Etrasimod, an Oral, Selective Sphingosine 1-phosphate Receptor Modulator Improves Skin Inflammation in a Contact Hypersensitivity Model of Dermatitis

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INTRODUCTION

Introduction:

Etrasimod (APD334) is an orally administered, selective sphingosine 1-phosphate receptor 1,4,5 (S1P_{1,4,5}) modulator¹ in development for multiple immune-mediated inflammatory disorders, including a Phase 2 trial in atopic dermatitis (AD) that is expected to be initiated in 2019. S1P₁ is a cell surface G protein-coupled receptor (GPCR) that has been shown to regulate lymphocyte egress from lymph nodes² and dendritic cell trafficking³.

Goal of Study:

The goal of this pre-clinical study was to evaluate the effect of etrasimod on skin inflammation and immune cell cellularity in the fluorescein isothiocyanate (FITC)-induced dermatitis mouse model.

Overall Conclusions:

Etrasimod effectively reduced ear skin inflammation and dermatitis in FITC-induced hypersensitivity. After challenge, etrasimod significantly reduced the activation and expansion of immune cells in the draining lymph node and ear skin. Notably, the dose-dependent reduction of immune cells in ear skin correlated with improvements in disease. This data encourages further study of etrasimod as a novel therapy for AD.

METHODS

Experimental Design and Treatment Groups

Beginning on Day -1, female BALB/c mice were orally dosed daily with the indicated treatments in phosphate buffered saline (PBS). Etrasimod doses were based on potency determined by previous *in vivo* studies.

On Days 0 and 5, mice were sensitized with 1% fluorescein isothiocyanate (FITC) in Acetone: Dibutyl phthalate (ADBP) on the hind flank skin, and subsequently challenged on the ear skin on Days 11, 12, and 13.

Treatment Group	Time Course Analysis	Day 2 Analysis (50% of mice)	Day 13 Analyses (50% of mice)
Sham (n=10)	Ear thickness measured using a dial caliper	Inguinal lymph node cellularity analyzed by flow cytometry	<ul style="list-style-type: none">Blood analyzed by complete blood count (CBC) with differentialCervical lymph node cellularity analyzed by flow cytometryOne ear fixed, stained with Hematoxylin & Eosin, and histologically scoredOne ear enzymatically digested, homogenized, and cellularity analyzed by flow cytometry
Vehicle Control (PBS) (n=20)			
Dexamethasone 1 mg/kg (n=20)			
Etrasimod 1 mg/kg (n=20)			
Etrasimod 3 mg/kg (n=20)			

RESULTS

Etrasimod Dose-Dependently Reduced Ear Skin Thickness and Histopathological Score

Ear Thickness Change

*p=.0044, **p<.0001. Two-Way ANOVA vs. Vehicle.

Figure 1A: Etrasimod treatment resulted in a significant, dose-dependent reduction in ear thickness. On Day 13, the difference in ear thickness between etrasimod 3 mg/kg and dexamethasone were not significantly different.

Histopathological Score

*p=.0014, **p<.0001. One-Way ANOVA with Tukey's multiple comparisons test vs. Vehicle.

Figure 1B: Etrasimod treatment resulted in a significant, dose-dependent reduction in histopathological score. There was no significant difference between etrasimod 3 mg/kg and dexamethasone.

SENSITIZATION Day 2: Inguinal Draining Lymph Node

Total Immune Cells and Subsets Similarly Increased in the Inguinal Lymph Node in All Treatment Groups

A. Total CD45+ Cells

B. Total B Cells

C. Total T Cells

D. Total Dendritic Cells

Figure 2: Total A) CD45+ cells, B) B cells, C) T cells, and D) dendritic cells significantly increased upon sensitization. Dexamethasone and etrasimod (1 and 3 mg/kg) had no significant impact on the increased cellularity in the lymph nodes.

CHALLENGE Day 13: Blood

Etrasimod Reduced Circulating White Blood Cells and Lymphocyte Frequency

A. White Blood Cell Count

*p=.0026, **p<.0001. One-Way ANOVA with Tukey's multiple comparisons test vs. Vehicle.

B. Lymphocyte Frequency of WBC

Figure 3: Etrasimod reduced circulating A) white blood cell (WBC) count and B) lymphocyte frequency of WBC similarly at both the 1 and 3 mg/kg doses.

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CHALLENGE Day 13: Cervical Draining Lymph Node

Etrasimod Reduced Dendritic Cell Influx Into the Cervical Lymph Node

A. Langerin+ Dendritic Cells

B. Langerin- Dendritic Cells

*p=.012, **p=.0012, ***p=.0003, ****p<.00001. One-Way ANOVA with Tukey's multiple comparisons test vs. Vehicle.

Figure 4: Etrasimod 1 and 3 mg/kg treatments similarly reduced the trafficking of both A) Langerin+ and B) Langerin- dendritic cells.

Etrasimod Similarly Reduced T Cells, B Cells, and Eosinophils in the Cervical Lymph Node

A. Total B Cells

B. Total Eosinophils

C. Total αβ T Cells

D. CD69+ αβ T Cells

*p<.0008, **p<.00006, ***p<.00001. One-Way ANOVA with Tukey's multiple comparisons test vs. Vehicle.

Figure 5: Etrasimod 1 and 3 mg/kg treatments reduced the expansion of A) B cells and B) Eosinophils to a similar magnitude as dexamethasone. Etrasimod similarly reduced the C) expansion and D) activation of αβ T cells.

CHALLENGE Day 13: Ear Skin

In the Ear Skin, Etrasimod Reduced T Cells, B Cells, and Eosinophils in Dose-dependent Manner

A. Total B Cells

B. Total Eosinophils

C. Total αβ T Cells

D. CD69+ αβ T Cells

E. CLA+ αβ T Cells Ear Skin

*p<.05, **p<.008, ***p<.001. One-Way ANOVA with Tukey's multiple comparisons test vs. Vehicle.

Figure 6: Etrasimod 1 and 3 mg/kg doses significantly reduced the activation and expansion of total αβ T cells, including CD69+ and skin-homing CLA+ αβ T cells in the ear skin (C-E). However, only the 3 mg/kg treatment significantly reduced the expansion of (A) B cells and (B) eosinophils in the skin.

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DISCLOSURES

CC, KK, and JA are employees of Arena Pharmaceuticals, Inc.

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CONCLUSIONS

Sensitization Phase

- Dendritic Cells, T cells, and B cells increased in number in the inguinal draining lymph node on Day 2, and etrasimod had no effect on the number of cells. This suggests the sensitization phase was relatively intact in this model.

Challenge Phase

- Etrasimod treatment reduced the trafficking of dendritic cells to the cervical draining lymph node. The effect was greater in the 3 mg/kg vs 1 mg/kg doses.
- In the cervical draining lymph node, etrasimod reduced T cells, B cells, and eosinophils. The effect was similar with the 1 mg/kg and 3 mg/kg doses for T and B cells, while the eosinophil reduction was only observed with the 3 mg/kg treatment.
 - There was also a dose-dependent reduction in activated CD69+ T cells in the lymph node after etrasimod treatment
- In the skin, etrasimod reduced T cells, B cells, and eosinophils. The reduction for each subset occurred in a dose-dependent manner.
 - There was a dose-dependent reduction in activated CD69+ and skin-homing CLA+ αβ T cells

The dose-dependent reduction of inflammatory cells in the skin correlated with a dose-dependent improvement in ear skin inflammation and histologic scoring following FITC challenge.

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