Presented at:

TIDES USA Oligonucleotide and Peptide Therapeutics 2021

Boston, MA
20–30 September 2021

Probing the Role of the Melanocortin Receptor Agonists in Experimental Immune-Mediated Diseases

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Introduction

- The anti-inflammatory benefits of the melanocortin system suggest that melanocortin receptor (MCr) agonists have great promise in the treatment of inflammatory diseases[1,2]
- The synthetic selective MC1r agonist PL8177, and the synthetic MC1r/MC3r/MC4r/MC5r pan-agonists PL9654 and PL9680 have been investigated in distinct settings of experimental pathologies[3,4]
- To demonstrate the utility of targeting the melanocortin system in inflammatory diseases, we determined the effects of these melanocortin agonists as potential treatments for diabetic retinopathy (DR), multiple sclerosis (MS), and rheumatoid arthritis (RA)

Methods

PL8177/PL9654 Streptozotocin (STZ)–Treated Rat Model of DR

- Potential beneficial effects of PL8177 and PL9654 on visual function in an STZ-treated rat model of DR were investigated in a 114-day study
- Rats were randomly assigned to 5 separate study arms (**Table 1**) and dosed on Days 4–113

Table 1. Study Arms for PL8177/PL9654 STZ–Treated Rat Model of DR

Treatment	Arm	Dose (SC BID)
Vehicle control (placebo)	1	(0.9% NaCl)
PL9654	2	0.05 mg/kg
	3	0.1 mg/kg
	4	0.5 mg/kg
PL8177	5	1 mg/kg

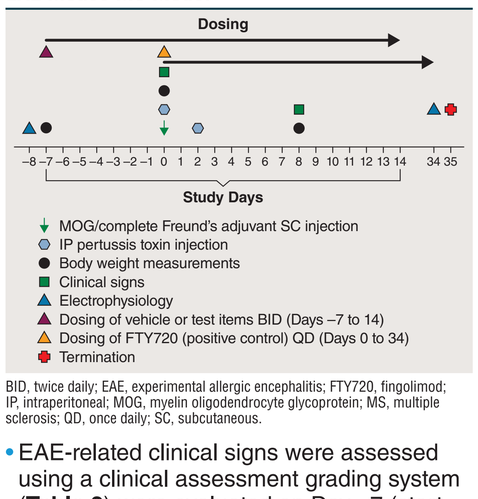
BID, twice daily; DR, diabetic retinopathy; SC, subcutaneous; STZ, streptozotocin.

- Visual function was measured by optokinetic tracking every 2 weeks starting on Day 43 and continuing to Day 113
  - –Cataract images were acquired after each optokinetic session
- Rats were euthanized on Day 114
  - –Right-eye retinas were dissected and snap-frozen
  - –Left eyes were enucleated and fixed for histology. Retinal thickness and photoreceptor degeneration/loss was measured

PL9680 Mouse Myelin Oligodendrocyte Glycoprotein (MOG)-Induced Experimental Allergic Encephalitis (EAE) Model of MS

- The effect of PL9680 in the EAE/MS mouse model was evaluated
- PL9680 solution (0.3 and 3 mg/kg) or vehicle (placebo) was administered orally via a tube once daily, starting on study Day –7 and continuing through Day 4, whereas the positive control (fingolimod [FTY720], 3 mg/kg) was administered once daily on study days 0–34 (**Figure 1**)

Figure 1. Study Design for PL9680 MOG-Induced EAE Mouse MS Model



BID, twice daily; EAE, experimental allergic encephalitis; FTY720, fingolimod; IP, intraperitoneal; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; QD, once daily; SC, subcutaneous.

- EAE-related clinical signs were assessed using a clinical assessment grading system (**Table 2**) were evaluated on Day –7 (start of PL9680 dosing), Day 0 (start of MOG inoculation), and daily from Day 8 (8 days after start of PL9680 and FTY720 dosing)

Table 2. Grading Systems for Clinical Assessment of EAE[5]

Score	Clinical Signs/Symptoms
0	No signs
1	Limp tail; weakness of tail; paralysis of tail
2	Abnormal gait
3	Severe hind-limb weakness, partial hind-limb paralysis
4	Complete hind-limb paralysis
5	Death

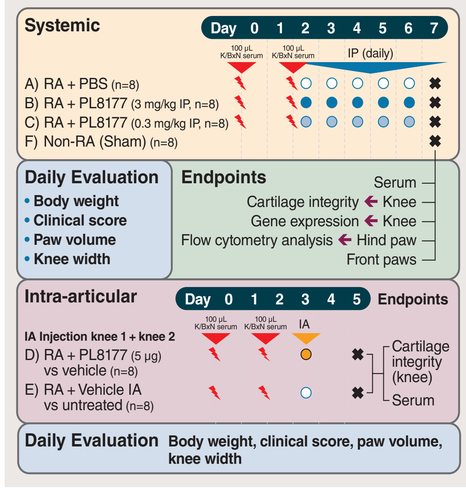
EAE, experimental allergic encephalitis.

- The area under the curve (AUC) was calculated for clinical score data for Days 8–35
- Body weight was measured at Day –7 and daily from Day 8
- Electrophysiological transcranial evoked potentials (tcMEPs) were assessed at Day –8 and Day 34

PL8177 K/BxN Serum Transfer Mouse RA Model

- Study objective was to determine the efficacy of PL8177 using the K/BxN serum transfer model in mice that mimics the active phases of RA

Figure 2. Study Design for K/BxN Mouse RA Model



IA, intra-articular; IP, intraperitoneal; PBS, phosphate-buffered saline; RA, rheumatoid arthritis.

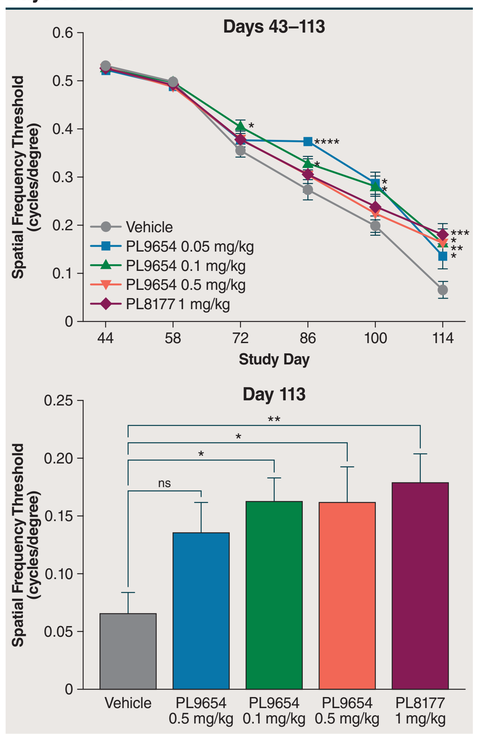
- Arthritis was induced in 40 C57BL6/j male mice (10 weeks old) by 2 intraperitoneal (IP) injections of 100 µL of K/BxN serum (diluted 1:1 in phosphate-buffered saline [PBS]) on Days 0 and 2 and mice were randomized into 8 cages
- 3 groups of mice received intraperitoneal IP injections of vehicle (PBS), PL8177 3 mg/kg, or PL8177 0.3 mg/kg for 5 days
- 2 further groups received single 5-µg intra-articular injections of PL8177: 1 group received PL8177 in 1 knee and vehicle (placebo) in the other, and the other group received either vehicle or no injection (untreated) in both knees
- Development of systemic arthritis was monitored daily by measuring body weight, clinical score, paw volume (plethysmometer), and knee width
- A clinical score was assessed by evaluating signs of inflammation in wrist/ankle, paw, and digits
  - –To obtain the clinical score, each limb was evaluated for signs of inflammation in wrist/ankle, paw, and digits (one point each of these 3 parts), thus reaching a maximum of 12 points (3 per limb)
- Mice were euthanized at day 5 or day 7, and tissue samples were collected for endpoint evaluations. Cartilage integrity was evaluated by histologic analysis

Results

PL8177/PL9654 Streptozotocin (STZ)–Treated Rat Model of DR

- PL9654 doses of 0.05–0.5 mg/kg twice daily (BID) and PL8177 at 1 mg/kg BID showed significant efficacy in reducing vision loss in STZ-treated rats compared to vehicle
- All treatment arms showed a progressive decline in spatial frequency threshold (visual acuity) and contrast threshold over the course of the study
- PL9654 0.1 and 0.5 mg/kg, and PL8177 1 mg/kg had significantly reduced loss of visual acuity vs vehicle on Day 113 (**Figure 3**)

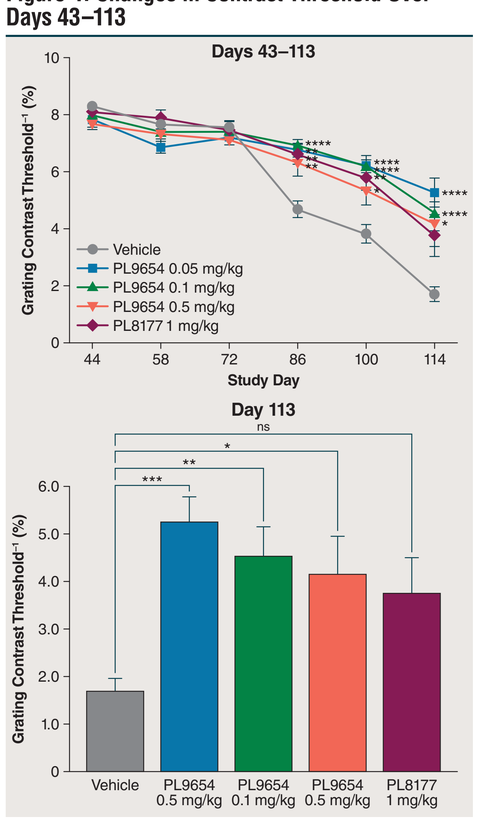
Figure 3. Changes in Visual Acuity Over Days 43–113



Values are mean (SEM).
*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs vehicle (Mann-Whitney U test).
ns, not significant.

- All treatment arms also showed a progressive decline in contrast threshold values. However, the rate was much slower in the PL9654 and PL8177 treatment groups compared to the vehicle group, and at Day 113 all PL9654 treatments had significantly reduced loss of contrast threshold (**Figure 4**)

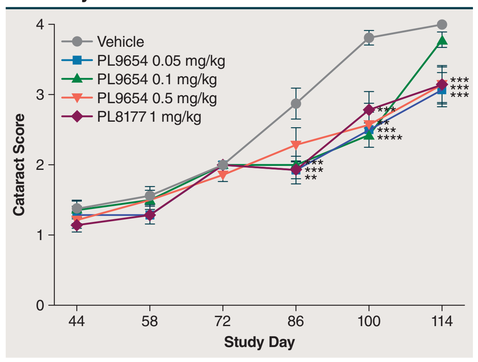
Figure 4. Changes in Contrast Threshold Over Days 43–113



Values are mean (SEM).
**P<0.01, ***P<0.001, ****P<0.0001 vs vehicle (Mann-Whitney U test).

- Lens cataracts were scored at 2-week intervals beginning at Day 44. The rate of increase in cataract severity (scored from 1 [mild] to 4 [total opacity]) was greatest in the vehicle administered rats with a lower rate observed in the PL9654 and PL8177 treatment groups (**Figure 5**)

Figure 5. Changes in Cataract Severity Scores Over Days 43–113



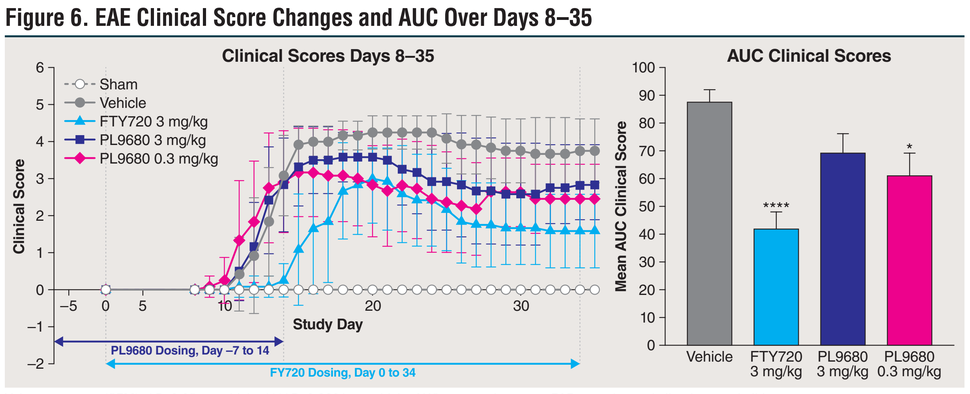
Values are mean (SEM). **P<0.01, ***P<0.001, ****P<0.0001 vs vehicle (Mann-Whitney U test).

- Histopathology showed that there was significantly less photoreceptor degeneration with PL9654 0.1 mg/kg vs vehicle (P<0.05). Retinal thickness was also significantly improved for the PL9654 (P<0.05) and PL8177 (P<0.05) groups vs vehicle
- There were no adverse events resulting from administration of either PL9654 or PL8177

PL9680 Mouse MOG-Induced EAE Model of MS

- In the MS model, oral PL9680 solution did not produce significant change (improvement) in body weight compared to vehicle, except on Days 18 and 21 for the 0.3-mg/kg solution. The positive control (FTY720) generally improved body weight through Day 28
- EAE clinical scores showed that PL9680 0.3 mg/kg produced a statistically significant mean AUC change in clinical score vs vehicle over the study duration (mean, 61.1 vs 87.6, respectively; P<0.05) (**Figure 6**)

Figure 6. EAE Clinical Score Changes and AUC Over Days 8–35



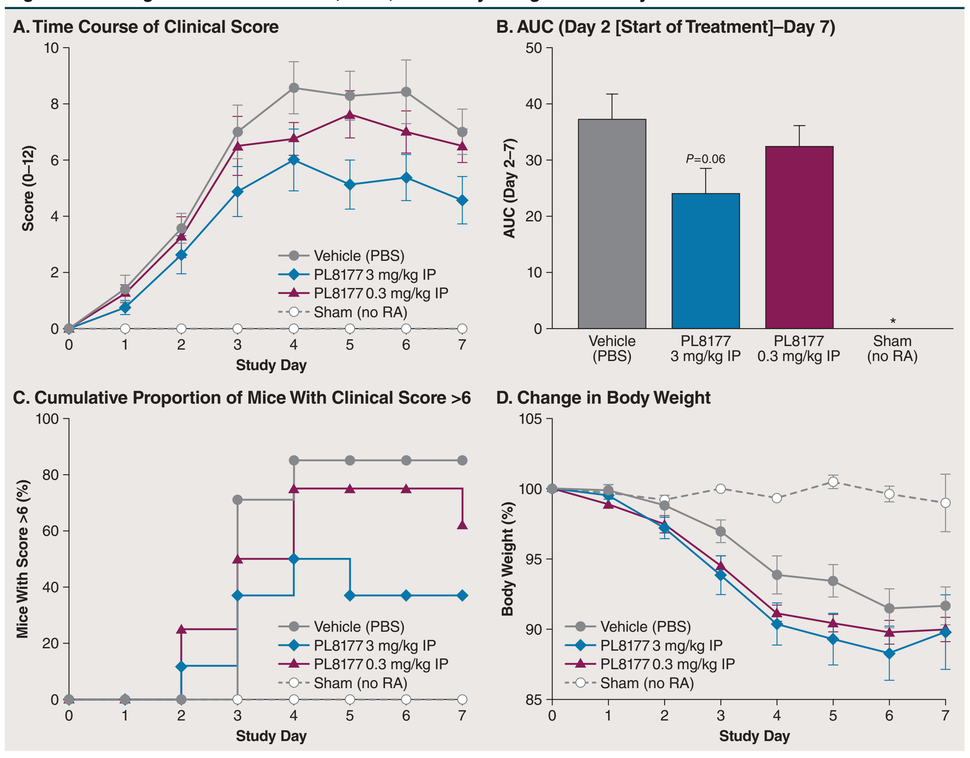
Values are mean (SEM). *P<0.05 vs vehicle. ****P<0.0001 vs vehicle. AUC, area under curve; EAE, experimental allergic encephalitis.

- PL9680 3-mg/kg solution did not achieve mean AUC clinical score significance vs vehicle
- The AUC clinical score for FTY720 was 42.0 (P<0.0001 vs vehicle)
- Electrophysiology showed improvements in tcMEPs for mean duration on Day 34 for both PL9680 solution doses vs vehicle (P<0.05). The 0.3-mg/kg solution also showed improvement in mean latency on Day 34 vs vehicle (P<0.05)
  - –FTY720 showed similar improvements in these parameters to PL9680 solution
  - –Other tcMEP changes were not significant for PL9680 or FTY720

PL8177 K/BxN Serum Transfer Mouse RA Model

- Systemic administration of PL8177 3 mg/kg resulted in a reduction of the signs of arthritis with an AUC 35% reduction of clinical score as compared to the vehicle arthritic group, although statistical significance was not achieved (P=0.06) (**Figure 7**)

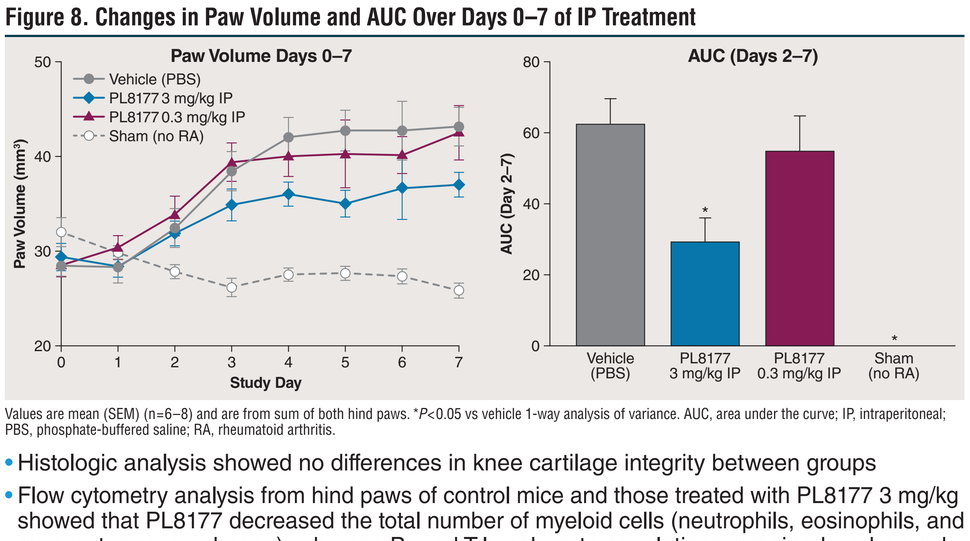
Figure 7. Changes in Clinical Score, AUC, and Body Weight Over Days 0–7 of IP Treatment



Values are mean (SEM). *P<0.05 vs vehicle 1-way analysis of variance; AUC, area under the curve; IP, intraperitoneal; PBS, phosphate-buffered saline; RA, rheumatoid arthritis.

- In the vehicle-treated group, 80% of mice developed a moderate to severe form of arthritis (defined as clinical score >6), vs 40% of those treated with PL8177 3 mg/kg
- The lower dose of PL8177 (0.3 mg/kg) had no statistically significant effect on clinical score
- Accelerated weight loss in PL8177 vs vehicle correlates with its effect on joint pathology
- Measures of joint edema (knee volume and width) showed that PL8177 3 mg/kg significantly ameliorated inflammation vs vehicle as measured by plethysmometry (AUC volume reduction was >50%, P<0.05), and via caliper measurements of knee width (P<0.05)
  - –The 0.3-mg/kg dose also attenuated swelling but did not reach significance
- Paw volume was significantly reduced vs vehicle for the PL8177 3-mg/kg dose with the AUC of paw volume achieving a >50% reduction (**Figure 8**)

Figure 8. Changes in Paw Volume and AUC Over Days 0–7 of IP Treatment



Values are mean (SEM) (n=6–8) and are from sum of both hind paws. *P<0.05 vs vehicle 1-way analysis of variance. AUC, area under the curve; IP, intraperitoneal; PBS, phosphate-buffered saline; RA, rheumatoid arthritis.

- Histologic analysis showed no differences in knee cartilage integrity between groups
- Flow cytometry analysis from hind paws of control mice and those treated with PL8177 3 mg/kg showed that PL8177 decreased the total number of myeloid cells (neutrophils, eosinophils, and monocytes-macrophages), whereas B- and T-lymphocyte populations remained unchanged
  - –This indicates that PL8177 treatment of mice reduced innate immune infiltration in the arthritic paws, which could explain the effects on inflammation and arthritis reported above

Conclusions

- Subcutaneous BID administration of PL9654 or PL8177 showed efficacy in reducing vision loss in a rat model of DR
- PL9680 solution (0.3 mg/kg), administered orally, was effective in reducing the clinical score of EAE in a mouse model of MS
  - –PL9680 (3.0 and 0.3 mg/kg) also showed efficacy in reducing action potential duration, with the 0.3-mg/kg dose also reducing latency
- Daily PL8177 administered with systemic IP injections displayed antiarthritic properties in the K/BxN serum transfer model of RA, with effects more evident with the 3-mg/kg dose
- In these preclinical model systems, these melanocortin agonists show promise in the treatment of DR, MS, and RA by reducing inflammation and by providing protection from or amelioration of symptoms

Funding These studies were funded by Palatin Technologies Inc. (Cranbury, NJ).

Acknowledgments Editorial support was provided by Robin Smith, PhD, of The Curry Rockefeller Group, LLC (Tarrytown, NY), and was funded by Palatin Technologies Inc. (Cranbury, NJ).

Disclosures Mauro Perretti and Andrew W. Taylor received consulting fees and a sponsored research agreement from Palatin Technologies, Inc. Carl Spana, Alison Obr, Paul S. Kayne, and John Dodd are employees of Palatin Technologies. Wei H. Yang and Marie Makhlina are former employees of Palatin Technologies.

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