LX-101, a Novel Payload-bearing IGF-1R Targeted Therapy, Inhibits Thyroid Eye Disease Inflammation

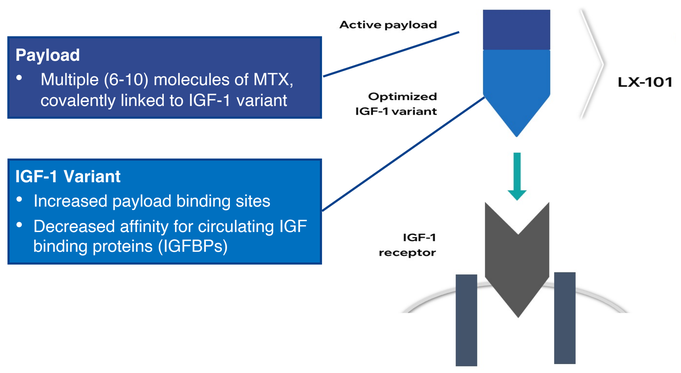
Husain, F[1]; Roztocil, E[1]; Patrick, CP[1]; Hoberman, M[2]; McDonald, P[2]; Feldon, SE[1]; Woeller, CF[1]; [1]: Flaum Eye Institute, Department of Ophthalmology, University of Rochester, Rochester, New York 14642, USA; [2]: Lirum Therapeutics, New York, New York 10020, USA

INTRODUCTION

- In thyroid eye disease (TED), excessive inflammation driven by activated orbital fibroblasts (OFs) and infiltrating T lymphocytes leads to orbital tissue expansion, pain, diplopia, vision impairment, and potentially sight-threatening outcomes for patients.
- The insulin-like growth factor 1 receptor (IGF-1R) pathway plays a key role in the pathogenesis of TED and is a clinically validated target for the treatment of patients with TED.
- Current treatment approaches in TED have limitations, such refractoriness, response duration, relapse and adverse events. There may also be unique opportunities in chronic TED, where signaling is potentially reduced. Aberrant signaling via other pathways (e.g., thyroid stimulating hormone receptor [TSHR]) is largely unaddressed. All of which highlights a need for novel treatment options.

LX-101: Targets IGF-1R

- LX-101 is a novel, next generation IGF-1R-directed agent that delivers a methotrexate (MTX) payload with high precision. MTX is widely used in many autoimmune diseases, including a history of use in TED.
- 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 nor maximum tolerated dose was reached leaving room for additional regimen optimization.[1]
- LX-101 consists of an optimized variant of the IGF-1 ligand, covalently conjugated to MTX, a drug that is systemically used to treat patients with a variety of autoimmune diseases.



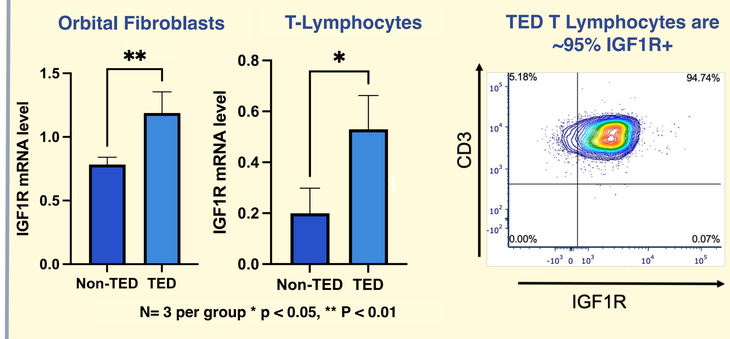
- **Herein, we evaluate LX-101's activity on IGF-1R+ OFs and T lymphocytes, the key effector cells of TED.**

METHODS

- OFs and T lymphocytes derived from TED patients were treated separately or in co-cultures with LX-101 (0.01 - 2.5 µM).
- Cell viability was measured using redox-sensitive dyes, and Western blot was used to evaluate markers of apoptosis.
- Inflammatory cytokine levels were analyzed by Luminex assay, ELISA, and RT-qPCR.
- Cell migration was assessed via Scratch assays. Open areas were quantified by ImageJ software and normalized to time 0.

RESULTS

IGF-1R Expression is Elevated in TED



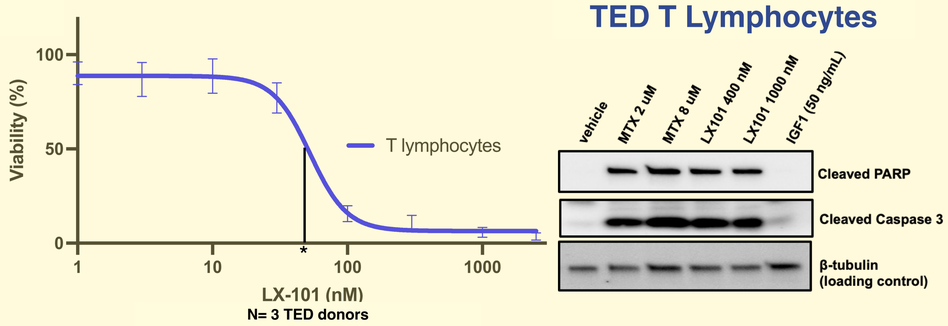
N= 3 per group * p < 0.05, ** P < 0.01

➢ **TED OFs and T lymphocytes over-express IGF-1R**

LX-101 Targets Key Pathogenic Processes in TED

Modulates Activity of IGF-1R+ T Cells

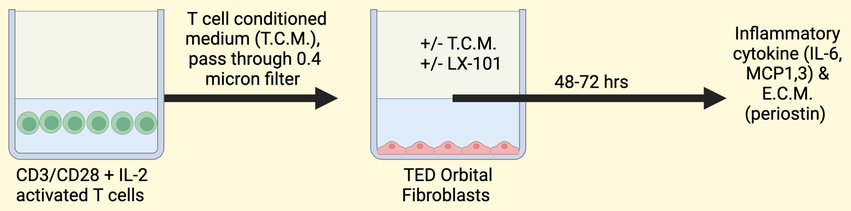
- TED T cells expanded from PBMC isolates or TED Orbital fibroblasts grown from tissue explants were treated with varying doses of LX-101 for 48 hours.



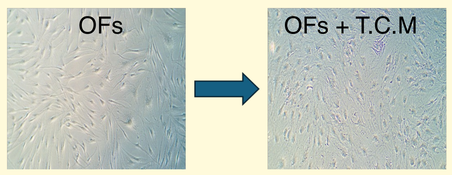
➢ **LX-101 modulates activity of IGF-1R+ T cells with an IC_{50} of ~52 nM(*), a clinically achievable concentration[1]**

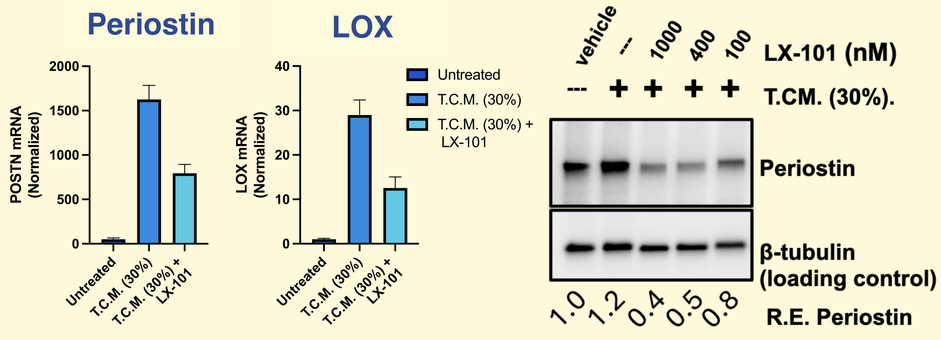
Reduces Production Pro-Fibrotic ECM Components

- T cell conditioned medium (T.C.M.) activates TED orbital fibroblasts.



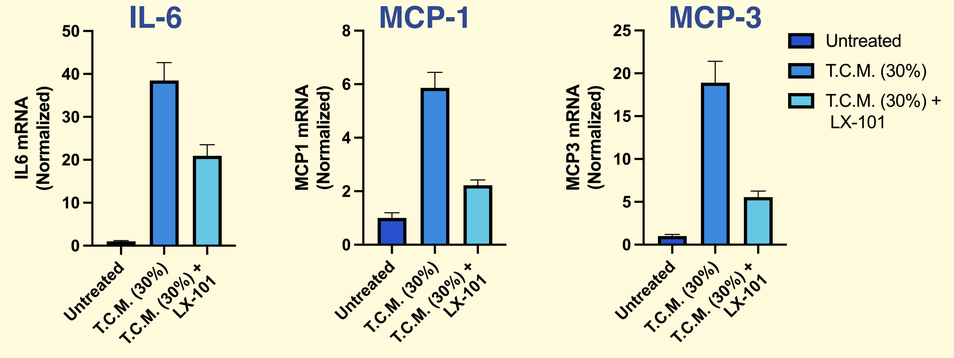
- Conditioned medium from TED T cells induces changes in TED OF morphology and production of extracellular matrix and cytokines.





➢ **LX-101 mitigates the production of pro-fibrotic extracellular matrix components, periostin and LOX, by TED OFs activated by T cell medium**

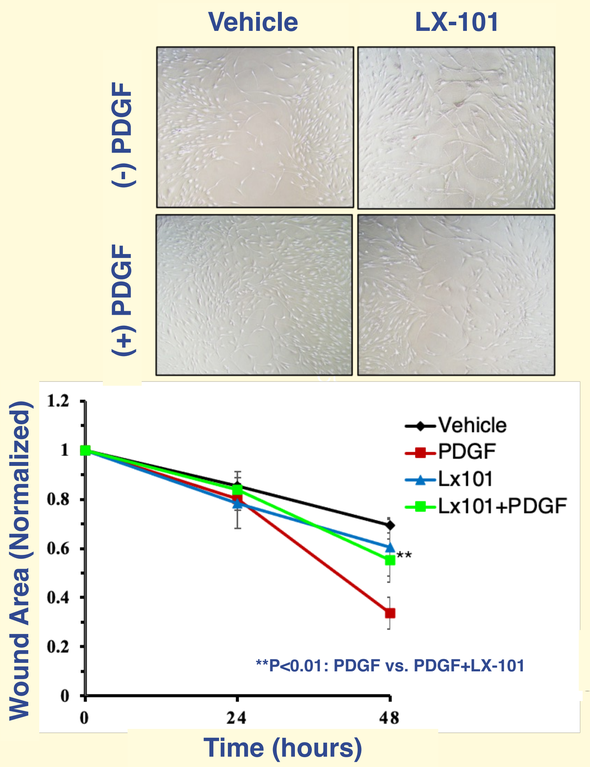
Decreases Production of Key Inflammatory Cytokines



➢ **LX-101 decreases production of IL-6, MCP-1 and MCP-3, key pro-inflammatory cytokines, by orbital fibroblasts**

Inhibits Migration of TED OFs

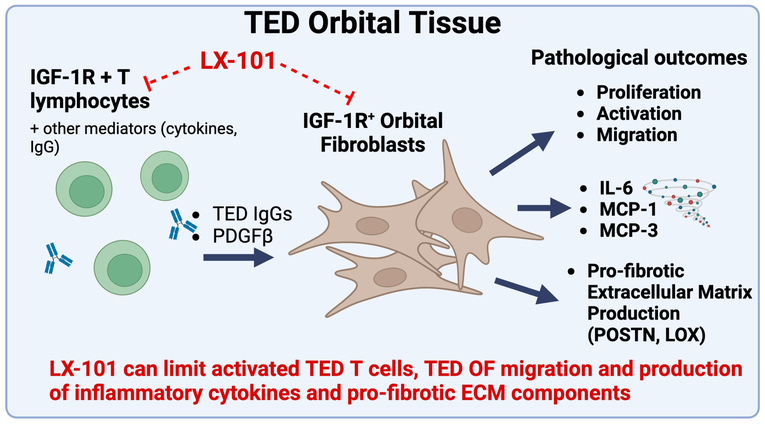
- OFs grown to confluence and subjected to scratch-wound followed by treatments as shown. Images captured at 0 and 48 hours (representative images shown).



➢ **LX-101 inhibits OF migration capacity, a marker of orbital tissue expansion / fibrosis**

CONCLUSIONS

- TED is marked by excessive inflammation driven by infiltrating T lymphocytes and activated orbital fibroblasts (OFs), resulting in pain, eye protrusion, fibrosis and vision impairment.
- These results highlight the ability of LX-101, a novel IGF-1R-directed agent, to effectively target the underlying inflammatory processes that drive TED pathogenesis.



LX-101 can limit activated TED T cells, TED OF migration and production of inflammatory cytokines and pro-fibrotic ECM components

Key findings demonstrate that LX-101 can:

- ➢ Inhibit migration of orbital fibroblasts,
- ➢ Decrease production of key inflammatory cytokines (IL-6, MCP-1, MCP-3),
- ➢ Reduce production of pro-fibrotic ECM components (Periostin, LOX), and
- ➢ Modulate activity of TED T lymphocytes at clinically achievable concentrations.

- **Given these encouraging results, strong scientific rationale and prior positive clinical experience, further clinical development of LX-101 in thyroid eye disease is planned.**

ACKNOWLEDGEMENTS

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Reference: [1] Venepalli et al Am J Clin Oncol 2019;42:862–869

CONTACT INFORMATION: MHOBERMAN@LIRUMTX.COM