# Lebrikizumab Directly Reverses IL-13 Driven Neuronal Gene Regulation and Neuronal Excitability


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## INTRODUCTION

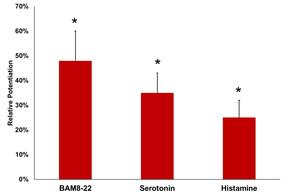
- IL-13 is a key mediator of multiple pro-inflammatory processes in atopic dermatitis (AD)
- Lebrikizumab, a high affinity monoclonal antibody targeting IL-13, showed dose-dependent, statistically significant improvement vs placebo measures of AD severity (Eczema Area Severity Index [EASI], Investigator's Global Assessment [IGA]) and itch (numeric rating scale [NRS]) at Week 16[1]
  - Randomized, double blind, placebo-controlled, phase 2b clinical trial in adults with moderate-to-severe AD (NCT03443024)
  - Anti-itch effect in clinical trials as early as day 2, implicative of a direct effect on human sensory neurons that mediate itch
- Previous findings suggest a direct neuroactive role for IL-13 to sensitize itch pathways[2]
  - Direct neuroactive role of IL-13 may support the mechanistic basis for lebrikizumab's anti-itch effects observed in clinical trial in moderate-to-severe AD

## MECHANISTIC INSIGHT

- **IL-13 potentiates neuronal responses elicited by multiple pruritogens and neuronal voltage gated excitability, which are inhibited by lebrikizumab**
- **IL-13 induces inflammation-related gene transcripts in human sensory neurons which are reversed by lebrikizumab**

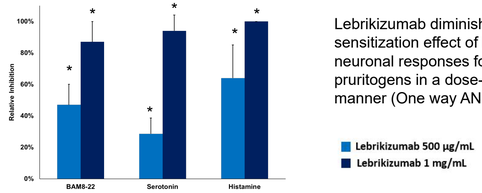
## KEY RESULTS

**Figure 1. Acute (15 min) IL-13 stimulation significantly potentiated neuronal responses of multiple pruritogens.**



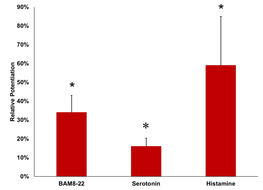

IL-13 potentiation effects in BAM8-22, serotonin, and histamine elicited neuronal responses expressed as relative percentage compared to vehicle controls use as the baseline reference response on which the relative effect is evaluated (One way ANOVA *p<0.05).

**Figure 2. Lebrikizumab significantly inhibited the potentiation response driven by IL-13.**



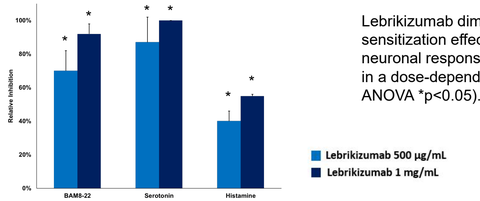

Lebrikizumab diminished the sensitization effect of IL-13 elicited neuronal responses for all pruritogens in a dose-dependent manner (One way ANOVA *p<0.05).

**Figure 3. Prolonged (24 hour) IL-13 stimulation significantly potentiated neuronal responses of multiple pruritogens.**




IL-13 potentiation effects in BAM8-22, serotonin, and histamine elicited neuronal responses expressed as relative percentage compared to vehicle controls (One way ANOVA *p<0.05).

**Figure 4. Prolonged Lebrikizumab exposure potently reduces potentiation effects driven by prolonged IL-13 stimulation.**




Lebrikizumab diminished the sensitization effect of IL-13 elicited neuronal responses for all pruritogens in a dose-dependent manner (One way ANOVA *p<0.05).

## SUMMARY

- IL-13 potentiated the neuronal responses elicited by serotonin, histamine, and BAM8-22
- Lebrikizumab dose-dependently reversed the IL-13 driven neuronal sensitization of histaminergic and non-histaminergic itch pathways
- In human sensory neurons, acute and prolonged stimulation with IL-13 increased neuronal excitability and firing to voltage increments. The increased neuronal firing by IL-13 was shifted to levels below vehicle control by lebrikizumab
- IL-13 stimulated human neurons showed upregulation of AD-associated gene transcripts which are reversed by lebrikizumab

## CONCLUSIONS

- **Our findings suggest that IL-13 might be a potent neuronal enhancer to multiple pruritogens potentially driving the chronicity of itch in type-2 polarized diseases**

## METHODS

- Experiments were conducted on isolated and cultured human dorsal root ganglion (hDRG) neurons derived from ethically consented organ donors
- Fluo 8-AM was used to monitor cytoplasmic calcium transients in the hDRG neurons following either direct application of pruritogens or following Electrical Field Stimulation (EFS)
- Image acquisition and data analysis were performed using MetaMorph.

### IL-13 SENSITIZATION & LEBRIKIZUMAB INHIBITION STUDIES

**Acute Pruritogen Protocol**

- BAM8-22 (2 µM), serotonin (100 µM) or histamine (10 µM) were applied twice in a dual challenge protocol. The first dose identified pruritogen responsive hDRG neurons. The second dose compared the pruritogen responses following acute application (15 min) of vehicle control, 500 nM IL-13 or a combination of IL-13 and lebrikizumab (500 µg/mL or 1 mg/mL).
- Fig. 1 compares the percentage potentiation of IL-13 to vehicle controls.
- Fig. 2 compares the percentage inhibition of the lebrikizumab + IL-13 compared to the normalized IL-13 response derived from the data in Fig 1.

**Prolonged Pruritogen Protocol**

- hDRG neurons were treated with vehicle, 500 nM IL-13 or a combination of IL-13 and lebrikizumab (500 µg/mL or 1 mg/mL) for 24h prior to a pruritogen challenge with either BAM8-22 (2 µM), serotonin (100 µM) or histamine (10 µM).
- Fig. 3 compares the percentage potentiation of IL-13 to vehicle controls.
- Fig. 4 compares the percentage inhibition of the lebrizikumab+IL-13 compared to the normalized IL-13 response derived from the data in Fig 3.

**Acute EFS Protocol**

- An EFS response was first established at 5 increasing voltages (500, 1000, 1500, 2000, 2500 mV), then evaluated again after 20 min of treatment with vehicle, 500 nM IL-13 or a combination of IL-13 and lebrikizumab (500 µg/mL) at the same 5 voltages. EFS responses were normalized against responses before treatment (Fig. 5A)

**Prolonged EFS Protocol**

- hDRG neurons were treated with vehicle, 500 nM IL-13 or a combination of IL-13 and lebrikizumab (500 µg/mL) for 24h prior to EFS stimulation at the same 5 increasing voltages used in the acute EFS study. EFS responses were normalized against vehicle control. (Fig. 5B)

### RNA-SEQ ANALYSES

- hDRG neurons were treated with vehicle, 500 nM IL-13 or a combination of IL-13 and lebrikizumab (500 µg/mL or 1 mg/mL) for 24h prior to fixation in RNALater
- Total RNA was isolated by phenol based (TRIzol) method
- 1 ug of total RNA was processed for preparing an mRNA sequencing library using TruSeq stranded mRNA sample preparation kit (Illumina, San Diego, CA)
- A cDNA library was synthesized from poly-A mRNA fragments and used in the final sequencing process.
- Sequencing of the prepared library was conducted on the Nextseq system (Illumina, San Diego, CA)


### DISCLOSURES

- **YM, PM, CH** are employees of AnaBios Corporation; **EL** is on the Scientific Advisory Board of Escient Pharmaceuticals; **FC** is an formar employee and minor shareholder of Eli Lilly and Company
- This study was sponsored by Dermira, a wholly-owned subsidiary of Eli Lilly and Company and conducted with AnaBios Corporation. Medical writing services were provided by Nancy Tan, PharmD, an employee of Eli Lilly and Company.
- This poster was previously presented at Society for Investigative Dermatology (SID) Virtual Meeting, May 3-8, 2021

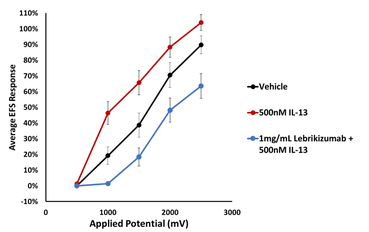

### REFERENCES


1. Guttman-Yassky E., et al. *JAMA Dermatol.* 2020; 156 (4): 411-420
2. Oetjen L., et al. *Cell.* 2017 Sep 21;171(1):217-228


## RESULTS

**Figure 5. Average EFS response of hDRG following** A) acute (20 min) incubation or B) prolonged (24 h) incubation with vehicle (black), IL-13 alone (red) or a mixture of IL-13 and Lebrikizumab (blue).

5A




5B

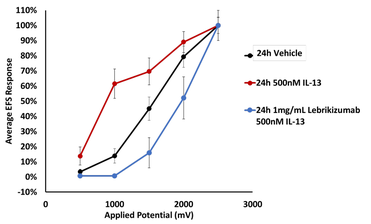
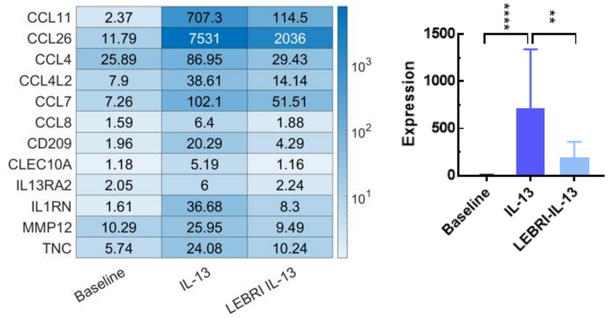



**Figure 6. Prolonged IL-13 stimulation (24 hours) in hDRG neurons induced a transcriptional potentiation in inflammation related genes that was reversed by the presence of lebrikizumab.**
Average gene expression in hDRGs derived from 3 donors following treatment with vehicle (baseline), IL-13, or IL13 + Lebrikizumab, ** p< 0.01, **** p< 0.001.







Study was sponsored by Dermira, a wholly-owned subsidiary of Eli Lilly and Company