LX-101, a Novel, Clinical Stage, Payload-bearing Targeted Therapy Directed to the IGF-1R Demonstrates Potent Anti-tumor Activity in Ewing Sarcoma Animal Models and Desmoplastic Small Round Cell Tumor

67P

M. HOBERMAN[1], P. MCDONALD[1], R. CARDENAS ZUNIGA[2], D. TRUONG[2], J. LUDWIG[2]

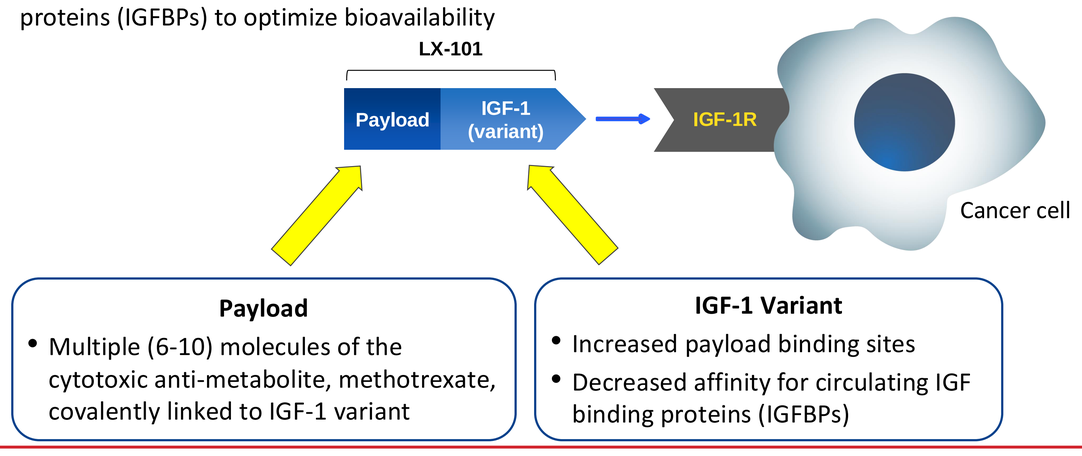
[1]Lirum Therapeutics, New York, USA; [2]MD Anderson Cancer Center, Houston, Texas, USA

BACKGROUND

- The insulin-like growth factor-1 receptor (IGF-1R) pathway is well-established in a wide range of cancers, and is associated with many aspects of oncogenesis, proliferation, resistance, and metastasis
- Certain cancers, including Ewing sarcoma (ES) and desmoplastic small round cell tumor (DSRCT), harbor epigenetic and/or genetic alterations that affect IGF-1R expression and pathway signaling
- Specifically, the *EWSR1* gene is found in the *EWSR1-FLI1* and *EWSR1-WT1* oncogenic fusions in ES and DSRCT respectively; both lead to IGF-1R overactivity and overexpression
- Prior IGF-1R targeting attempts consisted of non-payload bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors. These agents saw a range of clinical outcomes, including some partial and complete responses, but none were ultimately approved in oncology
- These previous approaches may not have been potent enough and allowed cancer cells to evade receptor blockade with work-arounds via redundant signaling pathways and other escape mechanisms
- In contrast to 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 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. 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 but produced encouraging data nonetheless
- 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 evaluated the preclinical anti-tumor activity of LX-101 against ES cell lines and mouse models and DSRCT cell lines**

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

- LX-101 is a next generation IGF-1R-directed agent that delivers a potent payload 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 diseases
- 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

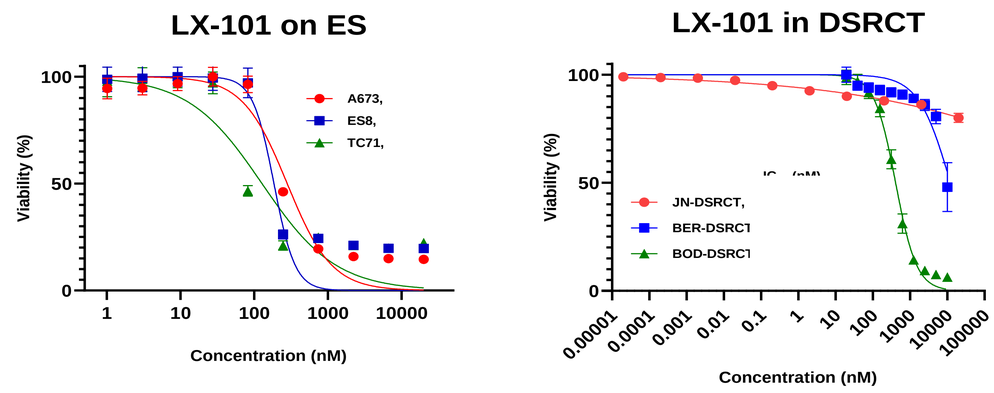
Cancer Type	Epigenetic and Genetic Alterations	
Ewing's sarcoma	IGF-1R overexpression	EWSR1-FLI1
DSRCT	IGF-1R and upregulation	EWSR1-WT1
Rhabdomyosarcoma	IGF-1R and short survival	PAX3/7-FKHR/FOXO1
GIST	High IGF-1R in peds (wild-type)	NBF1-IGF1R
Synovial Sarcoma	IGF-1R and more aggressive	SYT-SSX1/2
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

- **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 – 10,000 nM for 3 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. Pretreatment IGF-1R expression levels were analyzed by RNA-seq & Western blot.
- **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).
- **In Vivo:** NOD-SCID mice bearing subcutaneous A-673 tumors were treated i.v. with vehicle or LX-101 (16 uEq/kg, twice a week, for 3 weeks). Tumor volume and body weight were measured 3 times a week.

RESULTS

Figure 1 and Table 2. LX-101 in Ewing Sarcoma and DSRCT cell lines



Indication	Cell lines	Absolute IC_{50} (nM MTX)	Absolute IC_{50} (nM LX-101)
Ewing Sarcoma	A673	281	35
	ES8	185	23
	TC71	128	16
DSRCT	BOD	410	51
	JN	N/A	N/A
	BER	N/A	N/A

Figure 2. Pretreatment DSRCT IGF-1R mRNA expression

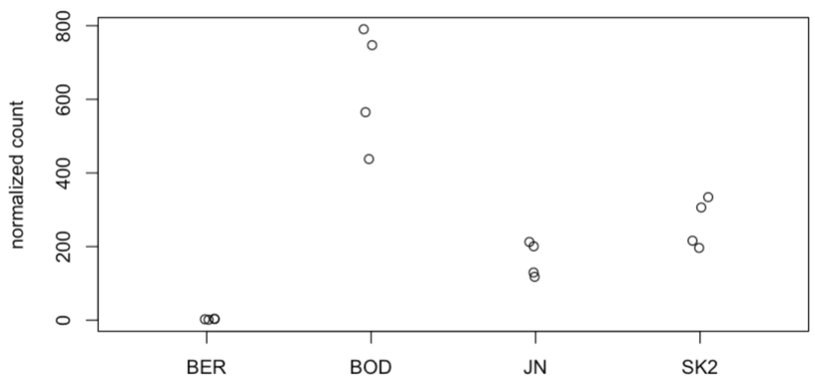
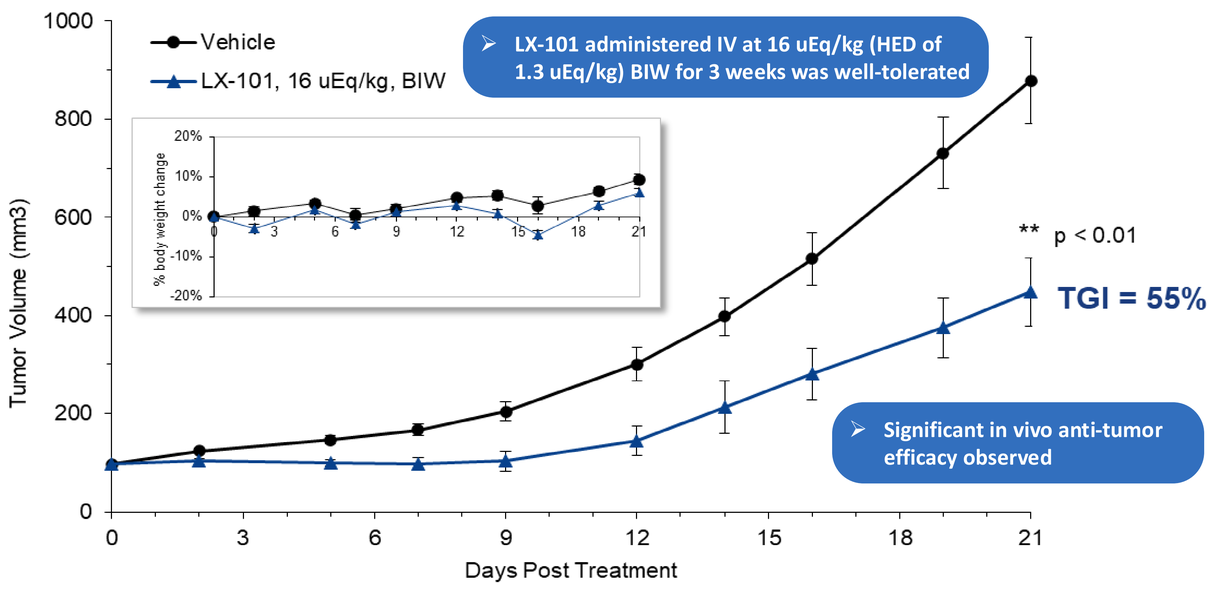


Figure 3. LX-101 In Ewing Sarcoma A-673 Xenograft Model



SUMMARY AND CONCLUSIONS

- **LX-101, a clinical stage next-generation, targeted therapy directed to IGF-1R, was previously evaluated 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**
  - **Notably, 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 and definitive therapeutic approach to targeting IGF-1R$^+$-expressing cancers versus past approaches**
- **LX-101 demonstrated potent preclinical anti-tumor activity in both in vivo and in vitro experiments against Ewing Sarcoma and DSRCT in vitro, showing high potency in the highest IGF-1R expressing DSRCT cell line**
- **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 1, with a particular focus on ES and DSRCT**

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)

M. Hoberman is a full-time employee of Lirum Therapeutics

CONTACT INFORMATION: MHOBERMAN@LIRUMTX.COM