#1918 LX-101, a Novel, Clinical Stage, Payload-bearing Targeted Therapy Directed to the Insulin-like Growth Factor 1 Receptor (IGF-1R), Demonstrates Potent Preclinical Anti-Tumor Activity Against Multiple Cancer Types with Well-Established Ties to the IGF-1/IGF-1R Pathway

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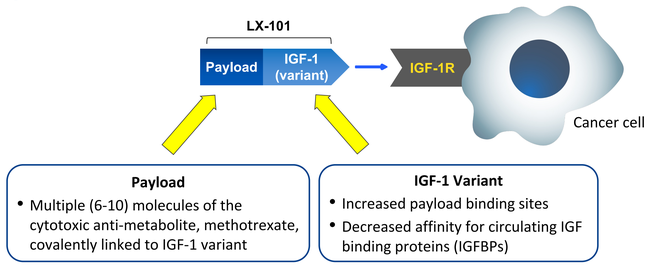
BACKGROUND

- 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-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 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
- 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:** FADU, BT-20 cells, and IMR32 cells were cultured in MEM + 0.01 mM NEAA + 10% FBS. 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. SK-N-AS cells were cultured in DMEM (high glucose) + 1% NEAA + 10% FBS. SH-SY5Y cells were cultured in 1:1 MEM + 0.01 mM NEAA / Ham's F12K + 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 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. Head and Neck Squamous Cell Carcinoma (HNSCC) and Triple Negative Breast Cancer

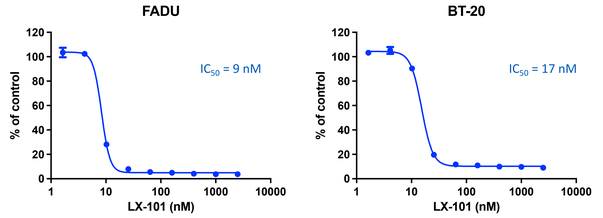


Figure 3. Ewing's Sarcoma and Rhabdomyosarcoma

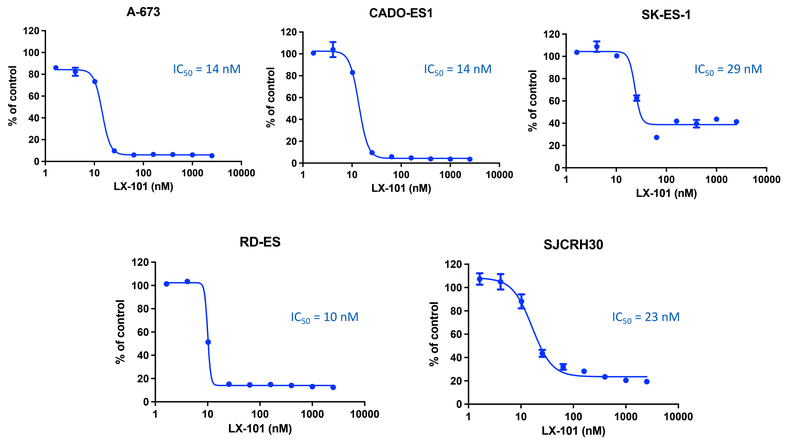


Figure 4. Osteosarcoma

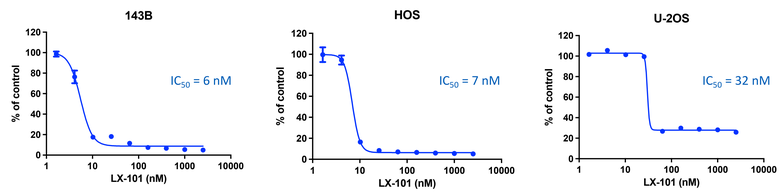
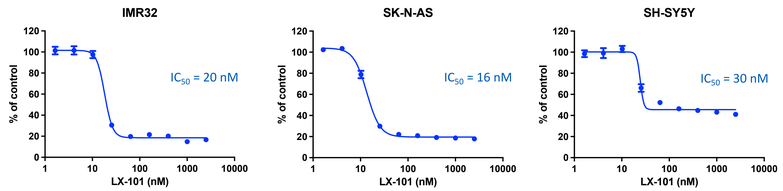


Figure 5. Neuroblastoma



RESULTS (CONT.)

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

Indication	Cell lines	Absolute IC_{50} (nM)
HNSCC	FaDu	9
Triple Negative Breast	BT-20	17
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
Neuroblastoma	SK-N-AS	16
	IMR-32	20
	SH-SY5Y	30

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 and poor prognosis	EWSR1-FLI1
Rhabdomyosarcoma	IGF-1R and short survival	PAX3/7-FKHR/FOXO1
Gastorintestinal stromal tumor (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
Desmoplastic small round cell tumor (DSRCT)	IGF-1R and upregulation	EWSR1-WT1
Adrenocortical carcinoma	IGF-2 overexpression	
Adenoid cystic carcinoma	IGF-2 overexpression	MYB-NF1B
Head and neck squamous cell carcinoma, 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 cancer cells with well-established ties to the IGF-1R pathway, including those with oncogenic gene fusions and other alterations 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 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)

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