NHLBI AsthmaNet

ALfA—ALendronate for Asthma

Study Protocol

June 30, 2015

I. RESEARCH QUESTION:

- Primary: Does alendronate prevent loss of bronchoprotection after regular use of long-acting beta-2 adrenoreceptor agonists?

- Secondary: Do measures of beta-2 adrenoreceptor density or functional activation correlate with alendronate's effect on bronchoprotection?

II. TRIAL OVERVIEW

In this proof of concept trial we aim to determine if alendronate, which diminishes beta-2 adrenergic receptor internalization, can reduce the loss of bronchoprotection that occurs with regular use of long-acting beta agonists (**LABAs**), even when used in combination with inhaled corticosteroids (**ICS**). We propose a 10 week, randomized, double-blind, placebo-controlled trial in patients with asthma aged 18 and older. Seventy-six (76) participants with moderate asthma will be run-in for 2 weeks on ICS, then randomly assigned to regularly use LABA plus alendronate or LABA plus placebo for 8 weeks (38 participants per study group), with both groups maintaining their run-in phase ICS. In order to prove the underlying concept – that agents can be used to prevent the loss of bronchoprotection to bronchoprovocative stimuli that occurs when LABAs are used regularly -- we will use the loss of bronchoprotection against methacholine (salmeterol-protected methacholine challenges (**SPMCh**)) as our marker. We will also determine whether the prevention of down-regulation of cell surface beta-2 adrenergic receptor density and function is associated with the preservation of bronchoprotection, utilizing *ex vivo* assays on peripheral blood mononuclear cells obtained from study participants. In exploratory aims we will examine: (1) the effect of regular use of LABAs, and the intervention, on transcription and translation of the gene encoding for the beta-2 adrenergic receptor (**ADRB2**); (2) whether salivary alpha amylase (**sAA**) can be used as a functional marker of ADRB2 downregulation; and (3) whether fractional-expired concentration of nitric oxide (**FeNO**) predicts patients more likely to experience loss of bronchoprotection.

III. BACKGROUND AND SIGNIFICANCE

A. Introduction

Beta-2-agonists are effective in reducing airway narrowing in asthma and protecting against stimuli that produce bronchoconstriction. Short-acting beta-2-agonists have become the reliever medication of choice for acute relief of asthma symptoms. National and international guidelines recommend the addition of a LABA when ICS fail to produce adequate symptom control. The combination, **LABA/ICS**, are prescribed to be used regularly and has become the most commonly used asthma controller medication class in the United States. Unfortunately, even when LABAs are added to ICS and used regularly, 58-81% of patients with asthma fail to achieve total control[1].

Regular use of beta-agonists (short-acting and long-acting) is associated with tachyphylaxis, which involves decreased expression of ADRB2 on the cell surface. This tachyphylaxis occurs due to increased rates of internalization of ADRB2 after binding the beta-agonist ligand.

Several lines of evidence suggest that, as would be expected from the data on tachyphylaxis, regular use of beta-agonists may reduce their effectiveness. In particular, regular use of beta-agonists, both short and long-acting, reduces the ability of these agents to protect against the airway narrowing that occurs in asthma in response to bronchoconstrictor stimuli. We refer to this reduced effect as **loss of bronchoprotection**.

Below, we review the loss of bronchoprotection as it relates to long-acting beta-agonists. We then present data regarding the ability of alendronate to reduce beta-receptor downregulation. We present *ex vivo* data from human lung fragments that suggest that alendronate can in fact blunt the loss of bronchoprotection that occurs with regular use of beta-agonists. We subsequently detail a proof-of-concept trial to examine whether we can reduce the loss of bronchoprotection that occurs with regular use of LABA/ICS.

B. Loss of Bronchoprotection with Use of LABAs

Multiple lines of evidence suggest that the efficacy of LABAs is reduced with their regular use as it relates to their ability to protect against bronchoprovocative stimuli. This phenomenon was first demonstrated by Cheung et al who showed that there was almost complete loss of bronchoprotection against methacholine (**Fig 1**)[2]. As shown in **Fig 1**, salmeterol initially increased the concentration of methacholine required to reduce the FEV_1 by 20% from ~1.5 mg to almost 16 mg. However, when retested after 28 days of regular salmeterol use, the concentration of methacholine required to reduce the FEV_1 by 20% one hour after salmeterol (SPMCh) had now fallen back to

Fig 1 Regular use of salmeterol leads to loss of bronchoprotection to methacholine challenge

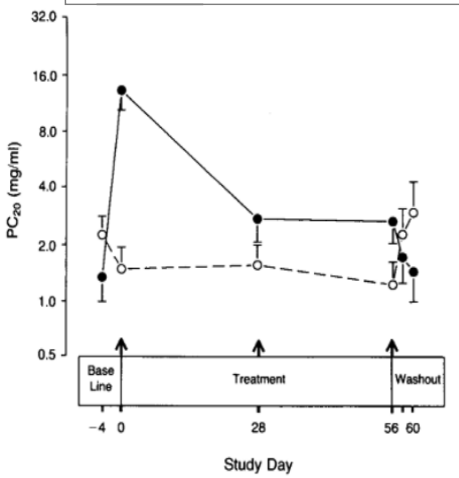

Fig 1. Loss of salmeterol's ability to protect against methacholine provocation after 28 days of regular salmeterol use. Open circle: placebo; Close circle: salmeterol. (See text for further explanation) Cheung, 1992

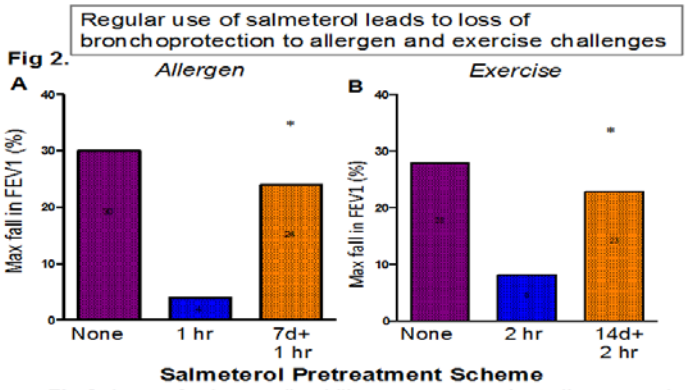

Fig 2. Loss of salmeterol's ability to protect against allergen and exercise challenges after 7 (A) and 14 (B) days of regular salmeterol use. * p<0.05. Bonini 2013, Giannini 1996

near the level of protection provided by administering placebo one hour prior to the methacholine. These findings demonstrating loss of bronchoprotection against the bronchoconstricting properties of methacholine with regular salmeterol use (in as few as 4 days) have subsequently been reproduced multiple times[3, 4]. Furthermore, multiple studies have now shown that the loss of bronchoprotection is a generalized effect that occurs in response to all tested bronchoconstricting agents, whether direct or indirect. Regular use of LABAs reduces their ability to protect against exercise- and allergen-induced provocations[5-8] (**Fig 2**).

While it was hoped that concomitant use of inhaled corticosteroids might eliminate loss of bronchoprotection attributed to beta-agonists, several studies have demonstrated that loss of bronchoprotection occurs even in the setting of ICS use[9, 10] as seen in **Fig 3**.

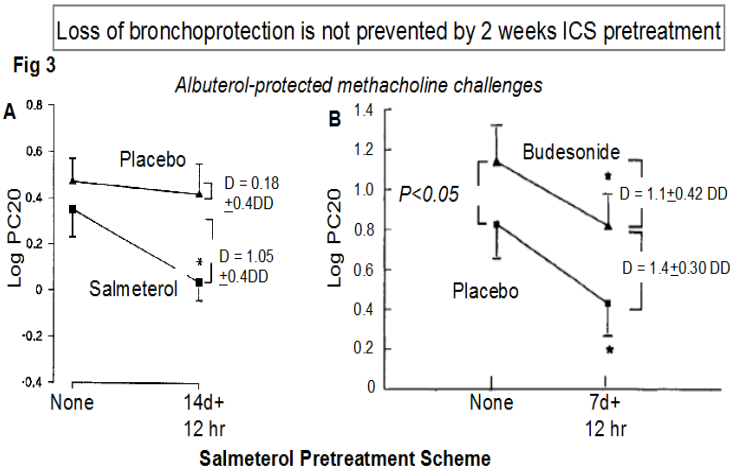

Fig 3. Regular use of salmeterol leads to the loss of albuterol-induced bronchoprotection without (**A**) or with (**B**) concomitant use of ICS. DD: doubling dose dilution; ICS: inhaled corticosteroids. *: p<0.05 vs. baseline. Yates 1996 and 1997

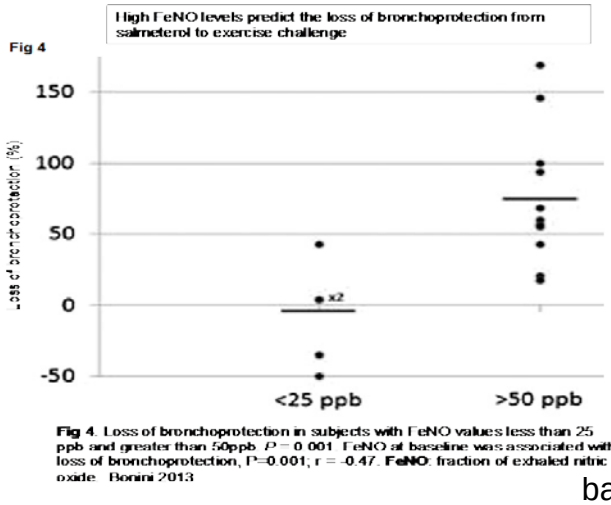

Fig 4. Loss of bronchoprotection in subjects with FeNO values less than 25 ppb and greater than 50ppb. $P = 0.001$. FeNO at baseline was associated with loss of bronchoprotection, $P=0.001$; $r = -0.47$. **FeNO**: fraction of exhaled nitric oxide. Bonini 2013

While the loss of bronchoprotection is a fairly widespread phenomenon, we recently examined whether polymorphisms of the beta-receptor are associated with greater degrees of loss of bronchoprotection[8]. We examined protection against exercise-induced bronchospasm in patients **not on ICS**. We found that the group as a whole experienced almost complete loss of bronchoprotection. While we did not find an association between ADRB2 polymorphisms and loss of bronchoprotection, we found that high baseline FeNO was predictive of loss of bronchoprotection (**Fig 4**).

In summary, the bronchoprotective effect of beta-agonists (short- and long-acting), whether used with or without ICS, is reduced after regular use of LABAs. Further, in patients not using ICS, high FeNO is associated with greater loss of bronchoprotection.

Fig 5

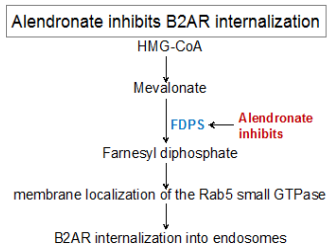

Fig 5. Alendronate specifically inhibits the synthesis of mevalonate pathway precursors required for B2AR internalization. B2AR: beta-2 adrenergic receptor; FDPS: farnesyl diphosphate synthase. Adapted from Bergstrom 2000, Fisher 1999, Jiang 2012

C. Alendronate and Loss of Bronchoprotection

The precise mechanism for the loss of bronchoprotection has not been elucidated. However, it appears to be linked to beta-receptor down-regulation[11, 12], up-regulation of bronchoconstrictive signaling[13, 14], uncoupling[11, 12], and possibly reduced ADRB2 transcription[15]. Several studies have suggested that a major event in loss of bronchoprotection includes internalization and degradation of the ADRB2, leading to down-regulation of the receptor's density on human airway smooth muscle cell (**HASM**) surfaces[16].

In an attempt to understand the mechanisms underlying receptor internalization, Jiang and colleagues performed an expressed sequence tag (**EST**)-derived RNAi screen that revealed a critical role for farnesyl diphosphate synthase (**FDPS**) in B2AR agonist (**BA**)-induced B2AR internalization and down-regulation[17]. FDPS synthesizes farnesyl diphosphate from precursors in the mevalonate pathway (**Figure 5**) and is required for membrane localization of the Rab5 small GTPase[18] needed for ADRB2 internalization into endosomes[19]. Nitrogen-containing bisphosphonates are specific inhibitors of farnesyl diphosphate synthase[20, 21]. Using multiple *in vitro* and immunofluorescence microscopic techniques, Jiang et al showed that alendronate, a nitrogen-containing bisphosphonate approved for use in osteoporosis, can indeed prevent both agonist-induced desensitization and loss of functional activation (cAMP production) of ADRB2 (**Figure 6**)[17].

Fig 6

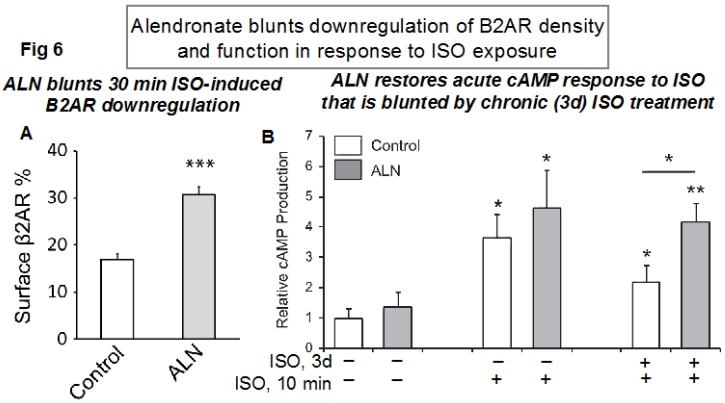

Fig 6. Flow cytometry of HEK cells transfected with B2AR, treated with Iso (to induce receptor internalization) and alendronate or control (**A**). cAMP ELISA of HASM cells Treated with or without Iso for 3 days, and alendronate or control. ALN: alendronate; B2AR: Beta-2 adrenergic receptor; HASM: human airway smooth muscle; ISO: isoproterenol. *P < 0.05 **P < 0.01 ***P < 0.001. Jiang 2012

More importantly, *ex vivo* experiments with intact human lung slices showed that alendronate was able to reduce the loss of protection against smooth muscle constrictors that occurred after long-term, continuous exposure to beta-agonists[22] (**Fig 7).** In this study, human lung slices were exposed to acetylcholine (**Ach**), which caused narrowing of airway lumens. The beta-agonist isoproterenol (**Iso**) reduced the airway narrowing (seen in the "None" section of Fig 7). Modeling chronic BA exposure, these slices were exposed to 36 hours

Fig 7

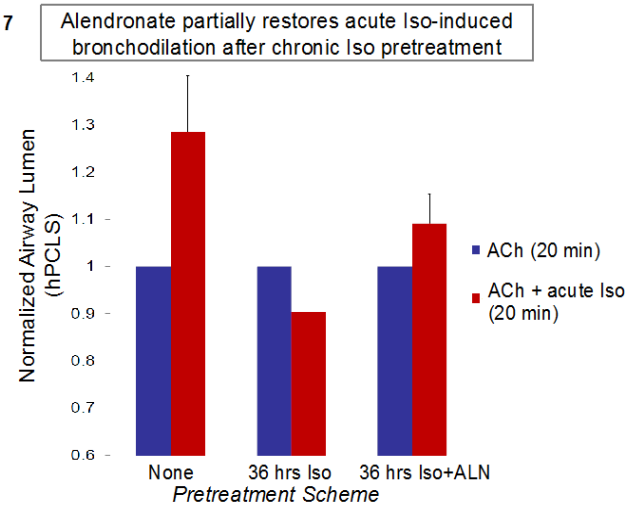

Fig 7. Alendronate's effect *ex vivo* on intact human airways exposed for 36 hours to short-acting beta agonists (see text for further explanation). ACh: acetylcholine; ALN: alendronate; Iso: isoproterenol. Rajendran 2012

of Iso or control and challenged with Ach. The sections exposed to 36 hours of Iso now had **narrower** airway lumens than those that had not been exposed (36 hour Iso bars—left bar Ach without Iso, right bar acetylcholine with Iso). Alendronate moderated this excess bronchoconstriction (36 hrs Iso+ALN bars)[22].

In summary, *in vitro* and *ex vivo* data suggest that alendronate reduces ADRB2 down-regulation and appears to moderate the magnified constrictor response that can occur after regular beta-agonist exposure.

D. Salivary alpha amylase and BAs

While we propose measuring beta-receptor density directly and performing functional studies of the beta-receptor *ex vivo* in mononuclear cells, we propose a possible functional biomarker of beta-agonist activation that we can examine in an exploratory manner in our trial—salivary alpha amylase (**sAA**).

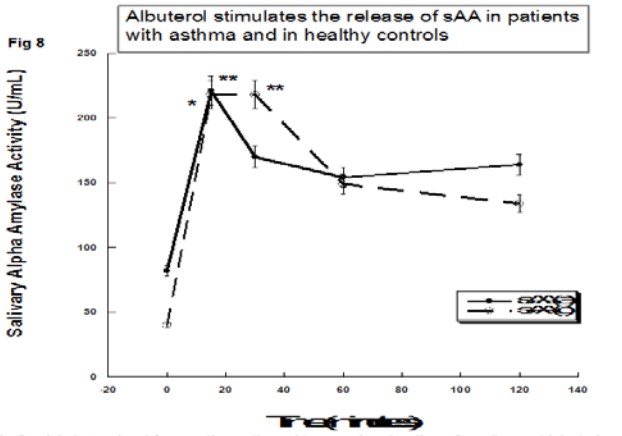

Fig 8. sAA determined from saliva collected by passive drooling after albuterol inhalation. *p<0.001 vs. baseline; *p<0.05.a: asthmatics; c: controls; sAA: salivary alpha amylase. Moy 2013 (unpublished data)

sAA is synthesized mainly in the parotid glands, and is secreted in response to physical[23] and psychological stress[24]. Preliminary data from our group show that albuterol stimulates the release of sAA; in both asthmatics and healthy controls, sAA rises 15 minutes after inhalation of albuterol **(Figure 8)**. Interestingly, sAA secretion is reduced in response to regular BA exposure. In rats, regular use of salmeterol was similar to albuterol in blunting sAA secretion by 20-30% versus saline control[25]. More importantly, regular use of SABAs was found to blunt the secretion of sAA by 40% in asthmatic children versus healthy controls[26], suggesting that BA-induced increases in sAA may be subject to ADRB2 desensitization. We therefore propose, in an exploratory manner, to examine the relationship between sAA and our proposed *ex vivo* measurements of beta-receptor density and function and changes in degree of bronchoprotection.

In summary, the data presented above indicate that:

1. Regular use of LABAs results in loss of bronchoprotection to bronchoprovocative stimuli.
2. *In vitro* alendronate prevents beta-agonist-induced decreases in ADRB2 density.
3. In *ex vivo* studies using human lung slices, alendronate inhibits beta-agonist-exposure-induced loss of bronchoprotection.
4. Beta-agonists stimulate the release of sAA, but their regular use blunts sAA secretion.
5. Elevated FeNO associates with loss of bronchoprotection seen with regular use of LABAs.

These data raise the following questions:

- Does alendronate prevent the loss of bronchoprotection from beta-2 adrenergic receptor agonists that occurs after regular use of LABAs?
- Does alendronate prevent the down-regulation of surface ADRB2 density, and if so, does it correlate with the preservation of bronchoprotection?
- Can sAA serve as a biomarker for reduced beta-receptor number or function or loss of bronchoprotection due to regular use of LABAs?
- Does elevated FeNO associate with greater loss of bronchoprotection to LABAs?

E. Anticipated Significance

If this proof-of-concept study demonstrates that an intervention associated with reduced down-regulation of beta-receptors reduces the loss of bronchoprotection associated with regular LABA/ICS use there are several potential therapeutic implications:

-Intervention to increase the effectiveness of ICS/LABA – The GOAL study suggested that even after the addition of LABA to ICS 58-81% of patients with asthma fail to achieve total control[1]. These patients become candidates for potentially expensive and/or toxic Stage V and Stage VI therapies. Positive results in ALfA would suggest that trials should be conducted to see if drugs that inhibit ADRB2 down-regulation might improve the effectiveness of ICS/LABA. We therefore anticipate follow-on trials in larger populations using LABA/ICS in patients who have not achieved complete control to examine whether drugs with such a mechanism of action improve control.

-Reducing the morbidity associated with regular use of short-acting beta-agonists - Regular use of beta-agonists is associated with paradoxical reduction in asthma control in some patients. Epidemiological data point to direct associations of morbidity and mortality associated with beta-agonist use even after correcting for asthma severity. Additionally, severe asthma, which accounts for a large proportion of health care expenditures, is frequently characterized by excess use of beta-agonists which also has been associated with reduced bronchoprotection against provocative stimuli. Thus, it is possible that agents with a mechanism of action similar to alendronate's might extend the therapeutic range of beta-agonists and reduce the morbidity that excess beta-agonist use may contribute to severe asthma. We therefore anticipate follow-on trials in specific populations where excess beta-agonist use may be contributing to poor control (e.g. severe asthmatics with excess beta-agonist use).

Further, if this approach is effective, we can envision studies as to whether such agents should be routinely co-administered with beta-agonists. If we demonstrate that we can moderate loss of bronchoprotection, we believe our data will stimulate a search for more agents that act in this manner and stimulate studies to examine the ramifications of this effect. Alendronate itself may not necessarily be the agent of choice in the future. However, a successful proof of concept study would encourage development of compounds with similar modes of action.

We therefore propose the Proof of Concept ALfA (ALendronate for Asthma) trial described below.

IV. HYPOTHESES TO BE TESTED

Primary hypothesis:

1. Alendronate prevents the loss of bronchoprotection against methacholine-induced bronchoconstriction that occurs with regular use of ICS/LABA.

Secondary hypothesis:

1. Alendronate's effect on bronchoprotection is associated with alendronate's effect on ADRB2's cell surface concentration.
2. Alendronate's effect on bronchoprotection is associated with alendronate's effect on ADRB2's signaling function.

Exploratory hypotheses:

1. As regards sAA:
    - Regular use of LABAs blunts the secretion of sAA
    - Changes in secretion of sAA correlate with LABA-induced loss of bronchoprotection
    - Changes in secretion of sAA correlate with ADRB2's cell surface density and signaling function
2. FeNO predicts alendronate's effect on bronchoprotection in patients with asthma regularly using LABAs for asthma treatment.
3. The addition of alendronate as compared to placebo will result in higher ACT scores when LABA is added to ICS.

V. PROTOCOL SUMMARY AND SCHEMA

To attempt to answer the questions raised above, we propose a 10-week, randomized, double-blind, placebo-controlled, parallel-arm trial examining the effect of oral alendronate as compared to placebo in asthmatics who will be treated with ICS/LABA. Through this study design **(Figure 9)** we will be able to ascertain whether the addition of oral alendronate is more effective than placebo in preventing the loss of bronchoprotection caused by regular LABA use as determined by SPMCh.

Study design—Figure 9

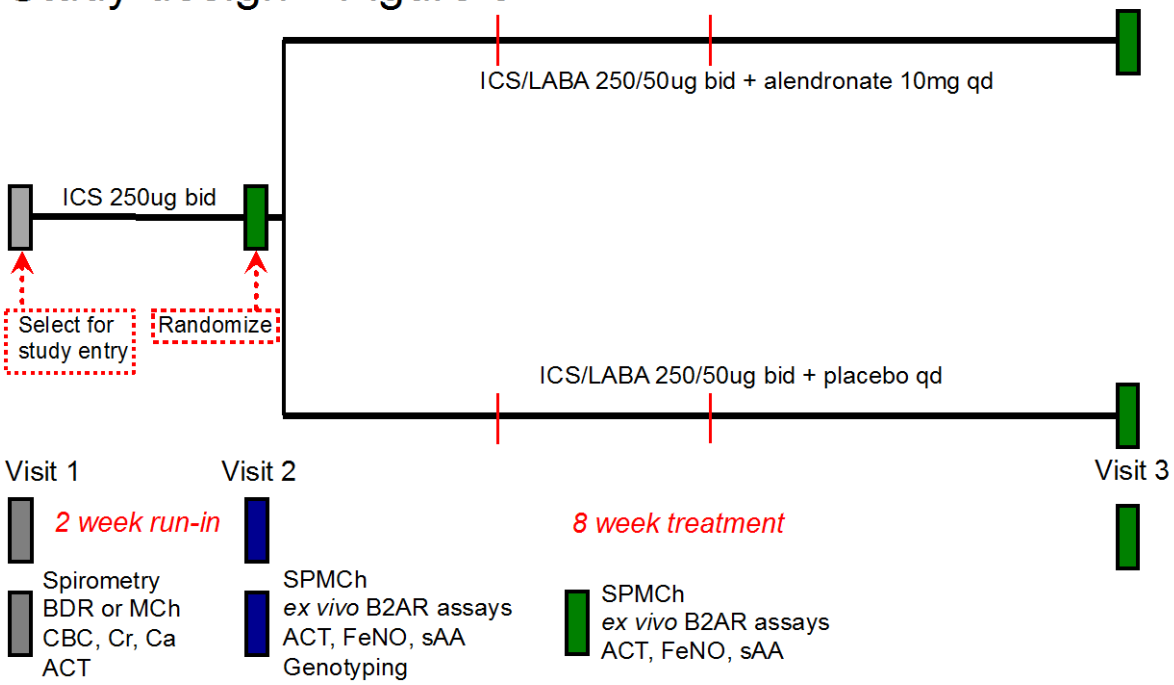

Figure 9. *ex vivo* B2AR assays will be done on peripheral blood mononuclear cells, and include radioligand binding assays, western blots, RNA extractions, and cAMP ELISAs. | Phone calls for monitoring study subjects' symptoms (ACT scores) and adherence to medications will be made 2 and 4 weeks after randomization. **ACT**: asthma control test; **B2AR**: beta-2 adrenergic receptor **BDR**: bronchodilator response; **Ca**: calcium; **Cr**: creatinine; **FeNO**: fraction of exhaled nitric oxide; **GFR**: glomerular filtration rate; **ICS**: inhaled corticosteroid (fluticasone propionate); **LABA**: long-acting beta-2 adrenergic receptor (salmeterol); **MCh**: methacholine challenge; **sAA**: salivary amylase; **SPMCh**: salmeterol-protected methacholine challenge; **WB**: western blot

Entry: At Visit 1, asthmatic participants will undergo spirometry and either a test for bronchial reversibility (if FEV_1 <80% predicted only) or a methacholine challenge (MCh). Participants with FEV_1 <80% predicted who perform reversibility at Visit 1 and do not reverse ≥12% will perform methacholine challenge at Visit 1A (see section VI-A). Asthma control test (ACT) scores will be obtained, and blood drawn to determine serum creatinine, calcium and CBC.

Run in: Following successful completion of the eligibility assessment at Visit 1, participants will be entered into the 2-week run-in period and switched from their controller asthma therapy to ICS (fluticasone propionate (**FP**) 250 mcg BID) with the purpose of standardizing the daily dose of ICS taken. In addition, participants will be asked to use ipratropium for rescue for the duration of the trial, although albuterol can be used if ipratropium fails to relieve symptoms (see section XVII)**.** Since the maximum daily dose of ICS allowed as controller therapy will be 1000 mcg daily of FP or equivalent, no participant will undergo a more than 2-fold decrease in ICS dose at the time of run-in. Participants will be monitored closely for safety during this 2-week run-in period. After these two weeks, participants will return for Visit 2, where adherence to

ICS will be determined from the dose counter on each Diskus device; those meeting this and other eligibility criteria will be randomized. SPMCh will be performed, blood will be collected for *ex vivo* assays on ADRB2's density and function and for genotyping, saliva will be collected for sAA assays, and FeNO levels will be determined.

Treatment period: At Visit 2, following the run-in period with ICS, participants will be randomly assigned to receive ICS/LABA and either alendronate or matching placebo for 8 weeks. After 2 and 4 weeks of treatment participants will receive phone calls where medication tolerance and adherence and any possible issues will be assessed prior to their next study visit. Additional contacts will be made to encourage adherence. After 8 weeks of treatment they will return to the study site for Visit 3, where adherence to ICS will again be determined from the dose counter on each Diskus device, while adherence to the alendronate or placebo capsules will be determined from a MEMSCap™ counter that tracks when a vial is opened. SPMCh, blood draw, salivary sampling, and FeNO determination will be repeated. At the end of this visit, participants will be asked to return to regular medications, thus completing the trial.

VI. INCLUSION AND EXCLUSION CRITERIA (TO ENTER CHARACTERIZATION PERIOD)

A. Inclusion criteria at Visit 1 *(to enter run-in)*

1) Male and female participants, age 18 years and older at enrollment

2) Clinical history consistent with moderate asthma for >1 year

3) Asthma is controlled with ICS, with an FP dose ≤ 1000mcg/day and >100mcg/day (or equivalent)

4) Able to perform reproducible spirometry according to ATS criteria

5) Baseline FEV_1 ≥ 50% of predicted and ≥1L.

6) If FEV_1 <80%, a minimum 12% increase in FEV_1 post-bronchodilator OR MCh PC_{20} ≤ 8 mg/mL at Visit 1. The decision to perform bronchodilator response or MCh testing during Visit 1 will be made at the discretion of the study site's principal investigator, in consultation with their study coordinator. Participants with FEV_1 <80% predicted who perform reversibility at Visit 1 and do not reverse ≥12% will perform methacholine challenge at Visit 1A.

7) If FEV_1 ≥80%, a MCh PC_{20} ≤ 8 mg/mL

8) Ability to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the participant's respective study institution.

9) If intranasal steroids might be needed, willingness to take a single agent at a stable dose throughout the trial, starting prior to or on enrollment at Visit 1.

B. Exclusion criteria at Visit 1 *(to enter run-in)*

1) MCh PC_{20} >8 mg/mL
2) Uncontrolled asthma, as suggested by an ACT score <18 *while* on high-dose ICS (FP daily dose >500mcg or equivalent)
3) Medical contraindication to LABA or history of adverse reactions to ICS or LABA preparations or any of their ingredients
4) LABA use within 4 weeks of study entry.
5) Bisphosphonate use within 6 months of study entry
6) History of intolerance or hypersensitivity to bisphosphonates
7) Regular use of aspirin or non-steroidal anti-inflammatory medications (NSAIDs) and inability to stop using aspirin and/or NSAIDs during the course of the study
8) History of esophageal ulcers
9) History of hematemesis
10) Uncontrolled gastro-esophageal reflux disease
11) History of delayed esophageal emptying caused by esophageal abnormality such as stricture or achalasia
12) Inability to stay erect for 30 minutes after oral drug
13) History of osteonecrosis of the jaw
14) Dental extraction or root canal in prior 8 weeks, or anticipated during the study
15) Women of childbearing age unwilling to practice birth control or unwilling to stop nursing during the course of the study or in the 6 months after study completion
16) Intolerance to anticholinergic inhalers
17) History of bladder-neck obstruction
18) History of urinary retention
19) History of benign prostatic hypertrophy
20) History of narrow angle glaucoma
21) History of significant cardiovascular disorders or arrhythmias
22) Major medical problems prohibiting study participation, i.e. presence of chronic or active lung disease other than asthma or history of *unstable* significant medical illness other than asthma, including (but not limited to) thyroid disease, diabetes mellitus, Cushing's disease, Addison's disease, hepatic disease, or concurrent medical problems that could require oral corticosteroids during the study or that would place the participant at increased risk
23) History of smoking (cigarettes, cigars, pipes, marijuana or any other substances) within the past 1 year, or > 10 pack-years total if ≥ 18 years of age
24) Systemic corticosteroid treatment for any condition within 4 weeks of enrollment at Visit 1
25) History of significant asthma exacerbation requiring systemic corticosteroids

within 4 weeks of Visit 1 or more than five courses of systemic corticosteroids in the past year

26) History of a life-threatening asthma exacerbation requiring intubation, mechanical ventilation, or resulting in a hypoxic seizure within the last 2 years

27) History of a respiratory tract infection within 4 weeks of Visit 1

28) Evidence that the participant may be non-adherent to medication regimen, or may move from the performance site area before trial completion

29) Inability or unwillingness to perform study procedures

30) Pregnancy or planning to get pregnant during the course of the study or in the 6 months after study completion

31) Receiving hyposensitization therapy other than an established maintenance regimen defined as a continuous regimen for ≥ 3 months prior to enrollment

32) Participation in an intervention trial or use of investigative drugs in the past 30 days or plans to enroll in such a trial during the study

33) Use of any drug prohibited during the study or within the washout period prior to Visit 1

VII. INCLUSION AND EXCLUSION CRITERIA FOR RANDOMIZATION

A. Inclusion criteria for randomization

1. display a SPMCh PC_{20} ≤ 16 mg/mL *and* ≥0.25 mg/mL

2. demonstrate adherence with study medications (≥80% of scheduled doses)*

3. Serum calcium ≥ 8.5 mg/dL

B. Exclusion criteria for randomization

1. Asthma exacerbation during the run-in: For participants who have an exacerbation during the run-in period (i.e. worsening asthma symptoms resulting in treatment with systemic corticosteroids - see exacerbation definition in section XVII below), their participation in the trial will be terminated due to safety concerns.

2. Calculated GFR of less than 35 mL/min

3. Hypocalcemia (serum calcium < 8.5 mg/dL, and ionized calcium < 4.4 mg/dL (or < 1.1 mmol/L))

4. Sum of absolute lymphocytes and monocytes < 900 cells/ µL

5. Inability to provide adequate blood sample for biochemical assay

*Individuals who do not meet inclusion criterion 2 after 2 weeks in the trial will be retrained and allowed to continue in the run-in for another 2 weeks. Visit 2 will be deferred until adequate adherence is demonstrated. If, after 4 weeks in the run-in, the participant cannot meet the adherence requirements, then his or her participation in the study will be terminated. Participation of individuals who show lack of adherence to medication dosing on two separate evaluations at any point during the run-in will be terminated. Depending on the circumstances, these individuals may be allowed to re-enroll starting at Visit 1 at a later time.

Intention-to-treat principles will apply following randomization. Thus, participants will be dropped after randomization for safety reasons only. These may include pregnancy or the development of a significant asthma exacerbation (as defined in section XVII, "Adverse Events Related to Asthma: Asthma Exacerbations") found not to be, in the opinion of the investigator, responsive to protocol treatment as defined in section XVII.

VIII. OUTCOMES

Primary outcome:

1. Change in SPMCh PC_{20} after 8 weeks of treatment.

Secondary outcomes:

1. Changes in peripheral blood mononuclear cell (PBMC) ADRB2 cell surface density

2. Changes in PBMC ADRB2 signaling function as measured by beta-2 adrenergic receptor agonist-induced cAMP production

Exploratory outcomes:

1. Salivary alpha amylase levels as affected by regular use of LABAs and alendronate

2. ACT scores

3. FeNO

IX. PROTOCOL DETAIL AND VISIT STRUCTURES

Specific elements for each study visit are provided in **Table 1**, below.

Visit 1.

- Participants will first be told the purposes, risks, and alternatives to participation and will sign an IRB-approved document for informed consent.
- An ACT score will be obtained.
- If female and of reproductive age, a urine pregnancy test must be negative and appropriate methods of contraception must be reported.

- Standardized questionnaires used in prior AsthmaNet studies will be administered to characterize asthma onset, severity, treatment, exacerbation history, and current control.
- Baseline measurements will include spirometry (testing for reversibility of bronchoconstriction OR bronchial hyperreactivity with MCh if FEV1<80%; testing for bronchial hyperreactivity with MCh if FEV_1≥80%) all by methods used in previous AsthmaNet studies. If FEV_1 <80% and reversibility performed but <12%, MCh will be performed at Visit 1A.
- Blood will be drawn for CBC, calcium, and creatinine.
- Study participants will be supplied with ipratropium inhalers for as-needed rescue. An albuterol inhaler will also be given for as-needed rescue should ipratropium fail to relieve symptoms.
- They will be asked to switch their controller asthma therapy at this point to FP 250mcg twice daily, for which they will be provided with dry powder inhalers (DPIs).
- Participants who meet the inclusion/exclusion criteria will be allowed to enter the run-in.

Visit 1A

For individuals with FEV_1 <80% predicted who performed reversibility at Visit 1 but do not meet the bronchodilator reversal criterion at Visit 1, this visit serves to confirm the diagnosis of asthma.

- Pregnancy test
- Spirometry
- Methacholine Challenge
- Remaining procedures from Visit 1 (See Table 1)

Visit 2.

- Medication withholds will be verified and visits rescheduled if appropriate
- An ACT score will be obtained.
- A pregnancy test will be repeated in women of child-bearing potential.
- Medication use will be reviewed.
- 80mL of peripheral blood will be drawn from the antecubital vein of study participants and used for the biochemical assays and other tests as detailed in Table 1 below.
- Blood will be drawn for Phadiatop testing.
- Spirometry will be performed.
- FeNO will be measured.
- Study participants will supply samples of saliva through passive drooling into test tubes, before and 1 hour after salmeterol administration.

- Study participants will receive 2 puffs of open-label fluticasone/salmeterol (115/21 mcg), and after 1 hour will undergo a SPMCh. Performance of this challenge will follow the steps outlines in the protocol section below (XIX).
- At the time of discharge, study participants will be assigned at random in a 1:1 ratio to receive alendronate 10mg capsules or identical-appearing placebo capsules, with instructions to take once daily for eight weeks.
- All study participants will be supplied with ICS/LABA (250/50 mcg) DPIs and will be asked to continue using ipratropium as-needed for rescue. Albuterol can be used if ipratropium fails to relieve symptoms.
- Phone calls will be made 2 and 4 weeks after randomization to monitor symptoms and address any potential issues or concerns. ACT scores will be obtained during those calls.
- Additional contacts will be made to encourage adherence.

Visit 3.

- Medication withholds will be verified and visits rescheduled if appropriate.
- An ACT score will be obtained.
- A pregnancy test will be repeated in women of child-bearing potential.
- Medication use will be reviewed.
- All biochemical and physiological assays done in Visit 2 will be repeated, including spirometry, FeNO analysis, a blood draw, sampling of saliva before and 1 hour after salmeterol administration, and a SPMCh challenge.
- At the end of this visit, study participants will be asked to discontinue taking study medications and return to home medications, thus concluding the study.

Table 1. Study visit schedule[1].

Visit	1	2	Telephone call #1	Telephone call #2	3
Study week	0	2	4	6	10
Informed Consent	X				
Characterize	X				
Randomize		X			
Clinical					
Full medical history	X				
Body measurements (ht, wt, waist, hip, neck)	X				
Asthma/general questionnaires	X[4]				
Long physical exam	X				
Urine pregnancy test	X	X			X
ACT	X	X	X	X	X

Visit	1	2	Telephone call #1	Telephone call #2	3
Phadiatop		X			
Physiologic					
Spirometry	X	X			X
BDR	X[2]				
MCh	X[2,3]				
SPMCh		X			X
Biochemical assays					
PBMC B2AR (surface)		X			X
PBMC B2AR (total)		X			X
PBMC agonist-stimulated cAMP		X			X
PBMC mRNA		X			X
sAA		X			X
FeNO		X			X
Miscellaneous					
CBC	X[4]				
Calcium	X[4]				
Creatinine	X[4]				
Genotyping (optional)		X			
Adverse Event query		X	X	X	X
Satisfaction Questionnaire					X
Adherence					
Dispense ICS	X[4]				
Dispense ipratropium	X[4]				
Dispense albuterol	X[4]				
Dispense Alendronate or placebo		X			
Dispense ICS + LABA		X			
Record Diskus counter and MEMS cap data (Visit 3 only)		X			X
Review medication use		X	X	X	X

[1] Actual visit times may vary slightly. ACT: Asthma Control Test; PBMC: peripheral blood mononuclear cell; B2AR: Beta-2 adrenergic receptor; BDR: bronchodilator response; cAMP: cyclic adenosine monophosphate; FeNO: Fractional exhaled Nitric Oxide; ICS: inhaled corticosteroid; LABA: long-acting beta-2 adrenergic receptor agonist; MCh: Methacholine challenge; sAA: salivary amylase; SPMCh: Salmeterol-protected methacholine challenge.

[2] Albuterol reversal or methacholine challenge will be performed at Visit 1 for participants with FEV_1 <80%

predicted. If reversal is performed but <12%, methacholine challenge will be performed at Visit 1A.

[3] Methacholine challenge will be performed at Visit 1 for participants with FEV_1 ≥80% predicted.

[4] Done at Visit 1A for participants with FEV1 <80% predicted who do not qualify by BDR at Visit 1 and perform MCh at Visit 1A.

X. RATIONALE

Clinical Variables:

1. Asthma Control Test (ACT) will be used to monitor asthma control throughout the study and will be analyzed as an exploratory outcome.

Physiologic Variables:

1) Spirometry and bronchodilator response. These standard physiologic parameters will be collected to characterize participants during Visit 1. In order to enter the study, participants will be required to have an FEV_1 ≥ 50% of predicted. For those with an FEV1 <80%, a minimum 12% increase in FEV1 post-bronchodilator will be required.

2) MCh PC_{20}. This assay will be an entry criterion to confirm the diagnosis of asthma at the time of screening in those with an FEV_1 ≥80%, using a threshold PC_{20} value of ≤8 mg/mL.

3) SPMCh PC_{20}. Changes in this outcome from randomization to the end of the study will be used to assess the loss of bronchoprotection.

Biologic Variables:

1. Surface ADRB2 density in PBMCs will be determined to test the consequences of LABA treatment and alendronate treatment.

2. Intracellular concentrations of cAMP in PBMCs stimulated with beta-2 adrenergic receptors agonist will assess the functional consequences of LABA treatment and alendronate treatment on ADRB2.

3. Total quantity of ADRB2 protein by Western blot in PBMCs will be assessed to determine the effect of LABA treatment and alendronate on ADRB2 translation.

4. ADRB2 mRNA in PBMCs will be assessed to determine the effect of LABA treatment and alendronate on the regulation of transcription of ADRB2.

5. sAA will be measured as an exploratory outcome to find out whether changes in sAA production track with salmeterol-induced changes in expression of the ADRB2 and bronchial reactivity as determined through SPMCh.

6. FeNO will be analyzed as an exploratory outcome, to investigate whether levels of FeNO predict and/or correlate with the degree of loss of bronchoprotection.

As mentioned in the Background and Significance, we had seen such a correlation in patients not treated with ICS.

XI. SPECIAL CONSIDERATIONS FOR SPECIFIC ASPECTS OF THE STUDY

1. Age of the study population: This study will be conducted in adults because of the unacceptable risk of adverse effects on skeletal maturation in children, and because safety studies have only been performed in adults.

2. Biological Effects: While alendronate is concentrated in bone it produces effects in other tissues. Such effects have been noted in breast cancer. In animals bisphosphonates have been shown to induce apoptosis of breast cancer cells and to reduce visceral metastases[28]. In a study in women with breast cancer, bisphosphonates have also been shown to reduce visceral metastases, suggesting that the biological effects occur at levels of pharmacologic dosing[29]. Additionally, *in vitro* and *in vivo* data show that alendronate is a specific inhibitor of FDPS[21, 30-32] at drug concentrations achieved in plasma with oral administration[33].

3. Choice of tissues for analysis of beta-receptor function: Lymphocyte beta-receptor density has generally been used to assess systemic beta-receptor function[34]. In addition we have added a potential tissue functional marker – salivary amylase.

4. Early disclosure of treatment allocation to women of childbearing potential: The safety of alendronate in pregnancy and lactation has not fully been established. To minimize the potential risk of harm to the fetus and/or newborn, we will ask women of childbearing potential who received alendronate during the study to continue contraception and avoid lactation for 6 months after completion of the study. Because this is a double-blind placebo-controlled study, neither study participants nor investigators will know whether alendronate was taken or not, making women who received placebo needlessly continue contraception or refrain from breastfeeding. To avert that situation, we will disclose treatment allocation to women of childbearing potential when they exit the study through a sealed, confidential envelope. These study participants will be asked to not reveal their treatment to anyone in order to preserve the integrity of the study design and the quality of the data, following precedents from other asthma randomized controlled trials.

XII. STATISTICAL DESIGN AND ANALYSES

A. Randomization

Participants will be randomized in a one-to-one ratio to the alendronate and placebo groups. The only stratification factor will be Clinical Center partnership (nine levels), and there will be permuted blocks of size four within each stratum. When a participant at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will access the AsthmaNet Randomization Module. After entering the participant's pertinent information, the Clinic Coordinator will be asked to verify that all of the entered

information is correct. If so, the Clinic Coordinator will be given an appropriate drug package number for that participant. In order to maintain security of the randomization schedules, DCC data management and coordination staff will receive automatically a notice from the AsthmaNet server that a participant has been randomized.

B. Masking

This will be a double-masked study in which participants and AsthmaNet personnel will be masked with respect to treatment identity.

C. Statistical Analysis Plan

The primary hypothesis is that alendronate prevents the loss of bronchoprotection against methacholine-induced bronchoconstriction that occurs with regular use of ICS/LABA.

The primary outcome for statistical analysis is the change in logarithm base 2 of the PC_{20} from the SPMCh challenge from the time of randomization to the end of the study (clinical researchers refer to this change in the logarithm base 2 of the methacholine PC_{20} as the number of doubling dilutions of methacholine). A blocked analysis of variance (**ANOVA**) will be applied to compare the alendronate and placebo groups, in which the blocks consist of the nine clinical center partnerships in AsthmaNet. The justification for this statistical approach is as follows:

1. The logarithm base 2 of the methacholine PC_{20} is well documented as following a normal distribution[35].
2. A blocked ANOVA applied to the change in the logarithm base 2 of the methacholine PC_{20} from the SOCS trial of the Asthma Clinical Research Network[36] revealed that clinical center effects (the blocks) were nearly statistically significant ($p = 0.07$).

Given that the maximum concentration of methacholine in AsthmaNet studies is 32 mg/ml, it is possible that some methacholine challenges at the end of the study will not lead to a calculated value of the PC20. In such situations, the PC20 is right-censored at 32 mg/ml, so the blocked ANOVA needs to account for these right-censored values. This type of analysis is comparable to a time-to-event analysis (in this case, a concentration-to-event analysis), and can be accommodated by constructing an appropriate likelihood function in SAS PROC NLMIXED.

Treatment $\times$ clinical center partnership interactions will not be included in the primary statistical analysis, but the interactions will be examined qualitatively and graphically in this proof-of-concept study. The treatment differences will be adjusted for the clinical centers in the blocked ANOVA. The adjusted means, and their corresponding standard errors and 95% confidence intervals, will be reported for each treatment group and between the treatment groups.

To test the secondary hypotheses, we will examine the changes in peripheral blood mononuclear cells (**PBMCs**) ADRB2 surface receptor density and PBMC beta-agonist-induced cAMP production after 8 weeks of treatment. The two secondary outcomes are relatively new, but it is anticipated that they will follow lognormal distributions. Therefore,

the secondary outcomes will be analyzed in the same manner as the primary outcome, i.e., a blocked ANOVA to examine the effect of alendronate vs. placebo.

Additional analyses will consist of:
(1) estimating the correlations, and their 95% confidence intervals, between the primary outcome (change in logarithm base 2 of the PC_{20}) and the secondary outcomes across both treatment groups
(2) determining whether baseline FeNO is predictive of the change in logarithm base 2 of the PC_{20}, within each treatment group and across both treatment groups, via the inclusion of FeNO as a covariate in a blocked analysis of covariance (**ANCOVA**).

Intention-to-treat analyses

All of the analyses described above will follow the intention-to-treat paradigm whereby all available data from randomized participants are included in the analyses regardless of information about deviations from study protocol. Per-protocol analyses will be conducted as a secondary set of analyses in order to determine the impact of medication adherence to the study results.

Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses that are planned for the primary and secondary outcomes assume that the data are missing-at-random (MAR). Because we are applying likelihood-based methods for the data adjustment with the primary outcome and for all of the secondary outcomes, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, then we will invoke shared parameter models to simultaneously model the time to drop-out and the individual secondary outcome[37].

Interim Analyses

This is a proof-of-concept study with an eight-week treatment period on a sample size of 76 participants. Therefore, a formal interim analysis is not planned.

D. Power Calculations

Effect Size: As reviewed in the Background and Significance, regular use of beta-agonists decreases the initial protection against provocative stimuli such as allergen, exercise, and methacholine by 50 to 100%. In specific, for methacholine, Yates and colleagues demonstrated a 1.1 doubling dilution decrease in beta-agonist-protected methacholine PC_{20} after one week of adding LABA to ICS therapy[9]. In this proof-of-concept study, we wish to determine whether alendronate will moderate this loss of bronchoprotection. The Steering Committee determined that if alendronate were to be effective, then it should decrease the loss of bronchoprotection by at least 50% of the effect observed by Yates and colleagues. This corresponds to a 0.55 reduction in the loss of bronchoprotection.

Power: The standard deviation of the change in the salmeterol-protected methacholine PC_{20} from the Yates et al study[9] was 0.4, although this standard deviation was based on a two-week treatment period. The standard deviation of the change in the methacholine PC_{20} from the SOCS trial[36] was 1.1, although the SOCS methacholine challenge was not salmeterol-protected and the treatment period endured for 16 weeks. Nevertheless, a standard deviation of 0.75 is used for the ALfA sample size calculation because it lies midway between 0.4 and 1.1. For the blocked ANOVA, homogeneity of variance (square of the standard deviation) across all treatment group $\times$ clinical center partnerships is assumed. In addition, the blocked ANOVA yields a loss of eight degrees of freedom for error due to the blocking effect of the nine clinical center partnerships. Therefore, applying the standard sample size calculation for comparing two groups with respect to a normally-distributed outcome variable, while assuming homogeneity of variance, requires a subtraction of 8 when extending the sample size calculation to the blocked ANOVA.

Based on the data and assumptions described above, a sample size of 76 randomized participants will provide 80% statistical power, allowing for a 10% drop-out rate, to detect a 0.55 doubling dose difference in the salmeterol-protected methacholine PC_{20} between the drug and placebo arms with a two-sided, 0.05 significance level test.

E. Timeline

Each participant will be in the study for 10 weeks. If 5 sites randomize 1 participant per month, total study duration from first participant screened to last participant exited will be 21 months. Nine sites have indicated an interest in participating, so we should be well within the timeline.

XIII. DRUG SUPPLIES

Blinded alendronate sodium 10mg/placebo capsules will be supplied by the AsthmaNet Data Coordinating Center (DCC). Each bottle contains 66 capsules and is labeled with a unique, 3-digit identifier for randomization purposes. The alendronate sodium 10mg/placebo capsules should be stored in a well-closed container at 77°F (25°C) with excursions permitted to 59°-86°F (15-30°C). The storage conditions are included on the product label.

If a participant meets the criteria for randomization at Visit 2, the ALfA randomization module will be accessed to assign one bottle of blinded alendronate sodium 10mg/placebo capsules. The following commercially available, open-label products will also be supplied by the AsthmaNet DCC:

- Fluticasone propionate (Flovent®) 250 mcg/puff 60-count Diskus®
- Fluticasone propionate and Salmeterol (Advair®) 250/50 mcg/puff 60-count Diskus®
- Ipratropium Bromide (Atrovent® HFA) 17mcg/puff 12.9g Inhaler
- Albuterol Sulfate (Ventolin® HFA) 90mcg/puff 8g Inhaler
- Fluticasone propionate and Salmeterol (Advair® HFA) 115/21 mcg/puff Inhaler

Additional information pertaining to the label text and dispensing procedures are included in the ALfA Pharmacy Manual of Operations.

Prednisone is not provided by the AsthmaNet DCC. The site must acquire and dispense per institutional policy/guidelines.

XIV. ADHERENCE AND MONITORING

The following mechanisms will be employed to determine adherence and measure outcomes:

1. Medications: The AsthmaNet Network has explored various published methods of assessing adherence to asthma treatment, including pharmacy records, canister weights, and self-report. No single adherence measure is currently deemed to provide complete accuracy. Each study participant will be dispensed MEMSCap™ medication counters, which, in addition to the Diskus® counter, will be recorded from visit to visit.

2. Inhalation techniques: To minimize the variability in the dose of both the ICS and LABA delivered to the lungs, the participant's inhalation technique will be reviewed at each study visit. Objective feedback will be given to each participant to improve performance.

XV. RECRUITMENT AND FEASIBILITY

This study benefits from the concurrent recruitment for the BARD study. It can use the non-Black participants excluded from BARD, and can use those who fail BARD run-in. Further, based on prior ACRN recruitment in SOCS/SLIC, we predict more than 60% of the patients screened will be eligible. Thus, we should easily exceed a randomization rate of 1 participant/month/center.

We expect the duration of the screening visit to be approximately 2 hours and the randomization and final visits to be approximately 4 hours each. Based on the SOCS/SLIC experience it is likely that >90% of the participants who pass the screening visit will have a SPMCh $PC_{20} \leq 16$mg/mL.

We will use off-label alendronate, ipratropium bromide as rescue inhaler, and methacholine challenges on participants with $FEV_1 \geq 50\%$. An IND has been obtained from the FDA (#123059) for the ALfA study.

We will target enrollment at 50% female and 33% of minority race or ethnicity. It is likely that there will be variation between performance sites in terms of race/ethnicity of participants recruited. However, all attempts will be made to recruit proportionately among sites as much as possible. Methods for accounting for potential asymmetric recruitment are discussed in the section on Statistics. Participants will be recruited from established cohorts, by advertisement, and by physician referral, by the recruitment methods and procedures found effective at the various participating AsthmaNet Centers.

XVI. RISKS AND BENEFITS

Risks and Benefits of Study Procedures

***Venipuncture**:* Blood samples will be obtained by venipuncture of an antecubital vein to obtain peripheral blood mononuclear cells as well as mRNA.

***Risks**:* The risks of venipuncture are minimal. The possible risks include bruising and/or infection at the site of the venipuncture and vasovagal episodes experienced by the blood donors. Pressure will be applied to the venipuncture site to prevent bruising. Aseptic technique will be used to prevent infection. Blood will be obtained while the donors are in a seated position and medical and nursing personnel will be available at the study sites to treat and manage vasovagal episodes.

***Benefits**:* The peripheral blood mononuclear cells are used for assessment of ADRB2 density and signaling function (cAMP production). The RNA will be used to identify differences in gene transcription that provide important insights into potential modifiers of the response to LABAs and alendronate.

The potential benefits justify the potential risks.

Pulmonary function testing (spirometry): Spirometry will be performed to determine the participants' pulmonary function and bronchodilator reversibility.

***Risks**:* The risks of spirometry are minimal. The possible risks include precipitation of bronchospasm and light-headedness from repeated blowing attempts. Medical and nursing personnel and medications will be available at the study sites to treat and manage bronchospasm. Inhalation of a short acting beta-2 adrenergic agonist (albuterol) will be used to assess reversibility. The possible risks of inhaled beta-2 adrenergic agonists include tachycardia and hand tremors.

***Benefits**:* Spirometry with assessment of reversibility to a short acting beta-2 adrenergic agonist will be used to characterize the participants and to determine eligibility for the study.

***The potential benefits justify the potential risks**.*

Methacholine inhalation challenge: Methacholine challenge will be used to assess airway reactivity for study eligibility. Methacholine challenge will also be performed at several times during the study as salmeterol-protected methacholine reactivity is the primary outcome variable of the study.

***Risks**:* The major risk of methacholine challenge is severe bronchoconstriction. As a precaution, participants will not undergo methacholine challenge if their FEV_1 is less than 50% of predicted. Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any bronchoconstriction episodes.

***Benefits**:* There are two benefits to this procedure. First, participants who have an FEV_1 greater than 80% of predicted must demonstrate a positive methacholine challenge test

to be included in the study. Second, for participants who have not previously undergone a methacholine challenge test, they will learn new information about one aspect of their asthma, airway hyper-reactivity.

The potential benefits justify the potential risks.

Saliva collection: Saliva will be collected for the measurement of sAA activity. The saliva will be collected by passive drooling into a plastic collection tube.

Risks: There are no risks anticipated with the collection of saliva.

Benefits: Will allow for the analysis of sAA levels, potentially supporting the use of sAA as a biomarker for loss of bronchoprotection with regular LABA use, ADRB2 expression and function.

The potential benefits justify the potential risks.

Risks of Study Design

Risks*:* Participants in the study have well-controlled asthma on a regular dose of ICS. In the Run-In Period they will be switched to a medium dose of ICS (fluticasone 250 mcg twice daily). Considering the relatively flat nature of the ICS dose-response curve, it is expected that participants will maintain good control. Rescue medications and a treatment action plan will be provided, which will include who to contact. During the randomization phase all participants will receive both an ICS and LABA in a single inhaler, which should provide equal or better asthma control. Again, rescue medication and a treatment plan will be provided, plus regular phone contact will be maintained during the 8-week active study period.

Participants who experience an asthma exacerbation will be treated with prednisone. Participants will be seen within 72 hours (± 48 hours) to ascertain the severity of the event and ensure appropriate treatment.

Benefits*:* There are no direct benefits for participants in this study. However, the information obtained may identify individuals in whom regular use of salmeterol increases their susceptibility to bronchoconstrictor stimuli. They would be informed of this and be counseled.

With multiple safeguards in place and the short study duration, we believe we have designed a study where ***the potential benefits justify the potential risks****.*

Risks and Benefits of Study Drugs

Alendronate: Half of the study participants will receive alendronate at a dose of 10 mg once daily for the 8-week study period.

Risks*:* Generally, alendronate is well tolerated and there is extensive experience with its use and that of other bisphosponates. Adverse effects usually have been mild and include abdominal pain, nausea, dyspepsia, constipation, diarrhea, and flatulence.

Regurgitation, esophageal ulcer, vomiting, dysphagia, abdominal distention, and gastritis also have occurred. Rarely, taste perversion has been reported. The frequency of adverse effects increases with higher dosages. Patients with preexisting esophageal disorders and those who take alendronate with little or no water and lie down immediately following ingestion may be at increased risk for esophageal ulcers.

There have been case reports of people bleeding inside their stomach and gut when taking alendronate. Such problems are rare and it is not clear whether alendronate is the cause. Postmenopausal women who take non-steroidal pain relievers, estrogens, or steroids may be at higher risk for general stomach side effects when taking alendronate. Alendronate also seems to cause more ulcers when people are taking aspirin or non-steroidal pain relievers (for example, Motrin or Aleve). Therefore, patients taking aspirin or non-steroidal pain relievers will not be enrolled in the study. Participants should not take aspirin or non-steroidal pain relievers (for example, Motrin or Aleve) during the study. Instead, participants should use acetaminophen (for example, Tylenol) for pain or fever.

Other side effects include reductions in serum calcium and phosphate levels as a result of the inhibition of bone resorption. These reductions generally have been mild, asymptomatic, and transient. Musculoskeletal side effects include bone, muscle or joint pain in approximately 4% of patients. Severe and occasionally incapacitating bone, joint and/or muscle pain, have been infrequently reported. Localized osteonecrosis of the jaw, generally associated with tooth extraction and/or local infection, often with delayed healing, has been reported rarely.

Headaches occur in fewer than 3% of patients. There are also rare reports of rash, erythema, Stevens-Johnson syndrome, toxic epidermal necrolysis, iritis, scleritis, uveitis, and nonspecific transitory conjunctivitis. Asthma exacerbations are also rare and it is unclear if they are related to the medication.

Benefits: There are no direct benefits to participants in this study. However, alendronate and similar drug are used in this population and the information gained from this study may inform subsequent treatment guidelines.

The benefits justify the risks.

Long-acting beta Agonist (LABA): All study participants will receive the LABA, salmeterol, as part of the ICS/LABA combination (Advair Diskus) 250/50 mcg one puff twice daily, for the 8-week study period.

Risks*:* The use of LABA in the treatment of asthma remains controversial. The SMART study suggested that there may be a risk of life threatening events in patients treated with salmeterol, particularly in Blacks[38]. Nonetheless, treatment with a LABA and an inhaled corticosteroid continues to be an accepted part of treatment for asthma of moderate severity. In addition, the SMART study suggested that the risk of LABA treatment might be diminished by the concomitant use of inhaled corticosteroids. All participants in this study will be receiving concomitant inhaled corticosteroids and we will closely monitor them for worsening asthma control.

Asthma symptoms will be monitored through phone calls and visits to the clinic, as well as by calls to the performance site as needed should safety concerns arise. Medications

and instructions will be given at the beginning of the study by study physicians or study staff so that any asthma attack may be treated promptly.

Other side effects of long-acting beta-agonists (LABA) include tremors, nervousness, rapid heart rate, headaches, dizziness, lightheadedness, sweating, nausea, and less frequently, insomnia, chest pain, and irregular heartbeat. This study hopes to find out if alendronate can reverse the heightened response to bronchoconstrictor stimuli during regular use of a LABA.

Benefits: There are no direct benefits to participants in this study. However, LABAs are frequently prescribed in this population and the information gained from this study may inform subsequent treatment guidelines.

The benefits justify the risks.

Inhaled Corticosteroid (ICS): All study participants will be assigned to receive a moderate dose of ICS. ICS is the standard treatment for chronic persistent asthma and all participants entering the study will already be on a regular dose of ICS. Although the preparation and dose administered during the run-in period and double-blind treatment phase may differ from what a participant was previously taking, the study design is such that the ICS preparation and dose should be within one doubling dose of what the participant has been receiving.

Risks*:* The potential risks of ICS are well-known and include oropharyngeal candidiasis, thinning of skin, osteoporosis, and cataracts. These risks are already known to the individuals participating in this research study and are outweighed by the beneficial effects of ICS for treatment of their asthma.

Benefits: Continued use of an ICS is necessary for maintaining control of their asthma.

The benefits justify the risks.

Short-acting Muscarinic Antagonist (SAMA): All study participants will be assigned to receive the SAMA ipratropium (Atrovent® HFA), an anticholinergic bronchodilator that is FDA approved for the treatment of chronic bronchitis, emphysema and chronic obstructive pulmonary disease (COPD). Although ipratropium has not been FDA-approved for use in asthma, it is widely used for asthma, and an NIH Task Force[39] and US and International guidelines[40] all recommend ipratropium in this dose for characterization of asthma. Ipratropium will be the first rescue bronchodilator used to treat increased symptoms during the Run-In and treatment period to minimize the confounding effect albuterol, a short-acting beta2 agonist, may have on alendronate-induced protection in the setting of regular LABA use. If symptoms do not improve with ipratropium therapy, albuterol will then be used (see section XVII).

Risks: Ipratropium has a well-established safety profile in COPD and substantial use in patients with asthma. The most common adverse events are upper respiratory infection, headache, dry mouth, taste perversion, hypotension, palpitations, urinary retention, tachycardia, constipation and paradoxical bronchospasm.

Ipratropium should not be taken by patients with narrow angle glaucoma, prostatic hypertrophy, bladder-neck obstruction, or renal insufficiency. There are no data to indicate that ipratropium increases the risk of stroke, heart attack, and death in patients with asthma or COPD. Participants with history of urinary retention, elevated intraocular pressure, and significant cardiovascular disease will be excluded from the study.

Benefits: Ipratropium is an effective short-acting bronchodilator in patients with asthma Although not approved as a treatment for asthma, ipratropium is an effective short-acting bronchodilator in patients with asthma[41].

As the risks are small and well-known, while the benefits as a bronchodilator are substantial, ***the potential benefits justify the risks.***

XVII. ADVERSE EVENTS

Definition and reporting

Participants are at risk of developing adverse events during study enrollment. A clinical adverse event is any unintended worsening in the structure (signs) or function (symptoms) of the body, whether or not considered to be study-related. This includes any side effect, injury, or sensitivity reaction, as well as any intercurrent event. An adverse event is deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

Documentation of an adverse event will be recorded on the Clinical Adverse Event Report Form and will include the following information: Description of the condition, dates of condition, treatment of condition (e.g., medications, doses, dates), whether hospitalization or emergency treatment was required, treatment outcome, relationship of the adverse event to the study medication(s), and severity of the event.

Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the participant is no longer able to effectively participate in the study. Participants experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

Termination criterion for study subjects requiring systemic corticosteroids for reasons other than asthma exacerbations: If any participant receives systemic corticosteroids after the first 4 weeks of blinded treatment, their participation will be extended so that the final visit occurs at least 4 weeks after they complete this treatment. If this extension goes beyond 12 weeks of alendronate, their participation in the study will be terminated.

Adverse Events Related to Asthma: Asthma Exacerbation

Since participants have well-controlled asthma and will receive regular ICS corticosteroids during the 2-week run in period and the 8-week intervention period, we anticipate that asthma exacerbations will be rare. Between in-person study visits participants will be contacted by telephone by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the participant. If, between phone contacts or in-person visits, an asthma exacerbation occurs, the participant should contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as quickly as possible (within 72 hours) for initiation of rescue prednisone. Extra phone visits will be conducted to monitor exacerbation recovery.

Asthma exacerbations will be defined as:

- the development of an increase in symptoms of cough, phlegm/mucus, chest tightness, wheezing or shortness of breath in association with one or more of the following

    1. an increase in rescue ipratropium or albuterol of ≥ 8 inhalations/day over baseline use for a period of 48 hours or ≥ 16 actuations per 24 hours, with baseline defined for the period between V2 and V3 as the average daily use of ipratropium during the week prior to randomization; baseline for the run-in phase will be defined as the average daily use of home rescue inhaler during the week prior to V1.

    2. a fall in FEV_1 to < 80% of baseline (visit 1)

    3. FEV_1 < 45% predicted

    4. if a participant receives systemic corticosteroids for an exacerbation.

- Participants who are potentially experiencing an exacerbation will be instructed to contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as rapidly as possible.

AsthmaNet rescue algorithms for participants with exacerbations of asthma are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma[39]:

Home care of exacerbations: Asthma exacerbations will be identified by the criteria described above. Participants will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity. Participants who recognize an exacerbation will be instructed to use ipratropium by MDI, 4 puffs initially, then 2 puffs every 20 min up to 60 minutes, if needed, and then every 4 hours, or less, if needed to reduce symptoms. If symptoms are not improved after the first 60 minutes of ipratropium therapy, the participant will be instructed to use the same treatment scheme substituting open-label albuterol rescue MDI for the ipratropium bromide rescue. If

symptoms are not improved after the first 60 minutes of albuterol therapy, the participant should contact the study coordinator, investigator, their primary physician, or seek care in the emergency department.

*Physician's Office or Emergency Room Treatment of exacerbations***:** Participants will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the participant's PEF or FEV_1 are less than 25% predicted or if the participant shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained. When treated in the physician's office or the hospital emergency room, participants should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEF increases to ≥70% of predicted (or of known best PEF value) after the first 60-90 min, the participant can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy. If symptoms persist and PEF remains <70% baseline, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (e.g. prednisone 40 mg orally; methylprednisolone 40 mg IV bolus). Monitoring of PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding participant disposition. If PEF increases to ≥70% baseline within 4 hours, the participant can be discharged to continue treatment at home. Home treatment should include a 5-day course of prednisone (see below). If PEF remains >40% but <70%, an individualized decision should be made to hospitalize the participant for more aggressive therapy or to continue therapy at home with a course of prednisone. If PEF is ≤40% baseline after repeated albuterol treatments, the participant should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

Prednisone Treatment: In this protocol, prednisone will be used when acute exacerbations cannot be controlled by increased ipratropium or albuterol therapy alone. The dose of prednisone used during an acute exacerbation shall consist of 40 mg as a single oral dose every day for 5 days. The decision to initiate or to continue a course of prednisone beyond 5 days is left to the discretion of the physician.

Termination criteria for study subjects experiencing asthma exacerbations requiring prednisone:

- If any participant receives prednisone for an asthma exacerbation after the first 4 weeks of blinded treatment, their participation will be extended so that the final visit occurs at least 4 weeks after they complete their prednisone burst. If this extension goes beyond 12 weeks of alendronate, their participation in the study will be terminated.
- If a participant requires a second burst of prednisone while in the treatment phase, his or her participation in the study will be terminated.

XVIII. SAFETY MONITORING

A Data and Safety Monitoring Board (DSMB) has been established for this study to monitor data and oversee participant safety. The DSMB consists of physicians skilled in both pediatric and adult asthma management, asthma pharmacology, and/or asthma clinical research, as well as a statistician and a bioethicist experienced in clinical trials. The Study Chair, the Director and a senior staff member of the Data Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the AsthmaNet Manual of Operations.

The current study will request DSMB review of study data every 6 months. The DSMB will assess the following:

- Study performance, including assessment of performance sites' adherence to protocol, adequate participant accrual, and quality control of data collection and management

- Adverse event reports. These data will be presented to the DSMB in a fashion blinded to treatment group assignment. However, the DSMB will have the option of unblinding when and if this action is deemed appropriate. Reports of serious adverse events will also be summarized. The DSMB will be notified within 72 hours of any serious adverse events that are unexpected and deemed related to the study procedures or drugs.

Serious adverse events

A serious adverse event is defined as any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly/birth defect or other medically important condition. A life-threatening event is one in which, in the study physician's opinion, the participant was at immediate risk of death from the reaction as it occurred. Although not unexpected as an outcome in asthma clinical trials, hospitalizations for asthma will be included in the listing of adverse events as identified in the AsthmaNet Network Manual of Operations. Summary reports of the DSMB's review of serious adverse events will be distributed to each AsthmaNet PI by the DCC within 30 days following each DSMB meeting. The Summary Reports will include the following: a statement that a DSMB review of the data and outcomes across all performance sites took place on a given date; a summary of the DSMB review of the cumulative serious adverse events without specific disclosure by treatment group unless safety considerations require such disclosure; and the DSMB's conclusion with respect to progress or need for potential protocol modification. The AsthmaNet PIs are required to forward the Summary Reports to their local IRBs.

Cost, Liability and Payment

All tests will be performed without cost to the participants. Since this is a trial comparing established asthma treatments, liability for participant care costs incurred by participants during the course of the trial will, in most cases, be borne by the participant or their insurer. Details of the NIH policies concerning this issue can be found in NIH

Documents #5305 and 6352-2, Research Participant Care Costs Supported Agreements, in the AsthmaNet Network Manual of Operations. Each participant will be paid a specified amount for study reimbursement that will be equivalent across clinical center partnerships. For participants who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

XIX. METHODS AND STUDY PROTOCOLS

SPMCh

- Study participants who meet the inclusion and exclusion criteria for randomization will undergo SPMCh during Visit 2, and again after the 8 week treatment period during Visit 3. If participant has not withheld ipratropium or ICS/LABA as required prior to Visits 2 or 3 for SPMCh, participants will be asked to reschedule the visit.
- The day of the SPMCh, participants will receive 2 puffs of open-label fluticasone/salmeterol (115/21 mcg), and after 1 hour will undergo a SPMCh. Increasing concentrations of methacholine will be delivered until the FEV_1 falls by 20% or more compared to baseline. The Provocative Concentration that causes a 20% fall in FEV_1 is referred to as a PC_{20}. When a PC_{20} is achieved (20% or greater reduction in FEV_1), the challenge is stopped. If the FEV_1 reduction is exactly 20%, the PC_{20} is equal to the last concentration of methacholine that was administered. If the reduction is greater than 20%, the PC_{20} is calculated from the last two concentrations administered. Since it is uncommon that a particular concentration causes exactly a 20% reduction in FEV_1 the PC_{20} is usually a calculated value.
- The inhalation method used will be the dosimeter method, which uses an electronically controlled valve (Salter Labs dosimeter) to deliver a controlled amount of methacholine from a nebulizer to be inhaled during the course of an inspiratory capacity breath. Doses are followed by a short waiting period and then spirometry. Increasing concentrations of methacholine are administered until a PC_{20} is met or the maximum concentration is given.
- Study participants will inhale increasing concentrations of methacholine for a maximum of 11 steps, starting with diluent control, followed by 0.0625mg/mL and increasing by doubling dose dilutions up to 32mg/mL.
- The PC_{20} will be calculated by plotting concentrations of methacholine on a log scale along with their associated FEV_1, and interpolating the change between the last two concentrations.
- All study sites will follow the same manual of operations allowing for standardization.

Blood sample preparation

- 80mL of blood from study participants' antecubital vein will be drawn into BD Vacutainer CPT tubes, allowing for the isolation of peripheral blood mononuclear

cells with centrifugation.

Radioligand binding assay for ADRB2

- ADRB2 density will be determined using iodinated radioligand (I-^{125} cyanopindolol, Fisher Scientific) on peripheral blood mononuclear cells[39].

RNA extraction and Quantitative Real-time RT-PCR Analysis

- RNA from peripheral blood mononuclear cells will be extracted using the Qiagen RNeasy Mini Kit according to the manufacturer's protocol. Quantitative real-time RT-PCR (qPCR) analyses will be done using the *ΔΔCT* method, using β-actin as internal control.

Protein extraction and Immunoblot Analysis

- Peripheral blood mononuclear cells will be lysed using an immunoprecipitation kit from Roche Applied Sciences. 60µg of total protein will separated by SDS-PAGE electrophoresis, followed by immunoblotting and detection using anti-ADRB2 antibodies from Santa Cruz, with anti-β-actin as positive control.

cAMP Assay

- Peripheral blood mononuclear cells cAMP concentrations will be measured using an assay kit from Biovision, using IBMX to prevent cAMP breakdown by phosphodiesterases, isoproterenol as a ADRB2 agonist, and forskolin as a positive control.

XX. REFERENCES

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Instruct patients not to take alendronate sodium at bedtime or before arising for the day. Patients should be informed that failure to follow these instructions may increase their risk of esophageal problems.

Instruct patients that if they develop symptoms of esophageal disease (such as difficulty or pain upon swallowing, retrosternal pain or new or worsening heartburn) they should stop taking alendronate sodium and consult their physician.

If patients miss a dose of once weekly alendronate sodium, instruct patients to take one dose on the morning after they remember. They should not take two doses on the same day but should return to taking one dose once a week, as originally scheduled on their chosen day.

Manufactured for: Northstar Rx LLC
Memphis, TN 38141
Toll Free: 1-800-206-7821

Manufactured by: Aurobindo Pharma Limited
Hyderabad-500 072, India

M.L.No.: 19/HD/AP/95/F/R

Revised: 02/2014

MEDICATION GUIDE

Alendronate Sodium Tablets, USP
(a len'droe nate soe'dee um)

Read the Medication Guide that comes with alendronate sodium tablets before you start taking them and each time you get a refill. There may be new information. This Medication Guide does not take the place of talking with your doctor about your medical condition or treatment. Talk to your doctor if you have any questions about alendronate sodium tablets.

What is the most important information I should know about alendronate sodium tablets?

Alendronate sodium tablets can cause serious side effects including:

1. Esophagus problems
2. Low calcium levels in your blood (hypocalcemia)
3. Bone, joint, or muscle pain
4. Severe jaw bone problems (osteonecrosis)
5. Unusual thigh bone fractures

1. Esophagus problems.

Some people who take alendronate sodium tablets may develop problems in the esophagus (the tube that connects the mouth and the stomach). These problems include irritation, inflammation, or ulcers of the esophagus which may sometimes bleed.

- **It is important that you take alendronate sodium tablets exactly as prescribed to help lower your chance of getting esophagus problems. (See the section "How should I take alendronate sodium tablets?")**
- **Stop taking alendronate sodium tablets and call your doctor right away if you get chest pain,**

new or worsening heartburn, or have trouble or pain when you swallow.

2. Low calcium levels in your blood (hypocalcemia).

Alendronate sodium tablets may lower the calcium levels in your blood. If you have low blood calcium before you start taking alendronate sodium tablets, they may get worse during treatment. Your low blood calcium must be treated before you take alendronate sodium tablets. Most people with low blood calcium levels do not have symptoms, but some people may have symptoms. Call your doctor right away if you have symptoms of low blood calcium such as:

- Spasms, twitches, or cramps in your muscles
- Numbness or tingling in your fingers, toes, or around your mouth

Your doctor may prescribe calcium and vitamin D to help prevent low calcium levels in your blood, while you take alendronate sodium tablets. Take calcium and vitamin D as your doctor tells you to.

3. Bone, joint, or muscle pain.

Some people who take alendronate sodium tablets develop severe bone, joint, or muscle pain.

4. Severe jaw bone problems (osteonecrosis).

Severe jaw bone problems may happen when you take alendronate sodium tablets. Your doctor should examine your mouth before you start alendronate sodium tablets. Your doctor may tell you to see your dentist before you start alendronate sodium tablets. It is important for you to practice good mouth care during treatment with alendronate sodium tablets.

5. Unusual thigh bone fractures.

Some people have developed unusual fractures in their thigh bone. Symptoms of a fracture may include new or unusual pain in your hip, groin, or thigh.

Call your doctor right away if you have any of these side effects.

What are alendronate sodium tablets?

Alendronate sodium tablets are a prescription medicine used to:

- Treat or prevent osteoporosis in women after menopause. It helps reduce the chance of having a hip or spinal fracture (break).
- Increase bone mass in men with osteoporosis.
- Treat osteoporosis in either men or women who are taking corticosteroid medicines.
- Treat certain men and women who have Paget's disease of the bone.

It is not known how long alendronate sodium tablets work for the treatment and prevention of osteoporosis. You should see your doctor regularly to determine if alendronate sodium tablets are still right for you.

Alendronate sodium tablets are not for use in children.

Who should not take alendronate sodium tablets?

Do not take alendronate sodium tablets if you:

- Have certain problems with your esophagus, the tube that connects your mouth with your stomach
- Cannot stand or sit upright for at least 30 minutes
- Have low levels of calcium in your blood
- Are allergic to alendronate sodium tablets or any of their ingredients. A list of ingredients is at the end of this leaflet.

What should I tell my doctor before taking alendronate sodium tablets?

Before you start alendronate sodium tablets, be sure to talk to your doctor if you:

- Have problems with swallowing
- Have stomach or digestive problems
- Have low blood calcium
- Plan to have dental surgery or teeth removed
- Have kidney problems
- Have been told you have trouble absorbing minerals in your stomach or intestines (malabsorption syndrome)
- Are pregnant, or plan to become pregnant. It is not known if alendronate sodium tablets can harm your unborn baby.
- Are breastfeeding or plan to breastfeed. It is not known if alendronate sodium passes into your milk and may harm your baby.

Especially tell your doctor if you take:

- antacids
- aspirin
- Nonsteroidal Anti-Inflammatory (NSAID) medicines

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements. Certain medicines may affect how alendronate sodium tablets work.

Know the medicines you take. Keep a list of them and show it to your doctor and pharmacist each time you get a new medicine.

How should I take alendronate sodium tablets?

- Take alendronate sodium tablets exactly as your doctor tells you.
- **Alendronate sodium tablets work only if taken on an empty stomach.**
- Take alendronate sodium tablets, **after** you get up for the day and **before** taking your first food, drink, or other medicine.
- Take alendronate sodium tablets while you are sitting or standing.
- **Do not chew or suck on a tablet of alendronate sodium.**
- Swallow alendronate sodium tablet with a full glass (6 to 8 oz) of plain water only.
- **Do not** take alendronate sodium tablets with mineral water, coffee, tea, soda, or juice.
- If you take **Alendronate Daily**:
  - Take 1 alendronate tablet one time a day, every day **after** you get up for the day and **before** taking your first food, drink, or other medicine.

- If you take **Once Weekly alendronate sodium tablets**:
  - Choose the day of the week that best fits your schedule.
  - Take 1 dose of alendronate sodium tablet every week on your chosen day **after** you get up for the day and **before** taking your first food, drink, or other medicine.

After swallowing alendronate sodium tablet, wait at least 30 minutes:
- Before you lie down. You may sit, stand or walk, and do normal activities like reading.
- Before you take your first food or drink except for plain water.
- Before you take other medicines, including antacids, calcium, and other supplements and vitamins.

Do not lie down for at least 30 minutes after you take alendronate sodium tablets and after you eat your first food of the day.

If you miss a dose of alendronate sodium tablets, do not take it later in the day. Take your missed dose on the next morning after you remember and then return to your normal schedule. Do not take 2 doses on the same day.

If you take too much alendronate sodium, call your doctor. Do not try to vomit. Do not lie down.

What are the possible side effects of alendronate sodium tablets?

Alendronate sodium tablets may cause serious side effects.
- See **"What is the most important information I should know about alendronate sodium tablets?"**

The most common side effects of alendronate sodium tablets are:
- Stomach area (abdominal) pain
- Heartburn
- Constipation
- Diarrhea
- Upset stomach
- Pain in your bones, joints, or muscles
- Nausea

You may get allergic reactions, such as hives or swelling of your face, lips, tongue, or throat.

Worsening of asthma has been reported.

Tell your doctor if you have any side effect that bothers you or that does not go away.

These are not all the possible side effects of alendronate sodium tablets. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How do I store alendronate sodium tablets?
- Store alendronate sodium tablets at room temperature, 20° to 25°C (68° to 77°F).

- Keep alendronate sodium tablets in a tightly closed container.

Keep alendronate sodium tablets and all medicines out of the reach of children.

General information about the safe and effective use of alendronate sodium tablets.

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. Do not use alendronate sodium tablets for a condition for which it was not prescribed. Do not give alendronate sodium tablets to other people, even if they have the same symptoms you have. They may harm them.

This Medication Guide summarizes the most important information about alendronate sodium tablets. If you would like more information, talk with your doctor. You can ask your doctor or pharmacist for information about alendronate sodium tablets that is written for health professionals.

For more information, call 1-800-206-7821.

What are the ingredients in alendronate sodium tablets?

Active ingredient: alendronate monosodium salt trihydrate

Inactive ingredients: corn starch, magnesium stearate, microcrystalline cellulose, povidone, and sodium starch glycolate.

This Medication Guide has been approved by the U.S. Food and Drug Administration.

Manufactured for: Northstar Rx LLC
Memphis, TN 38141
Toll Free: 1-800-206-7821

Manufactured by: Aurobindo Pharma Limited
Hyderabad-500 072, India

M.L.No.: 19/HD/AP/95/F/R

Revised: 02/2014

PACKAGE LABEL-PRINCIPAL DISPLAY PANEL - 10 mg (30 Tablets Bottle)

Rx only
NDC 16714-631-01
Alendronate Sodium
Tablets, USP 10 mg*
PHARMACIST: Dispense the accompanying
Medication Guide to each patient.
30 Tablets
Northstar Rx LLC

AsthmaNet	ALfA DAILY ACTIVITIES (Visit 2-3)	Part. ID: 9 - __ __ __ - __ __ __ Visit: __ Visit Date: __ __ / __ __ / 20 __ __

When you first awake (between 5 AM and Noon)…

- Take [ ] from your study capsule vial.
  - You must take capsule just after you get out of bed in the morning, before you eat or drink anything.
  - Swallow capsule with a full glass (6 to 8 ounces) of plain water. Never take capsule with tea, coffee, juice, milk, mineral water, sparkling water, or anything other than plain water.
  - For at least 30 minutes after taking capsule, do not eat, drink or take medication, and do not lie down.
  - Sit upright or stand upright until at least 30 minutes have passed since taking capsule and you have eaten your first food of the day.
- Take [ ] from your Advair® Diskus® ***at least 30 minutes after taking study capsule***.
  - Exception: Do not take Advair® the morning of Visit 3.

Before you go to bed (between 5 PM and 1 AM)…

- Take [ ] from your Advair® Diskus®.
- Record your daily rescue inhaler puffs (green RESCUE1, blue RESCUE2) on your Asthma Monitoring Log. Do not include preventive puffs.

Call the study coordinator…

- If your asthma gets worse (coughing, wheezing, having trouble breathing, etc.) and using your rescue inhalers (green RESCUE1, blue RESCUE2) as described in the handout "If Your Asthma Gets Worse" do not relieve symptoms.
- If you have used at least _____ puffs/day for the past 2 days from your rescue inhalers (total of green RESCUE1 and blue RESCUE2 combined).
- If you have used at least 16 puffs/day from your rescue inhalers (total of green RESCUE1 and blue RESCUE2 combined).