Characterization of VRDN-001, a High Affinity and Potent anti-IGF-1R Inhibitory Antibody for the Treatment of Thyroid Eye Disease

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INTRODUCTION

VRDN-001 is an antagonist antibody to insulin-like growth factor-1 receptor (IGF-1R) under development by Viridian Therapeutics for potential treatment of Thyroid Eye Disease (TED). TED is driven by Thyroid Stimulating Hormone Receptor (TSHR) agonistic autoantibodies and crosstalk between TSHR and IGF-1R. TED is characterized by recruitment of fibrocytes that express IGF-1R and TSHR in orbital tissues, where they mediate deposition of hyaluronan and expansion of orbital muscle and fat[1]. IGF-1R antagonism has been found to reverse this orbital tissue expansion and robustly relieve symptoms in TED patients[2].

VRDN-001 is a humanized monoclonal antibody targeting IGF-1R. VRDN-001 shares the same amino acid sequence as AVE1642, previously developed for oncology. Clinical data from this prior program showed a robust PK/PD and safety profile in oncology patients, with suggested receptor saturation at doses as low as 3 mg/kg[3, 4]. Here, we evaluated the IGF-1R binding and antagonist characteristics of VRDN-001.

METHODS

Surface plasmon resonance (SPR): Antibodies were captured by immobilized anti-Fc, and recombinant IGF-1R extracellular domain (ECD) was flowed as analyte. Association and dissociation rate constants (k_a and k_d, respectively), and equilibrium dissociation constant K_D were derived by global fit of data to single site model.

Epitope binning: VRDN-001 was immobilized on a chip surface by amine coupling and used to capture IGF-1R-ECD, after which teprotumumab was flowed over the chip.

Cell binding: A549 human lung adenocarcinoma cells or primary human ocular choroid fibroblasts (HOCF) were incubated with varying concentrations of VRDN-001 or teprotumumab. A single dose 50 nM IgG1 isotype control was used as negative control. Unbound antibody was removed by washing, and the cells were incubated with an Alexa Fluor 488- goat anti-human antibody and a cell impermeable dye to gate live cells. The median fluorescence intensity (MFI) of viable cells was measured by flow cytometry and the data were analyzed using FlowJo software. Dose curves were fitted using a non-linear regression model; log(agonist) vs response- variable slope (four parameters).

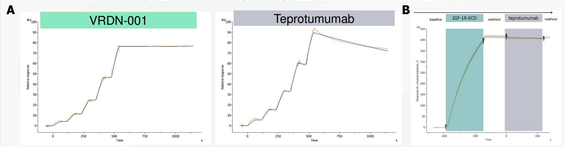
Internalization: Cells were incubated with various concentrations of antibodies of interest at 4ºC and 37ºC for 60 minutes. Cells were then washed 3X and incubated with FITC-labeled goat anti-human Fc secondary antibody for 30 minutes at 4ºC. The MFI of viable cells was measured by flow cytometry and the data were analyzed using FlowJo software.

Cell surface marker expression: HOCF cells were incubated with directly labeled antibodies or IgG isotype control at 10 ug/mL. The median fluorescence intensity (MFI) was measured by flow cytometry and the data were analyzed using FlowJo software.

Antagonism: Serum starved A549 or HOCF cells were preincubated with varying concentrations of test antibody for one hour at 37°C, then stimulated by addition of 100 ng/mL (A549s) or 200 ng/mL (HOCFs) IGF-1 for 7 minutes at 37°C. Phosphorylated IGF-1R (pIGF1R) of biological duplicates was measured using the R&D Systems pIGF-1R ELISA according to the manufacturer's protocol and pIGF-1R concentrations were normalized to the lowest test antibody concentration. Dose curves were fit using a non-linear regression model; log(inhibitor) vs response- variable slope (four parameters)).

BINDING CHARACTERISTICS

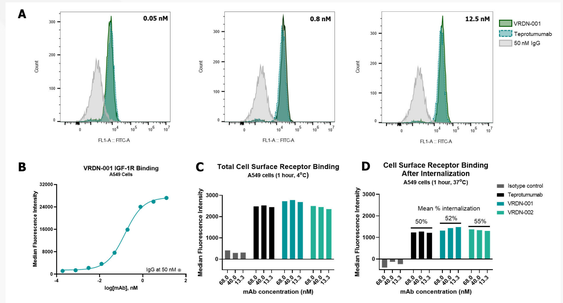
VRDN-001 BINDS IGF-1R WITH SUB-NANOMOLAR AFFINITY



	Rmax	k_a Ms^{-1}	k_d s^{-1}	K_D nM
VRDN-001	118.1	$8.8X10^4$	$5.1X10^{-5}$	0.57
Teprotumumab	109.7	$1.8X10^5$	$3.9X10^{-4}$	2.2

(A) Increasing concentrations of IGF-1R-ECD bound to anti-FC captured VRDN-001 or teprotumumab reveal a stepwise increase in SPR signal, enabling a global fit to a binding model. Following IGF-1R washout, VRDN-001 shows a more sustained binding interaction. **(B)** IGF-1R-ECD bound robustly to immobilized VRDN-001. Teprotumumab showed no binding to the IGF-1R:VRDN-001 complex, suggesting that teprotumumab and VRDN-001 have overlapping epitopes.

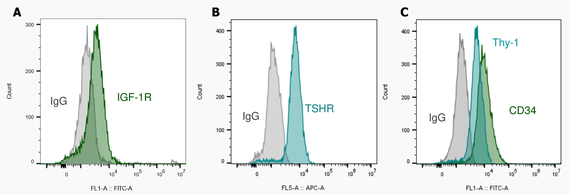
VRDN-001 BINDS WITH HIGH AFFINITY TO IGF-1R ON A549 CELLS



(A) VRDN-001 binding to A549 cells was assessed by flow cytometry and found to have similar binding distribution as teprotumumab at three different concentrations. **(B)** Binding dose response curve demonstrated VRDN-001 EC50 = 0.1 nM. **(C)** VRDN-001, VRDN-002*, and teprotumumab show comparable binding at temperatures that block IGF-1R receptor internalization. **(D)** VRDN-001, VRDN-002, and teprotumumab cause comparable levels of internalization (~50%) measured by reduction in membrane IGF-1R receptor levels at 37ºC vs 4ºC.

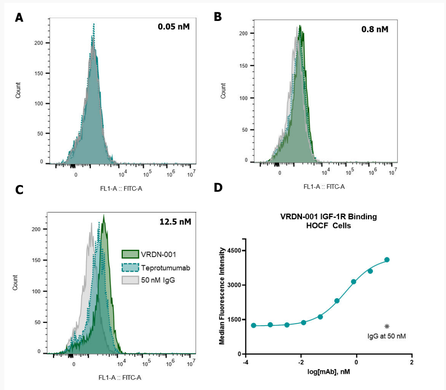
VRDN-001 AFFINITY ON HOCFs

HOCFs AS AN IN VITRO MODEL FOR TED PATHOLOGY



CD34+,Thy-1+ orbital fibroblasts are implicated in extracellular matrix deposition and pathogenic fibrosis in TED[5]. Here, HOFCs are shown to express **(A)** IGF-1R and **(B)** TSHR, as well as **(C)** CD34 and Thy-1, suggesting potential utility as an *in vitro* model system for IGF-1R function in TED.

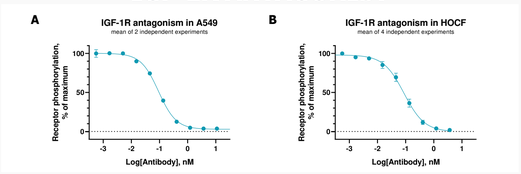
VRDN-001 BINDS WITH HIGH AFFINITY TO IGF-1R ON HOCF CELLS



(A, B, C) VRDN-001 binding to HOCF cells was assessed by flow cytometry and found to have largely similar binding as teprotumumab at three different concentrations. **(D)** Binding dose response curve demonstrated VRDN-001 EC_{50} = 0.4 nM.

IN VITRO ANTAGONISM

VRDN-001 IS A SUB-NANOMOLAR IGF-1R ANTAGONIST



VRDN-001 potently inhibits IGF-1 stimulated IGF-1R phosphorylation on **(A)** A549 cells (IC_{50} = 0.09 nM) and **(B)** HOCF cells (IC_{50} = 0.09 nM).

DISCUSSION

- VRDN-001 and teprotumumab epitopes on IGF-1R overlap
- VRDN-001 binds to IGF-1R on cells with sub-nanomolar EC_{50}
- VRDN-001 promotes IGF-1R internalization
- VRDN-001 inhibits IGF-1R phosphorylation with sub-nanomolar IC_{50}

VRDN-001 binds, antagonizes, and internalizes IGF-1R at sub-nanomolar concentrations, suggesting potential for potent inhibition of the pathophysiology driving TED. Viridian plans to interrogate VRDN-001's potential to reverse TED pathophysiology and improve symptoms in a randomized placebo-controlled trial in TED patients.

ACKNOWLEDGEMENTS

SPR, epitope binning, and internalization assays conducted at FairJourney Biologics.

References:

[1] Smith, T. *et al. Endocr Rev* (2018), 40:236-267
[2] Douglas, R. *et al. New Engl J Med* (2020), 382:341-352
[3] Soria, J.C. *et al. Eur J Cancer* (2013), 49:1799-1807.
[4] Moreau, P. *et al. Leukemia* (2011), 25:872-874
[5] Koumas, L. *et al. Am J Pathology* (2003), 163:1291-1300.

*VRDN-002 is another anti-IGF-1R monoclonal antibody being developed by Viridian Therapeutics for investigation in TED. See poster #37.