

# The targeted eosinophil-lowering effects of dexpramipexole in clinical studies



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## ABSTRACT

Dexpramipexole, an orally bioavailable small molecule previously under clinical development in amyotrophic lateral sclerosis, was observed during routine safety hematology monitoring to demonstrate pronounced, dose- and time-dependent eosinophil-lowering effects, with minor reductions on other leukocyte counts. Analysis of hematology lab values across two double-blind, randomized placebo-controlled clinical trials at total daily doses ranging from 50 mg to 300 mg demonstrated that dexpramipexole consistently and markedly lowered peripheral blood eosinophils. This effect developed after 1 month on treatment, required 3–4 months to reach its maximum, remained constant throughout treatment, and partially recovered to baseline levels upon drug withdrawal. All doses tested were well tolerated. The overall adverse event rate was similar for dexpramipexole and placebo, and notably with no increase in infection-related adverse events associated with eosinophil-lowering effects. Given the reliance on and insufficiency of off-label chronic corticosteroid therapy for hypereosinophilic syndromes and other eosinophil-associated diseases (EADs), a need exists for less toxic, more effective, targeted therapeutic alternatives. Further clinical studies are underway to assess the eosinophil-lowering effect of dexpramipexole in the peripheral blood and target tissues of EAD patients and whether such reductions, if observed, produce clinically important benefits.

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## 1. Introduction

Eosinophils are white blood cells of myeloid lineage for which data continue to emerge demonstrating their involvement in both health and disease [1,2]. Eosinophils have multiple beneficial roles, including in the innate response to parasitic infection, modulation of adaptive immune responses, and maintenance of tissue homeostasis [1,2]. However, under a variety of pathophysiological conditions, eosinophils accumulate to abnormally high levels in the blood and infiltrate target organs and tissues, secreting inflammatory proteins that may cause serious, potentially irreversible, tissue damage [3,4].

Eosinophil-associated diseases (EADs) are a heterogeneous group of disorders with poorly understood pathogenic mechanisms. The pathologic consequences of eosinophilia, both direct and indirect, span a wide clinical spectrum from gastrointestinal and respiratory disorders such as colitis and asthma to hematological disorders such as hypereosinophilic syndromes (HES) and chronic eosinophilic leukemia

(CEL). Many EADs remain treated with chronic corticosteroid administration, which has a well-established, deleterious side-effect profile [5]. Monoclonal antibodies against IL-5, an eosinophil-promoting cytokine, have recently been approved for the treatment of severe eosinophilic asthma [6] and also show promise in other EADs. However, treatment options remain limited and thus a need remains for effective, easily administered, and cost-effective treatments for EADs.

Dexpramipexole is a small synthetic molecule with high oral bioavailability, linear pharmacokinetics, and a well-characterized safety profile [7]. Its eosinophil-lowering effect, observed initially in a dose-ranging phase 2 study, was confirmed in a large phase 3 trial ( $n=942$ ) in amyotrophic lateral sclerosis (ALS) subjects, a generally non-atopic population with a normal hematologic status. An agent that safely, significantly, and durably lowers circulating eosinophils with a potential for a decrease in target tissues merits investigation for its potential to reduce the eosinophilia characteristic of many EADs and to improve clinical outcomes.

## 2. Methods

Hematology laboratory data sets were reviewed from two randomized, double-blind, placebo-controlled clinical trials in ALS [8,9]. Baseline hematology values were compared to the reference ranges

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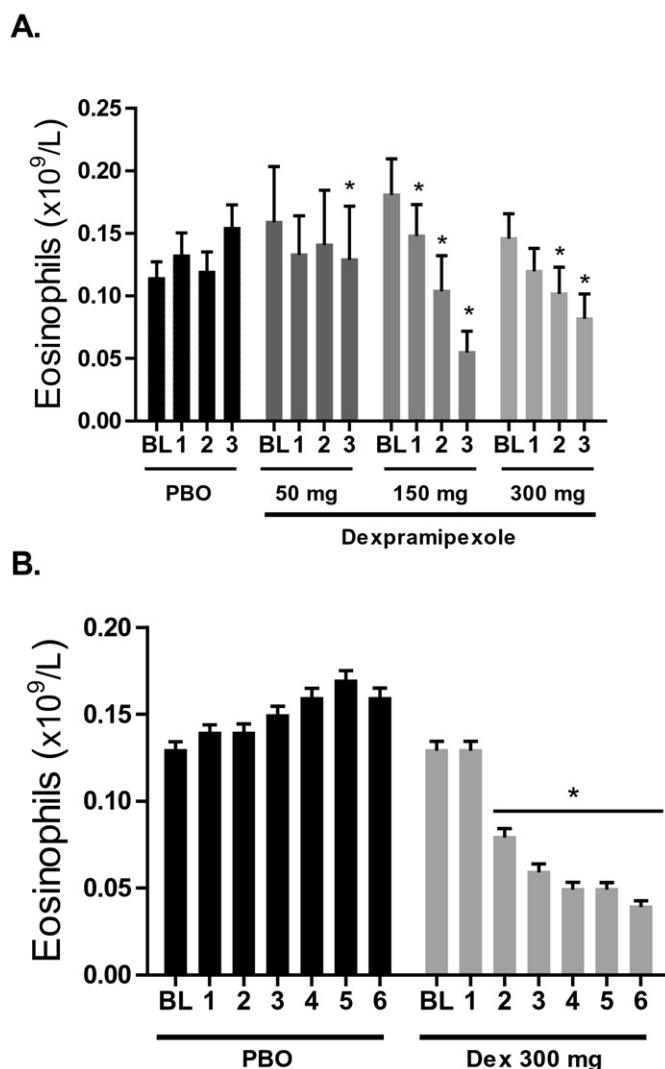
prespecified for each study. Eosinophil levels were summarized over available time points and statistically analyzed using an analysis of variance model (ANOVA) to compare means change from baseline between an active treatment and the placebo group. In the phase 2 and 3 studies, changes from baseline for other peripheral blood cells were also assessed to evaluate the broader hematologic effects of the drug. Adverse events were reviewed by preferred term and system organ class and their incidences compared between the treatment and placebo groups to assess the risk of dexpropimexole-associated infection rates as a potential side effect.

### 3. Results

The phase 2 clinical trial was a two-part, double-blind study that evaluated the safety, tolerability, and clinical effects of dexpropimexole in 102 ALS subjects [8]. In Part 1, subjects were randomized to placebo ( $n=27$ ), 50 mg/day ( $n=23$ ), 150 mg/day ( $n=26$ ), or 300 mg/day ( $n=26$ ) of dexpropimexole ( $n=26$ ) for 3 months. From baseline to month 3, mean blood eosinophils increased by 29.2% in the placebo group and declined by 17.7% ( $p = 0.038$ ), 69.9%, ( $p < 0.0001$ ), and 43.5% ( $p = 0.0008$ ) in the 50 mg, 150 mg, and 300 mg groups, respectively (Fig. 1A). In the 150 mg and 300 mg groups, statistically significant differences from baseline could first be observed at month 1 and month 2, respectively, with the magnitude of effect greatest at month 3 (Fig. 1A). During a one-month drug washout following month 3, mean eosinophils at month 4 recovered to 46.6% and 76.6% of baseline levels in the 150 and 300 mg/day groups, respectively (data not shown). Following the drug washout, subjects were re-randomized to 50 and 300 mg/day in part 2 of the study. Subjects on 300 mg/day had a greater decline in eosinophils from month 4 to month 10 than subjects re-randomized to 50 mg/day (78.9% vs. 17.6%), consistent with the dose-dependent effect on eosinophils observed in part 1. Also noted in part 1, a substantial reduction in peripheral blood basophils occurred at month 3 in the 150 mg and 300 mg groups (45.6%,  $p = 0.0004$  and 32.6%,  $p = 0.018$ ), respectively. In contrast, non-significant changes in peripheral blood neutrophils ( $-6.6\%$ ), lymphocytes ( $-15.8\%$ ), monocytes ( $-6.3\%$ ), platelets ( $+11.0\%$ ), or red blood cells ( $+1.9\%$ ) were observed at month 3 in the 300 mg group.

The phase 3 clinical trial was a double-blind study of dexpropimexole in 942 ALS subjects randomized 1:1 to placebo or dexpropimexole 300 mg daily treatment for up to 18 months [9]. As in the phase 2 study, the eosinophil-lowering effect developed slowly, and in this trial reached plateau at month 4 (Fig. 1B), and persisted through end-of-study. The eosinophil lowering onset was observed by month 2 with a 36.6% decrease in eosinophils for dexpropimexole,  $p < 0.0001$  compared to placebo. At month 6, the change from baseline in blood eosinophil counts was  $+18.7\%$  in the placebo group and  $-69.1\%$  in the dexpropimexole-treated group ( $p < 0.0001$ ). The effect of dexpropimexole in reducing eosinophil counts was observed in most subjects, with 76.8% of dexpropimexole-treated subjects experiencing a 50% or greater decline in eosinophil count after 6 months of treatment. Dexpropimexole also reduced basophil counts with a similar onset of action, reaching plateau by month 4 and persisting through end-of-study. As shown in Fig. 2A, at month 6, the change from baseline in blood basophil counts was  $+4.5\%$  in the placebo group and  $-46.7\%$  in the dexpropimexole-treated group ( $p < 0.0001$ ). At month 6, minor, but statistically significant reductions were observed in neutrophils ( $-6.6\%$ ), lymphocytes ( $-13.6\%$ ), and monocytes ( $-13.7\%$ ) in subjects receiving dexpropimexole (Fig. 2B–D), with no significant drug effect on red blood cells ( $+1.3\%$ ) or platelet counts ( $+7.5\%$ ).

The safety profile of dexpropimexole has been characterized in clinical studies of  $>1000$  ALS subjects. Dexpropimexole was well-tolerated in both the phase 2 and phase 3 studies, with no dose-limiting toxicities and with adverse event rates similar across treatment groups. In particular, the number of treatment-emergent adverse events classified in the

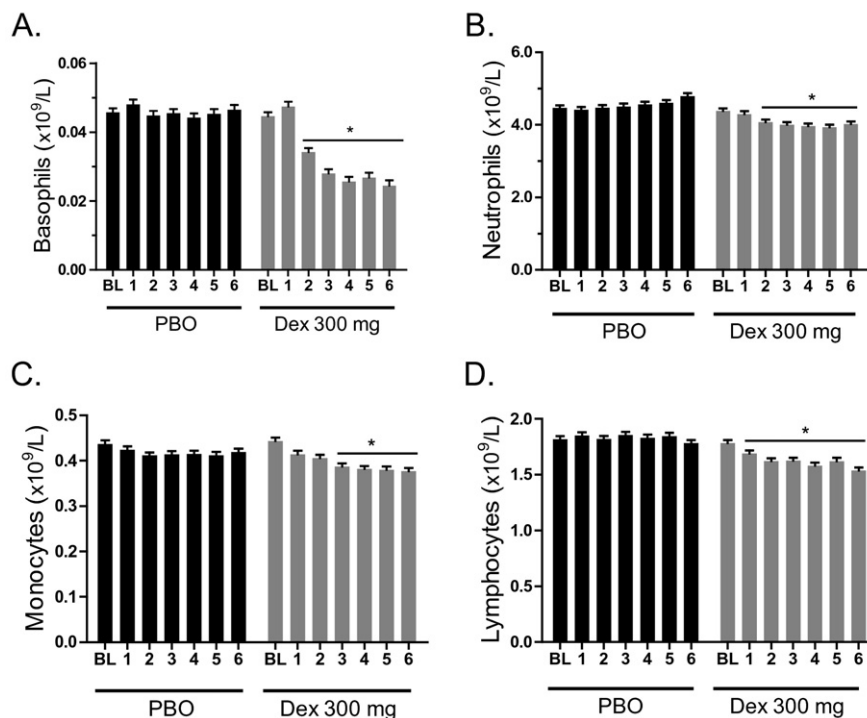


**Fig. 1.** Time- and dose-dependent eosinophil lowering effects of dexpropimexole in phase 2 and phase 3 ALS clinical trials. (A) In the phase 2 study, a 3-month dose ranging study in ALS subjects, mean blood eosinophils rose by 29.2% in the placebo group ( $n=27$ ) and declined by 17.7% ( $p = 0.038$ ), 69.9%, ( $p < 0.0001$ ), and 43.5% ( $p = 0.0008$ ) in the 50 mg ( $n=23$ ), 150 mg ( $n=26$ ), and 300 mg ( $n=26$ ) dexpropimexole treatment groups from baseline to week 12, respectively. (B) In the phase 3 trial, a marked decrease in peripheral blood eosinophil count was observed after 2 months of treatment with dexpropimexole that persisted for the duration of the trial. At month 6, eosinophil counts were reduced from baseline levels by 69.1% in the dexpropimexole-treated group ( $n=474$ ,  $p < 0.0001$ ) while there was an 18.7% increase in eosinophil count in patients receiving placebo ( $n=468$ ).

phase 3 trial as infections was similar in the placebo group (57%) compared with the dexpropimexole-treated group (56%). In phase 3, transient, laboratory-defined neutropenia ( $ANC < 1.5 \times 10^9$  cells/L) was observed in 6.1% of dexpropimexole-treated subjects ( $n=29$ ) vs. 1.7% receiving placebo ( $n=8$ ). Approximately 75% of ALS subjects in phase 3 received concomitant riluzole, which has a label warning for severe neutropenia. Of the phase 3 subjects with a laboratory event of neutropenia, 2/7 placebo subjects receiving riluzole (29%), 15/23 dexpropimexole subjects receiving riluzole (65%), and 0/6 subjects receiving only dexpropimexole had more than one episode.

### 4. Discussion

During the course of any clinical trial, hematology laboratories are routinely monitored for potential adverse events and safety signals. In the development of dexpropimexole for the treatment of ALS, we



**Fig. 2.** Effects of dexpramipexole treatment on circulating leukocytes in the phase 3 ALS trial. In the phase 3 trial, dexpramipexole treatment (300 mg daily) was associated with a marked reduction in peripheral blood (A) basophils and minor reductions in (B) neutrophils, (C) monocytes, and (D) lymphocytes through month 6. These changes were sustained through the end of study.

observed, across multiple studies, a consistent, robust reduction in peripheral blood eosinophils at doses well tolerated clinically. This effect was slow to develop, generally required 3–4 months to reach maximum effect and persisted for the duration of treatment. In the drug washout period of the phase 2 study, a slow recovery of eosinophil levels was observed, a reversibility of the eosinophil-lowering effect with kinetics similar to the onset of action.

Eosinophilia is not a typical feature of ALS. The mean baseline eosinophil counts across treatment groups in the phase 2 and phase 3 ALS studies ranged from  $0.12 \times 10^9/L$  to  $0.18 \times 10^9/L$ . However, two subjects enrolled in the ALS development program had baseline eosinophil counts  $\geq 0.50 \times 10^9/L$ . In part 2 of the phase 2 study, a subject randomized to dexpramipexole 300 mg daily with a baseline eosinophil count of  $0.97 \times 10^9/L$  had a reduction in eosinophil count to  $0.02 \times 10^9/L$  after 1 month of treatment that persisted through the end of the study (month 6). In phase 3, a subject randomized to dexpramipexole 300 mg daily with a baseline eosinophil count of  $0.58 \times 10^9/L$  had a reduction in eosinophil count to  $0.02 \times 10^9/L$  at month 6 that also persisted through the end of the study (month 12). While only two cases, the data suggest that dexpramipexole may have the potential to significantly lower eosinophils in subjects with elevated peripheral blood eosinophils.

The eosinophil has been a cellular target for treatment of EADs and clinical trials have been ongoing for the past 15 years. Mepolizumab and reslizumab, humanized monoclonal antibodies against interleukin-5, have recently been approved for the treatment of severe eosinophilic asthma, validating the approach that lowering peripheral blood eosinophils delivers clinical benefit. In the two ALS trials, the magnitude of dexpramipexole eosinophil-lowering was comparable to the anti-IL5 monoclonal antibodies and thus warrants further investigation of its activity in EADs.

In the phase 3 trial, there were statistically significant, but biologically modest, decreases in the number of peripheral blood monocytes, neutrophils, and lymphocytes without clinical effect in subjects randomized to dexpramipexole. This suggests that dexpramipexole may target

specific hematopoietic precursors during differentiation and maturation, with a predominant effect on eosinophils. Interestingly, basophils also showed about a 50% decrease in peripheral blood in both trials, with a similar time-course as seen for eosinophils. The magnitude of basophil-lowering suggests dexpramipexole may target the common human eosinophil-basophil (Eo/B) progenitor cell, and could prove to be beneficial especially in upper and lower airway diseases in which these Eo/B lineage-committed progenitor cells have been reported to be up-regulated [10]. Single-cell RNA sequencing in mice has recently identified an early myeloid lineage bifurcation of eosinophil-basophil progenitors from neutrophil-monocyte progenitors based on the expression of the transcription factor GATA-1 [11]. Also, in IL-5 transgenic mice [12] eosinophil increases >50-fold, are accompanied by a three-fold increase in neutrophils, monocytes, and lymphocytes, which may be either a primary effect from the increased IL-5 levels or a secondary effect associated with the increased eosinophil levels. It is interesting to speculate that a reciprocal effect may be occurring with dexpramipexole, such that a significant (>50%) decrease in eosinophils and basophils is associated with a modest decrease (~10%) in monocytes, lymphocytes, and neutrophils. As more clinical experience accumulates with the use of IL-5 targeted monoclonal antibodies, it would be valuable to learn if they exert indirect effects on other granulocytes and lymphocytes.

In both the phase 2 and 3 ALS trials, an increase in eosinophils was observed in the placebo groups. While there is no established connection associating eosinophils with the neuromuscular disorder ALS, the disease has been speculated to have a neuroinflammatory component, with the possibility of eosinophil involvement. While not measuring peripheral eosinophils directly, Liu et al. [13] showed an increase in EDN (eosinophil-derived neurotoxin) in sera from patients with ALS and Schroder et al. [14] demonstrated the histological presence of eosinophils in skeletal muscle biopsies from ALS subjects. As many ALS patients receive multiple medications, the elevated eosinophil level could be accounted for by drug interactions, or perhaps directly or indirectly related to loss of muscle function.

The eosinophil-lowering effect of dexpramipexole is most pronounced in humans. No effect of dexpramipexole on eosinophils or other white blood cell types was observed in a 26-week chronic toxicology study in rats at human equivalent doses up to 3000 mg/day. In a 39-week chronic toxicology study in Göttingen minipigs, a significant reduction in peripheral blood eosinophils was observed but only at human equivalent doses  $\geq 2850$  mg/day. This effect was unaccompanied by any pathologic bone marrow changes, including no change to myeloid or erythroid progenitors.

Current research is ongoing to study the effect of dexpramipexole on CD34<sup>+</sup> eosinophil and basophil precursors and whether the drug effects observed in normal hematologic states in the ALS population may be relevant in reducing increased levels of eosinophils in EADs. Two clinical trials are underway, one in HES and the other in CRS with nasal polyps (NCT02101138 and NCT02217332, respectively, at [clinicaltrials.gov](http://clinicaltrials.gov)) to test for eosinophil-lowering activity in subjects with peripheral blood and tissue eosinophilia, as well as to study exploratory clinical endpoints that could demonstrate clinical benefit in subjects with EADs.

## 5. Conclusions

In the ALS Phase 2 and Phase 3 studies, dexpramipexole demonstrated a highly significant and targeted, dose-dependent, eosinophil-lowering effect with a favorable safety and tolerability profile. With the recent approvals of injectable monoclonal antibodies against IL-5 for the treatment of severe eosinophilic asthma, as well as promise in other EADs, there is a need for new oral therapies that selectively mitigate eosinophilia in blood and tissue.

## Authorship contribution

All authors participated in designing and performing the post hoc analyses; S.D. prepared the figures; S.D. and M.E.B. wrote the paper. W.F. was the safety director of the phase 3 clinical trial and G.H. was the medical monitor for the phase 2 trial. D.A. performed statistical analyses.

## Conflict-of-interest disclosures

All authors except I.J.R. and W.F. are employees of and equity holders in Knopp Biosciences LLC, Pittsburgh, PA.

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