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Loss of bronchoprotection with ICS plus LABA treatment, β-receptor dynamics, and the effect of alendronate

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Abstract

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Trial registration: Clinicaltrials.gov identifier .

Background: Loss of bronchoprotection (LOBP) with a regularly used long-acting β_2-adrenergic receptor agonist (LABA) is well documented. LOBP has been attributed to β_2-adrenergic receptor (B2AR) downregulation, a process requiring farnesylation, which is inhibited by alendronate.

Objective: We sought to determine whether alendronate can reduce LABA-associated LOBP in inhaled corticosteroid (ICS)–treated patients.

Methods: We conducted a randomized, double-blind, placebo-controlled, parallel-design, proof-of-concept trial. Seventy-eight participants with persistent asthma receiving 250 μg of fluticasone twice daily for 2 weeks were randomized to receive alendronate or placebo while initiating salmeterol for 8 weeks. Salmeterol-protected methacholine challenges (SPMChs) and PBMC B2AR numbers (radioligand binding assay) and signaling (cyclic AMP ELISA) were assessed before randomization and after 8 weeks of ICS plus LABA treatment. LOBP was defined as a more than 1 doubling dose reduction in SPMCh PC_{20} value.

Results: The mean doubling dose reduction in SPMCh PC_{20} value was 0.50 and 0.27 with alendronate and placebo, respectively ($P = .62$). Thirty-eight percent of participants receiving alendronate and 33% receiving placebo had LOBP ($P = .81$). The after/before ICS plus LABA treatment ratio of B2AR number was 1.0 for alendronate ($P = .86$) and 0.8 for placebo ($P = .15$; $P = .31$ for difference between treatments). The B2AR signaling ratio was 0.89 for alendronate ($P = .43$) and 1.02 for placebo ($P = .84$; $P = .44$ for difference). Changes in lung function and B2AR number and signaling were similar between those who did and did not experience LOBP.

Conclusion: This study did not find evidence that alendronate reduces LABA-associated LOBP, which relates to the occurrence of LOBP in only one third of participants. LOBP appears to be less common than presumed in concomitant ICS plus LABA-treated asthmatic patients. B2AR downregulation measured in PBMCs does not appear to reflect LOBP.

Keywords

β_2-Adrenergic receptor; bronchoprotection; downregulation; bisphosphonate; loss of bronchoprotection; controller therapy; salmeterol; β_2-agonists

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β_2-Adrenergic receptor agonists (BAs) engage their receptor and stimulate a signaling pathway that results in smooth muscle relaxation, thereby reducing bronchial airway narrowing and protecting against bronchoconstrictors. Furthermore, loss of bronchoprotection (LOBP) is the reduction in the ability of BAs to protect against airway narrowing in response to bronchoconstrictors. LOBP occurs with regular BA use, both short-acting β_2-adrenergic receptor agonists and long-acting β_2-adrenergic receptor agonists (LABAs),[1–5] and is reflected in reactivity to “direct” bronchoconstrictors, such as methacholine[4] and histamine,[6] and “indirect” provocation, such as exercise[7] and allergen.[8]

International asthma guidelines recommend adding a LABA when inhaled corticosteroids (ICSs) inadequately control symptoms,[9] but 58% to 81% of asthmatic patients do not achieve optimal control with this strategy.[10] LOBP could be partially responsible for the incomplete effectiveness observed when LABAs are concomitantly used with ICSs.[11] Therefore medications that prevent LOBP could improve control in such patients.

The mechanism responsible for LOBP is unknown but might relate to β_2-adrenergic receptor (B2AR) downregulation through internalization,[12] B2AR phosphorylation by G protein–coupled receptor (GPCR) kinases,[13] and/or uncoupling from the adenylyl cyclase-mediated signaling pathway,[14] among other mechanisms.[15] Corticosteroids were initially hypothesized to preserve bronchoprotection based on *in vitro* studies demonstrating their ability to increase B2AR expression[16,17] but were later found in most *in vivo* studies to be unable to preserve bronchoprotection.[18–23]

Jiang et al[24] recently demonstrated a critical role for farnesyl diphosphate synthase in B2AR internalization. Farnesylation is required for translocation of the small GTPase Rab5 to the plasma membrane,[25] where it is required for BA-induced B2AR endocytosis.[26] Nitrogen-containing bisphosphonates, like alendronate, are specific inhibitors of farnesyl diphosphate synthase.[27,28] In human airway smooth muscle cell–based assays this group also showed that alendronate prevents both BA-induced internalization and loss of functional activation.[24] Furthermore, preliminary data on human lung slices suggest that alendronate preserves bronchoprotection against acetylcholine after long-term BA exposure (Rajendran, unpublished data).

We hypothesized that alendronate would reduce the LOBP that occurs with regularly administered LABAs despite concomitantly used ICSs. Therefore we conducted a randomized, controlled, proof-of-concept trial (Alendronate for Asthma [ALfA] trial) to evaluate changes in bronchoprotection after alendronate use measured with salmeterol-protected methacholine challenge (SPMCh) in participants with persistent, ICS-treated asthma for whom LABA treatment was added. We sought to identify the mechanism responsible for LOBP by quantifying B2AR cell-surface density and signaling in samples obtained from participants before and after exposure to regularly administered ICS plus LABA treatment. Additionally, because we previously showed that high fraction of exhaled nitric oxide (FENO) levels predict LOBP in ICS-naive patients,[7] we explored FENO's role in predict-ing LOBP in ICS plus LABA–treated asthmatic patients. Additionally, because salivary α-amylase (sAA) levels are B2AR regulated[29,30] and based on our preliminary data indicating that sAA increases acutely with salmeterol exposure (Moy, unpublished data), we also explored sAA as a potential biomarker for B2AR dynamics.

METHODS

Participants

Eligible participants (1) were 18 years or older, (2) had physician-diagnosed asthma, (3) had evidence of either bronchodilator reversibility (postbronchodilator FEV_1 ≥12%) or airway hyperresponsiveness (PC_{20} ≤8 mg/mL), (4) had a percent predicted FEV_1 of 50% or greater and FEV_1 of 1 L or greater, and (5) were taking stable ICS controller monotherapy for 4 or more weeks. The ALfA study protocol (Clinicaltrials.gov) was approved by the institutional review board at all participating institutions. All participants provided written informed consent. A data and safety monitoring board monitored the study. The full study protocol and additional details appear in this article's Online Repository at www.jacionline.org.

Study design and treatment

This was a 10-week, randomized, double-blind, placebo-controlled, parallel-arm trial. Participants with persistent asthma were treated with 250 μg of fluticasone propionate twice daily during a 2-week run-in period and then randomized to receive either 10 mg/d oral alendronate or placebo with 250 μg of fluticasone propionate and 50 μg of salmeterol in a combination Diskus device twice daily for 8 weeks during the treatment phase (Fig 1). Eligible participants were recruited from the community from January 2015 to May 2016 at 9 US sites from the National Heart, Lung, and Blood Institute's AsthmaNet research network.

Prior asthma treatments (including short-acting β_2-adrenergic receptor agonists) were discontinued to prevent potential confounding on B2AR dynamics. Participants used 17 μg per puff of ipratropium as a primary rescue inhaler during the study. SPMCh was used to test LOBP, as previously described,[4,22,23,31] with participants receiving 2 puffs of open-label fluticasone/salmeterol (115/21 μg) 1 hour before starting methacholine challenge. SPMCh PC_{20} values were measured at randomization and after 8 weeks of treatment. Participants were excluded if their SPMCh value was 16 mg/mL or greater at randomization (Fig 2).

Mechanistic procedures

Peripheral blood was collected at randomization and after 8 weeks of treatment, and biochemical assays were conducted on PBMCs to determine B2AR cell-surface density (radioligand binding assay; see Appendix E1 in this article's Online Repository) and signaling (BA-induced cyclic AMP [cAMP]; ELISA according to the manufacturer's instructions from Applied Biosystems, Foster City, Calif; see also additional details on cAMP ELISA in the ALfA protocol [Appendix E2] in this article's Online Repository). We determined sAA levels (Salimetrics, Carlsbad, Calif) from saliva samples before and 1 hour after salmeterol administration during these 2 study visits. F_{ENO} values were measured at both visits.

Outcome measures

The primary outcome was change in SPMCh PC_{20} value after 8 weeks of treatment expressed as logarithm base 2[4,18,23,31]; we defined LOBP as a doubling dose reduction in SPMCh PC_{20} value of greater than 1, no LOBP as no reduction in LOBP, and an indeterminate response as a doubling dose reduction of greater than 0 but less than 1. The 2 prespecified secondary outcomes were change in PBMC B2AR cell-surface density and B2AR signaling. Exploratory outcomes included the change in magnitude of acute salmeterol-induced sAA increases. We also explored whether F_{ENO} values predicted LOBP and whether alendronate improved asthma control using the Asthma Control Test (ACT).

Statistical analysis

Participants were randomized 1:1 to the alendronate and placebo groups. The only stratification factor was clinical partnership (8 levels), with permuted blocks of size 4 per stratum. Descriptive analyses were performed by using the *t* test or Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. The primary outcome for statistical analysis was the change in logarithm base 2 of the SPMCh PC_{20}

value.[32] The primary comparison was the visit 2 to visit 3 mean change in primary outcome for the alendronate versus placebo groups. We applied a blocked/stratified ANOVA to compare the alendronate and placebo groups, where blocks/strata consist of our 8 partnerships (representing 9 clinical sites). A similar analysis was performed to compare the LOBP and no LOBP groups. Logistic regression with adjustment for partnership was used to calculate the odds of LOBP for the alendronate versus placebo groups. Because of small sample sizes for the LOBP outcome, we pooled some of the partnerships. Finally, we estimated Pearson correlation coefficients to investigate linear relationships within pairs of variables. All analyses followed the intention-to-treat paradigm. We conducted a set of secondary analyses per protocol. We assumed missingness at random for all of the statistical analyses. We assumed that placebo-treated participants would experience an average of a 1.1 SPMCh PC_{20} doubling dose decrease with regular fluticasone/salmeterol treatment,[15] and we powered this study to detect at least a 50% decrease in alendronate-treated participants (approximately 0.55 doubling dose decrease in LOBP). Thus the effect size for the primary outcome was a difference of 0.55 on the logarithm base 2 scale. A total sample size of 76 participants would achieve 80% statistical power with a 2-sided .05 significance level test to detect this effect size, allowing for a 10% dropout rate.

RESULTS

We enrolled a total of 137 participants and randomized 78 (38 to the alendronate [34 completions] and 40 to the placebo [39 completions] groups, Fig 2). There was 1 medication-related adverse event per group. There were no significant differences in demographic, baseline clinical, or spirometric characteristics between treatment groups (*t* test, Wilcoxon rank sum test, or Fisher exact test, as appropriate; Table I).

Participants in both treatment groups experienced a statistically significant prebronchodilator FEV_1 increase during the 8-week treatment phase with addition of a LABA to an ICS. The percentage FEV_1 increase between visit 2 (randomization) and visit 3 (end of study) was 6.0% and 8.3% for the alendronate and placebo groups, respectively, but these increases were similar between groups ($P = .50$, blocked ANOVA; see Table E1 in this article's Online Repository at www.jacionline.org).

Effects on SPMCh PC_{20} values

As seen in Fig 3, the visit 2 to visit 3 mean doubling dose change in SPMCh PC_{20} value for participants receiving alendronate was −0.50(95% CI, −1.27 to 0.26; $P = .19$) and that for participants receiving placebo was −0.27 (95% CI, −0.78 to 0.24; $P = .29$, blocked ANOVA). The doubling dose decrease in SPMCh PC_{20} values between the alendronate and placebo groups was similar ($P = .62$, blocked ANOVA). Thirty-eight percent of participants receiving alendronate and 33% receiving placebo had LOBP (odds ratio, 1.2; 95% CI, 0.4-3.4; $P = .70$, logistic regression).

β-Receptor density and functional assays

We hypothesized that PBMC B2AR cell-surface density would decrease in participants regularly receiving ICS plus LABA treatment and that alendronate would attenuate this

decrease. However, the visit 2 to visit 3 mean B2AR cell-surface density did not significantly change in either group (visit 3/visit 2 ratio, 1.0 [95% CI, 0.8-1.2; $P = .86$] for the alendronate group; visit 3/visit 2 ratio, 0.8 [95% CI, 0.6-1.1; $P = .15$] for the placebo group [blocked ANOVA]). In addition, there was no difference in the visit 2 to visit 3 change in B2AR cell-surface density between the alendronate and placebo groups ($P = .31$, blocked ANOVA). Changes in B2AR cell-surface density during the treatment phase did not correlate with changes in SPMCh PC_{20} values (Pearson correlation, Fig 4). Also, the visit 2 to visit 3 change in PBMC B2AR cell-surface receptor density was not significantly different between participants with LOBP (defined as change in SPMCh $PC_{20} \leq -1$ doubling dose) and those without (defined as change in SPMCh $PC_{20} > 0$; Table II, blocked ANOVA).

Similarly, we hypothesized that PBMC B2AR signaling would decrease in participants regularly receiving ICS plus LABA treatment and that alendronate would attenuate this decrease. However, the visit 2 to visit 3 geometric mean in B2AR signaling did not significantly change in either group (visit 3/visit 2 ratio, 0.9 [95% CI, 0.7-1.2; $P = .43$] for the alendronate group; visit 3/visit 2 ratio, 1.0 [95% CI, 0.8-1.3; $P = .84$] for the placebo group [blocked ANOVA]). There was no difference in the visit 2 to visit 3 change in B2AR signaling between the alendronate and placebo groups ($P = .44$, blocked ANOVA). Changes in B2AR signaling during the treatment phase did not correlate with changes in SPMCh PC_{20} values (Pearson correlation, Fig 5). Also, the visit 2 to visit 3 change in PBMC B2AR signaling was similar between participants with and without LOBP (blocked ANOVA, Table II).

Effect of alendronate on sAA levels

sAA levels increased 1 hour after salmeterol administration, with a mean 1.9-fold increase (95% CI, 1.7-fold to 2.1-fold; $P < .001$) for all participants at visit 2 (blocked ANOVA). The placebo group did not exhibit a reduction in acute salmeterol-induced sAA increases (visit 3/visit 2 ratio, 0.9 [95% CI, 0.8-1.1; $P = .54$]). Although the alendronate group did show such a reduction (visit 3/visit 2 ratio, 0.8 [95% CI, 0.6-0.9; $P < .01$]), the visit 2 to visit 3 change in acute salmeterol-induced sAA level was not significantly different between treatment groups ($P = .09$, blocked ANOVA). Also, visit 2 to visit 3 changes in acute salmeterol-induced sAA levels did not correlate with changes in SPMCh PC_{20} values ($P = .80$, Pearson correlation, see Fig E1 in this article's Online Repository at www.jacionline.org). Participants without LOBP did not exhibit a reduction in acute salmeterol-induced increases in sAA levels (visit 3/visit 2 ratio, 0.9 [95% CI, 0.7-1.1; $P = .34$]). Although participants with LOBP did exhibit such a reduction (visit 3/visit 2 ratio, 0.8 [95% CI, 0.6-1.0; $P = .06$]), the visit 2 to visit 3 change in acute salmeterol-induced sAA level was similar between participants with and without LOBP (blocked ANOVA, see Table E2 in this article's Online Repository at www.jacionline.org).

Predictive value of F_{ENO} and effect of alendronate on asthma control

Baseline F_{ENO} values were similar between participants receiving alendronate (median F_{ENO}, 15 ppb) and those receiving placebo (median F_{ENO}, 17 ppb; $P = .57$, Wilcoxon rank sum test). F_{ENO} values did not predict LOBP in either group or in the groups combined (Pearson correlation, see Fig E2 in this article's Online Repository at www.jacionline.org).

Participants treated with alendronate did not experience a greater improvement in ACT scores relative to those assigned to the placebo group ($P = .14$, blocked ANOVA; see Fig E3 in this article's Online Repository at www.jacionline.org).

Characterization of participants who had LOBP with regular ICS plus LABA treatment

As mentioned above, visit 2 to visit 3 changes in PBMC B2AR cell-surface receptor density and signaling were similar between participants with and without LOBP (blocked ANOVA, Table II). We also found that participants with LOBP were not different from those who did not have LOBP in terms of pretreatment phase demographics (age, sex, and race), clinical features (body mass index and ACT score), lung function (FEV_1, bronchodilator response to albuterol, and airway responsiveness [MCh PC_{20}]), and biochemical features (FENO and sAA, t-test, Wilcoxon rank sum test or Fisher exact test, as appropriate; Table III). However, participants with LOBP experienced a smaller FEV_1 increase compared with those without (3.4% and 7.4% predicted FEV_1 for the LOBP and no LOBP groups, respectively; $P = .06$) after 8 weeks of ICS plus LABA treatment (blocked ANOVA, see Fig E4 and Table E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

We designed the ALfA trial to examine whether prevention of B2AR downregulation by alendronate would preserve bronchoprotection. Based on prior data, we expected an average 1.1 doubling dose decrease in SPMCh PC_{20} values. Instead, we only observed a 0.37-point decrease, with only 36% of participants experiencing a greater than 1 doubling dose decrease in SPMCh PC_{20} values. Thus we were not able to assess whether alendronate could prevent LOBP. However, we have made new observations concerning the frequency of LOBP and, more importantly, its apparent independence from B2AR downregulation.

Although prior data had suggested that as many as 75% of BA-treated patients experience LOBP,[18–22] we found LOBP in just over one third of our participants. Yates et al[18] reported that in patients regularly administered salmeterol without a concomitant ICS (n = 14), the mean SPMCh PC_{20} value decrease was 1.4 doubling dose dilutions; when these same patients were studied with concomitant ICSs, the mean decrease was 1.1 doubling dose dilutions. In contrast, we found that the mean SPMCh PC_{20} value decrease in participants receiving alendronate was 0.51 doubling dose dilutions, and in those receiving placebo it was 0.27 (0.37 for the entire group). Few studies have reported regular LABA use–associated LOBP in terms of proportions or described individual participant-level data to calculate proportions. Cheung et al[3] reported that 75% of participants regularly administered salmeterol without a concomitant ICS (n = 12) who underwent albuterol-protected methacholine challenge had LOBP. Kalra et al[19] described that 75% of participants regularly administered salmeterol with a concomitant ICS (n = 8) had LOBP.

The less frequent development of LOBP in this trial might be caused by several possible reasons. First, this trial consists of one of the largest groups (as detailed above) that received salmeterol to assess LOBP in the setting of concomitantly administered ICSs. Prior results might have been influenced by smaller sample sizes and been underpowered. Considering our sample size, it is unlikely for us to have failed to detect LOBP. Indeed, the posterior

probability of failure to detect LOBP, if it were truly occurring, is equal to 1% (data not shown).

Second, although many prior studies reported LOBP in the context of LABA monotherapy,[2,4,5,8,31,33] all participants in this trial used LABAs concomitantly with ICSs and did so for a treatment period longer than that of most studies. ICSs confer bronchoprotection,[34] which might counter LABA-associated LOBP. Therefore more prolonged concomitant ICS administration is a potential reason why our study showed reduced levels of LOBP.

Furthermore, it is possible that the timing of ICS therapy initiation might have counteracted our ability to detect an LOBP effect. Incremental bronchoprotection is observed several months after ICS therapy initiation.[35] Although all our participants reported receiving ICSs for more than 1 month before enrollment, participants more recently started on ICSs can have experienced incremental bronchoprotective ICS effects during the study, countering the LABA-associated LOBP. We would not have been able to detect such an effect because all our participants received ICS plus salmeterol and not ICS plus placebo as a comparator group. Also, even in patients with long-term ICS use before enrollment, ICS adherence might have increased with study entry; this behavior might also have conferred greater bronchoprotection and countered LABA effects.[36] In fact, in our study participants experienced a mean 70-mL FEV_1 increase during the run-in period ($P = .047$, data not shown), which suggests that they either recently started ICS therapy or became more adherent during the run-in period. Indeed, those participants who experienced the largest FEV_1 increases and F_{ENO} value decreases (both known ICS effects) had a lower likelihood of LOBP, although this only trended toward statistical significance ($P = .06$ and $P = .08$, respectively). Thus future studies on LOBP might benefit from longer run-in periods with ICSs and a history of LABA-associated LOBP as an inclusion criterion. Conversely, it is unlikely that a longer treatment duration would have yielded different trial results because the onset of LABA-associated B2AR downregulation and LOBP are both relatively quick.[4,15]

The possibility that increased ICS adherence in a trial can confound the ability to detect LOBP is reinforced in a trial by FitzGerald et al.[23] They conducted a study comparing the effects on bronchoprotection of regular formoterol versus regular albuterol versus as-needed albuterol in asthmatic participants who reported treatment with ICSs for at least 1 month. Reading off their graphical data, they observed only a 0.28 doubling dose decrease in bronchoprotection with formoterol after 3 months of therapy. However, the group that received intermittent albuterol had a simultaneous 0.42 doubling dose increase in their PC_{20} values, suggesting that there was indeed an incremental bronchoprotective effect occurring.

More than one third of our participants had LOBP. Changes in B2AR cell-surface density and signaling were similar in participants with and without LOBP. These results suggest that in patients with persistent asthma regularly using ICS plus LABA treatment, B2AR cell-surface density and signaling (determined in PBMCs) do not account for LOBP. We speculate that other means of B2AR regulation independent from B2AR cell-surface density or signaling through the Gαs subunit might account for LOBP development because of regularly administered ICS plus LABA treatment. These additional mechanisms of B2AR

regulation, including β-arrestin–dependent signaling,[37] B2AR signaling downregulation by GPCR-related kinases,[38] and heterodimerization between B2AR and other GPCRs,[39,40] might have divergent downstream effects without necessarily affecting B2AR numbers or intracellular cAMP levels. Alternatively, although prior studies on the clinical effects of BAs have used PBMCs as a proxy for target organ B2AR expression,[41,42] it is possible that PBMCs might not adequately reflect downregulation on airway smooth muscle. Future *ex vivo* studies using PBMCs might help ascertain whether alendronate regulates PBMC B2AR levels at the blood concentrations expected in human subjects with oral administration of alendronate.

Although PBMCs might be more removed from the target organ, sAA production might be more directly affected. For the first time, we have shown that sAA levels increase significantly with acute LABA exposure. Although we did not find any correlation between sAA level reductions and other measures of B2AR dynamics or changes in PC_{20} values, this again might be due to not having elicited LOBP as expected with regular ICS plus LABA treatment in the majority of participants. However, both participants with and without LOBP experienced a visit 2 to visit 3 reduction in acute salmeterol-induced sAA increases, but this reduction was not different between those with and without LOBP. Whether the finding of increases in sAA values with acute LABA exposure becomes clinically relevant in outcomes other than LOBP remains to be determined and should be the focus of future studies.

We did not observe a difference in change in B2AR number between those who were treated with alendronate versus placebo. However, fewer than half of participants experienced a greater than 20% decrease in B2AR cell-surface density (data not shown), which reduced our power to detect a difference between treatment groups. It is possible that alendronate did not reach adequate peripheral blood levels because alendronate is quickly absorbed from blood and preferentially taken up by bone. However, *in vitro*[43,44] data show that alendronate inhibits farnesylation at drug concentrations (inhibitory concentration of 50%, 10-460 nmol/L) achieved in plasma with orally administered alendronate (157 nmol/L).[45] Furthermore, patients with breast cancer have been reported to experience fewer visceral metastases with orally administered bisphosphonates,[46] suggesting that alendronate is biologically active in nonosseous tissue at pharmacologic dosing. On the other hand, the alendronate doses used to prevent BA-induced B2AR endocytosis *in vitro* and to preserve bronchoprotection *ex vivo* were approximately 2-log fold greater (10-50 μmol/L) than those quantified in human pharmacokinetic studies (157 nmol/L). Therefore we cannot rule out that the lack of a pharmacodynamic effect in our study is caused by insufficient drug exposure. Conversely, human biological complexity often explains why clinical trials have negative results despite supportive data derived from reductionist approaches.

Although nearly one third of participants regularly administered ICS plus LABA treatment had clinically significant LOBP, we did not note a difference in asthma symptoms (ACT scores) or baseline characteristics between participants with and without LOBP. Although F_{ENO} values predicted LOBP in studies of ICS-naive patients,[7] we did not see this in our study. Because our participants were using ICSs before enrollment, which reduces F_{ENO} values, we might have blunted the ability to detect this signal. In fact, the mean baseline

FENO value in our study was 21.3 ppb compared with a mean FENO value of 53 ppb in the study in which baseline FENO values predicted LOBP.

Finally, clinical outcomes were similar between participants with and without LOBP (see Table E3 in this article's Online Repository at www.jacionline.org). These data suggest that LOBP might not relate to the inability of patients receiving ICS plus LABA treatment to achieve complete symptom resolution. Our findings also reinforce trial data reporting the lack of a difference in serious asthma-related adverse events between those receiving ICS plus LABA treatment versus those using ICSs alone,[47,48] which prompted the US Food and Drug Administration to remove the boxed warning of LABAs in combination with ICSs.

In summary, LABA-associated LOBP appears to occur in fewer asthmatic participants than expected when used concomitantly with ICSs and might occur as a result of mechanisms other than B2AR downregulation. Because of the reduced incidence of LOBP, we were unable to determine whether alendronate could preserve bronchoprotection. Whether LABA-associated LOBP occurs in settings in which increases in adherence with ICSs do not confound its detection needs to be confirmed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ACT	Asthma Control Test
ALfA	Alendronate for Asthma trial
BA	β_2-Adrenergic receptor agonist
B2AR	β_2-Adrenergic receptor
cAMP	Cyclic AMP
F_{ENO}	Fraction of exhaled nitric oxide
GPCR	G protein-coupled receptor
ICS	Inhaled corticosteroid
LABA	Long-acting (β_2-adrenergic receptor agonist
LOBP	Loss of bronchoprotection
sAA	Salivary α-amylase
SPMCh	Salmeterol-protected methacholine challenge

REFERENCES

1. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled beta-2 agonists in asthma. N Engl J Med 1992;327:1204–8. [PubMed: 1357551]
2. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. Lancet 1993; 342:833–7. [PubMed: 8104272]
3. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting beta-2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. N Engl J Med 1992;327: 1198–203. [PubMed: 1357550]
4. Bhagat R, Kalra S, Swystun VA, Cockcroft DW. Rapid onset of tolerence to the bronchoprotective effect of salmeterol. Chest 1995;108:1235–9. [PubMed: 7587422]
5. Yates DH, Sussman HS, Shaw MJ, Barnes PJ, Chung KF. Regular formoterol treatment in mild asthma: effect on bronchial responsiveness during and after treatment. Am J Respir Crit Care Med 1995;152:1170–4. [PubMed: 7551366]

6. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. Lancet 1988;1:554–8. [PubMed: 2894492]
7. Bonini M, Permaul P, Kulkarni T, Kazani S, Segal A, Sorkness CA, et al. Loss of salmeterol bronchoprotection against exercise in relation to ADRB2 Arg16Gly polymorphism and exhaled nitric oxide. Am J Respir Crit Care Med 2013;188:1407–12. [PubMed: 24228710]
8. Giannini D, Carletti A, Dente FL, Bacci E, Di Franco A, Vagaggini B, et al. Tolerance to the protective effect of salmeterol on allergen challenge. Chest 1996;110: 1452–7. [PubMed: 8989060]
9. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018 Available at: www.ginasthma.org Accessed April 12, 2019.
10. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJH, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The gaining optimal asthma control study. Am J Respir Crit Care Med 2004;170:836–44. [PubMed: 15256389]
11. Sears MR, Taylor DR. The β2-agonist controversy: observations, explanations and relationship to asthma epidemiology. Drug Saf 1994;11:259–83. [PubMed: 7848546]
12. Ferguson SS, Downey WE, Colapietro A-M, Barak LS, Ménard L, Caron MG. Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. Science 1996;271:363–6. [PubMed: 8553074]
13. Premont RT, Inglese J, Lefkowitz RJ. Protein kinases that phosphorylate activated G protein-coupled receptors. FASEB J 1995;9:175–82. [PubMed: 7781920]
14. Penn RB, Panettieri RA, Benovic JL. Mechanisms of acute desensitization of the beta2AR-adenylyl cyclase pathway in human airway smooth muscle. Am J Respir Cell Mol Biol 1998;19:338–48. [PubMed: 9698608]
15. Johnson M Molecular mechanisms of beta-2-adrenergic receptor function, response, and regulation. J Allergy Clin Immunol 2006;117:18–24. [PubMed: 16387578]
16. Mak JCW, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of a glucocorticoid on downregulation of pulmonary beta-2-adrenergic receptors in vivo. J Clin Investig 1995;96:99–106. [PubMed: 7615841]
17. Profita M, Gagliardo R, Di Giorgi R, Pompeo F, Gjomarkaj M, Nicolini G, et al. Biochemical interaction between effects of beclomethasone dipropionate and salbutamol or formoterol in sputum cells from mild to moderate asthmatics. Allergy 2005;60:323–9. [PubMed: 15679717]
18. Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled beta-2-agonist. Am J Respir Crit Care Med 1996;154:1603–7. [PubMed: 8970342]
19. Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. Chest 1996;109:953–6. [PubMed: 8635376]
20. Boulet LP, Cartier A, Milot J, Côté J, Malo JL, Laviolette M. Tolerance to the protective effects of salmeterol on methacholine-induced bronchoconstriction: influence of inhaled corticosteroids. Eur Respir J 1998;11:1091–7. [PubMed: 9648961]
21. Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled beta-2 agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. Am J Respir Crit Care Med 1995;152:1485–9. [PubMed: 7582281]
22. Aziz I, Tan KS, Hall IP, Devlin MM, Lipworth BJ. Subsensitivity to bronchoprotection against adenosine monophosphate challenge following regular once-daily formoterol. Eur Respir J 1998;12:580–4. [PubMed: 9762783]
23. FitzGerald JM, Chapman KR, Della Cioppa G, Stubbing D, Fairbarn MS, Till MD, et al. Sustained bronchoprotection, bronchodilatation, and symptom control during regular formoterol use in asthma of moderate or greater severity. The Canadian FO/OD1 Study Group. J Allergy Clin Immunol 1999;103:427–35. [PubMed: 10069876]
24. Jiang X, Pan H, Nabhan JF, Krishnan R, Koziol-White C, Panettieri R, et al. A novel EST-derived RNAi screen reveals a critical role for farnesyl diphosphate synthase in 2-adrenergic receptor internalization and down-regulation. FASEB J 2012; 26:1995–2007. [PubMed: 22278941]

25. Gomes AQ, Bassam RA, Ramalho JSGR, Barral DC, Hume AN, Seabra MC. Membrane targeting of Rab GTPases is influenced by the prenylation motif. Mol Biol Cell 2003;14:1882–9. [PubMed: 12802062]
26. Seachrist JL, Anborgh PH, Ferguson SSG. Beta-2-adrenergic receptor internalization, endosomal sorting, and plasma membrane recycling are regulated by Rab GTPases. J Biol Chem 2000;275:27221–8. [PubMed: 10854436]
27. Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ, et al. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci U S A 1999;96:133–8. [PubMed: 9874784]
28. Yang J, Zhu HH, Chen GP, Ye Y, Zhao CZ, Mou Y, Hu SJ. Inhibition of farnesyl pyrophosphate synthase attenuates angiotensin II-induced cardiac hypertrophy and fibrosis in vivo. Int J Biochem Cell Biol 2013;45:657–66. [PubMed: 23277274]
29. Ryberg M, Möller C, Ericson T. Saliva composition and caries development in asthmatic patients treated with beta 2-adrenoceptor agonists: a 4-year follow-up study. Scand J Dent Res 1991;99:212–8. [PubMed: 1871531]
30. Ryberg M, Johansson I. The effects of long-term treatment with salmeterol and salbutamol on the flow rate and composition of whole saliva in the rat. Arch Oral Biol 1995;40:187–91. [PubMed: 7541622]
31. Drotar DE, Davis EE, Cockcroft DW. Tolerance to the bronchoprotective effect of salmeterol 12 hours after starting twice daily treatment. Ann Allergy Asthma Immunol 1998;80:31–4. [PubMed: 9475563]
32. Mauger EA, Mauger DT, James E, Chinchilli VM, Israel E. Summarizing methacholine challenges in clinical research. Control Clin Trials 2001;22(suppl): 244S–51S. [PubMed: 11728628]
33. Haney S, Hancox RJ. Rapid onset of tolerance to beta-agonist bronchodilation. Respir Med 2005;99:566–71. [PubMed: 15823453]
34. Haahtela T, Jarvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, et al. Comparison of a beta-2 agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. N Engl J Med 1991;325:388–92. [PubMed: 2062329]
35. Van Essen-Zandvliet E, Hughes M, Waalkens H, Duiverman E, Pocock S, Kerrebijn K, et al. Effects of 22 months of treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness and symptoms in children with asthma. Am Rev Respir Dis 1992;146:547–54. [PubMed: 1355640]
36. Erin EM, Zacharasiewicz AS, Nicholson GC, Tan AJ, Neighbour H, Engelstätter R, et al. Rapid effect of inhaled ciclesonide in asthma: a randomized, placebo-controlled study. Chest 2008;134:740–5. [PubMed: 18403668]
37. Shenoy SK, Lefkowitz RJ. beta-Arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol Sci 2011;32:521–33. [PubMed: 21680031]
38. Lefkowitz RJ, Shenoy SK. Transduction of Receptor Signals by beta-arrestins. Science 2005;308:512–7. [PubMed: 15845844]
39. Mcgraw DW, Mihlbachler KA, Schwarb MR, Rahman FF, Small KM, Almoosa KF, et al. Airway smooth muscle prostaglandin-EP 1 receptors directly modulate β2-adrenergic receptors within a unique heterodimeric complex. J Clin Invest 2006;116:1400–9. [PubMed: 16670773]
40. Ferre S. Building a new conceptual framework for receptor heteromers. Nat Chem Biol 2009;5:131–4. [PubMed: 19219011]
41. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. Lancet 1995;346:201–6. [PubMed: 7616798]
42. Busse WW, Sharpe G, Smith A, Arbabian M, Borgen L, Ruoho A. The effect of procaterol treatment on beta-adrenergic bronchodilation and polymorphonuclear leukocyte responsiveness. Am Rev Respir Dis 1985;132:1194–8. [PubMed: 3000233]
43. Duque G, Vidal C, Rivas D. Protein isoprenylation regulates osteogenic differentiation of mesenchymal stem cells: effect of alendronate, and farnesyl and geranyl-geranyl transferase inhibitors. Br J Pharmacol 2011;162:1109–18. [PubMed: 21077849]

44. Bergstrom JD, Bostedor RG, Masarachia PJ, Reszka AA, Rodan G. Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. Arch Biochem Biophys 2000;373:231–41. [PubMed: 10620343]

45. Rhim SY, Park JH, Park YS, Lee MH, Kim DS, Shaw LM, et al. Bioavailability and bioequivalence of two oral formulations of alendronate sodium 70 mg: an open-label, randomized, two-period crossover comparison in healthy Korean adult male volunteers. Clin Ther 2009;31:1037–45. [PubMed: 19539104]

46. Diel IJ, Solomayer EF, Costa SD, Gollan C, Goerner R, Wallwiener D, et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med 1998;339:357–63. [PubMed: 9691101]

47. Stempel DA, Raphiou IH, Kral KM, Yeakey AM, Emmett AH, Prazma CM, et al. Serious asthma events with fluticasone plus salmeterol versus fluticasone alone. N Engl J Med 2016;374:1–9. [PubMed: 26735989]

48. Peters SP, Bleecker ER, Canonica GW, Park YB, Ramirez R, Hollis S, et al. Serious asthma events with budesonide plus formoterol vs. budesonide alone. N Engl J Med 2016;375:850–60. [PubMed: 27579635]

Key messages

- In patients using concomitant moderate doses of ICSs, LOBP against bronchoconstricting stimuli after LABA use is less common than previously reported.
- Although β-receptor downregulation has been proposed as a mechanism for LOBP caused by LABAs, β-receptor numbers and function in PBMCs are not reduced in patients who experience salmeterol-induced LOBP while concomitantly using ICS.
- Alendronate, which has been shown to reduce β-receptor downregulation in *in vitro* and *ex vivo* models, did not attenuate β-receptor downregulation.

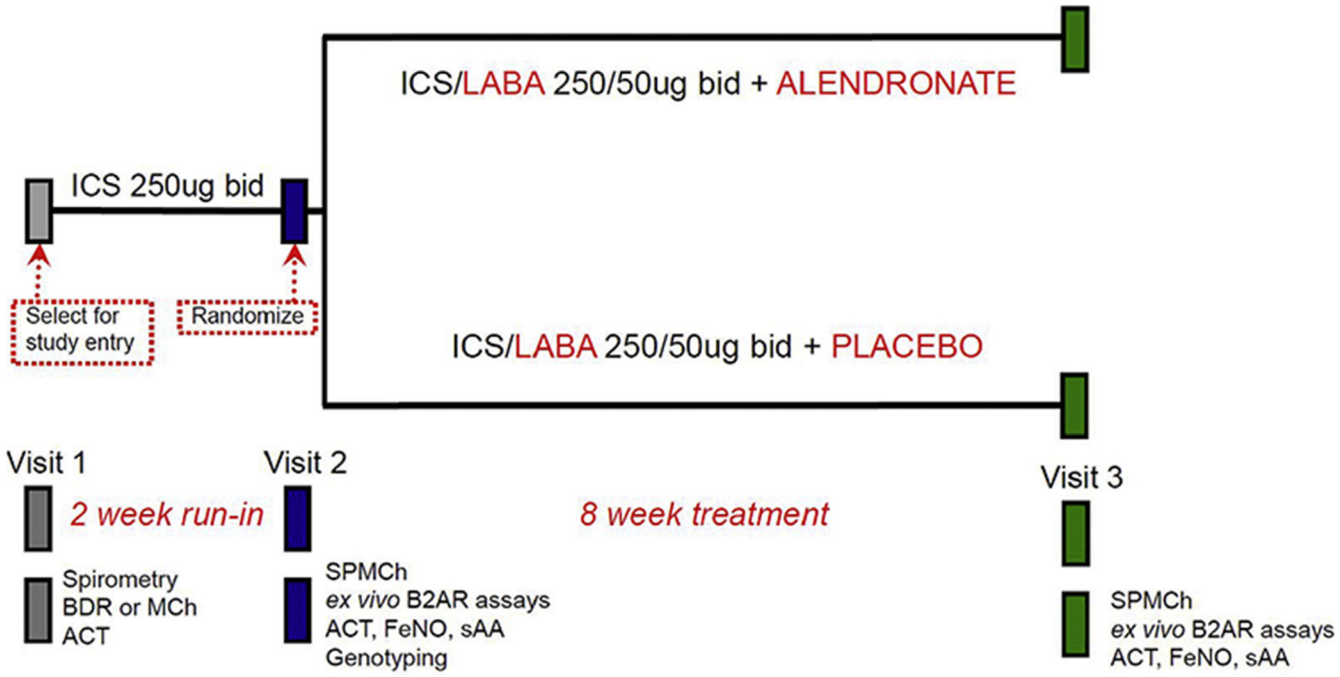

FIG 1.

ALfA trial study schema: *ex vivo* B2AR assays were conducted on PBMCs. These included radioligand binding assays to quantify B2AR cell-surface density and cAMP ELISAs to measure B2AR intracellular signaling. *MCh*, Methacholine challenge; *SPMCh*, modified methacholine challenge in which participants receive 2 puffs of open-label fluticasone/salmeterol (115/21 μg) 1 hour before the start of the challenge.

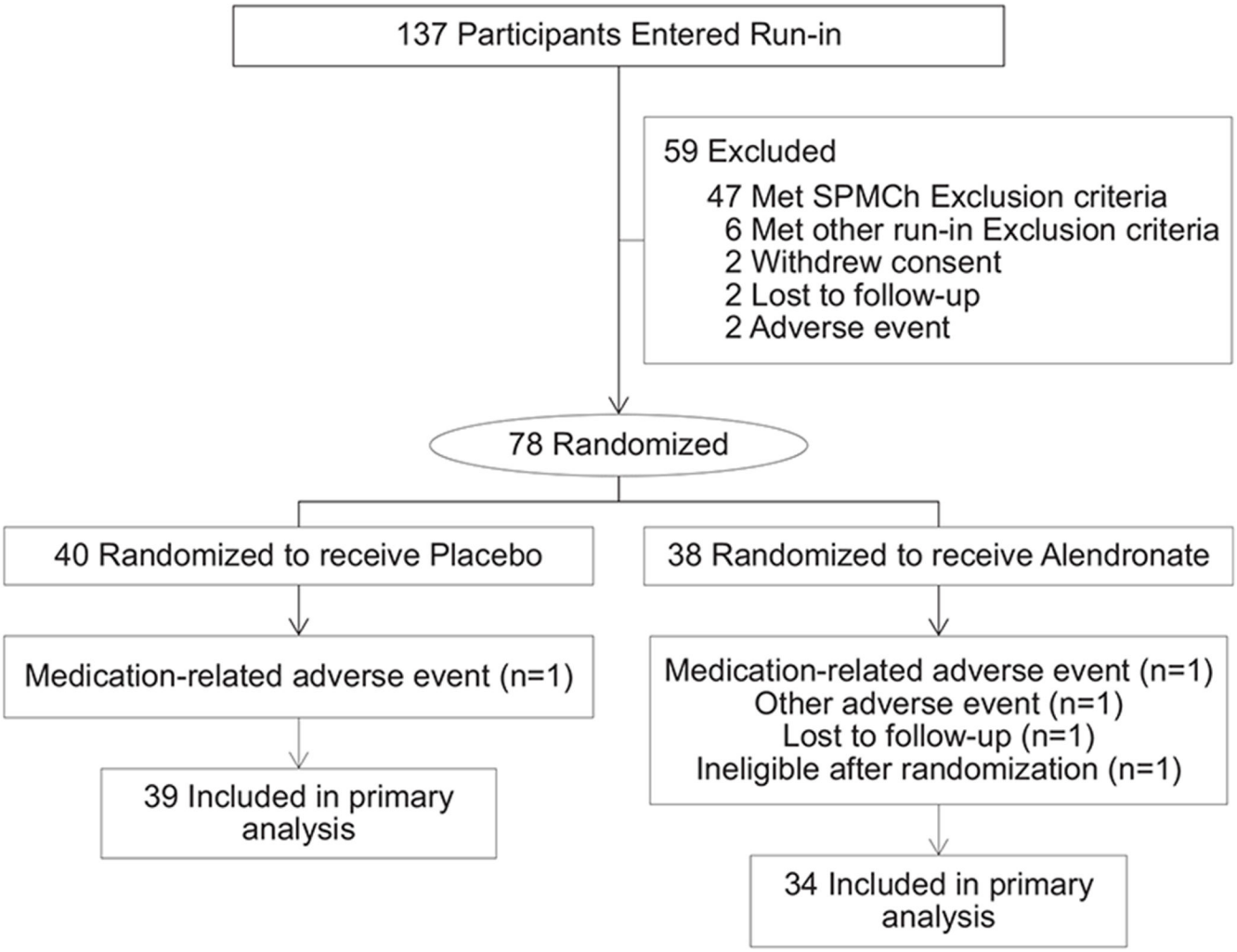

FIG 2.
Participant flow diagram.

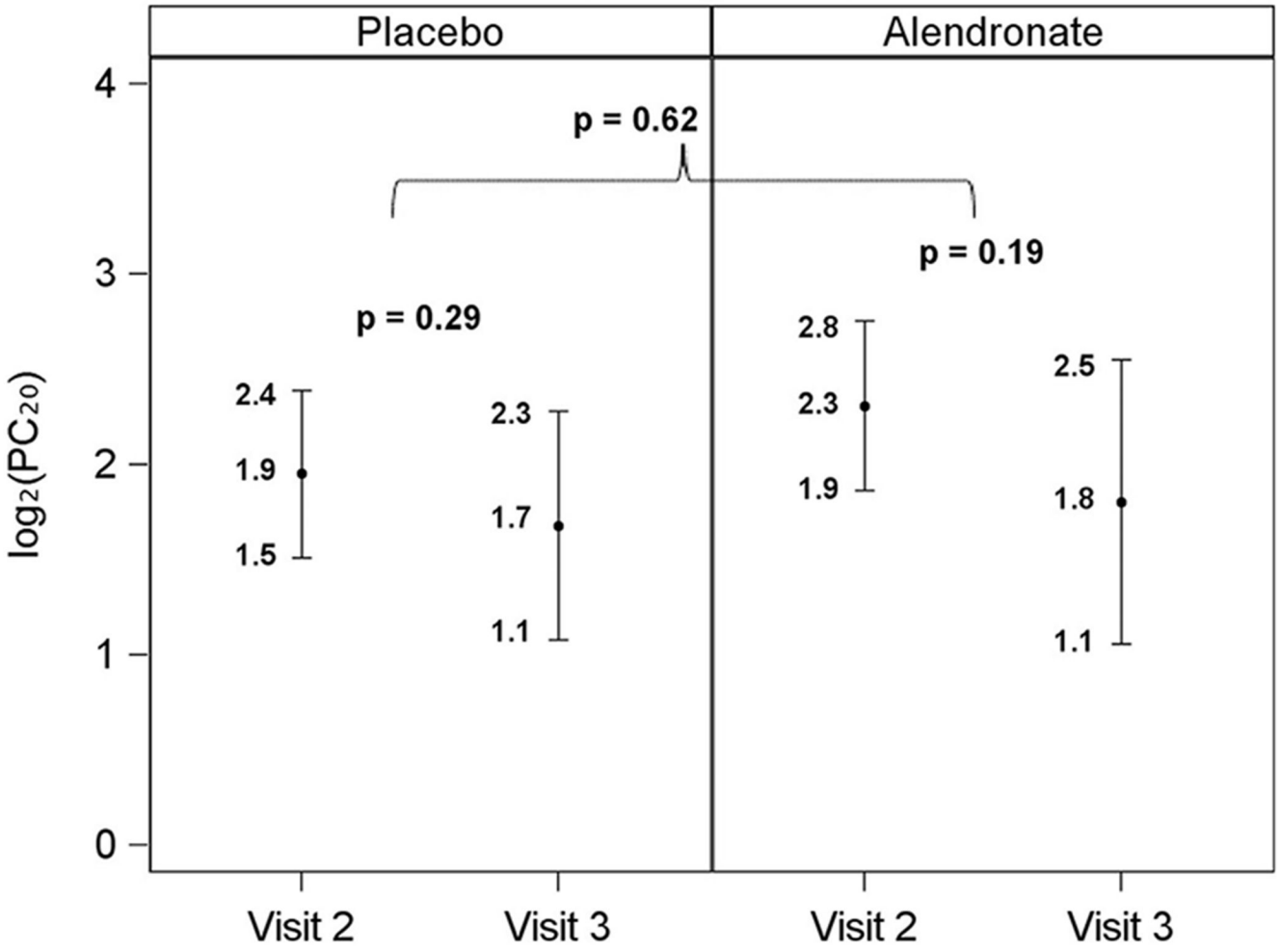

FIG 3.

Effect of alendronate and placebo on SPMCh PC_{20} values: change in SPMCh PC_{20} values from visit 2 to visit 3 for placebo and alendronate groups. Geometric means, 95% CIs, and *P* values from blocked ANOVAs are presented. *MCh*, Methacholine challenge.

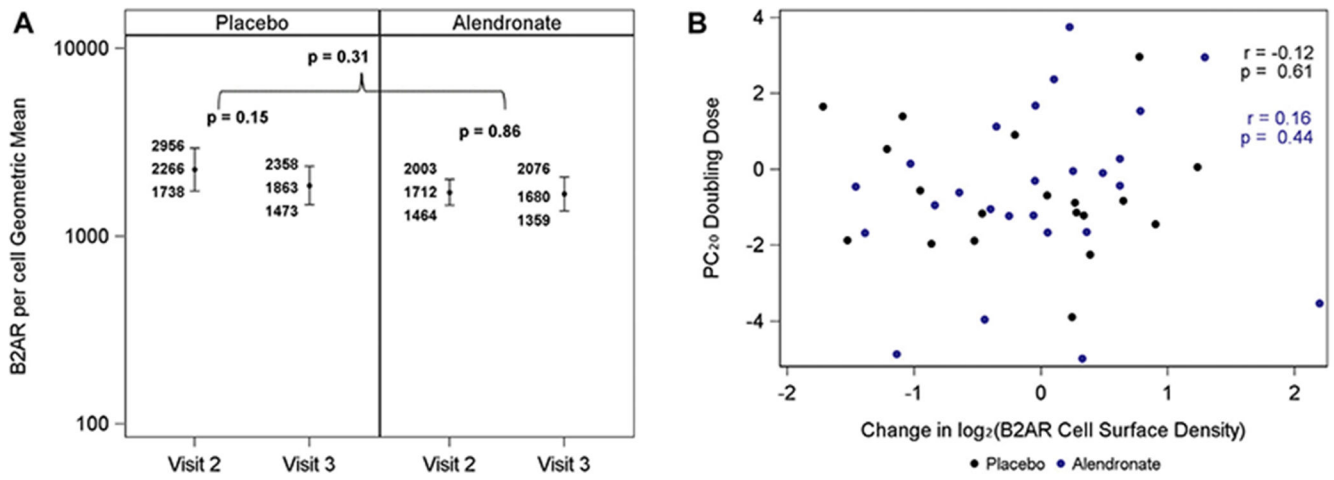

FIG 4.

Effect of alendronate on change in PBMC B2AR cell-surface density. **A**, Change in PBMC B2AR cell-surface density (determined by radioligand binding assay) from visit 2 to visit 3 for the placebo and alendronate groups. Geometric means, 95% CIs, and *P* values from blocked ANOVAs are presented. **B**, Correlation between change in PBMC B2AR cell-surface density and SPMCh PC_{20} doubling dose dilutions from visit 2 to visit 3. Pearson correlation coefficients and *P* values are presented for the alendronate and placebo groups, respectively.

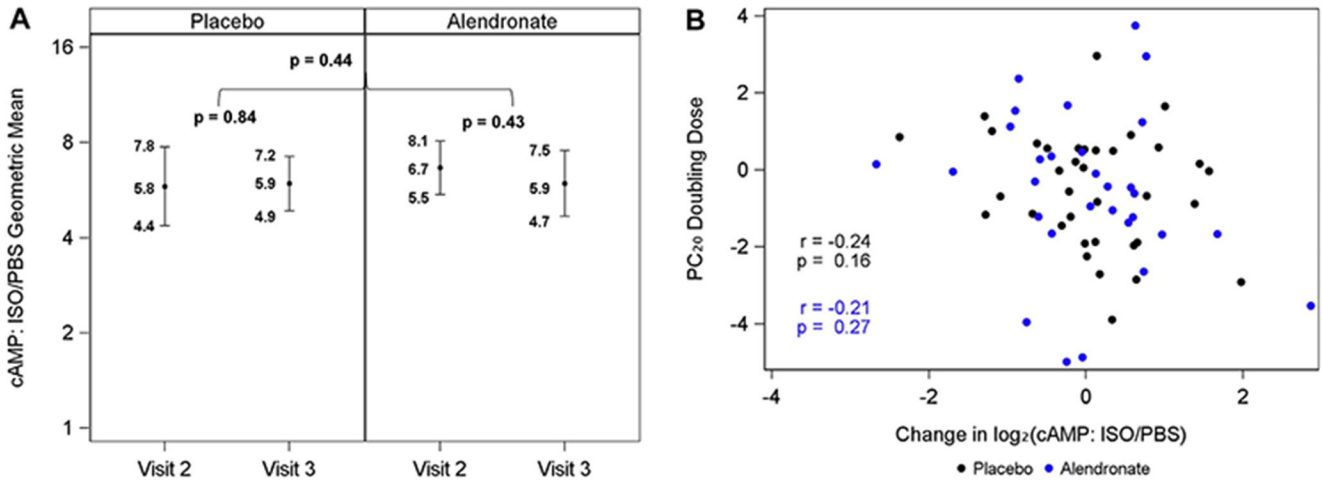

FIG 5.

Effect of alendronate on change in PBMC B2AR cell signaling (change in intracellular cAMP levels). **A**, Change in PBMC B2AR signaling (determined by using a cAMP ELISA) from visit 2 to visit 3 for the placebo and alendronate groups, as determined by using agonist-stimulated intracellular cAMP levels. Geometric means, 95% CIs, and *P* values from blocked ANOVA are presented. **B**, Correlation between change in PBMC B2AR signaling and SPMCh PC_{20} doubling dose dilutions from visit 2 to visit 3. Pearson correlation coefficients and *P* values are presented for the alendronate and placebo groups, respectively. *ISO*, Isoproterenol.

TABLE I.

Comparison of baseline participant characteristics by treatment assignment

Characteristic	Placebo group (n = 40)	Alendronate group (n = 38)	*P* value
Demographics			
Age (y)	39.3 (12.3)	38.3 (13.1)	.73‡
Male sex	13 (32.5%)	18 (47.4%)	.25¶
Race/ethnicity			.72¶
Asian/Pacific Islander	3 (7.5%)	1 (2.6%)	
Black	13 (32.5%)	10 (26.3%)	
White	21 (52.5%)	24 (63.2%)	
Hispanic	3 (7.5%)	3 (7.9%)	
Clinical and spirometric characteristics			
BMI (kg/m^2) *	29.4 (6.5)	27.9 (5.9)	.30‡
Median ACT score † (interquartile range)	21 (19-23)	21 (18-23)	.50‖
FEV_1 (%) †	83.1 (14.0)	81.3 (14.9)	.58‡
Reversibility (%), * no. qualifying by reversibility	27.6 (12.5), n = 15	25.0 (10.2), n = 17	.53‡
PC_{20} (mg/mL), * geometric mean (CV), no. qualifying with PC_{20}	0.96 (1.0), n = 25	0.93 (1.4), n = 21	.93§
Salmeterol-protected PC_{20} (mg/mL), † geometric mean (CV)	3.74 (1.0)	5.41 (0.8)	.08§
Median FENO (ppb [interquartile range]) †	17 (11-22.5)	15 (12-25)	.57‖

Results are presented as means (SDs) and numbers (percentages), unless otherwise noted.

BMI, Body mass index; *CV*, coefficient of variation.

* From enrollment visit (visit 1).

† From randomization visit (visit 2).

‡ *t* Test.

§ *t* Test on log scale.

‖ Wilcoxon rank sum test.

¶Fisher exact test.

TABLE II.

PBMC B2AR cell-surface density and signaling before and after regular use of ICS plus LABA treatment in participants with and without LOBP

	LOBP?						
	Yes			No			
Assay	**Visit 2 (n = 26)**	**Visit 3 (n = 26)**	**Visit 3/visit 2**	**Visit 2 (n = 46)**	**Visit 3 (n = 46)**	**Visit 3/visit 2**	**LOBP/no LOBP**
B2AR cell-surface density	1870 (1497-2337)	1730 (1351-2217)	0.9 (0.7-1.2), *P* = .57	2105 (1658-2673)	1923 (1482-2495)	0.9 (0.7-1.2), *P* = .52	1.0 (0.7-1.5), *P* = .95
B2AR signaling (cAMP [ISO/PBS])	6.23 (4.79-8.10)	6.41 (5.11-8.04)	1.03 (0.8-1.4), *P* = .83	6.17 (4.76-8.01)	5.36 (4.27-6.74)	0.9 (0.7-1.1), *P* = .26	1.2 (0.8-1.7), *P* = .33

Geometric means (95% CIs) and *P* values from blocked ANOVA are presented.

ISO, Isoproterenol.

TABLE III.

Comparison of participants' characteristics by development of LOBP*

Characteristic	LOBP†			*P* value, yes vs no
	Yes (n = 26)	Indeterminate (n = 15)	No (n = 32)	
Demographics				
Age (y)	41.3 (11.6)	39.2 (11.4)	36.9 (14.4)	.21‖
Male sex	11 (42.3%)	7 (46.7%)	10 (31.3%)	.42**
Race/ethnicity				.75**
Asian/Pacific Islander	2 (7.7%)	1 (6.7%)	1 (3.1%)	
Black	7 (26.9%)	5 (33.3%)	9 (28.1%)	
White	14 (53.8%)	9 (60.0%)	20 (62.5%)	
Hispanic	3 (11.5%)	0 (0%)	2 (6.3%)	
Clinical and spirometric features				
BMI (kg/m^2)‡	29.9 (6.4)	28.1 (5.5)	27.4 (6.0)	.14‖
Median ACT score§ (interquartile range)	20 (18-21)	22 (19-23)	21 (19-23)	.25#
Albuterol (4 puffs) reversibility (%), ‡ no. qualifying with reversibility	25.0 (9.9), n = 10	23.0 (8.2), n = 9	34.0 (13.5), n = 9	.11‖
PC_{20} (mg/mL),‡ geometric mean (CV), no. qualifying with PC_{20}	1.1 (1.3), n = 16	0.5 (0.5), n = 6	1.1 (1.2), n = 23	.96¶
FEV_1 (%)§	81.4 (13.8)	83.5 (14.2)	83.5 (14.6)	.58‖
Biochemical features§				
B2AR cell-surface density, geometric mean (CV)	1837.7 (0.3)	1632.7 (0.4)	2056.6 (0.6)	.45¶
cAMP (ISO/PBS), geometric mean (CV)	6.3 (0.9)	5.0 (0.9)	7.1 (0.8)	.57¶
Median FENO (ppb [interquartile range])	18.5 (11-25)	15 (12-22)	18 (12-28)	.80#
sAA (after/before salmeterol), geometric mean (CV)	1.9 (0.7)	2.4 (0.6)	1.8 (0.7)	.76¶

Results are presented as means (SDs) and numbers (percentages), unless otherwise noted.

BMI, Body mass index; *CV*, coefficient of variation; *ISO*, isoproterenol.

*Based on 73 participants who completed visit 2 and visit 3 SPMChs.

[†] LOBP was defined as a change in SPMCh PC_{20} value of −1 doubling dose or less, no LOBP was defined as a change in SPMCh PC_{20} value of greater than 0 doubling dose, and LOBP of indeterminate status was defined as a change in SPMCh PC_{20} value of greater than −1 but less than 0.

[‡] From enrollment visit (visit 1).

[§] From randomization visit (visit 2).

[//] *t* Test.

[¶] *t* Test on log scale.

[#] Wilcoxon rank sum test.

[**] Fisher exact test.