravenous infusion using the dosing schedule from the Phase I clinical study as a guide. Two dose levels of LOR-253 were chosen for testing: 40mg/kg, which is the no observed adverse effect dose (NOAEL) in rats, and 70 mg/kg, which is the maximum tolerated dose (MTD). NIH nude rats were injected in the lower mid-back with 1.5x10^7 human NSCLC H226 cells. When tumors reached the desired size, LOR-253 or vehicle control was administered by IV-infusion through a pre-cannulated femoral vein catheter. LOR-253 was infused for 4 doses (2 days dosing, 12 day break, 2 days dosing), corresponding to one treatment cycle in the clinical trial. Tumors were excised for analysis 24h after the final dose. **A and B**. Antitumor activity of LOR-253 in NSCLC tumors. Changes in tumor volumes over the course of the study are shown. Treatment with 4 doses of LOR-253 at 40 mg/kg (panel A) and 70 mg/kg (panel B) showed a significant difference in tumor growth compared to control vehicle treatment (*P<0.05). **C and D.** Mean body weights of control and LOR-253 treated animals in 40 mg/kg and 70 mg/kg groups. **E.** Antitumor activity of LOR-253 in another study showing a direct comparison of LOR-253 infused for 4 doses at 40 mg/kg or 70 mg/kg. **F.** Expression levels of KLF4 relative to GAPDH in tumors from the study shown in panel E. Tumor sample protein was analyzed by WB for KLF4 and GAPDH, and protein levels were determined by densitometry. The average KLF4/GAPDH ratio for each group is shown.

Summary

- LOR-253 is a novel anticancer small molecule that induces expression of the tumor suppressor KLF4. Preclinical studies were conducted to assess the use of KLF4 as a biomarker for LOR-253 in NSCLC
- In mouse models of NSCLC, LOR-253 shows a significant dose response antitumor effect that correlates with a dose dependent increase of KLF4 mRNA and protein in NSCLC xenografts
- KLF4 levels are increased in NSCLC xenografts following treatment of mouse models using the same dosing schedule that is being used in the LOR-253 Phase I clinical study.
- LOR-253 shows anticancer activity in a rat infusion model of NSCLC with minimal toxicity, and induces expression of KLF4 protein in tumor xenografts
- Analysis of tumors from the mouse model shows that KLF4 levels are highest after eight doses of LOR-253, corresponding to two treatment cycles in the clinical study
- Our data support the use of KLF4 as a biomarker for LOR-253 anticancer activity, and provide insight into the optimal time points for assessment of KLF4 expression in patient biopsies