105P: LX-101, A NOVEL, CLINICAL STAGE, PAYLOAD-BEARING, IGF-1R TARGETED THERAPY, HAS POTENT PRECLINICAL ANTI-TUMOR ACTIVITY AGAINST SARCOMAS AND OTHER IGF-RELATED CANCERS

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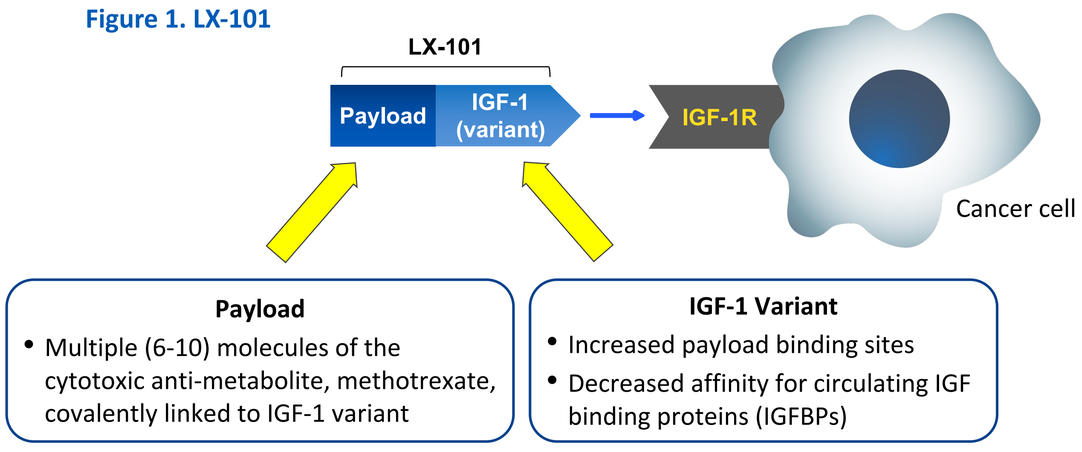
BACKGROUND

- 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 sarcomas and other IGF-related cancers.**

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
- The IGF-1 variant used in LX-101 designed to have reduced binding affinity to circulating serum IGF binding proteins (IGFBPs) to optimize bioavailability

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. 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. SW-13 cells were cultured in L-15 medium + 10% FBS. All cell lines were cultured at 37°C and 5% CO_2, except for SW-13 cells, which were cultured in 100% air.
- **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

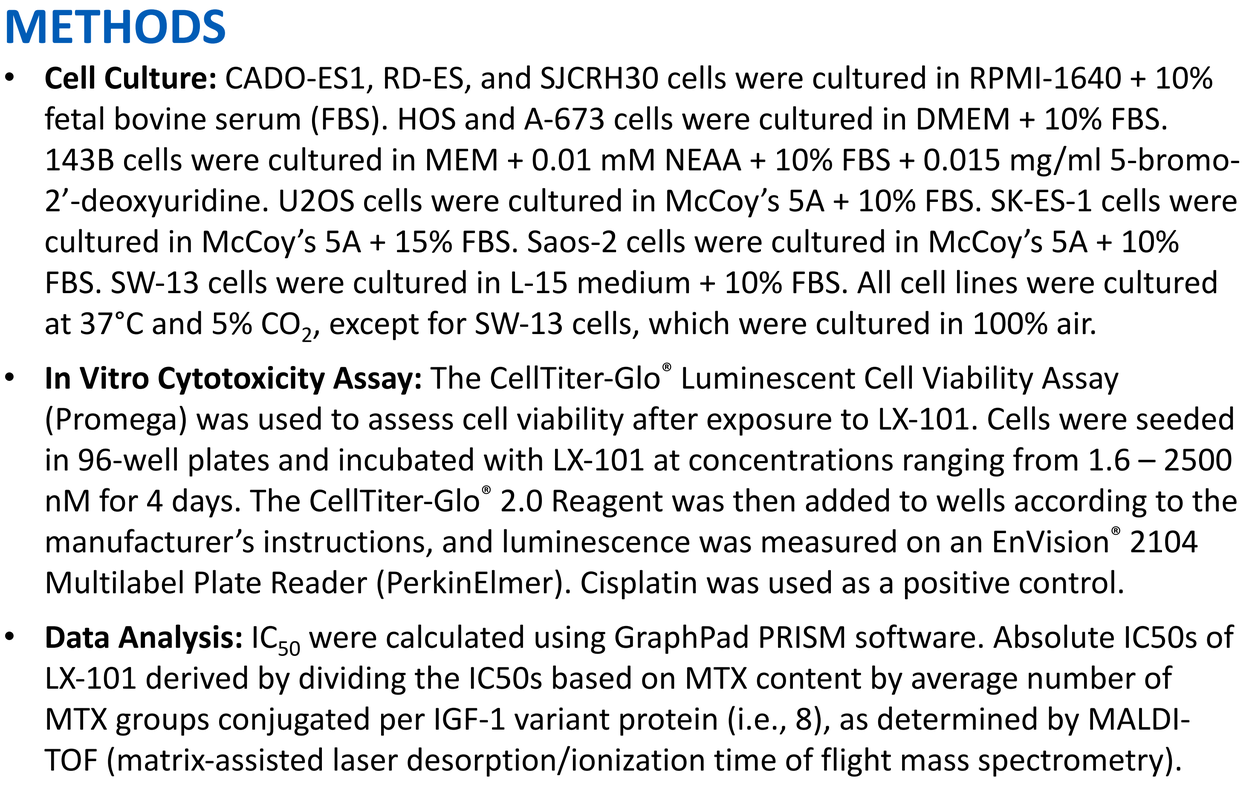
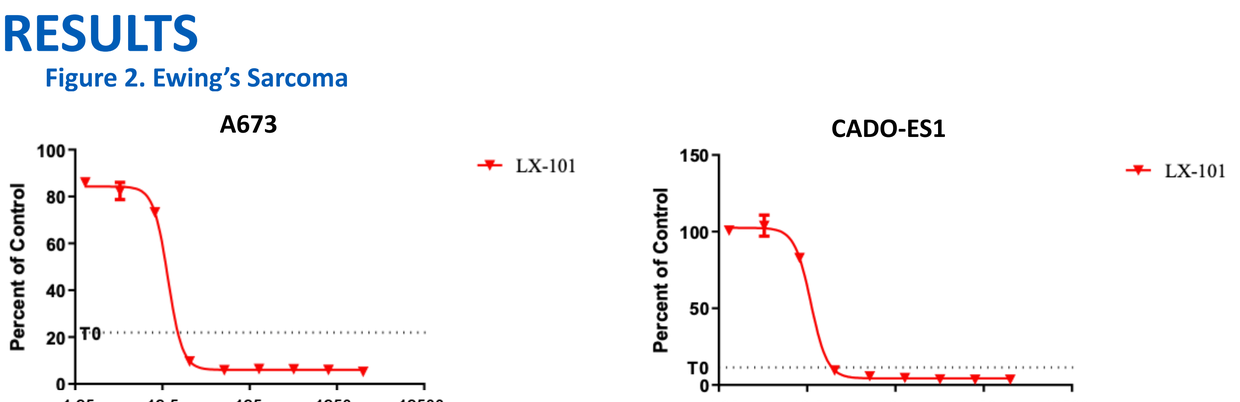


Figure 3. Rhabdomyosarcoma and Adrenocortical Carcinoma



RESULTS (CONT.)

Figure 4. Osteosarcoma

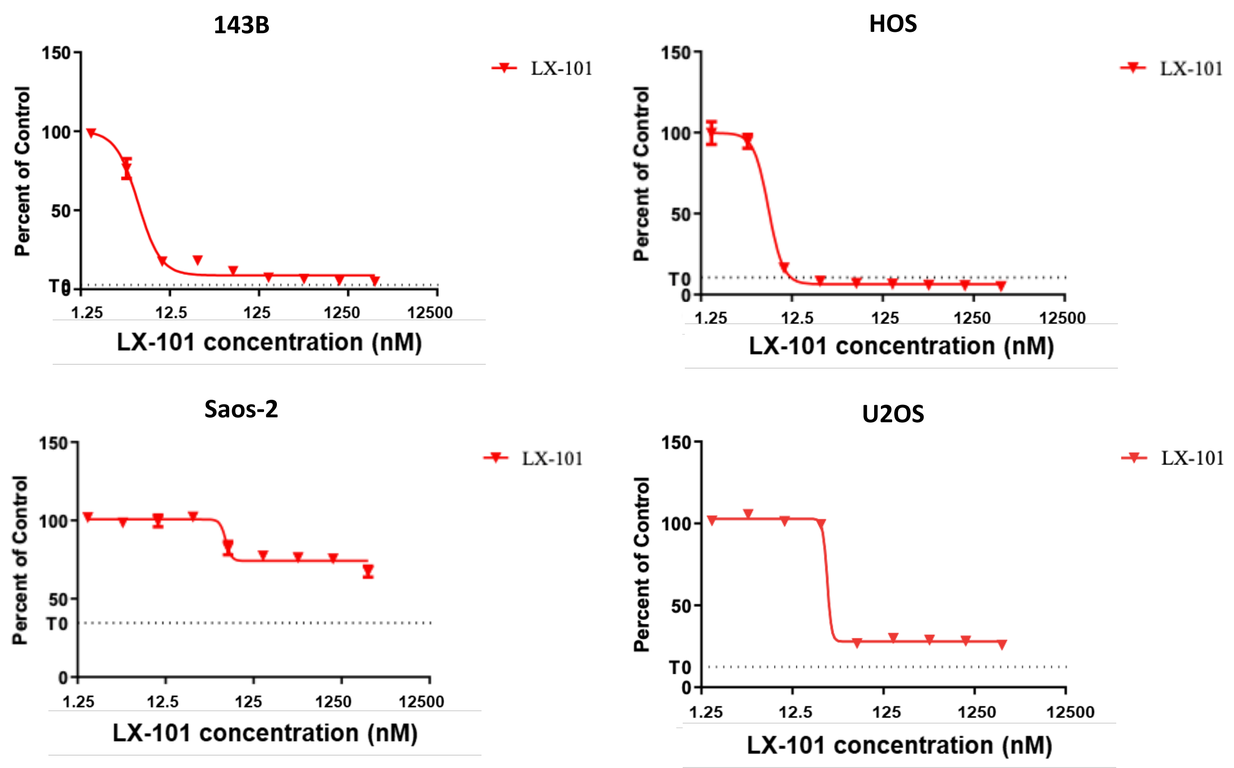


Table 1. LX-101 Absolute IC_{50} Summary

Indication	Cell line	Absolute IC_{50} (nM)
Ewing's sarcoma	RD-ES CADO-ES1 A673 SK-ES-1	10 14 14 29
Rhabdomyosarcoma	SJCRH30 (alveolar)	23
Adrenocortical Carcinoma	SW-13	9
Osteosarcoma	143B HOS U2OS Saos-2	6 7 32 >2500

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

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

Cancer Type	Epigenetic and Genetic Alterations	
Ewing's sarcoma	IGF-1R poor prognosis	EWSR1-FLI1
Rhabdomyosarcoma	IGF-1R and short survival	PAX3/7-FKHR/FOXO1
GIST	High IGF-1R in peds (WT)	NBF1-IGF1R
Synovial Sarcoma	IGF-1R aggressive disease	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
DSRCT	IGF-1R and upregulation	EWSR1-WT1
Adrenocortical carcinoma	IGF-2 overexpression	
Adenoid cystic carcinoma	IGF-2 overexpression	MYB-NF1B
H&N cancer, HPV(-) Bladder cancer, invasive Breast cancer, triple negative	IGF-1R associated with poor outcomes, mortality and shortened survival	
Many cancer type subsets, including lung, breast, colorectal, prostate, ovarian, gastric, esophageal, etc.	IGF-1R over-expression and poor outcome features	

SUMMARY AND CONCLUSIONS

- **LX-101, a clinical stage next-generation, targeted therapy directed to IGF-1R, was previously evaluated (as 765IGF-MTX) in Phase 1a 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**
  - **Also, an enrichment strategy was not employed which presents the opportunity for a more focused tumor-type-specific approach**
- **Prior IGF-1R-targeting drug candidates were non-payload-bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors, and thus may not have addressed redundant pathways and other escape mechanisms that enable cancer cells to evade therapy**
- **In contrast, LX-101, with its novel payload-bearing construction, could provide a more potent therapeutic approach to targeting IGF-1R$^+$-prominent cancers than has been employed in the past**
- **LX-101 demonstrated potent preclinical anti-tumor activity against sarcoma and other cancer cells with well-established ties to the IGF-1R pathway, including those with oncogenic gene fusions affecting the pathway.**
- **Given these encouraging data, new clinical trials with LX-101 are being planned in indications with strong ties to the IGF-1R pathway, focusing on cancers mentioned in Table 2 (above)**

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)

DISCLOSURE

M. Hoberman is an employee of Lirum Therapeutics, Inc.

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