Author’s response to reviews

Title: Efficacy and Safety of a Novel Naltrexone Treatment for Dry Eye in Type 1 Diabetes

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Author’s response to reviews:

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Dear Reviewer:

Thank you for your comments. Responses to each “Query” are cited in red font below and in the manuscript text identified as “red lined”.

Background:

Query: I have double checked your citation; I cannot see refs 5 and 6 showing this however ref 10 mentions punctal plugs that are “dexamethasone-loaded punctal plugs” so please insert “steroid-loaded” before punctal plugs on pg5; line 4 and remove refs 5 and 6.

RESPONSE: statement on pg 5 has been changed and refs removed.

Methodology:

Query: You reference #19; however my concern was altering the width to 1mm and this reference does not justify the validity of modifying the original strip width. Please defend why you are not concerned about this augmentation by recording in the methods section of the manuscript the potential implications on the test results. Lastly, however did you prepare 1mm width and how did you measure to 0.5mm accuracy?
RESPONSE: The 1 mm strips were cut from full-size strips under sterile conditions using two single-edged razor blades. Strips were handled with autoclaved forceps to maintain sterility and held in place in the rat cul-de-sac with forceps. Measurements were made using digital calipers. In some cases, images of “wetness” were taken and magnified to assess increments. We did not measure to the tenth of a millimeter – just whole numbers or between whole numbers – thus, any measurement between millimeters was read as 0.5 millimeter.

p11; line 49:
Query: Please clarify how did you measure IOP on the rats?
RESPONSE: IOP was measured by a tonopen (Tono-Pen XL Tonometer, Medtronic, Jacksonville, FL). This information has been included in the text. Measurements were taken on unanesthetized rats. Ocular pressure was obtained as 4 readings per eye and averaged. Statements were added in the METHODOLOGY and RESULTS.

RESULTS:
Query: Thanks for amending; considering that you augmented the manufacturer’s normal strip; would it not been more accurate to state “Modified Schirmer’s 1”. Again please convey to me why you are not concerned as I am?
RESPONSE: All of the animal experiments are using equipment that is designed for humans. As such, all of basic science research can be considered “modified”. In general, scientists do not designate tests as “modified for animals”. Given that all animals --- controls and treated – were handled in the same manner, the data are valid. Moreover, the repeated measures and biological significance of the study increases the reliability and validity of the data.

DISCUSSION:
p15; line 14: I am not convinced about the IOP unless it was high to start of with which was not shown anywhere. Response: published IOPs for male Sprague-Dawley rats indicate IOPs as high as 25 mm Hg.
Query: Please reflect this in the manuscript
RESPONSE: Additional comments were included on page 15 and a new reference (#27) that involved IOPs of diabetic rats has been included.

p15; line 29: "...most likely" this appear very vague and almost arbitrary;
sure the mechanism of action must be more concrete if not state why. Response: Sentence was reworded. We are convinced of the mechanism that we can prove, but it may not be the only pathway.

Query: There is no evidence of this- please state what the original sentence was and how it was reworded.

RESPONSE: Author does not understand the query.

Revised: At the end of 10 days, IOPs were normal for NTX-001 eyes. T1D rats had low tear volume several days after cessation of NTX treatment implying that this treatment does not cure dry eye, and that chronic treatment would be necessary for treatment of T1D associated dry eye.