LX-101, a Novel, Clinical Stage, Payload-bearing Targeted Therapy Directed to the IGF-1R Demonstrates Potent Preclinical Anti-tumor Activity Against Multiple Cancer Types With Elevated IGF-1R Expression

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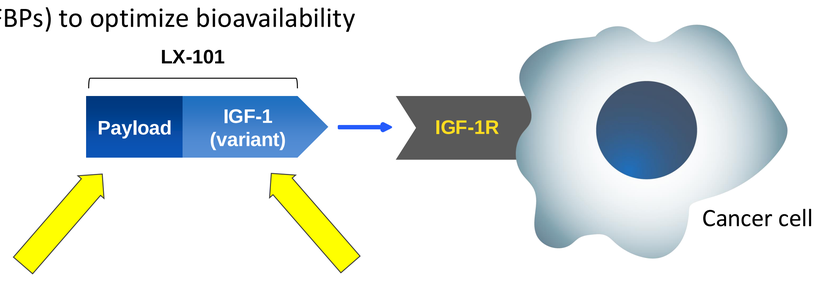
BACKGROUND

- The insulin-like growth factor-1 receptor (IGF-1R) pathway is well-established in a wide range of cancers, and is associated with cancer proliferation, migration, invasion, metastasis, treatment resistance, poor prognosis, and shortened survival.
- Prior attempts at targeting IGF-1R consisted of non-payload bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors. These agents produced a range of clinical outcomes, including some partial and complete responses, but none were ultimately approved in an oncology setting.
- These previous approaches may not have been potent enough thereby allowing cancer cells to evade receptor blockade via redundant signaling pathways and other escape mechanisms.
- In contrast to 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 methotrexate (MTX) payload.
- LX-101 was previously evaluated (as 765IGF-MTX) in a Phase 1a trial 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. Moreover, notably, while patients had some level of IGF-1R expression, the trials were not specifically designed to enrich for tumors with high IGF-1R expression and/or well-established ties to the IGF-1R pathway.
- **Herein, we tested the preclinical anti-tumor activity of LX-101 against a variety of IGF-1R-expressing cancer cell lines**

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

- Next generation IGF-1R-directed agent that delivers a potent payload with high precision to target cells
- Consists of an optimized variant of the IGF-1 ligand, covalently conjugated to MTX, a cytotoxic inhibitor of DNA synthesis, repair, and cellular replication that has been used to treat patients with a variety of cancers and autoimmune disease
- Designed with additional binding sites, via a proprietary N-terminal leader sequence, to allow for the conjugation of increased number of MTX molecules in an effort to enhance potency
- Targeted delivery of MTX directly to the cells of interest designed for increased precision versus systemically administered high dose MTX
- The IGF-1 variant used in LX-101 designed to have reduced binding affinity to circulating serum IGF binding proteins (IGFBPs) to optimize bioavailability



Payload
- Multiple (6-10) molecules of the cytotoxic anti-metabolite, methotrexate, covalently linked to IGF-1 variant

IGF-1 Variant
- Increased payload binding sites
- Decreased affinity for circulating IGF binding proteins (IGFBPs)

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

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

Cancer Type	Epigenetic and Genetic Alterations	
Ewing's sarcoma	IGF-1R overexpression	EWSR1-FLI1
DSRCT	IGF-1R and upregulation	EWSR1-WT1
Rhabdomyosarcoma	IGF-1R and short survival	PAX3/7-FKHR/FOXO1
GIST	High IGF-1R in peds (WT)	NBF1-IGF1R
Synovial Sarcoma	IGF-1R and more aggressive	SYT-SSX1/2
Neuroblastoma	IGF-1R and poor outcomes	
Osteosarcoma	IGF-1R and poor prognosis	
Wilms Tumor	IGF-1R and poor outcomes	IGF-1R gene amplification
Adrenocortical carcinoma	IGF-2 overexpression	
Head & Neck Cancers:		
○ HNSCC HPV(-)	IGF-1R and poor outcomes	
○ Adenoid cystic carcinoma	IGF-2 overexpression	MYB-NF1B
Bladder cancer, invasive	IGF-1R and higher mortality	
Breast cancer, triple negative	IGF-1R and short survival	
Many cancer type subsets, including lung, breast, colorectal, prostate, ovarian, gastric, esophageal, etc.	IGF-1R expression and over-expression linked to poor outcomes	

METHODS

- **Cell Culture:** A549 cells were cultured in Ham's F12K + 10% fetal bovine serum (FBS). NCI-H2122, NCI-H526, TE-1, KYSE-70, MKN74, and NUGC4 cells were cultured in RPMI1640 + 10% FBS. All cell lines were cultured at 37°C and 5% CO2.
- **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 a Tecan Spark microplate reader. 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 (CONT.)

Figure 1. Lung Cancer

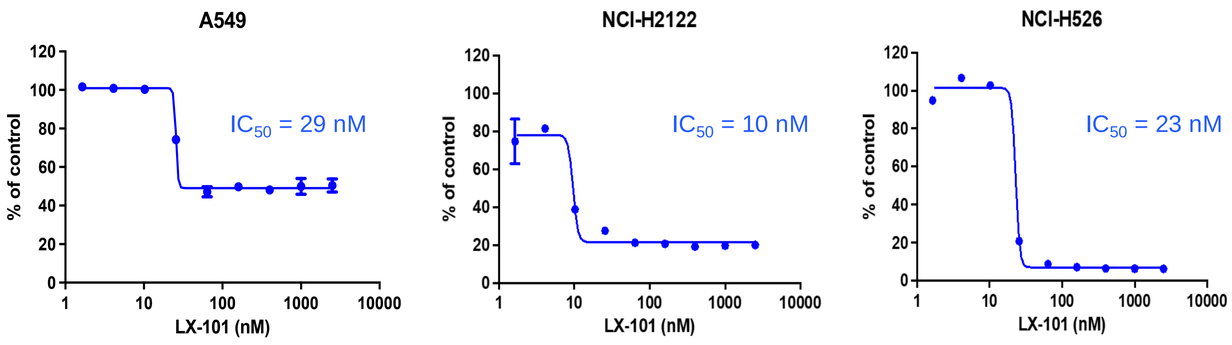


Figure 2. Esophageal Cancer

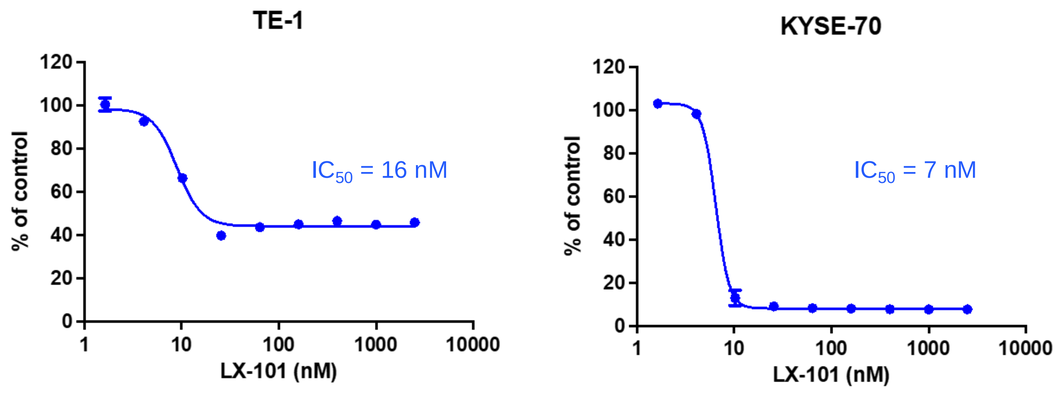


Figure 3. Stomach Cancer

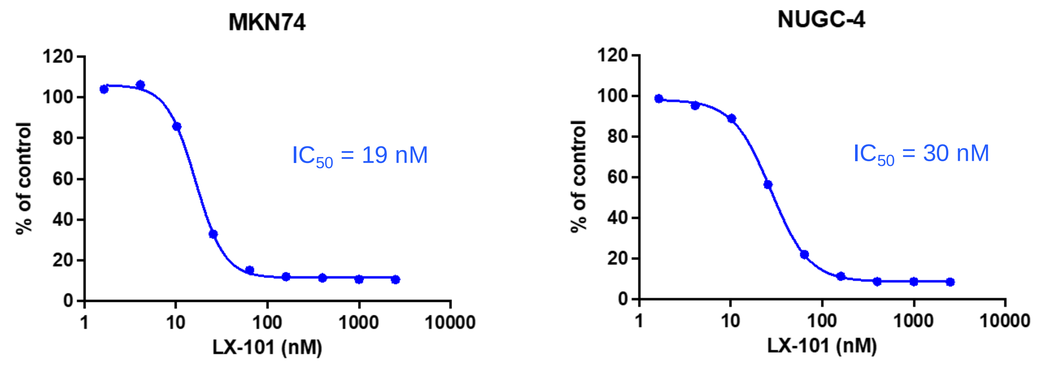


Table 2. LX-101 Absolute IC_{50} Summary

Indication	Cell lines	Absolute IC_{50} (nM)
Lung	A549	29
	NCI-H2122	10
	NCI-H526	23
Esophagus	TE-1	16
	KYSE-70	7
Stomach	MKN74	19
	NUGC-4	30

SUMMARY AND CONCLUSIONS

- **LX-101, a clinical stage next-generation, IGF-1R targeted therapy, demonstrated potent preclinical anti-tumor activity against cancer cells expressing IGF-1R in various tumor types including lung, esophageal, and stomach**
- **Clinical development of LX-101 may benefit from an enrichment strategy focused on enrollment on specific tumor types and/or on patients that express IGF-1R.**
- **Additionally, the utilization of a companion diagnostic and/or tumor agnostic clinical strategy could further enhance development**
- **Prior IGF-1R-targeting drug candidates were non-payload-bearing, naked monoclonal antibodies or small molecule tyrosine kinase inhibitors, and thus may not have effectively addressed redundant pathways and other escape mechanisms**
- **In contrast, LX-101's novel payload-bearing construction could provide a more potent therapeutic approach to targeting IGF-1R-expressing cancers**
- **LX-101 has been previously evaluated in Phase 1a trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity**
- **Given these encouraging data, new clinical trials with LX-101 are being planned in pediatric and adult cancer indications with strong ties to the IGF-1R pathway**

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