In vivo preclinical efficacy of a novel "payload-bearing" peptide LX-101 targeting IGF-1R in Ewing sarcoma

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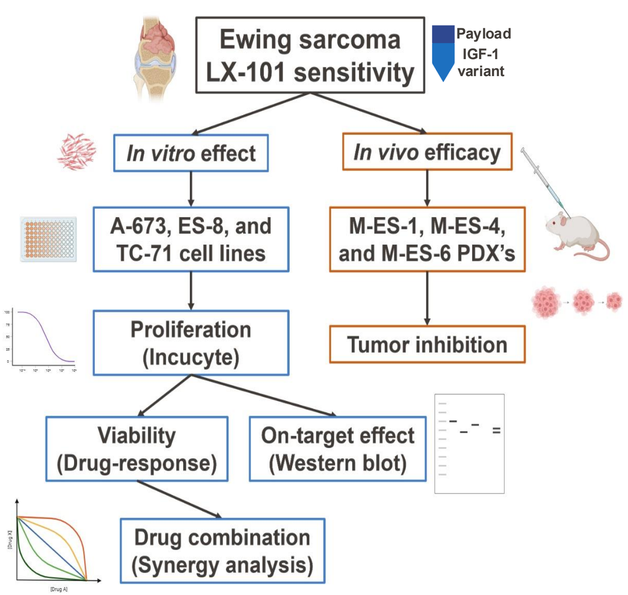
Abstract

Background: Ewing sarcoma (ES) is a highly aggressive and metastatic bone malignancy that primarily affects children and young adults, representing the second most common bone tumor in pediatric patients. While survival rates approach ~80% for patients with localized or regional disease, they decline to ~40% in metastatic cases. Standard-of-care treatment typically includes multimodal therapy with radiotherapy, surgery, and combination chemotherapy based on the VDC/IE regimen. In parallel, targeted therapies directed at key oncogenic pathways such as IGF-1/PI3K/mTOR have demonstrated promising clinical activity across multiple cancer types.

Rationale: Given the urgent need for improved therapies in ES, robust preclinical evaluation of novel agents is essential. In this study, we investigated the *in vitro* and *in vivo* efficacy of LX-101, a next-generation IGF-1R-targeted therapeutic that couples a proprietary IGF-1 variant to a cytotoxic Methotrexate (MTX) payload. LX-101 was evaluated as a single agent and in combination with Alpelisib (PI3K inhibitor) and Temsirolimus (mTOR inhibitor).

Findings: LX-101 demonstrated potent antitumor activity as a single agent in PDX ES models, with enhanced efficacy observed when combined with PI3K or mTOR inhibition. These findings highlight the therapeutic potential of LX-101 and support its further development.

Experimental design



Results

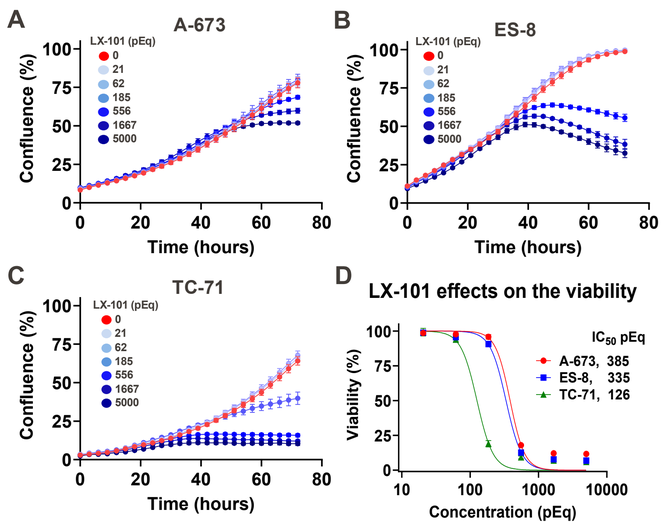


Figure 1. Effect of LX-101 on proliferation, viability, and IGF-1 pathway activation in ES cell lines. All three cell lines were exposed to serial dilutions of LX-101. Cell confluence was monitored every 3 hours for 72 hours using the Incucyte Live-Cell Imaging System (A–C). Cell viability was assessed 72 hours post-treatment (D). Confluence (%) and viability (%) data were collected, analyzed, and graphed using GraphPad Prism. Additionally, to confirm the in vitro on-target effects of LX-101, protein expression changes were evaluated using the JESS automated western blot system (E). Levels of phosphorylated and total IGF-1R (F) and S6 (G) were analyzed and quantified using GraphPad Prism. GAPDH was used as a loading control. All experiments were performed in three independent replicates

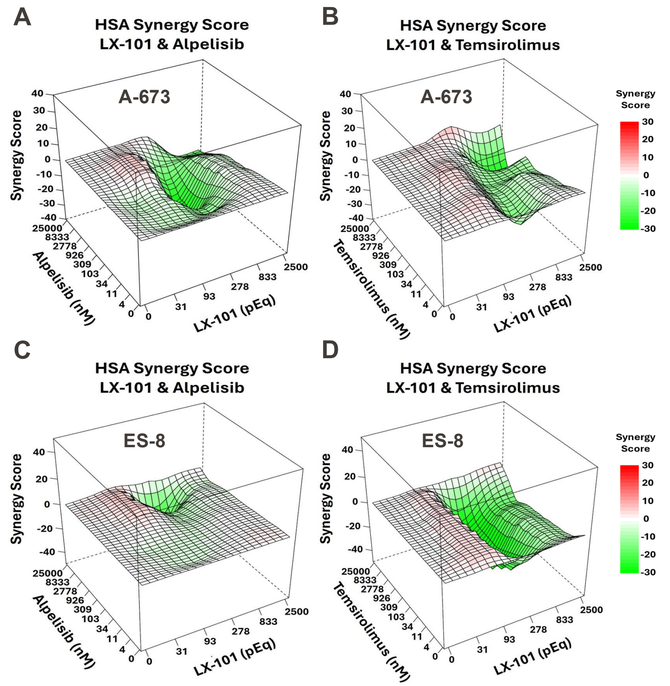


Figure 2. *In vitro* effect of LX-101 in combination with Alpelisib and Temsirolimus in ES cell lines. To evaluate the combinatorial effects of LX-101 with Alpelisib or Temsirolimus, 2D dose-response assays were performed at 72 hours post-treatment. Data were analyzed and visualized using the SynergyFinder tool. Synergy scores were calculated using the Highest Single Agent (HSA) model and represented as 3D plots, illustrating the interaction between LX-101 and Alpelisib (A, C, E) or Temsirolimus (B, D, F) across ES cell lines. In the plots, positive regions (red) indicate synergistic interactions, while negative regions (green) indicate antagonism. Graphs represent data from three independent experiments.

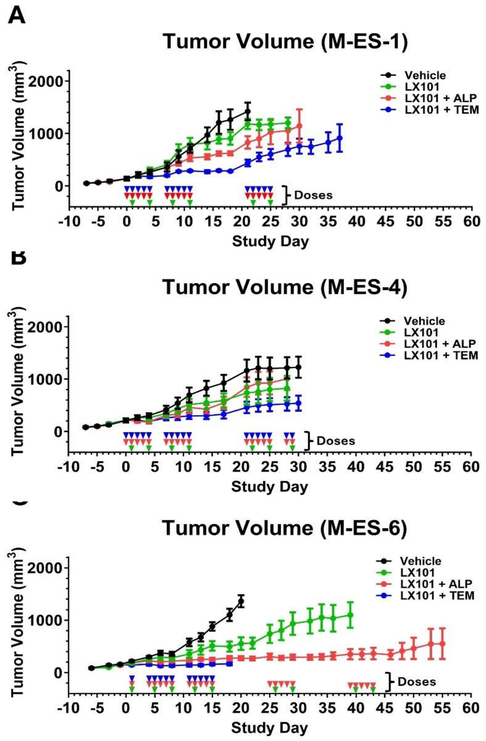


Figure 3. *In vivo* efficacy of LX-101 in combination with Alpelisib or Temsirolimus. The *in vivo* efficacy of LX-101 in combination with Alpelisib (ALP) or Temsirolimus (TEM) was tested on three ES PDX model. NSG mice were implanted with PDX tumor fragments S.Q. and then randomized into four treatment groups (n=5): Vehicle, LX101 (I.V. at 16 µEq/kg twice a week), LX101 + ALP (orally at 25 mg/kg daily), or LX101 + TEM (I.P. at 5 mg/kg daily) groups. Tumor dimensions were recorded thrice/week. Based on body weight or tumor size, mice were euthanized, and tumor samples were dissected.

Conclusion

- The LX-101 IGF1 ligand-drug conjugate shows superior preclinical single-agent activity in ES compared to other IGF-1R antibody approaches.
- Strong synergy with PI3K and mTOR inhibitors is evident, supporting the clinical development of LX-101 and LX-101-based drug combinations that effectively suppress the oncogenic IGF-1/PI3K/mTOR pathway.

References

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