ABSTRACT

Administration of blosozumab, a humanized monoclonal antibody that binds sclerostin, increases bone formation and bone mineral density (BMD) in postmenopausal women with low BMD. To evaluate the effect of discontinuing blosozumab, we studied women enrolled in a 1-year randomized, placebo-controlled phase 2 trial for an additional year after they completed treatment. Of the 120 women initially enrolled in the study, 106 women completed treatment and continued into follow-up; 88 women completed 1 year of follow-up. At the beginning of follow-up, groups remained balanced for age, race, and body mass index, but lumbar spine and total hip BMD were increased in prior blosozumab groups, reflecting an anabolic treatment effect. At the end of follow-up, 1 year after discontinuing treatment, lumbar spine BMD remained significantly greater than placebo in women initially treated with blosozumab 270 mg every 2 weeks (Q2W) and blosozumab 180 mg Q2W (6.9% and 3.6% above baseline, respectively). Total hip BMD also declined after discontinuation of treatment but at 1 year after treatment remained significantly greater than placebo in women initially treated with blosozumab 270 mg Q2W and blosozumab 180 mg Q2W (3.9% and 2.6% above baseline, respectively). During follow-up, median serum P1NP was not consistently different between the prior blosozumab groups and placebo. A similar pattern was apparent for median serum C-terminal telopeptide of type 1 collagen (CTx) levels, with more variability. Mean serum total sclerostin concentration increased with blosozumab, indicating target engagement, and declined to baseline after discontinuation. There were no adverse events considered related to prior treatment with blosozumab. Anti-drug antibodies generally declined in patients who had detectable levels during prior treatment. These findings support the continued study of blosozumab as an anabolic therapy for treatment of osteoporosis.

KEY WORDS: BLOSOZUMAB; SCLEROSTIN ANTIBODY; ANABOLICS; DXA; OSTEOPOROSIS

Introduction

Osteoporosis, a disease characterized by low bone mineral density (BMD) and reduced bone quality, affects approximately 200 million people worldwide, with an increasing incidence anticipated among the aging world population. Low BMD predisposes patients to fracture and accounts for an increasing global health burden. Patients experiencing a fracture are at higher risk for future fracture, morbidity, and mortality.

Blossozumab, a humanized monoclonal antibody targeted to sclerostin, is undergoing clinical study for the treatment of osteoporosis. Through binding sclerostin, an inhibitor of the Wnt signaling pathway, blosozumab exerts an anabolic effect on bone. Preclinical studies of blosozumab and a phase 1 clinical trial have demonstrated increases in BMD with...
Results from a randomized, double-blind phase 2 clinical trial of blosozumab in postmenopausal women with osteoporosis have recently been published. In this trial, significant increases in BMD of the lumbar spine and total hip were achieved in patients receiving 1 year of blosozumab treatment as compared with placebo. To evaluate the effect of discontinuing blosozumab, we studied the women enrolled in the 1-year phase 2 trial for an additional year after they completed treatment. The objectives of the phase 2 follow-up period were to conduct a longitudinal assessment of BMD response to stopping treatment and to extend the period of observation for possible emergent adverse events. This report describes the effects of discontinuing blosozumab treatment, observed during the 52-week post-treatment follow-up period of the phase 2 clinical trial.

Materials and Methods

Study design

The randomized, double-blind, parallel, placebo-controlled phase 2 clinical trial of blosozumab consisted of a 52-week treatment period and a post-treatment 52-week follow-up period (Fig. 1). During the treatment period, women with low BMD were randomly assigned to treatment with matching placebo, blosozumab 180 mg every 4 weeks (Q4W), blosozumab 180 mg Q2W, and blosozumab 270 mg Q2W, all given by subcutaneous injection. Calcium and vitamin D supplementation continued through the post-treatment period of the trial. The post-treatment follow-up period was composed of 12 weeks of observation per study protocol and an additional 40 weeks of observation by protocol addendum. Patients completing the treatment period of the phase 2 trial at sites where the investigator chose to participate in the optional addendum were eligible for inclusion. Patients were excluded from the follow-up study if they dropped out of the treatment period of the phase 2 trial for any reason or if they were noncompliant with study medication. Patients electing to participate in the additional follow-up provided written informed consent. This study was conducted according to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects and with approval of local ethical review boards. Further details about the study design and the results obtained during study treatment have previously been published. This study, ClinicalTrials.gov identifier NCT01144377, was sponsored by Eli Lilly and Company.

Laboratory assessments

During the study follow-up period, serial assessments of BMD by dual-energy X-ray absorptiometry (DXA) were performed at prespecified times at the lumbar spine, total hip, and femoral neck at 3, 6, 9, and 12 months after discontinuing study treatment. Serum samples were obtained at prespecified time points for measurement of biochemical markers of bone metabolism, total sclerostin concentrations, and immunogenicity. The methodologies for these laboratory assessments in the phase 2 clinical trial have been previously described, with the exception of the assay for serum total sclerostin concentrations, which is described below.

Biochemical markers of bone turnover were measured in fasting serum samples, which were requested to be collected at approximately the same time of day for each subject. Total P1NP and osteocalcin concentrations were measured in the serum by an electrochemiluminescent assay (ECLIA) (cobasTM; Roche Diagnostics, Indianapolis, IN, USA). Bone-specific alkaline phosphatase concentrations were measured by enzyme immunoassay (EIA) (Metra™ BAP immunoassay; QuidelTM Corporation, San Diego, CA, USA). C-terminal telopeptide of type 1 collagen (CTx) concentrations were measured by Beta CrossLaps ECLIA assay (b-CrossLaps/serum, cobas™, Roche Diagnostics).

Serum sclerostin concentration

Serum sclerostin concentrations were measured in all study participants during the treatment and follow-up periods using...
a blosozumab-tolerant sclerostin immunoassay (Eli Lilly and Company, Indianapolis, IN, USA) developed to evaluate total serum sclerostin concentrations. The Lilly total sclerostin assay was validated for measuring concentration of a biomarker (sclerostin) in the presence of a therapeutic antibody to that biomarker (blosozumab). The assay employed a capture-elution format to measure the total sclerostin concentration in the serum, consisting of the combined total of free sclerostin and sclerostin bound to blosozumab. The assay had a lower limit of quantitation of 57 pg/mL and was fully tolerant to as much as 500 mcg/mL of blosozumab. Precision was assessed during validation using three levels of quality control (QC) material (2.24 ng/mL, 9.33 ng/mL, and 16.68 ng/mL). Intra-assay coefficients of variation (CV) ranged from 4.1% to 5.5%, whereas interassay CVs ranged from 10.3% to 20.7% (data on file, Eli Lilly and Company). QC samples were routinely run in duplicate on every plate each time an assay was performed.

**Immunogenicity**

Patient serum samples for immunogenicity screening and neutralization assay were assayed in separate assays validated according to industry recommendations. Using rabbit anti-human IgG control antibody, the screening assay demonstrated sensitivity of at least 7.8 ng/mL. With 500 ng/mL of control antibody, the assay was able to generate a positive result even in the presence of up to 200 mcg/mL of blosozumab.

**Statistical analyses**

Data obtained during the follow-up period were analyzed according to a prespecified statistical analysis plan, using methods previously reported. Efficacy analyses were conducted using a mixed-effects repeated measures model, with treatment group comparisons based on least squares means of change or percent change from study baseline at each study visit. Factors in the model included treatment, time, treatment-by-time interaction, and the study baseline as a covariate. The percent change from baseline for BMD was analyzed by Wilcoxon rank sum test for each time point. The proportion of patients experiencing adverse events in the study follow-up period was compared among all treatment groups using Fisher’s exact test. Fractures were collected as adverse events; fractures of the fingers, toes, and teeth are not reported. Blinding was maintained through the follow-up period. All statistical analyses were conducted using SAS version 9 software (SAS Institute, Cary, NC, USA) or later, at a two-sided alpha level of 0.05.

**Access to material and methods**

The active treatment in this study, blosozumab, a humanized monoclonal antibody targeted to sclerostin, is regulated and restricted by the United States Food and Drug Administration.
Results

Patient recruitment and enrollment for this clinical trial began August 1, 2010, and ended February 6, 2011. Patients were treated between September 8, 2010, and February 25, 2011. The follow-up period was August 23, 2011, through February 22, 2013. The last patient visit, inclusive of the follow-up period, was February 22, 2013.

A total of 106 patients completed the study treatment period and entered the combined follow-up period. Of these patients, 88 completed the 52-week follow-up (Fig. 2). Baseline characteristics for the patient population entering the combined follow-up period are summarized in Table 1. At entry to the combined follow-up period, treatment groups were balanced with regard to age, race, ethnicity, and body mass index; however, there was an imbalance among treatment groups in lumbar spine and femoral neck BMD, reflecting dose-dependent increases in BMD from 1 year of blosozumab treatment.

Once study treatment had been discontinued and patients entered the follow-up period, the study protocol permitted patients to receive prescribed osteoporosis medication at the discretion of the clinical investigator or the patient’s physician. Only one patient was prescribed another osteoporosis medication, risedronate, with treatment beginning 2 months before the end of follow-up. This patient had been randomized to blosozumab 180 mg Q4W during the study treatment period. Hence, the overall results obtained during the combined 52-week follow-up and reported here are reflective of treatment exposure during the phase 2 clinical trial.

BMD

In these women, 1 year of blosozumab treatment increased BMD from baseline up to 17.7% at the lumbar spine, 6.7% at the total hip, and 6.3% at the femoral neck in the highest blosozumab dose group, blosozumab 270 mg Q2W. With treatment discontinuation, a decline in BMD was observed in all blosozumab treatment groups, which continued through the 1-year follow-up period (Fig. 3).

At the end of follow-up, 1 year after the last dose of blosozumab, lumbar spine BMD remained significantly greater compared with placebo in the group receiving prior blosozumab 270 mg Q2W. At the end of follow-up, BMD increase at the lumbar spine was 6.9% from baseline and 8.2% higher than placebo ($p < 0.001$). At the total hip, BMD remained significantly greater than placebo at the end of follow-up for this group, with a 3.9% increase from baseline and 5.2% increase compared with placebo ($p < 0.001$). For this group, BMD gains at the femoral neck remained statistically significant compared with placebo throughout the follow-up period, and, at the end of follow-up, were 5.3% above baseline and 4.6% higher than placebo ($p < 0.001$).

In the prior blosozumab 180 mg Q2W treatment group, BMD gains at the end of follow-up were 3.6% greater than baseline at the lumbar spine and 4.9% greater than placebo ($p = 0.002$). At the total hip, this group had a 2.6% increase from baseline at the end of follow-up, which was 3.9% greater than placebo ($p < 0.001$). At the femoral neck, a 2.7% increase from baseline and 2.0% increase versus placebo were noted at the end of follow-up.

For the prior blosozumab 180 mg Q4W group, BMD gains at the end of follow-up were 1.6% greater than baseline at the lumbar spine or 2.9% higher than placebo ($p = 0.096$). At the total hip, the increase in BMD was 0.08% greater than baseline or 1.4% greater than placebo. This group had a BMD increase from baseline of 1.8% at the femoral neck at the end of follow-up, which was a 1.1% increase compared with placebo.

Biochemical markers of bone formation and resorption

Serial serum measurements of biochemical markers of bone formation (procollagen type-1 N propeptide [P1NP], osteocalcin, and bone-specific alkaline phosphatase [BSAP]) and a marker of bone resorption (CTX) were obtained throughout the follow-up period (Fig. 4). Median concentrations of markers of bone formation and bone resorption were little changed for the first 3 months after stopping study treatment. Serum concentrations of markers of bone formation returned to baseline during the

Table 1. Patient Characteristics at Entry to Combined Follow-up

|                          | Placebo (n = 26) | Blosozumab 180 mg Q4W (n = 29) | Blosozumab 180 mg Q2W (n = 25) | Blosozumab 270 mg Q2W (n = 25) | p Value |
|--------------------------|-----------------|--------------------------------|--------------------------------|--------------------------------|---------|
| Age (years), mean (SD)   | 65.7 (9.4)      | 67.0 (8.8)                     | 64.3 (8.3)                     | 66.6 (7.8)                     | 0.650   |
| Race, n (%)              |                 |                                |                                |                                | 0.963   |
| White                    | 15 (57.7)       | 14 (53.8)                      | 17 (58.6)                      | 15 (60.0)                      |         |
| Black                    | 1 (3.8)         | 0 (0.0)                        | 0 (0.0)                        | 0 (0.0)                        |         |
| Japanese                 | 10 (38.5)       | 12 (46.2)                      | 12 (41.4)                      | 10 (40.0)                      |         |
| BMI (kg/m²), mean (SD)   | 24.0 (5.4)      | 23.3 (3.5)                     | 24.1 (3.9)                     | 25.4 (5.1)                     | 0.426   |
| Lumbar spine T-score, mean (SD) | -2.85 (0.55) | -2.17 (0.58)                  | -1.76 (0.56)                   | -1.4 (0.63)                    | <0.001  |
| Femoral neck T-score, mean (SD) | -2.0 (0.94) | -1.96 (0.73)                  | -1.90 (0.88)                   | -1.56 (0.49)                   | 0.180   |
| Total hip T-score, mean (SD) | -1.86 (0.95) | -1.59 (0.77)                  | -1.49 (0.93)                   | -1.09 (0.61)                   | 0.014   |

Q4W = every 4 weeks; Q2W = every 2 weeks; SD = standard deviation; BMI = body mass index.
follow-up period. Serum concentration of CTx increased above baseline by the end of the follow-up period and was significantly greater than placebo for the patients who had received blosozumab 180 mg Q4W and blosozumab 180 mg Q2W as study treatment ($p < 0.05$). However, interpreting this statistical difference must consider the substantial variability in CTx concentration observed in the placebo group. Median serum concentrations of osteocalcin and bone-specific alkaline phosphatase were not significantly different from placebo, except for osteocalcin in the blosozumab 180 mg Q4W group at 104 weeks.

Total sclerostin serum concentration
Total sclerostin (bound and free) concentrations were balanced across the treatment groups at baseline before treatment. Treatment with blosozumab resulted in an increase in total sclerostin concentration above baseline beginning at week 1 (Fig. 5). A peak increase in total sclerostin concentration occurred at week 4 for the blosozumab 180 mg Q2W group and at week 52 for the blosozumab 270 mg Q2W group. Total sclerostin concentrations generally remained elevated above baseline for the duration of blosozumab treatment. The higher blosozumab dose and more frequent blosozumab dosing regimens were associated with higher magnitude of sclerostin elevation. The largest mean increase in serum total sclerostin concentration from baseline to the end of treatment was observed in the blosozumab 270 mg Q2W treatment group. At the end of the treatment period, total sclerostin concentrations decreased quickly for all blosozumab treatment groups, with concentrations nearing baseline within 4 weeks of discontinuation for the blosozumab 180 mg treatment groups and within 12 weeks of discontinuation of treatment for the blosozumab 270 mg Q2W group. Total sclerostin concentrations remained near baseline throughout the follow-up period for study patients.

Safety
There were no patient deaths throughout the course of this clinical trial. Treatment-emergent adverse events and serious adverse events observed during the treatment phase have previously been reported.$^{(15)}$ During the follow-up period, 5 patients experienced a total of 6 serious adverse events, which were judged unrelated to prior treatment. These serious adverse events included cerebral infarction and dehydration in 1 patient (patient had received placebo); pneumonia in 1 patient (patient

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**Fig. 3.** Least squares mean percent change from baseline in BMD at the (A) lumbar spine, (B) total hip, and (C) femoral neck. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. LS = least squares mean; SE = standard error.
had received blosozumab 180 mg Q2W), retinal artery occlusion in 1 patient (patient had received blosozumab 270 mg Q2W), and breast cancer in 2 patients (patients were randomized 1 each to the blosozumab 180 mg Q4W and blosozumab 180 mg Q2W treatment groups). The two cases of breast cancer detected during follow-up have been previously reported.\(^\text{(15)}\) During the follow-up period, a fracture was reported by 3 patients who had been randomized to the blosozumab 180 mg Q4W group (rib, humerus, elbow). There was no statistical difference in serious adverse events observed among the prior treatment groups. Overall, adverse events during the follow-up period were similar among the prior treatment and placebo groups and did not appear related to previous blosozumab exposure.

**Immunogenicity**

A total of 32 patients developed anti-blosozumab antibodies after blosozumab exposure in this trial.\(^\text{(15)}\) Twelve patients had anti-drug antibody (ADA) detected for the first time during the follow-up period (sampled from week 64 through week 104). Twenty patients had ADA detected during the treatment phase, and 17 of those finished the treatment and had ADA samples collected in the follow-up period. By their last sampling event during follow-up, ADA from 10 of the 17 patients had turned negative, 5 still had positive ADA but experienced a decline in the ADA titers, and 2 remained ADA positive and experienced a stabilization of their titer. The ADA titers were generally low, in the range of 1:5 to 1:320. Overall, with the exception of one patient further described below, the anti-blosozumab antibodies did not appear to impact BMD. No adverse effect was found to be associated with the development of ADA in any patient during the follow-up period.

One patient with high ADA demonstrated reduced blosozumab exposure and efficacy, as previously reported.\(^\text{(15)}\) At the time when the anti-blosozumab antibodies were detected, the antibodies were found in this patient to be neutralizing, and the

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**Fig. 4.** Median percent change from baseline in serum concentrations of biochemical markers of bone metabolism. (A) Median percent change from baseline in serum PINP. The median percent change from baseline for serum PINP concentration was statistically significant at \(p < 0.001\) compared with placebo for all blosozumab treatment groups for weeks 1, 2, and 4. ***\(p < 0.001\); **\(p < 0.01\); *\(p < 0.05\). IQR = interquartile range. (B) Median percent change from baseline in serum osteocalcin concentration. The median percent change from baseline for serum osteocalcin concentration was statistically significant at \(p < 0.05\) versus placebo for all blosozumab treatment groups at week 1 and statistically significant at \(p < 0.001\) versus placebo for all blosozumab treatment groups at weeks 2 and 4. ***\(p < 0.001\); **\(p < 0.01\); *\(p < 0.05\). (C) Median percent change from baseline in serum bone-specific alkaline phosphatase concentration. The median percent change from baseline for serum bone-specific alkaline phosphatase concentration was statistically significant at \(p < 0.05\) versus placebo for the blosozumab 180 mg Q4W treatment group at week 1, and \(p < 0.001\) versus placebo for the blosozumab 180 mg Q2W and 270 mg Q2W treatment groups at week 1. At weeks 2 and 4, the median percent change from baseline for all blosozumab treatment groups was statistically significant at \(p < 0.001\) versus placebo. ***\(p < 0.001\); **\(p < 0.01\); *\(p < 0.05\). (D) Median percent change from baseline in serum C-terminal telopeptide (CTX) concentration. The median percent change from baseline for serum CTx concentration was statistically significant at \(p < 0.001\) compared with placebo for all blosozumab treatment groups at weeks 1 and 2. At week 4, the blosozumab 270 mg Q2W treatment group was statistically significant at \(p < 0.01\) versus placebo. ***\(p < 0.001\); **\(p < 0.01\); *\(p < 0.05\).
patient’s serum total sclerostin concentration had decreased to approximately one-fifth of its peak level. This patient’s total sclerostin concentration continued to decline toward baseline by the end of treatment. At the end of treatment, the patient’s antibody titers reached their maximum, which declined thereafter, but were still detected as neutralizing throughout the follow-up period. By the end of the follow-up period, the ADA titer had decreased by 32-fold from its maximal level. For this patient, there were no adverse effects associated with the presence of high titers of ADA.

Discussion

We have previously reported that administration of blosozumab every 2 weeks or every 4 weeks for 1 year results in significant increases in lumbar spine and hip BMD and total bone mineral content (BMC) in postmenopausal women with low BMD. Here, we report a decline in BMD after discontinuation of blosozumab. Yet, 1 year after study treatment was stopped, lumbar spine and hip BMD remained significantly higher than placebo and baseline in the groups treated every 2 weeks with 180 mg or 270 mg of blosozumab. BMD at the hip appeared to decline more gradually than at the lumbar spine, consistent with compartment-specific differences in response to treatment withdrawal.

During 1 year of treatment, median concentrations of the bone formation marker P1NP increased and then returned to baseline, and remained so during 52 weeks off treatment. After discontinuation of blosozumab, CTx concentration reflected a modest change in overall bone resorption without obvious rebound, as has been observed after discontinuation of some osteoporosis therapies. Serum samples measuring biochemical markers of bone metabolism were drawn in the fasting state, and patients generally had sampling at the same time of day, with most occurring in the morning. Diurnal variation of 40% to 66% has been reported with measures of CTx serum concentration and may lend to the variability noted with measures of CTx in this study. Overall, in the present study, with discontinuation of blosozumab, the measures of biochemical markers of bone metabolism, which reflect an integrated effect across the skeleton, show a slight imbalance favoring resorption, but may demonstrate that the overall bone mass gain with blosozumab treatment is better maintained across the total skeleton than in the spinal vertebrae. The observed pattern of changes in bone markers may signal resetting of bone mechanosensitivity.

Because of the overall pattern of response, evaluation of sequential antiresorptive therapy after treatment with blosozumab may be warranted.

After repeated blosozumab treatment, dose-dependent increases in total sclerostin levels (bound and free) were observed, which gradually returned to pretreatment levels after treatment discontinuation. Free sclerostin, the target of interest, could not be directly measured in our study because the circulating concentration of free sclerostin is extremely low in the presence of blosozumab. However, others have described a pattern of target antigen increase similar to what we observed, likely composed primarily of antibody-target complexes, and secondary to the relatively long half-lives of monoclonal antibodies and their high affinity and specificity to the target. This results in a sharp and dramatic decrease of the free target concentration during antibody treatment. Hence, it is likely that the temporal profiles of total sclerostin observed in the present study

![Fig. 5. Serum total sclerostin concentrations as measured by blosozumab-tolerant sclerostin immunoassay. Serum total sclerostin concentrations (bound and free) were measured in a blosozumab-tolerant sclerostin assay as described in Materials and Methods. Total sclerostin (bound and free) concentrations were balanced across the treatment groups at baseline before treatment. Treatment with blosozumab resulted in an increase in total sclerostin concentration above baseline beginning at week 1. A peak increase in total sclerostin concentration occurred at week 4 for the blosozumab 180 mg Q2W group and at week 52 for the blosozumab 270 mg Q2W group. Total sclerostin concentrations generally remained elevated above baseline for the duration of blosozumab treatment, representing target engagement. Higher blosozumab dose and more frequent blosozumab dosing regimens resulted in greater increases of total sclerostin elevation. Total sclerostin concentrations quickly returned to baseline post-treatment. SE = standard error.](image-url)
are indicative of sustained and dose-dependent target engagement during the dosing period. Further study is needed to determine the clinical impact of serum sclerostin concentration and the usefulness of monitoring serum sclerostin concentration as a potential biomarker of blosozumab treatment.

There were no observed adverse events attributed to prior blosozumab exposure in the study population during follow-up. ADA levels tended to decline in patients who had detectable levels during treatment and were not associated with any clinical or laboratory measures of concern during post-treatment follow-up, consistent with previous reports. In conclusion, discontinuing treatment with blosozumab resulted in a decline of BMD gained during treatment but little change in markers of bone formation and resorption. Based on the magnitude of increase in BMD during treatment and the rate of decline after stopping treatment, the BMD at the spine and hip remained significantly increased from baseline at 1 year post-treatment. Although it is possible that BMD might return to pretreatment levels over a longer duration of time, a prolonged positive effect on BMD after stopping treatment with blosozumab may be clinically advantageous. Further study is needed to determine optimal duration of blosozumab treatment. There were no significant adverse effects attributed to blosozumab exposure during the 1-year treatment and 1-year follow-up periods. Together, these results support continued study of blosozumab as an anabolic therapy for osteoporosis.

Disclosures

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Authors’ roles: Study design: CB, DR, AC, JA, LH, HS, JS, RK, BM, and AS. Study conduct: CR, RR, CB, DR, AC, JA, LH, TM, HS, BM, and AS. Data collection: CR, RR, LH, JS, and RK. Data analysis: AC, JA, LH, JS, and RK. Data interpretation: CR, RR, CB, DR, AC, JA, LH, TM, HS, JS, RK, BM, and AS. Drafting manuscript: CR, RR, CB, DR, AC, JA, LH, TM, HS, JS, RK, BM, and AS. Revising manuscript content: CR, RR, CB, DR, AC, JA, LH, TM, HS, JS, RK, BM, and AS. Approving final version of manuscript: CR, RR, CB, DR, AC, JA, LH, TM, HS, JS, RK, BM, and AS. AC takes responsibility for the integrity of the data analysis.

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