LONIGUTAMAB (ANTI–IGF-1R MONOCLONAL ANTIBODY) INDUCES EFFICIENT DEGRADATION OF IGF-1R IN THYROID EYE DISEASE ORBITAL FIBROBLASTS

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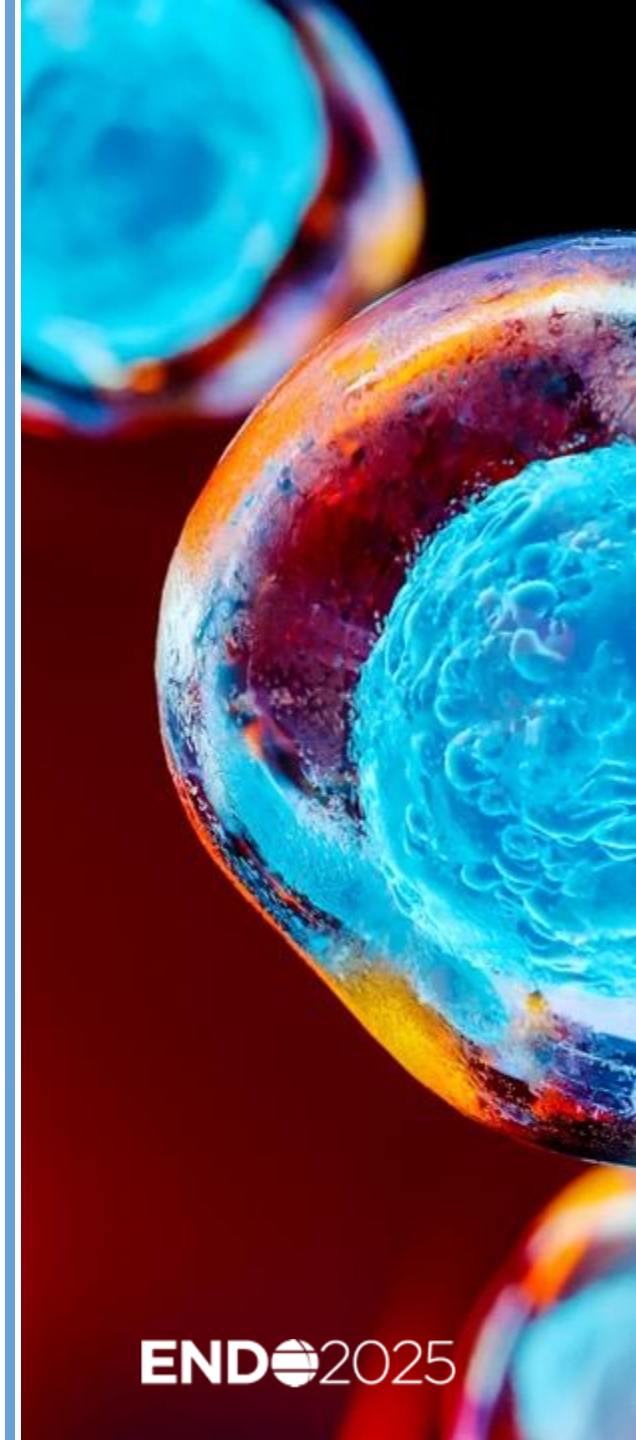
*AT THE TIME OF THE STUDY



DISCLOSURES

- **Brett Welch** and Andrew Nyborg are employed by and have stock/stock options from ACELYRIN, INC., a wholly owned subsidiary of Alumis, Inc.
- Shephard Mpofu was employed by ACELYRIN, INC., at the time of the study, and has stock/stock options from ACELYRIN, INC., a wholly owned subsidiary of Alumis, Inc.
- Collynn F. Woeller receives consulting fees from ACELYRIN, INC., a wholly owned subsidiary of Alumis, Inc.
- This study is sponsored by ACELYRIN, INC., a wholly owned subsidiary of Alumis, Inc. Lonigutamab is an investigational therapy not approved by any regulatory authority
- All authors met the ICMJE authorship criteria and had full access to relevant data
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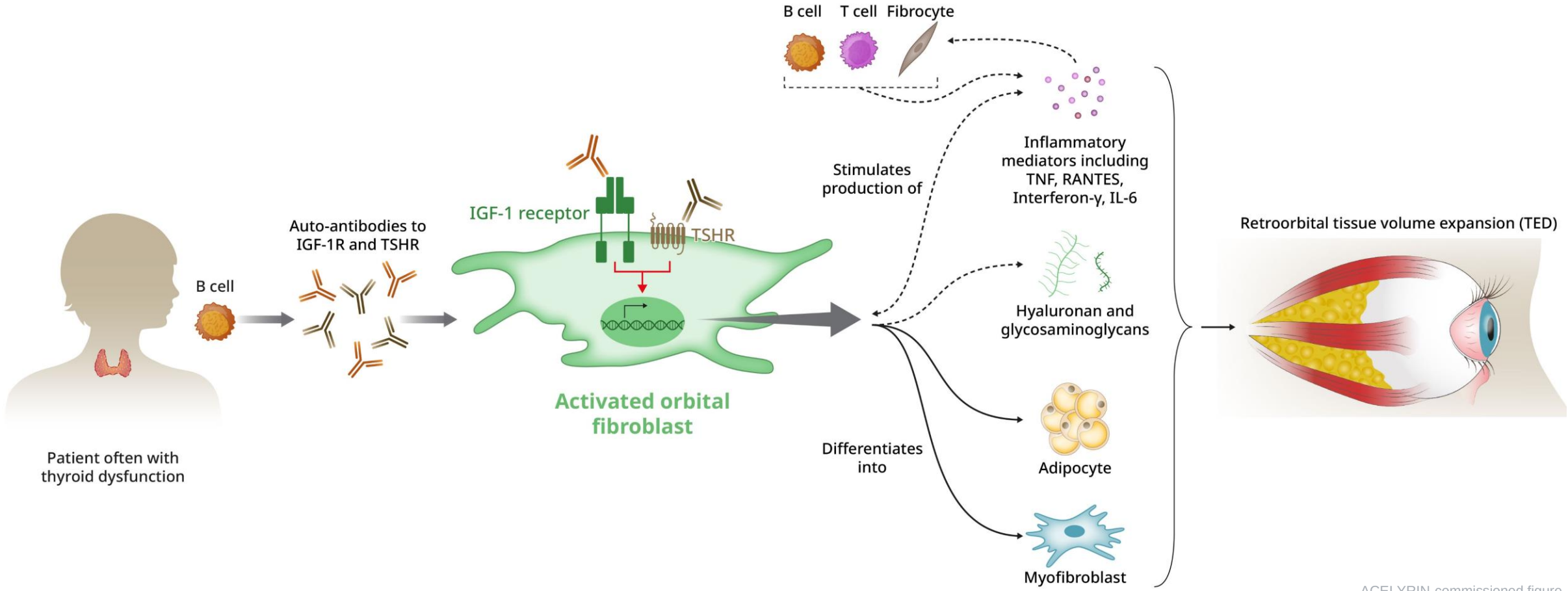
IGF-1R IS CENTRAL TO TED PATHOGENESIS

- **Thyroid eye disease (TED)** is a chronic, **debilitating,** and **vision-threatening** condition driven by aberrant stimulation of the **IGF-1R** pathway[1,2]
- **IGF-1R** is a clinically validated therapeutic target in TED[1,3]



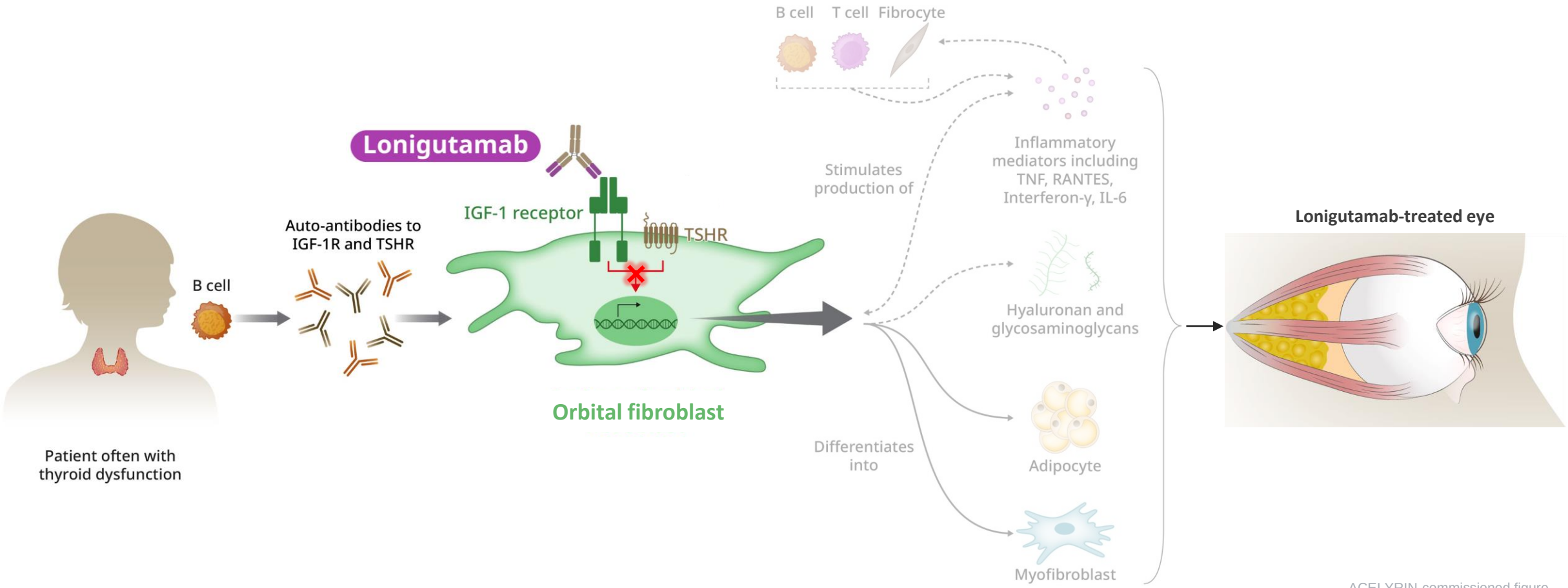
ACELYRIN-commissioned figure

IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; IL-6, interleukin-6; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; TED, thyroid eye disease; TNF, tumor necrosis factor; TSHR, thyroid-stimulating hormone receptor.

1. Smith TJ, et al. *Endocr Rev*. 2019;40(1):236-67. 2. Men CJ, et al. *Ther Adv Ophthalmol*. 2021;13:25158414211027760. 3. Douglas RS, et al. *N Engl J Med*. 2020;382(4):341-52. 4. Akla B, et al. *Mol Cancer Ther*. 2020;19(1):168-77. 5. Data on file. ACELYRIN, INC.

IGF-1R IS CENTRAL TO TED PATHOGENESIS

- **Lonigutamab** is a high-affinity, subcutaneously administered, next-generation **anti–IGF-1R monoclonal antibody**[4,5]



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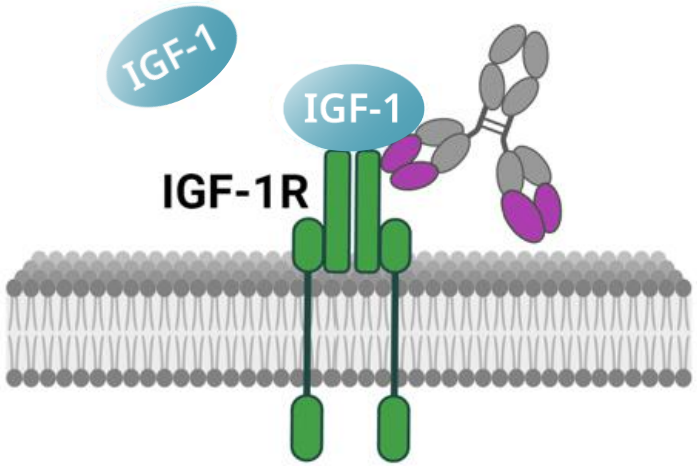
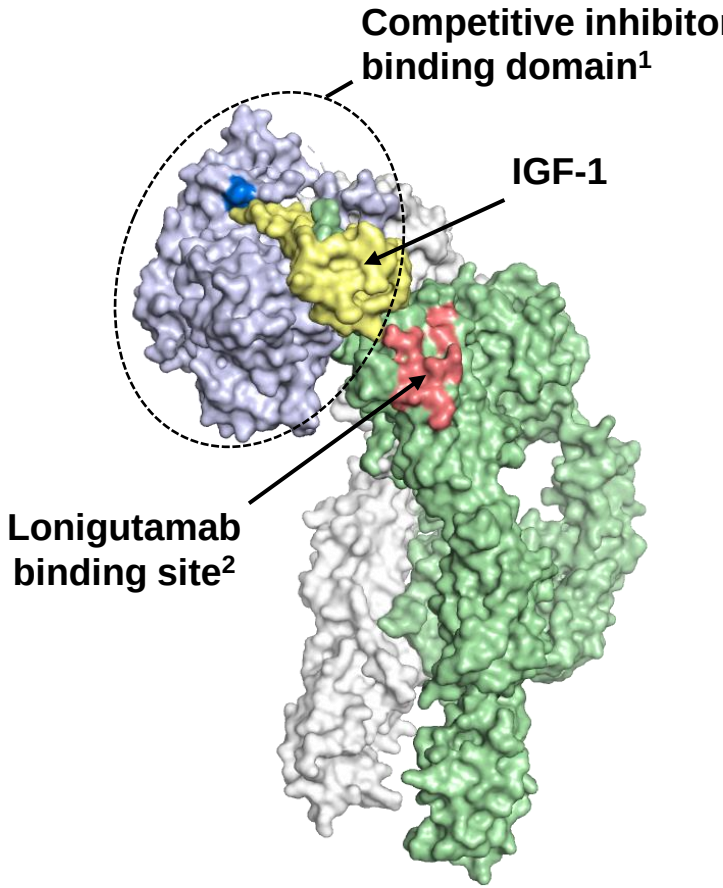
LONIGUTAMAB BINDS A UNIQUE IGF-1R EPITOPE AND DOES NOT COMPETE WITH IGF-1 BINDING

Lonigutamab



Noncompetitive IGF-1/IGF-1R inhibition may provide a positive clinical risk/benefit profile for patients with TED



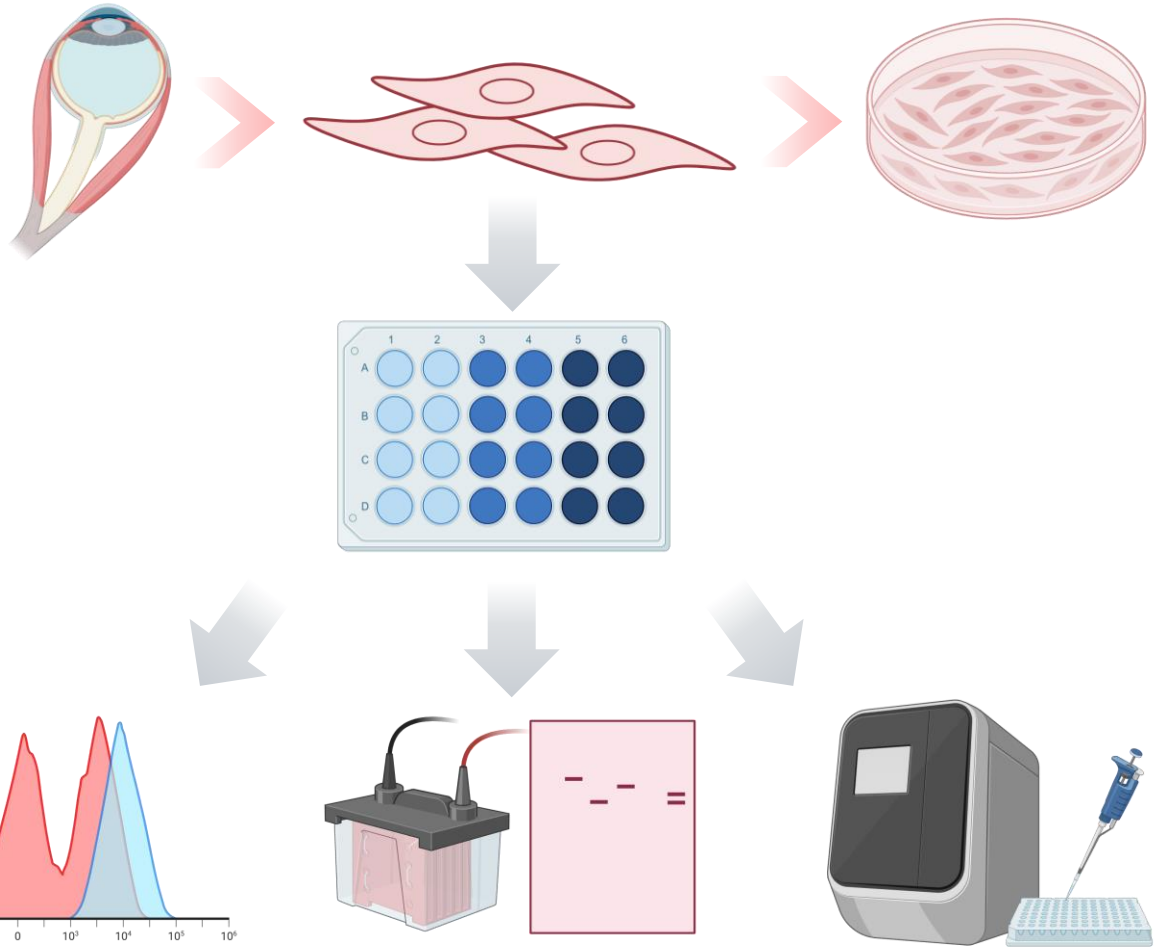
Ligand-bound IGF-1R dimer[3,4]

IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; TED, thyroid eye disease.

1. Bedian V, et al. *J Endocr Soc*. 2023;7(suppl 1):bvad114.2051. 2. Data on file. ACELYRIN, INC. 3. Li J, et al. 2019. https://doi.org/10.2210/pdb6PYH/pdb. 4. Li J, et al. *Nat Commun*. 2019;10(1):4567.

OBJECTIVE AND METHODS

OBJECTIVE: to describe studies that **establish the mechanism of action of lonigutamab** in TED orbital fibroblasts



Cell Culture

- Primary human orbital fibroblasts from patients with TED were harvested and cultured in DMEM supplemented with 10% FBS and antibiotics

Treatment

- Orbital fibroblasts (passages 4–10 post explant) were pretreated with vehicle (0.1% BSA-PBS) or lonigutamab
- Orbital fibroblasts were then incubated with vehicle or IGF-1 for 48 to 72 hours

Assessments

- Surface IGF-1R protein: flow cytometry
- Total cellular IGF-1R protein: Western blot
- *IGF-1R* mRNA: RT-qPCR
- Mechanism of IGF-1R degradation:
  - Addition of a proteosome inhibitor and/or an autophagy/lysosome inhibitor to cell culture
  - Western blot

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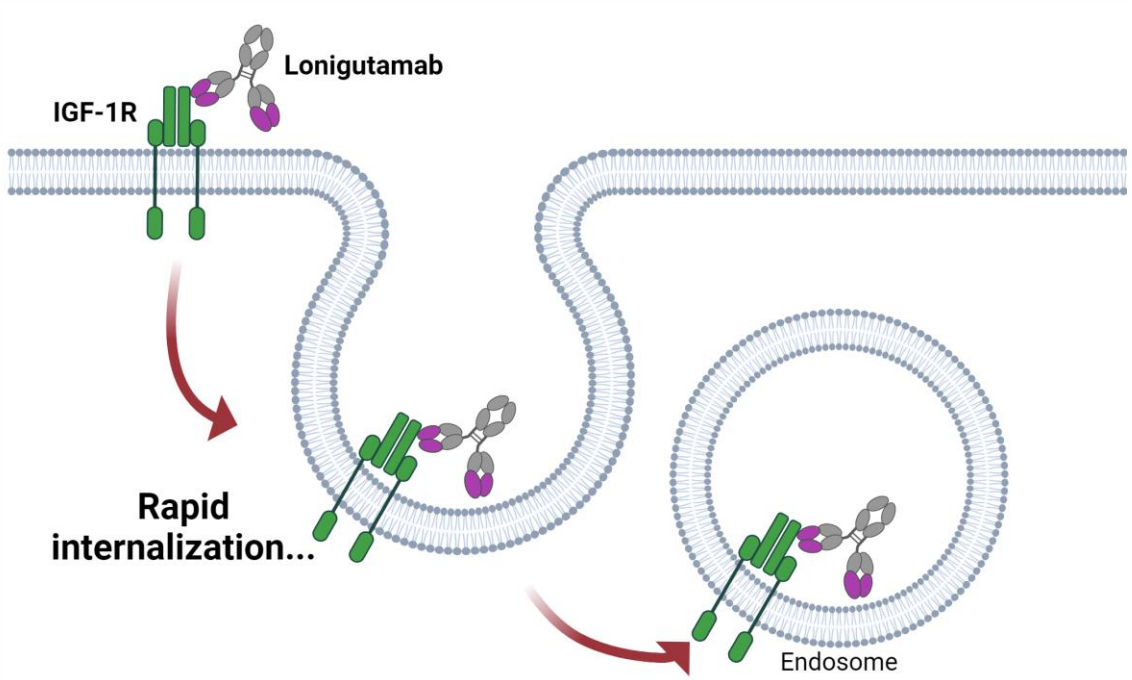
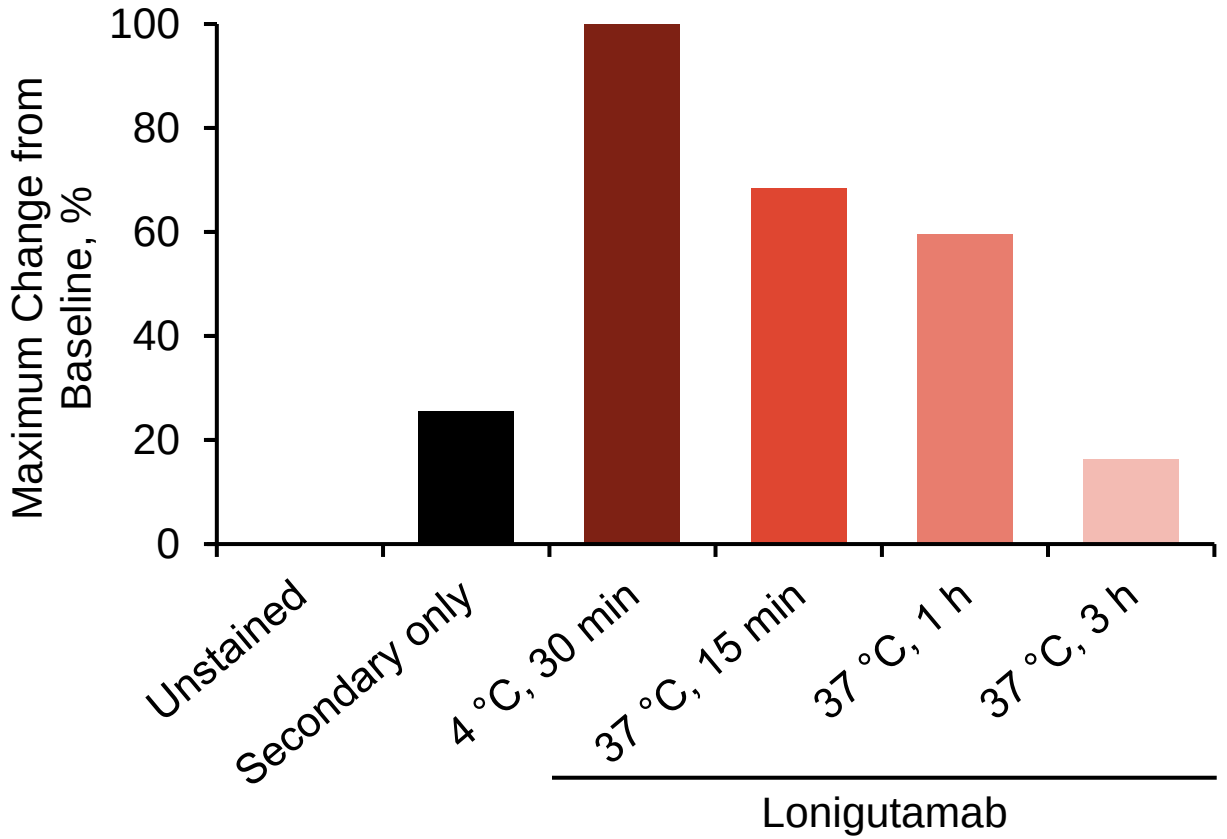
BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; mRNA, messenger RNA; PBS, phosphate-buffered saline; RT-qPCR, reverse transcription quantitative polymerase chain reaction; TED, thyroid eye disease.

LONIGUTAMAB TREATMENT ELICITED RAPID INTERNALIZATION OF IGF-1R FROM THE CELL SURFACE

Treatment with **lonigutamab resulted in rapid internalization of IGF-1R** from the surface of TED orbital fibroblasts

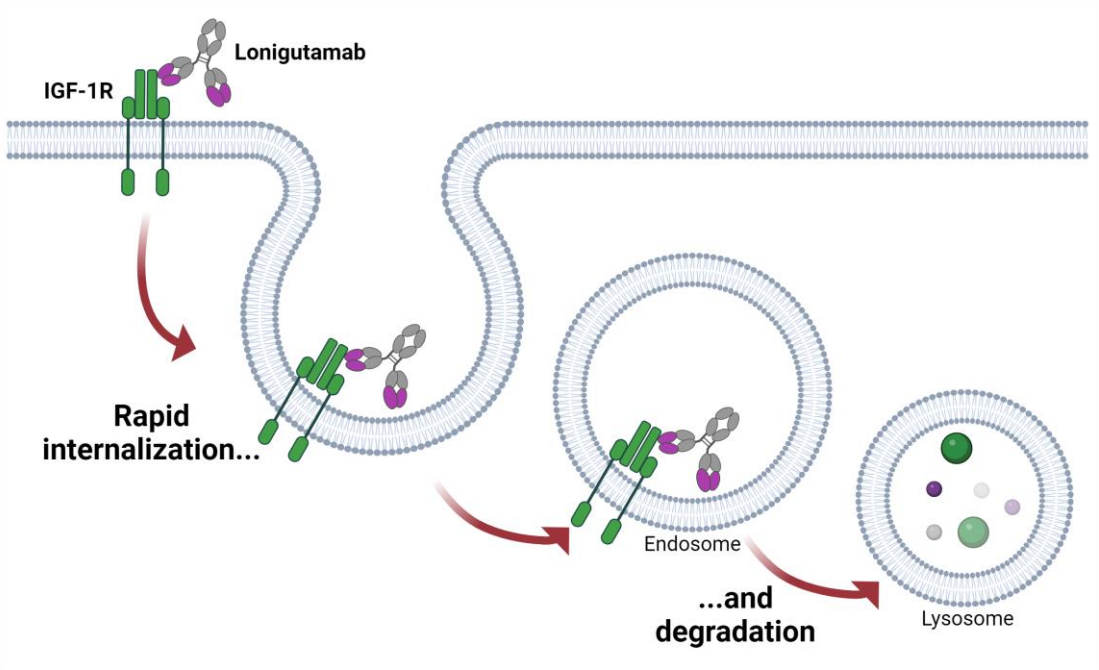


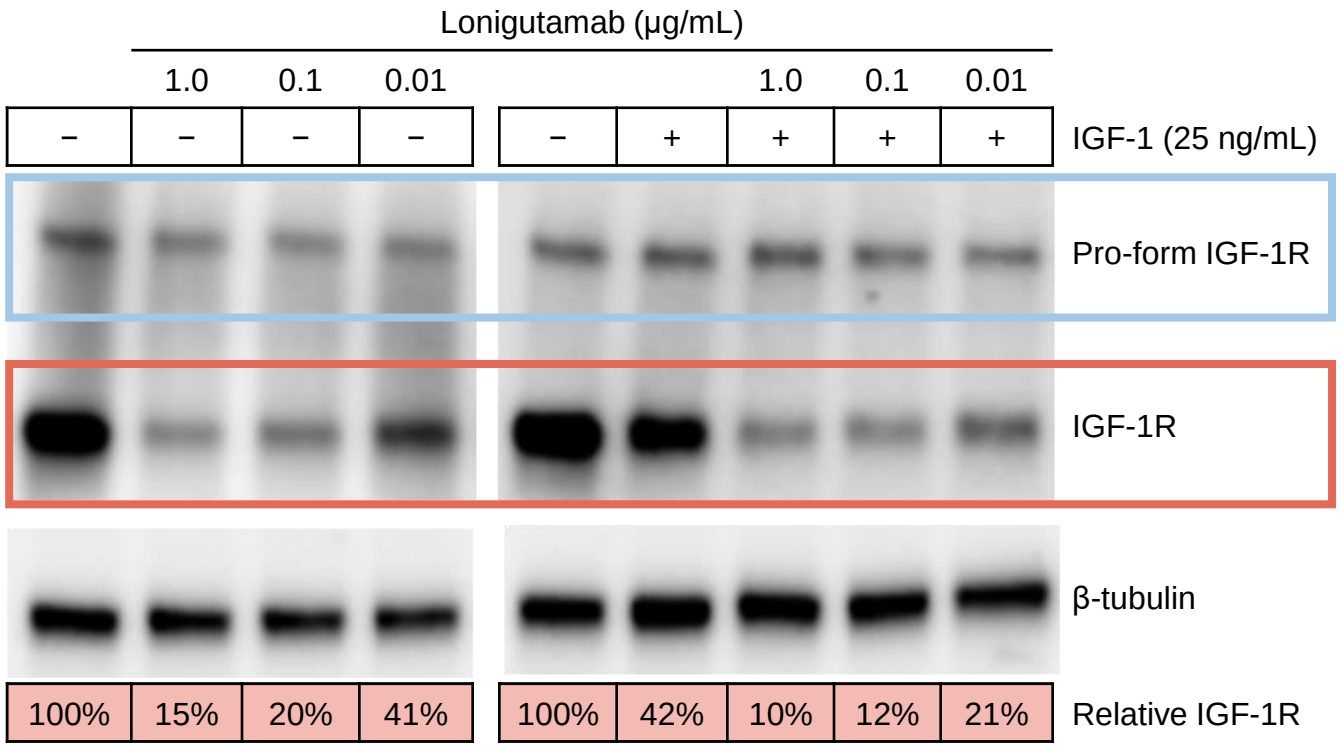


Cells were treated with lonigutamab (2 µg/mL) at 4 °C or 37 °C for up to 3 hours. Samples were then harvested, washed, stained at 4 °C with a fluorescently conjugated secondary antibody, and analyzed by flow cytometry.
IGF-1R internalization was quantified by comparing surface IGF-1R levels (MFI) at 37 °C vs 4 °C using the formula: % internalization = $100 \times (MFI_4°C - MFI_{37}°C) / MFI_4°C$
IGF-1R, insulin-like growth factor 1 receptor; MFI, mean fluorescence intensity; TED, thyroid eye disease.

LONIGUTAMAB TREATMENT REDUCED TOTAL IGF-1R PROTEIN LEVELS

Treatment with **lonigutamab substantially reduced total mature IGF-1R levels** in TED orbital fibroblasts in the absence and presence of IGF-1

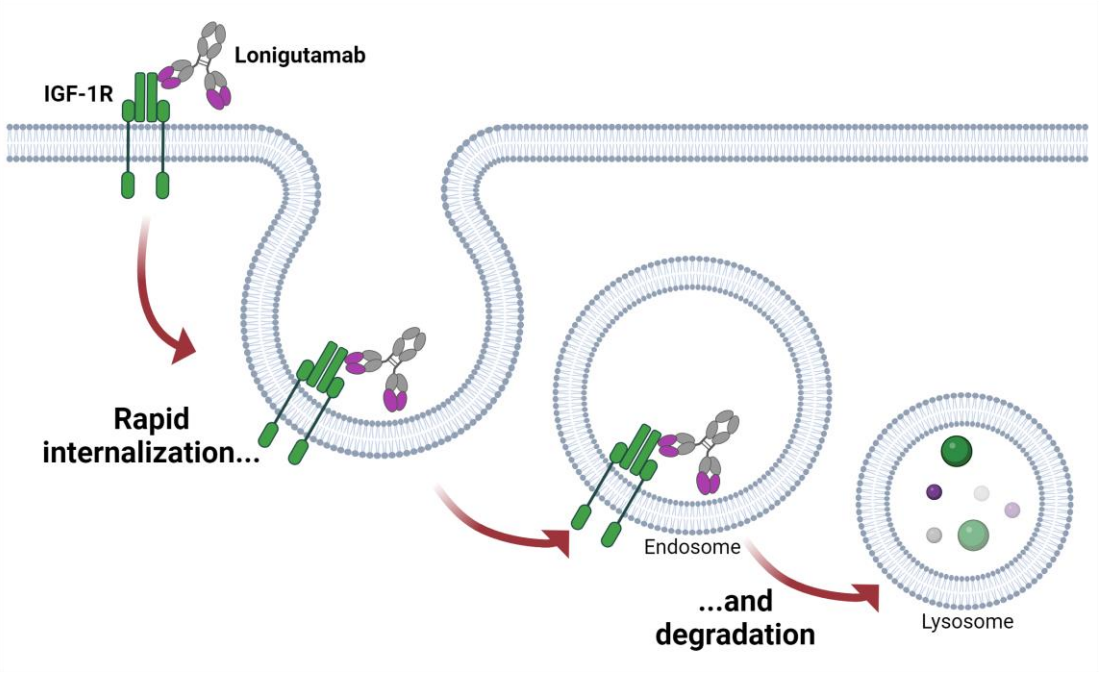


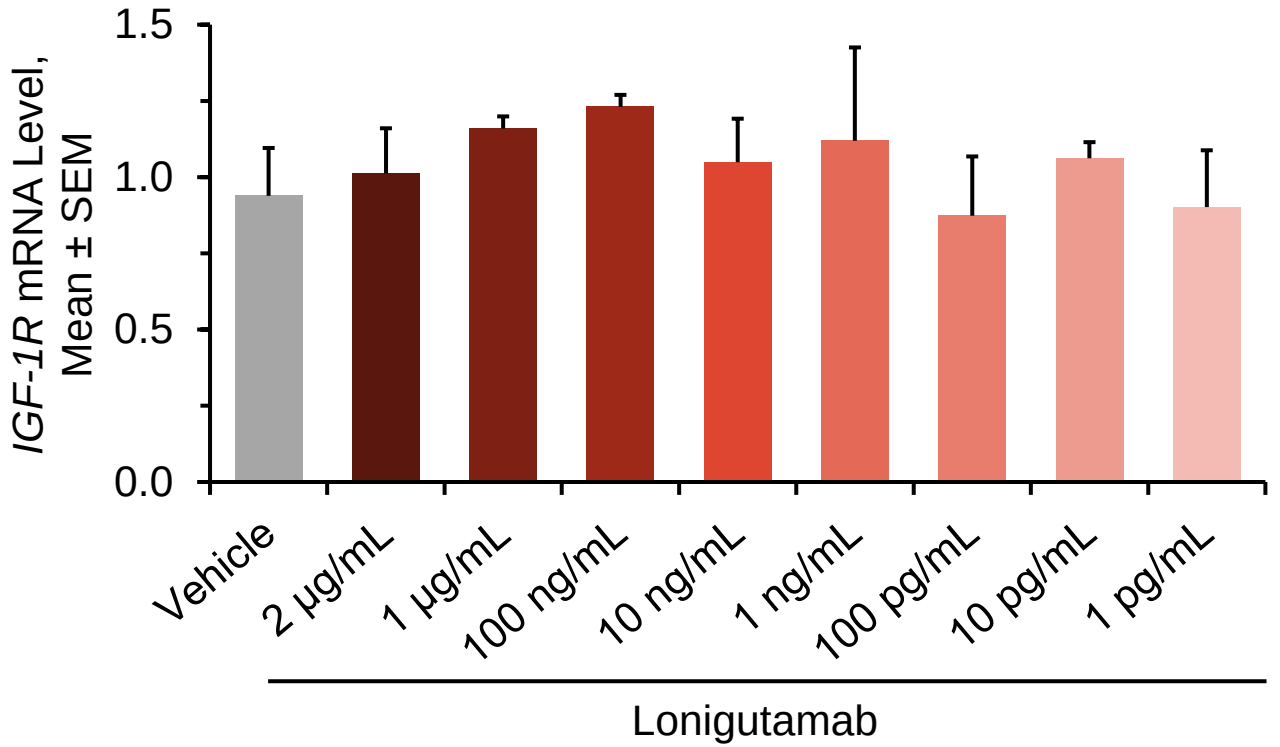


TED orbital fibroblasts were plated and grown to confluence. The media was replaced with fresh media containing vehicle or 1.0, 0.1, or 0.01 µg/mL lonigutamab. After 1 hour, IGF-1 (25 ng/mL) was added for an additional 48 hours. Then, cell lysate and cell culture supernatants were collected and analyzed for mature IGF-1R levels by Western blot.
IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; TED, thyroid eye disease.

EFFECTS OF LONIGUTAMAB WERE SPECIFIC TO MATURE IGF-1R PROTEIN

Treatment of TED orbital fibroblasts with **lonigutamab did not change mRNA levels of *IGF-1R***

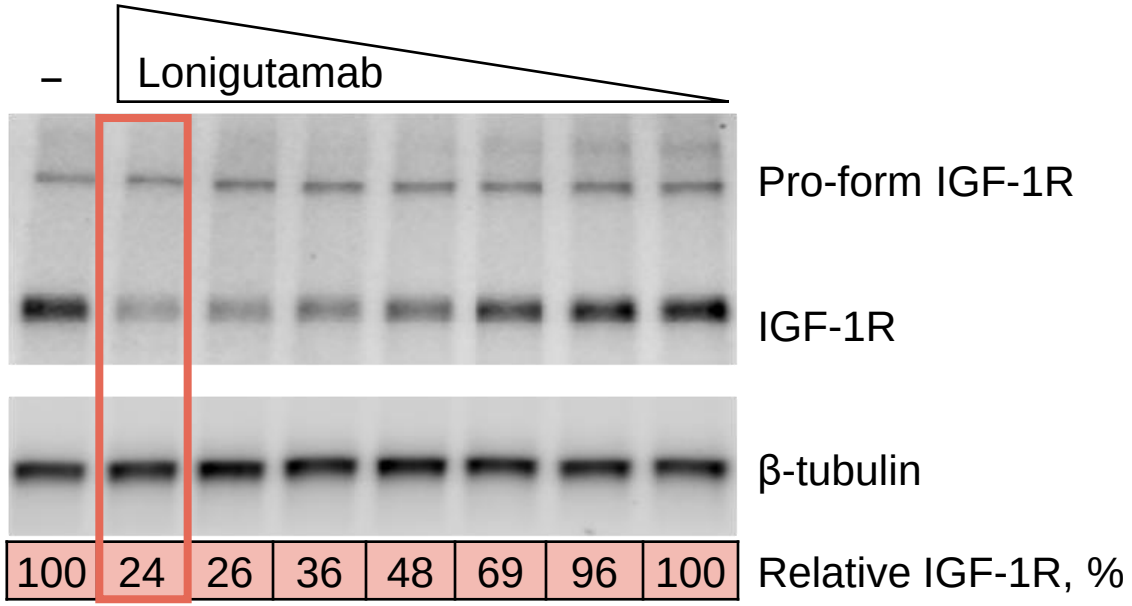




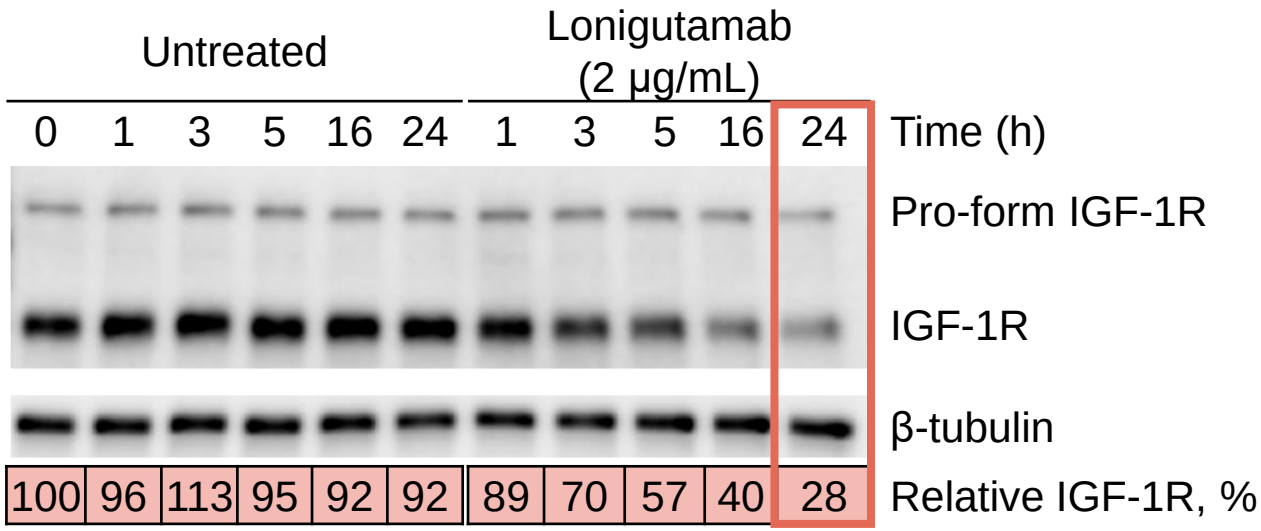
TED orbital fibroblasts were treated with varying amounts of lonigutamab for 24 hours. Cells were then lysed and total RNA isolated. RT-qPCR analysis was performed for *IGF-1R* mRNA and 18S rRNA (control gene). The levels of *IGF-1R* mRNA were normalized to 18S rRNA values.
IGF-1R, insulin-like growth factor 1 receptor; mRNA, messenger RNA; rRNA, ribosomal RNA; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SEM, standard error of the mean; TED, thyroid eye disease.

LONIGUTAMAB-MEDIATED REDUCTION IN IGF-1R WAS CONCENTRATION AND TIME DEPENDENT

The **reduction** in total IGF-1R levels in TED orbital fibroblasts **was dependent on lonigutamab concentration** (0.00001–2 µg/mL)



The **reduction** in total IGF-1R levels in TED orbital fibroblasts **was time dependent** (1–24 hours)

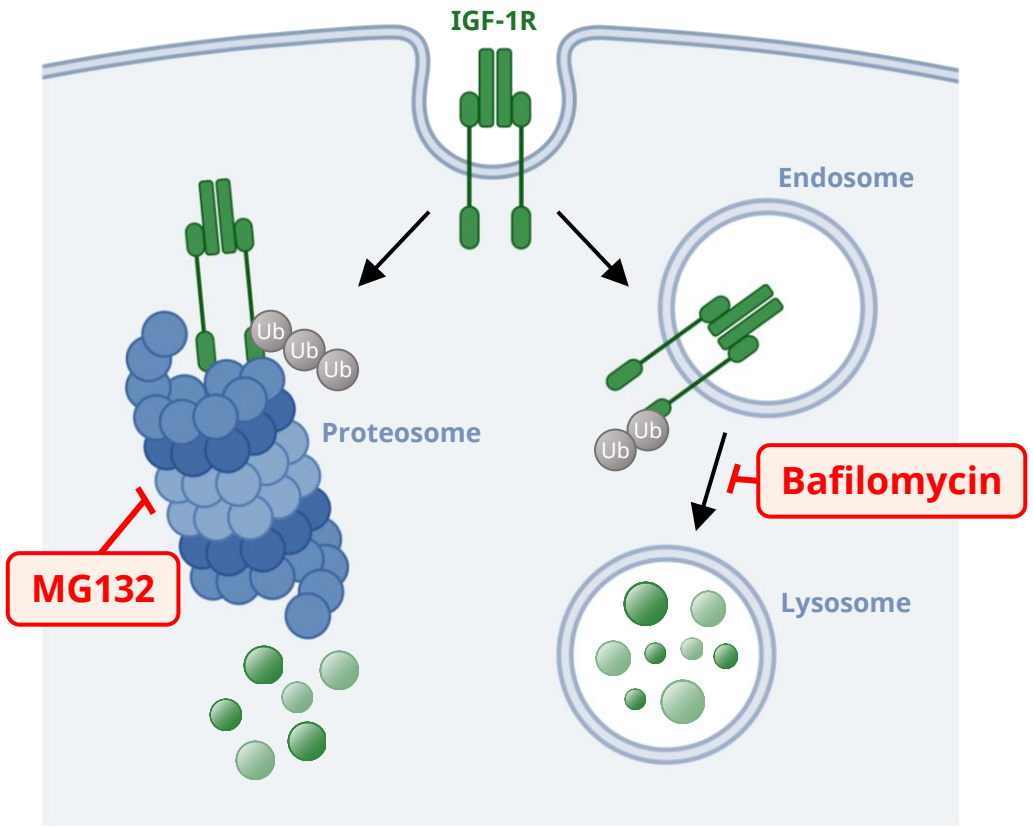


IGF-1R levels decrease by ~75% within 24 hours relative to control

Left: TED orbital fibroblasts were treated with varying amounts of lonigutamab (2.0, 1.0, 0.1, 0.01, 0.001, 0.0001, and 0.00001 µg/mL) for 24 hours. Cells were then lysed and analyzed by Western blot for IGF-1R and β-tubulin (loading control). The levels of mature IGF-1R were normalized to β-tubulin levels and densitometry values are presented below the blot.
Right: TED orbital fibroblasts were treated with vehicle (PBS) or lonigutamab (2 µg/mL) for the hours listed. Cells were then lysed and analyzed by Western blot for IGF-1R and β-tubulin (loading control). The levels of mature IGF-1R were normalized to β-tubulin levels and densitometry values are presented below the blot.
IGF-1R, insulin-like growth factor 1 receptor; PBS, phosphate-buffered saline; TED, thyroid eye disease.

LONIGUTAMAB-MEDIATED REDUCTION OF IGF-1R OCCURRED VIA THE PROTEOSOME AND LYSOSOME

Proteosome and/or lysosome inhibitors prevented lonigutamab-mediated IGF-1R degradation, **indicating a mechanism of degradation vs receptor recycling**



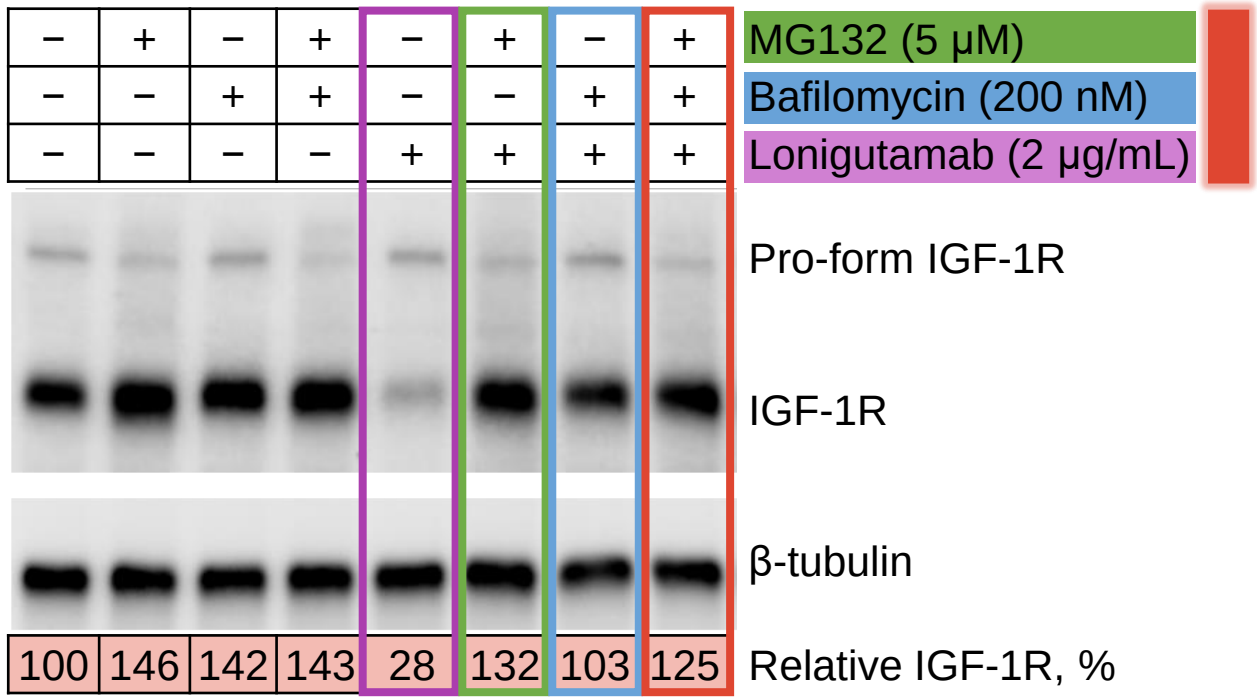
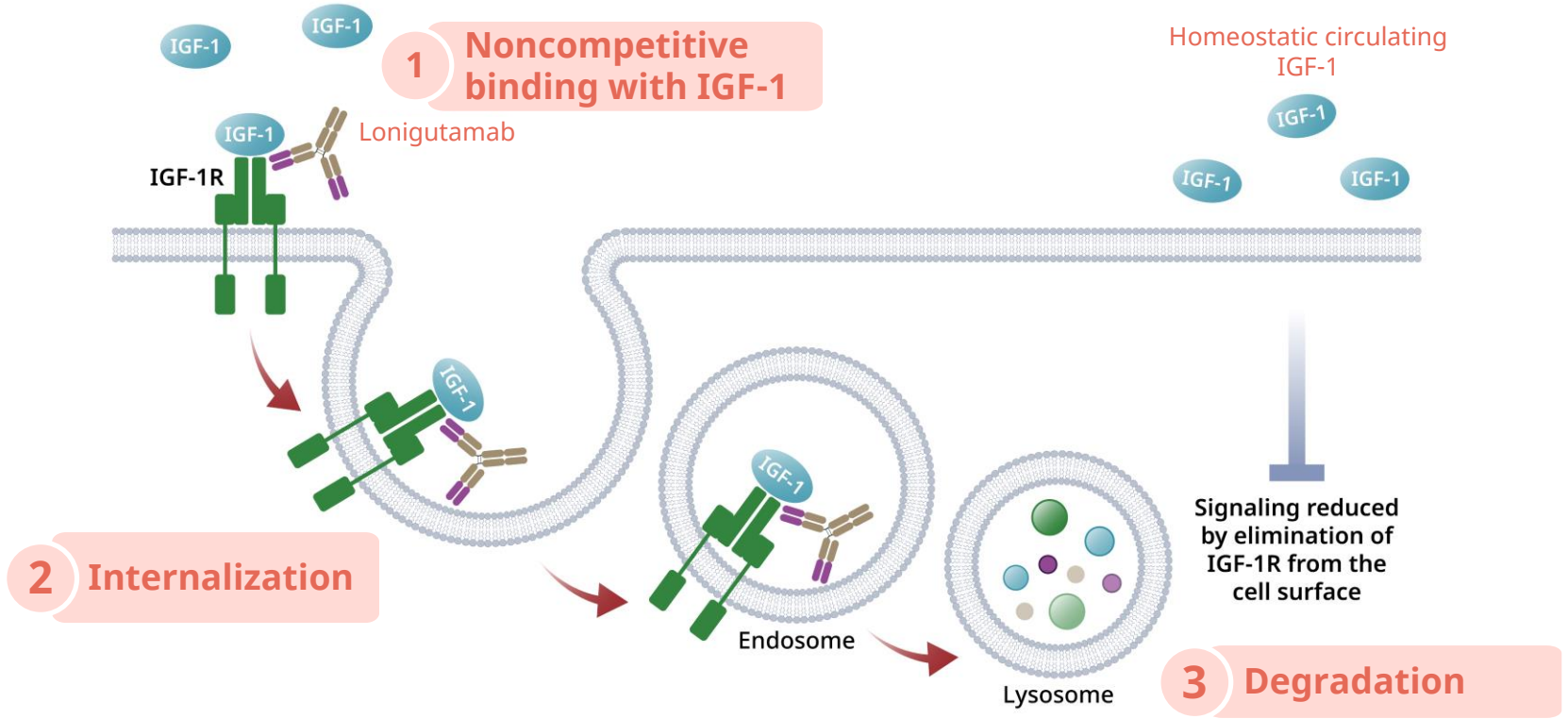


Figure created with BioRender.com.
TED orbital fibroblasts were treated with vehicle (DMSO), a proteasome inhibitor (MG132), or a lysosomal inhibitor (bafilomycin) for 1 hour before the addition of either lonigutamab (2 μg/mL) or vehicle for 24 hours. Then, the cells were lysed and analyzed by Western blot for IGF-1R and β-tubulin (loading control). The levels of mature IGF-1R were normalized to β-tubulin levels.
DMSO, dimethyl sulfoxide; IGF-1R, insulin-like growth factor 1 receptor; TED, thyroid eye disease; Ub, ubiquitin.

LONIGUTAMAB MECHANISM OF ACTION

- **Lonigutamab** is a novel, high-affinity, subcutaneously administered, **anti–IGF-1R monoclonal antibody** with a unique **noncompetitive** mechanism of action[1,2]



CLINICAL OBSERVATION: lonigutamab treatment did not result in increased circulating IGF-1 levels to the extent seen with competitive inhibitors of IGF-1R, possibly due to lonigutamab's unique mechanism of action[3]

IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor.

1. Akla B, et al. *Mol Cancer Ther*. 2020(1);19:168-77. 2. Data on file. ACELYRIN, INC. 3. Kahaly GJ, et al. Poster presented at ENDO 2025; July 12-15, 2025; San Francisco, CA, USA.

CONCLUSIONS

- Lonigutamab, a high-affinity, noncompetitive, next-generation, anti–IGF-1R monoclonal antibody, induced efficient internalization and degradation of IGF-1R in TED orbital fibroblasts
- Lonigutamab-mediated degradation was specific to mature IGF-1R, with no effect on pro-protein or mRNA levels, and occurred via both the proteosomal and lysosomal pathways

- Lonigutamab has also been shown to decrease basal and IGF-1–mediated hyaluronan production in TED orbital fibroblasts[1]
- Inhibition of the IGF-1/IGF-1R axis through elimination of IGF-1R from the surface of orbital fibroblasts has the potential to provide a meaningful benefit in TED
  - Lonigutamab has shown preliminary efficacy in a phase 1/2 study of patients with TED[2]

IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; mRNA, messenger RNA; TED, thyroid eye disease.

1. Nyborg A, et al. Poster presented at ENDO 2025; July 12-15, 2025; San Francisco, CA, USA. 2. Ugradar S, et al. *J Endocr Soc*. 2024;8(suppl):bvae163.2040.