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Presented at:

TIDES: Oligonucleotide and Peptide Therapeutics 2018 May 7–10, 2018 Boston, MA, USA

Probing the Role of the MC1 Receptor Agonists in **Diverse Immunological Diseases**

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Introduction

Biological Role of Melanocortin-1 (MC1) Receptor Agonists

- MC1 receptor agonism is an endogenous mechanism that downregulates inflammatory/immune responses¹
- MC1 receptors are upregulated in inflammatory bowel disease (IBD) and expressed on the cell surface of intestinal epithelia²
- Alpha-melanocortin stimulating hormone (α -MSH) is an endogenous agonist of 4 of the 5 melanocortin receptors (MC1, and melanocortin-3, -4, and -5), and was shown in numerous animal models to prevent and reverse intestinal inflammation^{1,2}
- α -MSH is also constitutively expressed in the healthy eye, stimulates regulatory actions, and has been shown to suppress experimental ocular autoimmune inflammation (uveitis)³⁻⁵
- The MC1 receptor has the highest affinity for α -MSH⁶
- PL-8177 and PL-8331 (Palatin Technologies, Inc.) are potent MC1 receptor agonists that demonstrate binding characteristics similar to those of α-MSH (**Table 1**)

• In the DNBS rat model of bowel inflammation, PL-8177 was as active as sulfasalazine (standard of care), and superior to untreated controls, in reducing parameters of bowel inflammation (colon weight and inflammation score) (Figure 2)

Figure 2. Effects of PL-8177 and Sulfasalazine on Colon Weight and Inflammation Score in Rats With DNBS-Induced Bowel Inflammation



PL-8177: EAU histology findings

- In comparison with healthy retinas from non-EAU mice, retinas of untreated EAU mice featured (**Figure 4**):
- -Cellular infiltration, uneven nuclear layers with folding, and loss of the outer limiting membrane
- —Missing retinal intervening plexiform layer between the inner and outer nuclear layers
- —Thinner photoreceptor layer, suggesting photoreceptor dropout
- —Vasculitis of central retinal vessels
- In contrast to retinas of untreated EAU mice, retinas of PL-8177-treated EAU mice showed (**Figure 4**):
- -Retention of even layers of retina with little evidence of photoreceptor loss
- —Some retention of outer limiting membrane
- —Clear outer plexiform layer between the inner and outer nuclear layers -No evidence of vasculitis

Figure 4. Histology of Retinas From Healthy Mice, EAU Mice Treated With PL-8177, and Untreated EAU Mice

Table 1. In Vitro MC1 Receptor Activity of Endogenous Melanocortins and Palatin (PL) MC1 Receptor Agonists

Action	Alpha-MSH	ACTH	PL-8177	PL-8331
MC1r binding affinity (nM)	0.095	4.0	0.04	0.01
MC1r functional activity (cAMP production, nM)	0.22	980	0.39	0.033

cAMP, cyclic adenosine monophosphate; MC1r, Melanocortin-1 receptor.

Objectives

• A series of preclinical studies was conducted to determine whether PL-8177 and PL-8331 exhibit actions and efficacy similar to those of α -MSH in preventing and reversing intestinal and ocular inflammation

Studies Overview

- PL-8177 and PL-8331
- —MC1 receptor lead profile in vitro assays
- PL-8177
- -Experimental IBD
- MC1 receptor in vitro activity
- Preclinical in vivo proof of principle
- -Experimental (murine) autoimmune uveitis (EAU)
- EAU scores by fundus examination
- Confirmatory histology
- PL-8331
- -Experimental (murine) dry eye
- Corneal fluorescein score

*P<0.05. DNBS, dinitrobenzene sulfonic acid; IC, intracolonic; PO, oral

PL-8177: EAU STUDIES/RESULTS

- EAU was induced by immunizing C57BL/6 mice with an emulsion of complete Freund's adjuvant with an additional 5 mg/mL desiccated *M. tuberculosis* and 2 mg/mL IRBP peptide amino acids 1-20
- —The course of EAU was evaluated every 3–4 days by fundus examination
- PL-8177, 0.3 mg/kg/mouse, was injected intraperitoneally into EAU study treatment mice on the first day of clinically positive uveitis (uveitis score ≥ 2) and 48 hours after first dose
- Native α -MSH, 50 µg/mouse, was injected in EAU positive control mice



EAU, experimental autoimmune uveitis.

PL-8331: SiccaSystem™ Model for Moderate Chronic Dry-Eye Disease

- Male C57BL/6 mice were exposed to a controlled desiccating environment with transdermal administration of scopolamine (SiccaSystem[™])
- Day 12:
 - -Corneal fluorescein staining was performed to assess corneal epithelial damage
 - —Based on fluorescein staining scores, mice were randomized into treatment arms such that each arm had a median score of 2
- 8 experimental arms (n=10 for each; n=5 in naïve group) were tested as follows:
- —Untreated mice
- —Vehicle treated mice
- -3 arms of PL-8331 treated mice: doses of 1×10^{-8} mg·ml⁻¹, 5×10^{-7} mg·ml⁻¹, 1×10⁻⁵ mg⋅ml⁻¹
- -Restasis[®] (cyclosporine ophthalmic emulsion) treatment
- —Xiidra[®] (lifitegrast ophthalmic solution) treatment

PL-8331: Chronic Dry-Eye Corneal Fluorescein **Staining Results**

• PL-8331 showed a reduction in corneal fluorescein staining scores at all 3 dosage levels, which was statistically significant (P=0.02) on Day 22 versus Day 12 for the highest dose (1×10⁻⁵ mg·ml⁻¹; **Figure 4** [**B**])

Studies/Results

PL-8177 and PL-8331 In Vitro Assays

- In a Eurofins lead profile (Eurofins Scientific 2018), PL-8177 and PL-8331 demonstrated no activity in any of 72 in vitro assays at 10 µм; highlights included no activities in:
- -Cytochrome P450 enzymes 1A2, 2C19, 2C9, 2D6, and 3A4
- —Potassium channel hERG
- —Any of 7 adrenergic receptor subtypes
- —Any remaining assays included in the panel

PL-8177: IBD In Vitro Activity

• PL-8177 demonstrated similar inhibition of lipopolysaccharide-induced tumor necrosis factor alpha inhibition compared with the endogenous MC1 receptor agonists α -MSH and adrenocorticotropic hormone (**Figure 1**)

PL-8177: IBD Proof of Principle Data

- PL-8177 was evaluated in a cannulated rat model of bowel inflammation
- —Dinitrobenzene sulfonic acid (DNBS) was administered rectally as a solution in male, 200g Wistar rats to induce inflammation of the bowel lumen
- —The rats were implanted with a catheter in the proximal part of the ascending colon, which exited out the nape of the neck for dosing access
- -In groups of 10, the rats were dosed at: 0.5 µg and 5.0 µg PL-8177 and vehicle (sterile water) via intracolonic injection at 24 h, 12 h, and 2 h before and 6 h after DNBS challenge, followed by twice-daily dosing for 5 consecutive days through day 7
- -Non-cannulated control rats were administered sulfasalazine (positive controls), and vehicle (untreated controls)

• Untreated EAU mice served as negative controls

PL-8177: EAU Inflammation Scores

• The 2 doses of PL-8177 significantly reversed EAU versus untreated controls (*P*=0.0001) over 3 to 5 weeks (**Figure 3**)

Figure 3. Effect of PL-8177 Treatment Versus Controls on Inflammation Scores of Mice With Induced Experimental Autoimmune Uveitis (EAU)



**P*=0.0001 by analysis of variance. α -MSH, alpha-melanocortin stimulating hormone.

• The reduction for PL-8331 was similar to that observed for the reference agent, Restasis[®] (P=0.03 for Day 22 versus Day 12) (Figure 4 [C]); Xiidra[®] had no statistically significant effect

Figure 4. Corneal Fluorescein Staining in Mice With Chronic Dry-Eye (SiccaSystem[™] Model)^a



^aData are presented as median±interquartile range; data were compared by paired nonparametric Wilcoxon test.

Conclusions

- PL-8177 and PL-8331 are potent MC1 receptor agonists with in vitro actions similar to those of endogenous MC1 receptors α -MSH and ACTH
- In a rat model of bowel inflammation, PL-8177 administered via catheter reduced inflammation and colon weight scores to a similar degree as sulfasalazine
- In a mouse model of autoimmune uveitis, PL-8177 administered via intraperitoneal injection significantly reduced retinal inflammation

Figure 1. Lipopolysaccharide-Induced Tumor Necrosis Factor Alpha Inhibition in Human Whole Blood



α -MSH, alpha-melanocortin stimulating hormone; ACTH, adrenocorticotropic hormone; TNF- α , tumor necrosis factor alpha.

versus untreated controls, and to a similar degree as α -MSH

- Based on these data, PL-8177 appears to have anti-inflammatory actions across body systems, similar to those observed with α -MSH
- PL-8331 demonstrated reduction (significant at dose of 1×10⁻⁵ mg·ml⁻¹) in corneal epithelial damage due to dry eye, and efficacy similar to Restasis®

Disclosures Andrew Taylor conducted the PL-8177 uveitis study supported by a sponsored research agreement with Palatin Technologies, Inc. Carl Spana, Marie Mahklina, Wei Yang, and John Dodd are employees of Palatin Technologies, Inc. David Yee has nothing to disclose.

Support These studies were funded by Palatin Technologies, Inc. Editorial assistance was provided by The Curry Rockefeller Group, LLC, which was funded by Palatin Technologies, Inc.

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