Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
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1. eMethods 1: Definitions

Exacerbation: Exacerbation was defined as an event of worsening asthma requiring oral or parenteral corticosteroids, or an asthma-related hospitalization.

Deterioration: Deterioration was defined as an episode of worsening asthma symptoms that did not reach the level of an exacerbation, but for which the patient increased any of his/her asthma-related medications for 2 or more days.

2. eMethods 2: Maximum bronchodilator reversibility; statistical analysis; other genetic analyses

Maximum Bronchodilator reversibility

Since many of the subjects enrolled were already on LABA/ICS at baseline, we were unable to assess classic bronchodilator reversibility without a withhold of subjects’ long acting bronchodilator at the baseline visit. Thus, we examined the “maximal achievable forced expiratory volume in 1 second (FEV₁)” following 4 puffs of albuterol at the baseline visit and at the end of the study. On visit two we assessed bronchodilator response after a withhold to obtain additional characterization information.

Statistical analyses

The primary analysis of the primary outcome of time to asthma exacerbation during follow-up was analyzed using a log-rank test. If a patient experienced more than one asthma exacerbation, the time to the first exacerbation was used. For the primary analysis, patients discontinuing the study without the event of interest were censored at the time of their discontinuation. For patients who missed their last study visit, were not lost to follow up, and did not report an exacerbation, the last time at which they were known not to have experienced an exacerbation (last questionnaire, visit, or contact) was used as their end of follow-up. A sensitivity analysis was conducted adapting the pattern-mixture idea with offset described by Little et al. (Little RJ, D’Agostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. The New England journal of medicine. 2012;367(14):1355-1360.). A simple parametric model (exponential or similar) was fit to the complete case data to calculate distributional parameters separately for each treatment group. Then these parameters were varied in the two treatment groups and time-to-event was simulated for subjects with incomplete follow-up.

A secondary analysis compared the two treatment arms after adjusting for age, gender, baseline FEV₁, and geographic location. Cox proportional hazards regression was employed. Interaction of treatment and geographic location, treatment and baseline age, and treatment and baseline body mass index (BMI) was tested to assess effect size modification.

Another secondary analysis of the primary outcome focused on the annualized mean rate of exacerbations and was based on a Poisson regression model with time as an offset. All exacerbations experienced by each patient during follow-up were in this analysis. However, if a patient experienced the onset of a second exacerbation within 21 days of the onset of the first exacerbation, this second event was not counted. The onset date of the exacerbation was the date steroids or a hospitalization started. Additional secondary analysis was performed adjusting for age, gender, baseline FEV₁, and geographic location.
Changes from baseline FEV\textsubscript{1}, percent FEV\textsubscript{1} and questionnaire (Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, asthma symptom-free days and Asthma Symptom Utility Index) mean outcomes were compared between the two treatments using a linear mixed effect model for repeated measurements. The model used questionnaire data from all available assessments. Linear and quadratic terms for the assessment of time, treatment, the interaction of treatment and the two time variables, age, gender, FEV\textsubscript{1}, and geographic location were included as fixed effects in the model. The intercept and linear and quadratic time variables were included as independent random effects. The significance of either of the two interaction terms was used to determine if treatment effect varied during the course of the treatment. For the purpose of this analysis, questionnaires with less than two-thirds of questions answered were considered not valid and treated as missing.

A 2-sided $\alpha = .05$ was selected for statistical significance for analyses of outcomes.

**Other Genetic Analyses**

Genetic analyses of FEV\textsubscript{1}

The primary hypothesis of this aim is that Arg/Arg homozygotes in the Tio/ICS arm will demonstrate higher FEV\textsubscript{1} as compared to Arg/Arg homozygotes in the LABA/ICS arm. Linear mixed model was employed to estimate changes in FEV\textsubscript{1} in the four treatment-genotype groups. Change was defined as the difference from the baseline value. The model considered the absolute values of FEV\textsubscript{1} from all available assessments, and the predictors (included as fixed-effects) included continuous time of assessment as a linear and quadratic term, treatment indicator, genetic indicator (Arg/Arg vs. Arg/Gly and Gly/Gly), and the interaction of the treatment, genetic group and the two time variables. Independent random-effects were included for intercept and linear and quadratic time. The model included covariate adjustment for age, gender, height and height$^2$. Only FEV\textsubscript{1} values that passed the quality control check were used in the analysis. The significance of either of the three way interaction terms were used to determine if the treatment effect over time differed across the four genetic sub-groups. To confirm that the recessive model was the most appropriate, we contrasted these associations with those under additive (using an ordinal variable coded 0, 1, or 2 corresponding to the number of Arg alleles) and dominant (Gly/Gly vs. Arg/Arg and Arg/Gly) models.
3. eMethods 3: Inclusion/Exclusion Criteria

Inclusion Criteria

Subjects meeting all of the following criteria could have been included:

1. Black (self-identified, with at least one biological parent identified as Black).
2. Subjects ages 18-75.
3. Ability to provide informed consent.
4. Clinical history consistent with asthma for > 1 year.
5. Ability to perform pulmonary function tests.
6. FEV$_1$ $\geq$ 40% of predicted.
7. Receiving inhaled corticosteroids (ICS)/long-acting beta-agonists (LABA) combination therapy, or moderate to high dose ICS monotherapy and baseline Asthma Control Questionnaire (ACQ) $>$ 1.25.
8. Non-smoker for past year (total lifetime smoking history < 10 pack-years). (While smoking is prevalent in asthma, we excluded smokers from participating in this study to avoid confounding with COPD, and perhaps a possible bias towards tiotropium.)

Exclusion Criteria

Subjects meeting any of the following criteria were to be excluded:

1. Use of greater than the equivalent of 1000 mcg inhaled fluticasone daily.
2. Chronic use of oral corticosteroids or Anti-IgE for asthma.
3. Lung disease other than asthma or diagnosis of vocal cord dysfunction.
4. Significant medical illness (other than asthma) that is not stable.
5. Pregnancy or lactation or an unwillingness to maintain effective birth control.
6. History of a significant exacerbation of asthma or respiratory tract infection in the prior 4 weeks.
7. History of life-threatening asthma requiring treatment with intubation and mechanical ventilation within 5 years.
8. Hypo sensitization therapy other than an established maintenance regimen.
9. Use of inhaled anticholinergic therapy (ipratropium, tiotropium) in prior month.
10. Known contraindication to inhaled tiotropium e.g. narrow angle glaucoma, history of bladder neck obstruction or significant symptoms related to prostatic hypertrophy.
11. Inability to speak and read English.
4. eMethods 4: Asthma Exacerbation Questionnaire.

The Asthma Exacerbation Questionnaire was developed specifically for the BELT study:

BELT
Asthma Exacerbation Questionnaire

Date: ___ ___/ ___ ___/ ___ ___

1. During the past month have you had worsening of your asthma that required steroids (pill or shot, for example, prednisone), or a visit to the doctor’s office, the emergency room or the hospital?
   - □ 1 Yes
   - □ 2 No

2. During the past month have you had worsening of your asthma (attack or flare with increased symptoms), and/or increased reliever /rescue/bronchodilator use (e.g., Ventolin, Proventil or Albuterol) that lasted for 2 days or more, but didn't require steroids (pill or shot) or a visit to the doctor’s office, the emergency room or the hospital?
   - □ 1 Yes
   - □ 2 No

3. During the past month have you had to stop taking any of your asthma medications due to side effects or problems?
   - □ 1 Yes
   - □ 2 No

4. During the past month have you, your doctor or other provider had to change your asthma medications because of:
   a. Problems with the medicine?
      - □ 1 Yes
      - □ 2 No
   OR
   b. New or continuing asthma symptoms?
      - □ 1 Yes
      - □ 2 No
In this study, you are receiving two different medications, a) an inhaled steroid and b) a study medication.

5. The next two questions relate to the use of your inhaled steroid medication (Flovent (fluticasone) or Asmanex (mometasone) or Pulmicort (budesonide) or Qvar (beclamethasone))
   a. In the past two weeks how often have you used your inhaled steroid medication?
   □₁ Not at all
   □₂ A few days (1-3 days)
   □₃ Several days (4-7 days)
   □₄ Most days (8-11 days)
   □₅ Almost every single day (12-13 days)
   □₆ Every single day
   b. In the past two weeks, how many times a day, on average, did you use your inhaled steroid medication?
   □₁ 2 times
   □₂ 1 time
   □₃ Not at all

6. The next two questions relate to the use of your prescribed study medication (Serevent (salmeterol) or Foradil (formoterol) or Spiriva (tiotropium)).
   a. In the past two weeks how often have you used your prescribed study medication?
   □₁ Not at all
   □₂ A few days (1-3 days)
   □₃ Several days (4-7 days)
   □₄ Most days (8-11 days)
   □₅ Almost every single day (12-13 days)
   □₆ Every single day
   b. In the past two weeks, how many times a day, on average, did you use your prescribed study medication?
   □₁ 2 times
   □₂ 1 time
   □₃ Not at all
5. eResults 1. Details of Asthma-related Deaths

Asthma death #1:

A 67 year old African American woman with asthma, diabetes, nephrotic syndrome and nephritis was enrolled in the BELT trial on July 1, 2011 and randomized to the tiotropium (18 mcg once daily) plus ICS (fluticasone propionate 250 mcg twice daily) arm. Previous asthma therapy was Advair Diskus 100/50 twice daily plus albuterol prn. At time of enrollment the patient’s Asthma Control Questionnaire score was 2.5. Baseline concomitant medications were reported as tiotropium, fluticasone 100 mcg and albuterol. All asthma medications were discontinued at the time of enrollment other than the study medications and albuterol prn. The patient died at home on November 22, 2011, 3 days after discharge from a 3 day hospitalization for shortness of breath deemed to be secondary to an asthma exacerbation. During hospitalization the patient was given low flow oxygen therapy, oral steroids and repeated nebulizer treatments with Duo Nebs. After discharge the patient refused all medications, leaving them with her granddaughter. The patient had been known to be non-compliant with her study medications filling the tiotropium and inhaled corticosteroid only on July 1, 2011 and August 8, 2011. On November 14, 2011 the patient returned to the pharmacy to obtain albuterol and refused her study medications but did obtain serevent from a previous prescription. Despite calls from the pharmacist and the local study coordinator, the patient refused any further medications. On November 22, 2011 the patient was visited by her son who stated that she appeared to be asleep with her oxygen tubing in her nose. He returned on November 23, 2011 to find that his mother was on the couch in a sitting position with the tubing still in place but was unresponsive and cold to the touch. No autopsy was performed and final cause of death was profound hypoxemia was the immediate primary cause of death with severe bronchial asthmatic exacerbation and medical non-compliance are secondary causes.

Asthma death #2:

A 47 year old African-American male with asthma, and environmental allergies was enrolled in the BELT trial on May 29, 2012 and randomized to the tiotropium (18 mcg once daily) plus ICS (budesonide 360 mcg twice daily) arm. Previous asthma therapy was Symbicort 160/4.5 twice daily, montelukast once daily, Zileuton once daily, theophylline in an unknown dosage daily, and albuterol prn. All asthma medications were discontinued at the time of enrollment other than the study arm medications and albuterol prn. At the time of enrollment the patient’s Asthma Control Questionnaire score was 5.3. Baseline concomitant medications were reported to be ziprasidone, fluoxetine and alprazolam each once daily. From pharmacy records, the patient appeared to refill the study medications on 11 of 13 possible opportunities. On January 4, 2013 the patient “stopped breathing” due what is wife reported as an “asthma attack”. She called an ambulance. The patient was resuscitated and transported to a hospital emergency department where he had a Glasgow coma score of 5 on a respirator. He was admitted to the hospital intensive care unit with a diagnosis of acute respiratory failure and cardiac arrest. The patient was pronounced dead on January 11, 2013 having never had any change in neurological or respiratory status with the immediate and primary cause of death reported as “acute cardiopulmonary failure”.

Asthma death #3 (non-asthma related):

A 47 year old African American female with asthma, hypertension and cardiovascular disease was enrolled in the BELT trial on December 21, 2011 and was randomized to the tiotropium (18 mg once daily) plus ICS (fluticasone propionate 250 mcg twice daily. Previous asthma therapy was Advair Diskus 250/50 twice daily plus albuterol prn. All asthma medications were discontinued at the time of enrollment other than the study medications and albuterol prn. The patient was admitted the local hospital on April 6, 2012. Hospital based medical history from admission states that the patient reported a 2-3 day history of
having abdominal distention, shortness of breath and severe headaches and admission BMI was 35.4. On admission her blood pressure was 283/154 and she was stated to have “volume overload”. Admission blood work included a troponin of 0.31 (elevated), a serum creatinine of 1.9, and a brain natriuretic peptide level of 582 (markedly elevated). The patient was seen by the cardiology consultation service and transferred to the cardiology service. Electrocardiogram showed a normal sinus rhythm, with left atrial enlargement and poor R-wave progression from leads V1-V6 that was stated to be consistent with morbid obesity and body habitus. Patient was transferred to the ICU and had an apparent episode of oxygen desaturation (<70%) and extreme shortness of breath while being moved onto the ICU bed. She was placed on a “nonrebreather” and given 80 mg of Lasix IV. Overnight in the ICU she apparently did some diuresis and blood pressure decreased. Patient apparently continued to have declining cardiac function, blood pressure and some acute event from which she could not be resuscitated. Hospital discharge summary sent to the enrolling physician’s office did not state the exact nature of episode. The death certificate states that the cause of death was “congestive heart failure” and asthma is not listed as a contributing factor.
6. eResults 2: Protocol Violations

Two types of protocol violations occurred. Several patients (n=13: 5 in the TIO + ICS group and 8 in the LABA + ICS group) were removed from the study shortly after their baseline enrollment visit due to failure to meet the enrollment criteria, either were less than 4 weeks from an exacerbation or were not taking daily maintenance medications at enrollment. Other patients (n=12; 8 in the TIO + ICS group and 4 in the LABA + ICS group) were removed from the study for inappropriate use of the funds on their medication cards that were used to buy non-medication items at the pharmacies. Patients were warned once and removed from the study if it happened a second time. After several episodes, additional safeguards were put in place to block this activity.
Figure 1. BELT Study Diagram

**BELT Entry Criteria**
- Physician diagnosis of asthma
- Self-identified Black
- Smoke ≤ 10 pack years
- Already on LABA/ICS or ICS alone with ACQ > 1.25

**Visit 1**
- Consent & History
- Max Rev
- ACQ, SFD
- AQLQ, ASUI
- Baseline spirometry
- Saliva for genotyping
- Randomize: TIO/ICS or LABA/ICS

**Visit 2**
- Spirometry, BDR4
- ACQ, SFD
- AQLQ, ASUI
- AE/SAE Review
- Medication Review

**Visit 3**
- Spirometry, Max Rev
- ACQ, SFD
- AQLQ, ASUI
- AE/SAE Review
- Medication Review

**Visit 4**
- ACQ, SFD, Asthma Exacerbation Questionnaire completed monthly in between or at study visits.

**Visit 5**
- Spirometry
- ACQ, SFD
- AQLQ, ASUI
- AE/SAE Review
- Medication Review

Abbreviations: ACQ, Asthma Control Questionnaire; AE, adverse event; AQLQ, Asthma Quality-of-Life Questionnaire; ASUI, Asthma Symptoms Utility Index; BDR4, bronchodilator response; ICS, inhaled corticosteroids; LABA, long-acting beta-agonists; Max Rev, Reversibility with 4 puffs of albuterol SAE, serious adverse event; SFD, Symptom Free Day Questionnaire; TIO, tiotropium.

Patients could enroll for ½ year, 1 year, or 1 ½ years depending on when they were enrolled.
8. eFigure 2 BELT Sites and relative enrollment.

eFigure 2. BELT Sites and relative enrollment. BELT participating sites and their relative subject enrollment – radius of the circle is proportional to the logarithm of the total number of subjects enrolled.
9. **eTable 1. Number of exacerbations per patient stratified by treatment**

<table>
<thead>
<tr>
<th>Number of Exacerbations per patient</th>
<th>All</th>
<th>LABA</th>
<th>TIO</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>78.2% (837/1070)</td>
<td>77.3% (416/538)</td>
<td>79.1% (421/532)</td>
<td>0.51</td>
</tr>
<tr>
<td>1</td>
<td>13.8% (148/1070)</td>
<td>13.6% (73/538)</td>
<td>14.1% (75/532)</td>
<td>0.86</td>
</tr>
<tr>
<td>2</td>
<td>5.0% (53/1070)</td>
<td>6.5% (35/538)</td>
<td>3.4% (18/532)</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>2.5% (27/1070)</td>
<td>1.9% (10/538)</td>
<td>3.2% (17/532)</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>0.5% (5/1070)</td>
<td>0.7% (4/538)</td>
<td>0.2% (1/532)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

10. **eTable 2. Number of asthma hospitalizations per patient stratified by treatment**

<table>
<thead>
<tr>
<th>All Patients</th>
<th>LABA</th>
<th>TIO</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with Hospitalization for Asthma Exacerbation*</td>
<td>1.9% (10/538)</td>
<td>3.6% (19/532)</td>
<td>0.09</td>
</tr>
<tr>
<td>Number of Hospitalizations for Asthma Exacerbation per Patient</td>
<td>98.1% (528/538)</td>
<td>96.4% (513/532)</td>
<td>0.09</td>
</tr>
<tr>
<td>0</td>
<td>1.5% (8/538)</td>
<td>3.2% (17/532)</td>
<td>0.07</td>
</tr>
<tr>
<td>1</td>
<td>0.4% (2/538)</td>
<td>0.2% (1/532)</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>0.0% (0/538)</td>
<td>0.2% (1/532)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* The adjusted rates of asthma-related hospitalizations were 0.046 vs. 0.018 hospitalizations/patient/year (Rate Ratio 2.60, P=0.023)