LX-101, a clinical stage, novel, payload-bearing targeted therapy directed to the insulin-like growth factor 1 receptor (IGF-1R), has potent anti-tumor activity against pediatric cancer cell lines

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BACKGROUND

The role of the insulin-like growth factor-1 receptor (IGF-1R) pathway in cancer is well-established. Increased pathway activity promotes cancer cell proliferation, migration, and invasion, and is associated with tumor metastasis, treatment resistance, poor prognosis, and shortened survival in certain cancers. Multiple aggressive pediatric cancers, including Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, neuroblastoma, and others, have genetic alterations activating the IGF-1R pathway and/or high IGF-1R expression that correlates with poor outcomes. Prior IGF-1R-targeting drug candidates were largely non-payload bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors. These agents produced a range of clinical outcomes, with some partial and complete responses, including in Ewing's and other related sarcomas, but none were ultimately approved in an oncology setting. These approaches may not have been potent enough and allowed for redundant signaling pathways and other escape mechanisms.

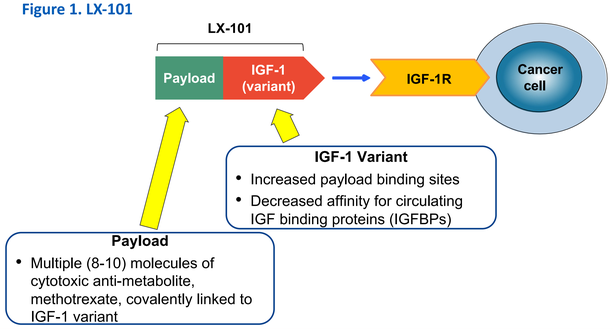
Unlike these past approaches, LX-101 is a novel, next-generation, payload-bearing targeted therapy directed to IGF-1R. LX-101 consists of a proprietary IGF-1 variant coupled to a cytotoxic payload methotrexate (MTX). LX-101 was previously evaluated (as 765IGF-MTX) in Phase 1 trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity. Neither a dose limiting toxicity (DLT), nor a maximum tolerated dose (MTD) were reached, leaving potential room for additional dose escalation and schedule optimization. Herein, we report the potent anti-tumor activity of LX-101 against cell lines of certain pediatric cancers with well established ties to the IGF-1/IGF-1R pathway.

LX-101, A NOVEL APPROACH TO TARGETING IGF-1R

LX-101 is a next generation IGF-1R-directed agent that delivers a potent payload with high precision to targeted cells. LX-101 consists of a proprietary, engineered variant of the IGF-1 ligand, covalently conjugated to MTX, a drug that has been used to treat patients with a variety of cancers and autoimmune diseases. Moreover, MTX is a cytotoxic inhibitor of DNA synthesis, repair, and cellular replication. We believe targeted, intracellular delivery of MTX by LX-101 makes it ideal for these indications.

LX-101 was designed to include additional binding sites, via a proprietary N-terminal leader sequence, to allow for the conjugation of an increased number of MTX molecules, in an effort to increase potency while ensuring safety by enabling delivery of more payload in a directed fashion to the targeted cell. In addition to enhanced efficacy, we believe the targeted delivery of MTX directly to the cells of interest should avoid the toxicities that are often seen with systemically administered high dose MTX. Notably the IGF-1 variant used in LX-101 is also designed to have reduced binding affinity to circulating serum IGFBPs which can interfere with IGF-1 ligand binding to IGF-1R.

Figure 1. LX-101



METHODS

Cell Culture: CADO-ES1, RD-ES, and SJCRH30 cells were cultured in RPMI-1640 + 10% fetal bovine serum (FBS). HOS and A-673 cells were cultured in DMEM + 10% FBS. SK-N-AS cells were cultured in DMEM (high glucose) + 10% FBS + 1% NEAA. IMR-32 cells were cultured in MEM + 0.01 mM non-essential amino acids (NEAA) + 10% FBS. 143B cells were cultured in MEM + 0.01 mM NEAA + 10% FBS + 0.015 mg/ml 5-bromo-2'-deoxyuridine. SH-SY5Y cells were cultured in MEM + 0.01 mM NEAA / Ham's F12K (1:1) + 10% FBS. U2OS cells were cultured in McCoy's 5A + 10% FBS. SK-ES-1 cells were cultured in McCoy's 5A + 15% FBS. All cell lines were cultured at 37°C and 5% CO_2.

In Vitro Cytotoxicity Assay: The CellTiter-Glo® Luminescent Cell Viability Assay (Promega) was used to assess cell viability after exposure to LX-101. Cells were seeded in 96-well plates and incubated with LX-101 at concentrations ranging from 1.6 – 2500 nM for 4 days. The CellTiter-Glo® 2.0 Reagent was then added to wells according to the manufacturer's instructions, and luminescence was measured on an EnVision® 2104 Multilabel Plate Reader (PerkinElmer). Cisplatin was used as a positive control.

Data Analysis: IC_{50} were calculated using GraphPad PRISM software. Absolute IC50s of LX-101 derived by dividing the IC50s based on MTX content by average number of MTX groups conjugated per IGF-1 variant protein (i.e., 8), as determined by MALDI-TOF (matrix-assisted laser desorption/ionization time of flight mass spectrometry).

RESULTS

Figure 2. Ewing's Sarcoma

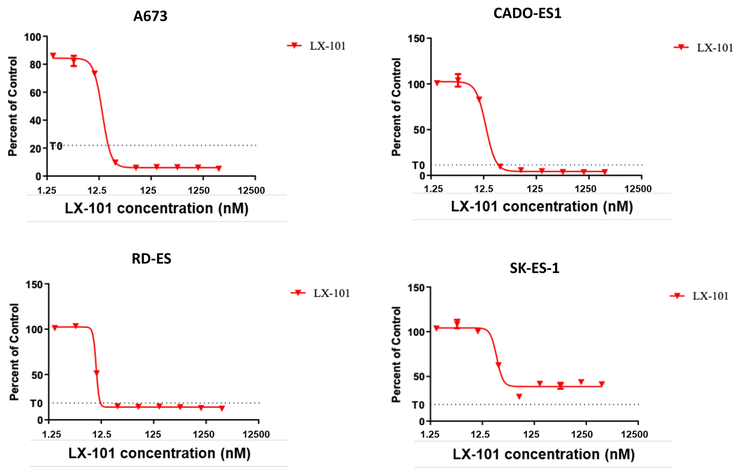


Figure 3. Rhabdomyosarcoma

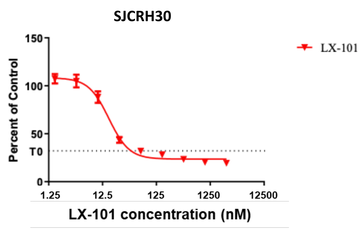
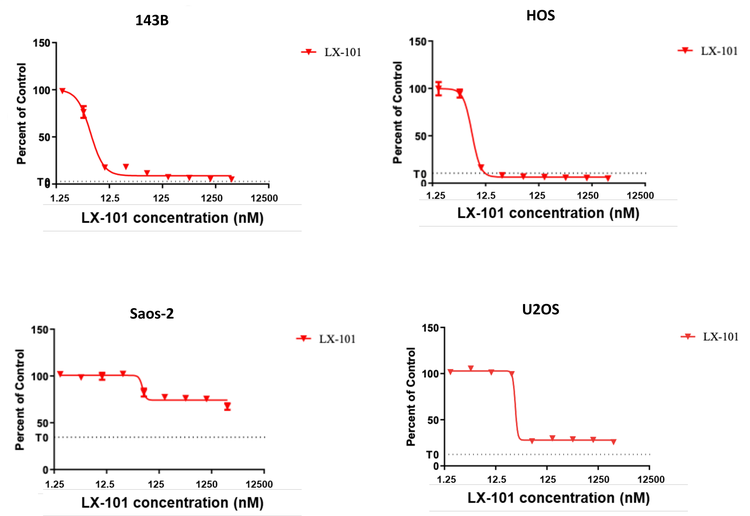


Figure 4. Osteosarcoma



RESULTS (CONT.)

Figure 5. Neuroblastoma

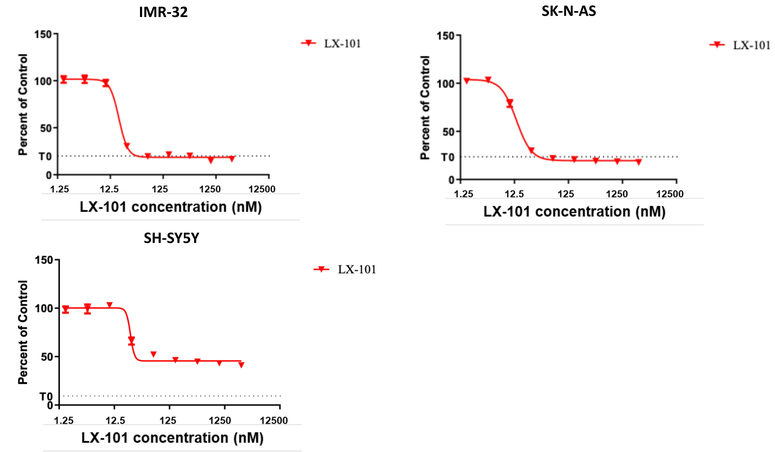


Table 1. LX-101 Absolute IC_{50} Summary

Indication	Cell lines	Absolute IC_{50} (nM)
Ewing's sarcoma	CADO-ES1	15
	RD-ES	11
	SK-ES-1	30
	A673	14
Rhabdomyosarcoma	SJCRH30 (alveolar)	24
Osteosarcoma	143B	6
	HOS	7
	Saos-2	>2500
	U2OS	33
Neuroblastoma	SK-N-AS	17
	IMR-32	21
	SH-SY5Y	31

SUMMARY AND CONCLUSION(S)

- LX-101, a next-generation, targeted therapy directed to IGF-1R, was previously evaluated (as 765IGF-MTX) in Phase 1 trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity. Neither a DLT nor an MTD were reached, leaving room for possible further dose escalation and schedule optimization
- The novel mechanism of LX-101, i.e. its targeted payload delivery to IGF-1R, could potentially provide a more potent therapeutic approach to target and kill IGF-1R$^+$ cancer cells than has been attempted in the past
- LX-101 displayed potent anti-tumor activity against cell lines derived from pediatric cancers with well established ties to the IGF/IGF-1R pathway including Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, and neuroblastoma
- Given the encouraging data from these experiments and the existing clinical data package, new clinical trials with LX-101 in these indications are being planned

REFERENCES

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