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Use of Epigenetic Cell Counting to Determine the Effect of Ozanimod on Circulating Leukocyte Subtypes in Patients With Relapsing Multiple Sclerosis

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INTRODUCTION

- Ozanimod is a sphingosine 1-phosphate (S1P) receptor modulator, which binds with high affinity selectively to S1P receptor subtypes 1 and 5[1]
- Ozanimod causes lymphocyte retention in lymphoid tissues[1]
  - The mechanism by which ozanimod exerts therapeutic effects in multiple sclerosis (MS) is unknown but may involve the reduction of lymphocyte migration into the central nervous system
- Ozanimod was effective and well tolerated as an oral treatment for relapsing multiple sclerosis (RMS) in phase 2[2,3] and 3 trials[4,5]
- An analysis of absolute lymphocyte count (ALC) and leukocyte subtypes in RMS patients treated with ozanimod was conducted using flow cytometry and supported dose-dependent reductions in ALC, primarily T and B cells, but with minimal changes in monocytes, natural killer (NK) cells, and NKT cells[6]
- This analysis was conducted to evaluate the effect of ozanimod on ALC and circulating leukocyte subtypes in patients with RMS using epigenetic cell counting, which allows precise, robust, and reproducible measurement of immune cells using frozen whole blood samples[7]

METHODS

- A phase 1 randomized, open-label, pharmacokinetic/pharmacodynamic study of ozanimod HCl 0.5 or 1 mg/d (equivalent to ozanimod 0.46 or 0.92 mg, respectively) was conducted in participants with RMS (NCT02797015)
- Following an initial 7-day dose escalation consisting of ozanimod HCl 0.25 mg/d (equivalent to ozanimod 0.23 mg) on days 1–4, then 0.5 mg/d on days 5–7, participants received their assigned dose of ozanimod HCl 0.5 or 1 mg/d for approximately 12 weeks, after which they were eligible for an open-label extension study (DAYBREAK; NCT02576717)
- The study enrolled adults aged 18–55 years with active, clinically stable RMS and an Expanded Disability Status Scale score of 0 to 6 who were otherwise generally healthy
  - Key exclusion criteria included active infection or history of chronic infections or immunodeficiency, recent live vaccination, previous lymphocyte-depleting or immunosuppressant therapy, and ALC <1.000 x 10^9/L or white blood cell count <3.500 x 10^9/L
- ALC was evaluated on days 1, 5, 8, 28, 56, and 85 (end of treatment) during the study
- In this exploratory analysis, epigenetic cell counting was performed on days 2, 5, 28, 56, and 85 (end of treatment) by Epiontis/Precision for Medicine as previously described,[7] using bisulfite converted DNA from frozen whole blood samples as substrate for quantitative polymerase chain reaction assays for selected cell type–specific demethylated loci for the following leukocyte subtypes:
  - B cells
  - T cells
  - Cytotoxic T cells (CD8+)
  - Helper T cells (CD4+)
  - Regulatory T cells (Treg)
  - Th17 cells
  - Naive CD8+ T cells
  - PD1+ cells (follicular T helper cells)
- Circulating leukocyte subset counts were compared with baseline using descriptive statistics
- The geometric mean, used to report percentage of baseline cell count, is the mean of the logged values, then transformed back to the original scale; the 95% confidence intervals (CIs) are computed on the log scale, then transformed back
- R version 3.6.1 (2019-07-05) (R Core Team 2018) was used for all analyses

RESULTS

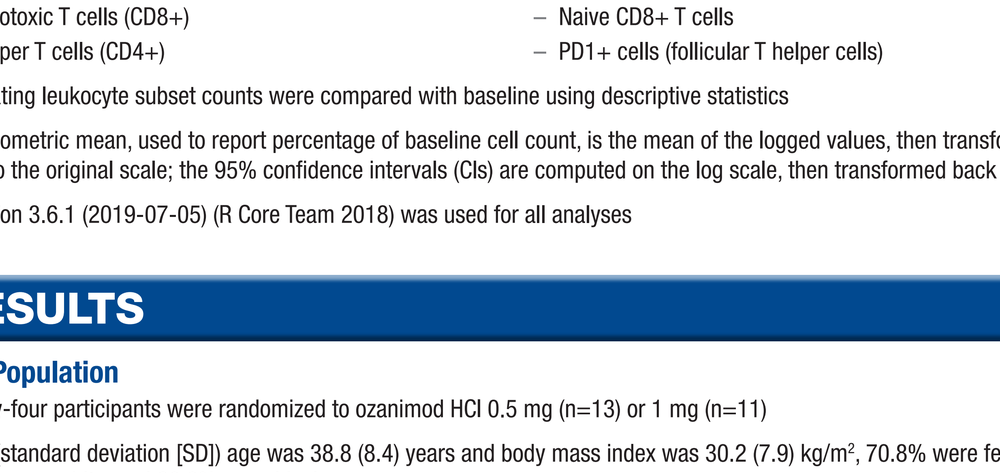
Study Population

- Twenty-four participants were randomized to ozanimod HCl 0.5 mg (n=13) or 1 mg (n=11)
- Mean (standard deviation [SD]) age was 38.8 (8.4) years and body mass index was 30.2 (7.9) kg/m^2, 70.8% were female, 75.0% were white, and 20.8% were black

Course of ALC

- Mean (SD) ALC at baseline was 1.754 (0.489) x 10^9/L in the ozanimod HCl 0.5 mg group and 2.118 (0.961) x 10^9/L in the ozanimod HCl 1 mg group
- Following dose escalation, ozanimod was associated with dose-dependent reductions in ALC (**Figure 1**)
- At the end of treatment (day 85), mean (SD) ALC was 1.055 (0.594) x 10^9/L (–42.2% change from baseline) in the ozanimod HCl 0.5 mg group and 0.536 (0.262) x 10^9/L (–73.3% change from baseline) in the ozanimod HCl 1 mg group

Figure 1. Absolute Lymphocyte Counts Over Time During Treatment With Ozanimod HCl 0.5 or 1 mg/d in a Population With RMS



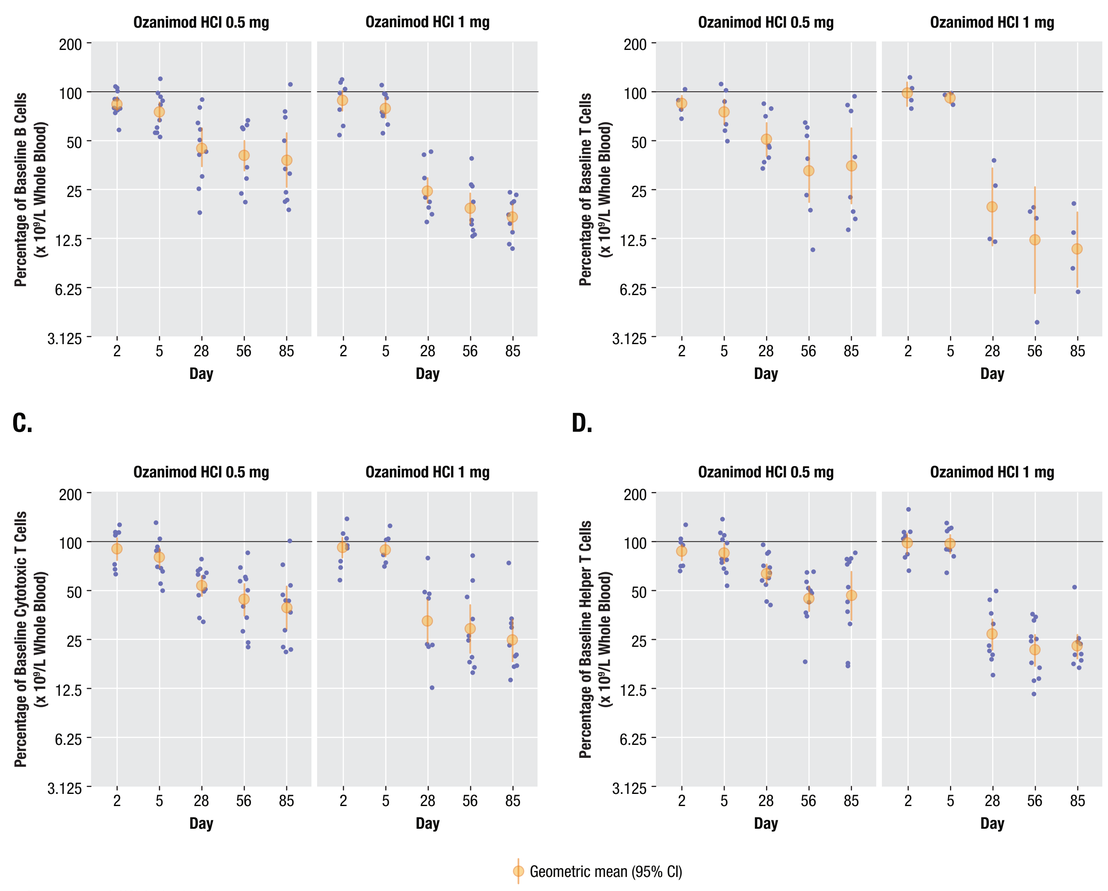
[a]LLN established by the reference laboratory for the study.
[b]Dose escalation consisted of ozanimod HCl 0.25 mg/d on days 1–4, 0.5 mg/d on days 5–7, then assigned dose (0.5 or 1 mg/d) for approximately 12 weeks.
Error bars represent ±1 SD.
ALC, absolute lymphocyte count; HCl, hydrochloride; LLN, lower limit of normal; RMS, relapsing multiple sclerosis; SD, standard deviation.

Effect of Ozanimod on Total Circulating Leukocytes and Leukocyte Subtypes

- Total circulating leukocytes at day 85 were reduced to 90% (95% CI: 78%, 104%) and 73% (95% CI: 55%, 97%) of baseline in the ozanimod HCl 0.5 and 1 mg groups, respectively
- Dose-dependent decreases were observed for total B cells (**Figure 2A**) and T cells (**Figure 2B**), and all other subtypes tested during ozanimod treatment, including cytotoxic T cells (**Figure 2C**) and helper T cells (**Figure 2D**)
- Decreases were greatest for total T cells (**Figure 2B**), Th17 (**Figure 3A**), and naive CD8+ T cells (data not shown) (11%–13% of baseline with ozanimod HCl 1 mg at day 85)
- Decreases were less for Treg (**Figure 3B**) and PD1+ cells (data not shown) (34%–35% of baseline with ozanimod HCl 1 mg at day 85)

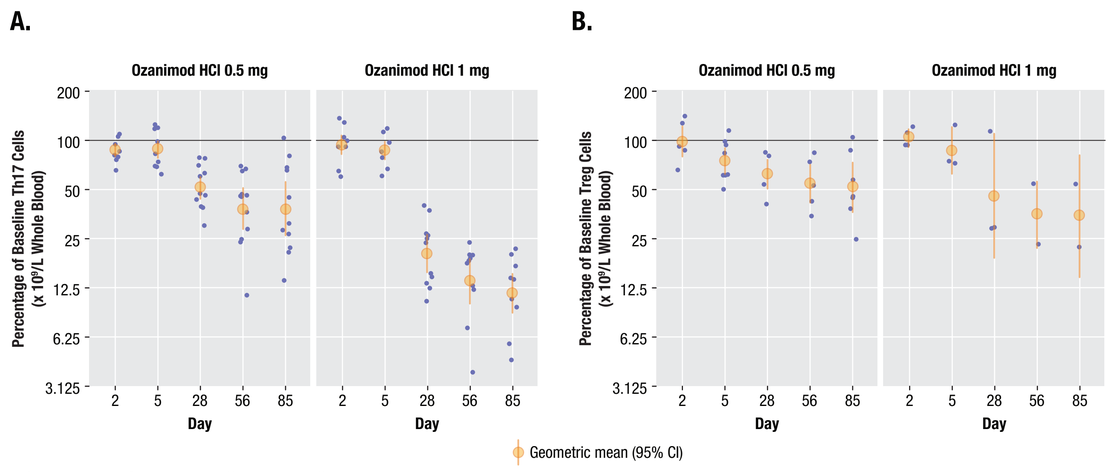
RESULTS (cont'd)

Figure 2. Total B Cells (A), Total T Cells (B), Cytotoxic T Cells (C), and Helper T Cells (D) During Treatment With Ozanimod



CI, confidence interval; HCl, hydrochloride.

Figure 3. Th17 Cells (A) and Treg Cells (B) During Treatment With Ozanimod



CI, confidence interval; HCl, hydrochloride; Treg, regulatory T cells.

CONCLUSIONS

- Epigenetic cell counting results were consistent with previously reported flow cytometry for all leukocyte subsets tested in both analyses, including decreases in total B cells and T cells as well as cytotoxic T cells, helper T cells, and naive CD8+ T cells
- Epigenetic cell counting also provided data on previously untested Th17, Treg, and PD1+ cells, further clarifying the differential effects of ozanimod on specific leukocyte subtypes in patients with RMS
  - Ozanimod had a greater effect on T cells, helper T cells, and Th17 cells that are associated with neuroinflammation and neurodegeneration[8]
  - In contrast, ozanimod had less effect on the leukocyte subtypes associated with maintenance of immune surveillance, Treg and PD1+ cells, which are also important immune regulators associated with stable MS[9,10]
  - These findings are consistent with the low rates of infection and serious opportunistic infections observed in phase 2 and 3 clinical trials of ozanimod in patients with RMS[2-5]
- Dose-dependent effects were observed across all leukocyte subtypes, with ozanimod HCl 1 mg producing greater reductions in B cells and T cells compared with ozanimod HCl 0.5 mg
  - These findings are consistent with clinical findings from the phase 3 studies, in which ozanimod HCl 1 mg consistently achieved a numerically lower annualized relapse rate and fewer brain MRI lesions compared with ozanimod HCl 0.5 mg[4,5]

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ACKNOWLEDGMENTS

This study was sponsored by Celgene International II Sàrl. The authors wish to thank Epiontis/Precision for Medicine for conducting the epigenetic cell counting. Support for third-party writing assistance for this poster was provided by Peloton Advantage, LLC, an OPEN Health company, and was funded by Celgene Corporation.

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DISCLOSURES

SH and **JQT** are employees of Celgene Corporation; **HS** has received compensation from Allecra Therapeutics, Alpha Medical Statistics Limited, Ardea Biosciences, AstraZeneca, Celgene Corporation, Clarostat Consulting Limited, CytomX Therapeutics, GlaxoSmithKline, Gossamer Bio, Stone Biostatistics Limited, University of Lancaster, and University of Manchester. **BACC** has received personal compensation for consulting from AbbVie, Akili, Alexion, Biogen, EMD Serono, Novartis, and TG Therapeutics.

Presented at the 27th Annual Meeting of the European Charcot Foundation; 21–23 November 2019; Baveno, Italy