LX-101, a Clinical Stage, Novel, Payload-bearing Targeted Therapy Directed to the Insulin-like Growth Factor 1 Receptor (IGF-1R), has Potent Preclinical Anti-Tumor Activity Against Pediatric Sarcomas

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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 thereby allowing cancer cells to evade receptor blockade via 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.

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. 143B cells were cultured in MEM + 0.01 mM NEAA + 10% FBS + 0.015 mg/ml 5-bromo-2'-deoxyuridine. U2OS cells were cultured in McCoy's 5A + 10% FBS. SK-ES-1 cells were cultured in McCoy's 5A + 15% FBS. Saos-2 cells were cultured in McCoy's 5A + 10% 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

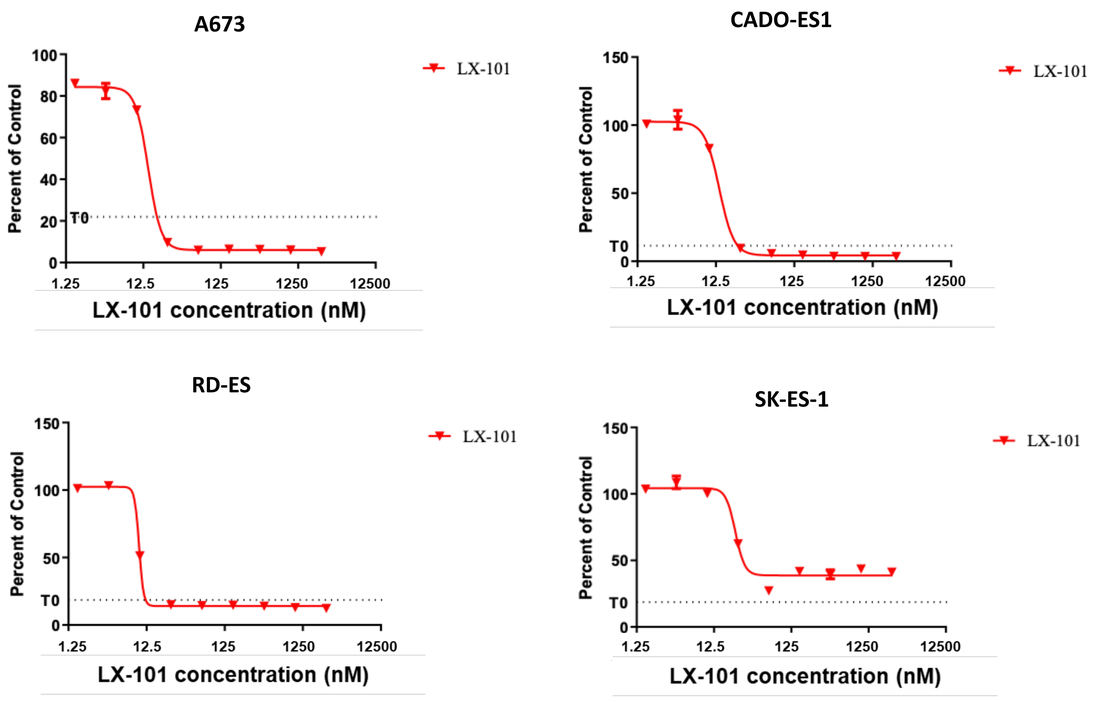


Figure 3. Rhabdomyosarcoma

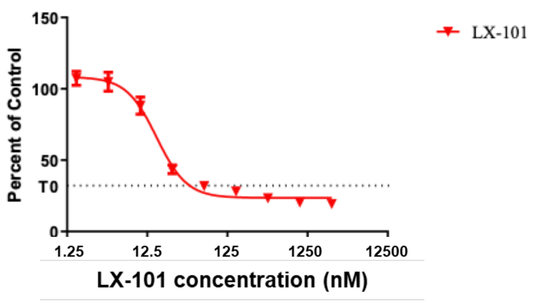
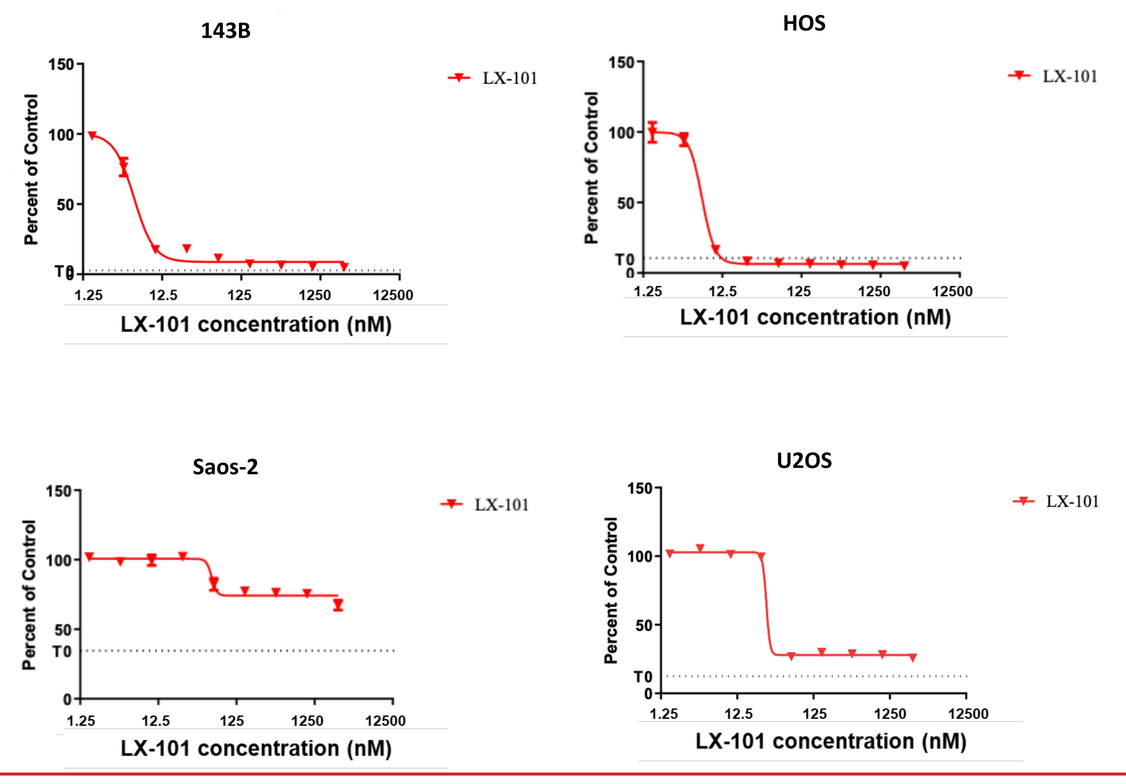


Figure 4. Osteosarcoma



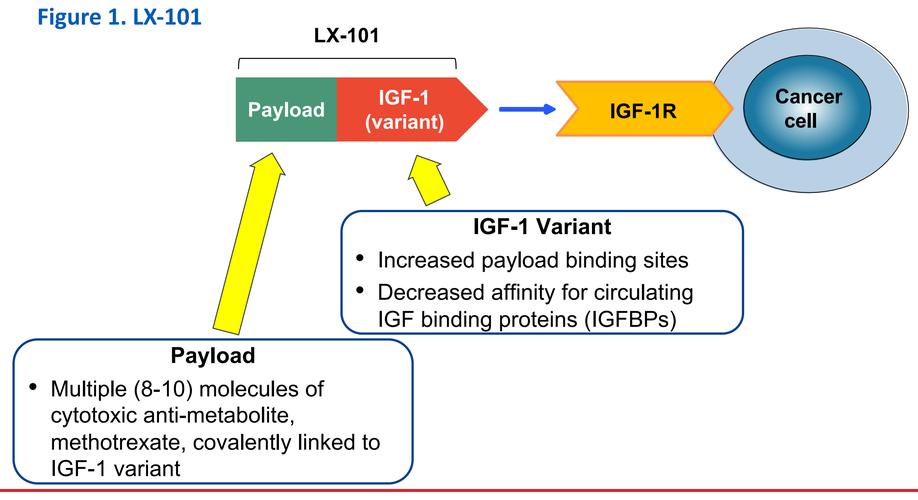
LX-101, A NOVEL APPROACH TO TARGETING IGF-1R

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

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 enhance potency. In addition, the targeted delivery of MTX directly to the cells of interest is designed to avoid the toxicities 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, to optimize bioavailablity.

Figure 1. LX-101



RESULTS (CONT.)

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

Indication	Cell lines	Absolute IC_{50} (nM)
Ewing's sarcoma	RD-ES	10
	CADO-ES1	14
	A673	14
	SK-ES-1	29
Rhabdomyosarcoma	SJCRH30 (alveolar)	23
Osteosarcoma	143B	6
	HOS	7
	U2OS	32
	Saos-2	>2500

CANCERS WITH TIES TO THE IGF-1 / IGF-1R PATHWAY

Table 2. Select Cancers with IGF-1 / IGF-1R Pathway Involvement

Cancer types	Genetic alterations affecting the IGF-1R pathway	IGF-1R pathway effects
Ewing's sarcoma	IGF-1R gene amplification	Shorter survival
Rhabdomyosarcoma	EWSR1-FLI1	Aggressive disease
Gastrointestinal stromal tumor (GIST)	PAX3/7-FKHR/FOXO1	Tumor metastasis
Synovial sarcoma	NBF1-IGF1R	Poor prognosis
Neuroblastoma	SYT-SSX1/2	Treatment resistance
Osteosarcoma	EWSR1-WT1	High mortality rates
Wilms Tumor	MYB-NF1B	
Desmoplastic small round cell tumor		
Adrenocortical carcinoma		
Adenoid cystic carcinoma		
Head and neck cancer, HPV(-)		
Bladder cancer, invasive		
Breast cancer, triple negative		

SUMMARY AND CONCLUSIONS

- **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**
- **Prior IGF-1R-targeting drug candidates were largely non-payload bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors, thus did not address redundant pathways and other related escape mechanisms for cancer cells to evade therapy**
- **The novel payload-directed delivery of LX-101 could provide a more potent therapeutic approach to target and kill IGF-1R$^+$ cancer cells than has been employed in the past**
- **LX-101 displayed potent preclinical anti-tumor activity against pediatric cancers with well-established ties to the IGF-1R pathway including those with specific genetic alterations affecting the pathway like Ewing's sarcoma, rhabdomyosarcoma, and osteosarcoma**
- **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

Andersson, Mattias K et al. "IGF2/IGF1R Signaling as a Therapeutic Target in MYB-Positive Adenoid Cystic Carcinomas and Other Fusion Gene-Driven Tumors." *Cells* vol. 8,8 913. 16 Aug. 2019, Hua, Hui et al. "Insulin-like growth factor receptor signaling in tumorigenesis and drug resistance: a challenge for cancer therapy." *Journal of hematology & oncology* vol. 13,1 64. 3 Jun. 2020, Venepalli, Neeta K et al. "Phase I Study of IGF-Methotrexate Conjugate in the Treatment of Advanced Tumors Expressing IGF-1R." *American journal of clinical oncology* vol. 42,11 (2019), Chen, Helen X, and Elad Sharon. "IGF-1R as an anti-cancer target--trials and tribulations." *Chinese journal of cancer* vol. 32,5 (2013), Yuan, Jingsheng et al. "Function of insulin-like growth factor 1 receptor in cancer resistance to chemotherapy." *Oncology letters* vol. 15,1 (2018), Larsson, O et al. "Role of insulin-like growth factor 1 receptor signalling in cancer." *British journal of cancer* vol. 92,12 (2005)

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