Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Peters SP, Kunselman SJ, Icitovic N, et al. Tiotropium bromide step-up therapy for adults with uncontrolled asthma. N Engl J Med 2010;363:1715-26. DOI: 10.1056/NEJMoa1008770.

Tiotropium Bromide Step-Up Therapy for Adults with Uncontrolled Asthma

On-Line Supplement

Table of Contents

Selected Methods

Study Patients

Beginning in June 2007 we enrolled 826 patients into a common run-in period with the Best Adjustment Strategy for Asthma over Long Term (BASALT) trial; 342 were randomized into BASALT, 210 subjects were randomized to TALC, with the last subject completing TALC on May 21, 2010 . Inclusion criteria for enrollment in the common run-in period included male and female subjects, ages 18 and older with a clinical history consistent with asthma, FEV_1 > 40% predicted, asthma confirmed either by: (1) beta-agonist reversibility to 4 puffs albuterol ≥ 12% OR (2) PC_{20} FEV_1 methacholine ≤ 8 mg/ml NOT on an inhaled corticosteroid, or ≤ 16 mg/ml ON an inhaled corticosteroid, need for daily controller therapy (i.e., inhaled corticosteroids, leukotriene modifiers, and/or long-acting beta-agonists) as shown by either: (1) used or received prescription for asthma controller during past year OR (2) symptoms more than twice a week if not on asthma controller, if on inhaled steroids (any drug at any dose not exceeding the equivalent of 1000 mcg fluticasone daily), subject must have been on a stable dose for at least 2 weeks, non-smoker (total lifetime smoking history < 10 pack-years, no smoking for at least 1 year), and ability to provide informed consent, as evidenced by signing a copy of the consent form approved by the Committee on Human Research of the study institution, which approved this study. Exclusion criteria included use of any prohibited drug including other asthma medications or medications contraindicated in the study insert of study drugs, significant medical illnesses or lung diseases other than asthma, vocal cord dysfunction, respiratory tract infection or significant asthma exacerbation in the previous 4 weeks, history of

life-threatening asthma in the past 5 years, pregnant or not using acceptable birth control methods if of childbearing potential, hyposensitization therapy other than an established maintenance regimen, and inability to effectively use drug delivery devices used in the study.

Outcome Measures

The predetermined primary outcome measure was morning peak flow (AM PEF).

Predetermined secondary outcomes included

1. additional measures of lung function (prebronchodilator FEV_1 measured in the morning at the end of the dosing interval for all drugs);
2. indices of asthma control and quality of life (asthma symptoms [scale 0 to 3, none, mild, moderate or severe], asthma-control days [days without any asthma symptoms or rescue albuterol use, except for preventative treatment for exercise], rescue inhaler use, asthma control assessed by the Asthma Control Questionnaire [ACQ, range 0-6, lower better asthma control, minimal clinically important difference (MID) 0.5] ,[11,12] Asthma Symptom Utility Index [ASUI, range 0-1, higher better asthma control, MID unknown but 0.3 between mild/moderate and moderate/severe asthma],[13] and asthma-specific quality of life [AQLQ, range 1-7, higher better asthma QOL, MID 0.5],[14] coordinator and patient preference for study medications (ACRN – CPQ), asthma exacerbations [increased asthma symptoms which result in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma], hospitalizations, ER visits, unscheduled visits to a medical provider, good asthma control defined by a composite index that takes into account measures of pulmonary function, asthma symptoms,

rescue beta-agonist use, need for additional asthma medications, and asthma exacerbations, similar to that used in the GOAL [Gaining Optimal Asthma Control] study;[15]

3. biomarkers of inflammation (exhaled nitric oxide [FENO], sputum eosinophilia/eosinophil cationic protein [ECP], and markers of oxidative stress [de-aerated pH] and inflammation in exhaled breath condensate.

Additional pre-specified exploratory hypotheses included associations of response variables with variants in genes in the beta-2 adrenergic receptor, the M_3 muscarinic receptor, and glucocorticoid pathways; whether bronchodilator reversibility with 4 puffs of albuterol or 4 puffs of ipratropium bromide could predict positive responses to salmeterol and tiotropium respectively; and whether a lower resting heart rate (lowest quintile versus highest quintile), could predict a positive response to tiotropium bromide. Pre-specified also was a "responder analysis" similar to that used in previous CARE Network protocols.[16,17]

Selected Results

Schedule of Evaluations

Figure S1 (Schedule of Evaluations) shows when various procedures were performed.

Baseline Characteristics of TALC Randomized Subjects

Baseline characteristics of TALC randomized subjects with all data presented are found in the Supplement **Table S1.**

Baseline Values for AM PEF, Asthma Control Days, and FEV_1 Before Each Treatment Period

Average (2 weeks before randomization) AM PEF was 377±117 L/min and proportion of ACDs 0.21±0.33 (2.97±4.64 days during the 2 weeks before randomization). Baselines before the each of the 3 active treatment periods were the similar for AM PEF (377±117, 384±118, 383±115 L/min, respectively), and FEV_1 (2.31±0.77, 2.36±0.77, 2.36±0.75 L), while proportion of ACDs increased from 0.21±0.33 to 0.34±0.40 and 0.34±0.41 before treatment periods 2 and 3. While minimal carry-over effects were observed for measures of lung function, an effect was seen for ACDs as shown in Supplement **Table S2 (Baseline Values for AM PEF, Asthma Control Days, and FEV_1 Before Each Treatment Period (V3, V7, and V11) for Each Treatment Sequence)**.

Results of All Outcome Variables

The results of all outcome variables are found in the Supplement **Table S3.**

Reasons for Patient Withdrawals from TALC

Reasons for withdrawal of randomized subjects from the trial (7 tio/1xbeclo, 14 2xbeclo, 15 salm/1xbeclo) are given in the Supplement **Table S4**. As can be seen in the Table, 23 of the 36 dropouts were due to a withdrawal of consent. As far as could be determined, no participant withdrew from TALC because of the FDA advisory concerning tiotropium and stroke, with the subsequent revision of the informed consent document, and appropriate counseling of study participants.

Figure S1. Schedule of Evaluations

Visit	**1a**	**1b**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Week	0	0	2	4	8	13	18	20	24	29	34	36	40	45	50	52
Study Phase	Open-Label ICS Run-In			14-Week Double-Blind Treatment Period 1				14-Week Double-Blind Treatment Period 2				14-Week Double-Blind Treatment Period 3				Wash out
Randomization				X												
Skin Testing			X													
Heart Rate Assessment/AQLQ				X			X	X			X	X			X	
FENO/EBC/Sputum				X			X				X				X	
Spirometry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Albuterol Reversal (4 puffs)	(X)	X		X			X				X				X	
Ipratropium Reversal (4 puffs)			X													
Methacholine	(X)															
ACQ/ASUI/Diary Data			X	X	X	X	X	X	X	X	X	X	X	X	X	X

The TALC and BASALT trials were companion trials which used a common run-in period in order to maximize the use of patients with asthma: better controlled asthmatics were allocated to BASALT, and less well controlled asthmatics were allocated to TALC. This figure shows when various procedures were performed. Scores on the Asthma Control Questionnaire (ACQ) range between 0 and 6, with lower score indicating better asthma control; minimal clinically important difference (MID) is 0.5. Asthma Symptom Utility Index (ASUI) scores range between 0 and 1, with higher score indicating better asthma control; MID unknown but a difference of 0.3 is suggested to distinguish between mild/moderate and moderate/severe asthma. Asthma-specific quality of life (AQLQ) scores range between 1 and 7, with higher score indicating better asthma quality of life; MID is 0.5. FENO refers to measuring the fraction of exhaled nitric oxide. EBC is the collection of exhaled breath condensate. Sputum refers to the induction and processing of induced sputum. Diary data include twice daily collection of asthma symptoms, PEF, and any other medications used. Subjects were required to have the diagnosis of asthma confirmed EITHER by bronchodilator reversibility testing to 4 puffs of albuterol OR by bronchial hyperresponsiveness to methacholine inhalation (hence the parentheses () around these two tests) in Visit 1a.

124 **Table S1. Baseline Characteristics of TALC Randomized Subjects with All Data**

125

Characteristic	
Male †	69 (32.9%)
Race †	
American Indian/Alaskan Native	1 (0.5%)
Asian/Pacific Islander	9 (4.3%)
African American	59 (28.1%)
Caucasian	115 (54.8%)
Hispanic	24 (11.4%)
Other	2 (1.0%)
Skin test atopic status (one or more skin tests positive) †	175 (87.5%)
Age at visit 1 *	210 42.2±12.3
Duration of asthma (years since doctor first diagnosed) *	210 26.1±14.1
Height at visit 1 (cm) *	210 167.8±9.5
Weight at visit 1 (kg) *	210 88.3±25.3
BMI at visit 1 (kg/m^2) *	210 31.4±8.8
Pre-Bronchodilator FEV_1 at visit 3 (liters) *	210 2.31±0.77
Pre-Bronchodilator FEV_1 at visit 3 (% predicted) *	210 71.5±14.9
FEV_1 Albuterol (4 puffs) Reversal at visit 3 (%) *	210 14.9±9.8
FEV_1 Post-Albuterol (4 puffs) at visit 3 (liters) *	210 2.64±0.82
FEV_1 Ipratropium (4 puffs) Reversal at visit 2 (%) *	202 12.43±9.48
FEV_1 Post-Ipratropium (4 puffs) at visit 2 (liters) *	202 2.62±0.80
AM Peak Flow 2-week average prior to visit 3 (liters/min) *	210 377.2±117.0
PM Peak Flow 2-week average prior to visit 3 (liters/min) *	210 383.6±119.0
Peak Flow Variability 2-week average prior to visit 3 (%) *	210 0.29±6.87
Daily Symptoms 2-week average prior to visit 3 (0 to 3 scale) *	210 0.46±0.44
Nighttime Symptoms (AM Symptom Diary) 2-week average prior to visit 3 *	210 0.41±0.45
Daytime Symptoms (PM Symptom Diary) 2-week average prior to visit 3 *	210 0.50±0.46
Albuterol Rescue Use 2-week average prior to visit 3 (puffs/day) *	208 1.71±2.09
Albuterol Rescue Use 2-week average prior to visit 3 (times dosed/day) *	208 1.01±1.22
Asthma control days 2-week average prior to visit 3 (proportion of controlled days) *	210 0.212±0.331
Asthma control days 2-week sum prior to visit 3 (# of controlled days) *	210 2.97±4.64
Shortened (without FEV_1 % predicted) ACQ Average at visit 3 *	210 1.36±0.84

Characteristic	
ACQ Average at visit 3 *	210 1.64±0.73
AQLQ Average at visit 3 *	205 5.43±1.05
ASUI Score at visit 3 *	210 0.78±0.15
IgE at visit 2 (IU/mL) ^	199 114.5 (1.4)
FENO at visit 3 (ppb) ^	201 18.8 (0.7)
Sputum over-read Eosinophils at visit 3 +	154 0.40 (0.00,1.20)
Exhaled Breath Condensate pH at visit 3 +	196 8.53 (8.27,8.66)
Blood EOS at visit 2 (/mm^3) +	200 182.0 (100.0,273.0)
Ventricular Heart Rate at visit 3 (beats/min) +	209 66.0 (59.0,75.0)
Medication Use (Ever Taken) †	
Inhaled Corticosteroids (ICS)	189 (90.0%)
Oral Steroids	141 (68.8%)
Long-Acting Beta Agonist (LABA)	163 (78.7%)
Leukotriene Receptor Antagonist (LTRA)	89 (42.8%)
Non-long-acting Beta-Agonists	199 (95.2%)
Asthma Medications via Nebulizer	156 (75.7%)
Oral Beta-2 Agonists	34 (16.8%)
Oral Theophylline	49 (24.6%)
Inhaled Anticholinergic Medications	48 (24.0%)
IgE Blocker (anti-IgE)	2 (1.0%)

† N (%) reported
* N Mean±SD reported
+ N Median (Q1,Q3) reported
^ N Geometric mean (CV) reported

The run-in medication was beclomethasone HFA 40 mcg 2 puffs bid. If a patient was using other asthma medications at the onset of the run-in, these were stopped. Scores for Daily Symptoms were on a scale from 0 to 3 (0 – none, 1 – mild, 2 – moderate, 3 – severe). Scores on the Asthma Control Questionnaire (ACQ) range between 0 and 6, with lower score indicating better asthma control; minimal clinically important difference (MID) is 0.5. Asthma Symptom Utility Index (ASUI) scores range between 0 and 1, with higher score indicating better asthma control; MID unknown but a difference of 0.3 is suggested to distinguish between mild/moderate and moderate/severe asthma. Asthma-specific quality of life (AQLQ) scores range between 1 and 7, with higher score indicating better asthma quality of life; MID is 0.5.

136 **Table S2. Baseline Values for AM PEF, Asthma Control Days, and FEV_1**
137 **Before Each Treatment Period (V3, V7, and V11) for Each Treatment Sequence**

	Drug **Sequence**	**Visit 3 Value (SE)**	**Visit 7 Value (SE)**	**Visit 11 Value (SE)**
AM Peak Flow (liters/min)	tio/1xbeclo → 2xbeclo → salm/1xbeclo	388.9 (7.9)	411.2 (7.8)	394.4 (6.4)
	tio/1xbeclo → salm/1xbeclo → 2xbeclo	439.2 (10.6)	446.2 (9.2)	439.2 (9.5)
	2xbeclo → tio/1xbeclo → salm/1xbeclo	299.3 (8.0)	323.7 (8.3)	340.6 (8.6)
	2xbeclo → salm/1xbeclo → tio/1xbeclo	364.6 (8.6)	352.4 (8.4)	358.1 (9.1)
	salm/1xbeclo → tio/1xbeclo → 2xbeclo	382.4 (7.7)	382.2 (8.6)	390.7 (8.1)
	salm/1xbeclo → 2xbeclo → tio/1xbeclo	401.3 (8.5)	406.1 (10.4)	392.5 (9.2)
Asthma control days (proportion of controlled days)	tio/1xbeclo → 2xbeclo → salm/1xbeclo	0.214 (0.040)	0.403 (0.050)	0.459 (0.048)
	tio/1xbeclo → salm/1xbeclo → 2xbeclo	0.199 (0.043)	0.648 (0.047)	0.623 (0.049)
	2xbeclo → tio/1xbeclo → salm/1xbeclo	0.145 (0.042)	0.258 (0.053)	0.225 (0.052)
	2xbeclo → salm/1xbeclo → tio/1xbeclo	0.178 (0.041)	0.398 (0.049)	0.393 (0.050)
	salm/1xbeclo → tio/1xbeclo → 2xbeclo	0.237 (0.042)	0.462 (0.051)	0.437 (0.054)
	salm/1xbeclo → 2xbeclo → tio/1xbeclo	0.146 (0.040)	0.395 (0.052)	0.318 (0.053)
Asthma control days (# of controlled days per 14 days)	tio/1xbeclo → 2xbeclo → salm/1xbeclo	3.0 (0.6)	5.6 (0.7)	6.4 (0.7)
	tio/1xbeclo → salm/1xbeclo → 2xbeclo	2.8 (0.6)	9.1 (0.7)	8.7 (0.7)
	2xbeclo → tio/1xbeclo → salm/1xbeclo	2.0 (0.6)	3.6 (0.7)	3.1 (0.7)
	2xbeclo → salm/1xbeclo → tio/1xbeclo	2.5 (0.6)	5.6 (0.7)	5.5 (0.7)
	salm/1xbeclo → tio/1xbeclo → 2xbeclo	3.3 (0.6)	6.5 (0.7)	6.1 (0.8)
	salm/1xbeclo → 2xbeclo → tio/1xbeclo	2.0 (0.6)	5.5 (0.7)	4.5 (0.7)
Pre-Bronchodilator FEV_1 (liters)	tio/1xbeclo → 2xbeclo → salm/1xbeclo	2.29 (0.11)	2.36 (0.11)	2.36 (0.11)
	tio/1xbeclo → salm/1xbeclo → 2xbeclo	2.43 (0.14)	2.50 (0.14)	2.44 (0.14)
	2xbeclo → tio/1xbeclo → salm/1xbeclo	2.17 (0.11)	2.23 (0.11)	2.33 (0.12)
	2xbeclo → salm/1xbeclo → tio/1xbeclo	2.36 (0.15)	2.45 (0.15)	2.34 (0.16)

	Drug **Sequence**	**Visit 3 Value (SE)**	**Visit 7 Value (SE)**	**Visit 11 Value (SE)**
	salm/1xbeclo → tio/1xbeclo → 2xbeclo	2.23 (0.10)	2.20 (0.10)	2.20 (0.10)
	salm/1xbeclo → 2xbeclo → tio/1xbeclo	2.46 (0.13)	2.46 (0.14)	2.48 (0.14)

This Table lists the baseline values for AM PEF, Asthma Control Days (in both proportion of controlled days, and controlled days per 14 days), and for pre-bronchodilator FEV1, before each treatment period, for each treatment sequence. Carry-over effects were not observed for AM PEF or pre-bronchodilator FEV_1, but were observed for Asthma Control Days.

143

Table S3. Results of All Outcome Variables

	tio/1xbeclo*	2xbeclo*	salm/1xbeclo*	(Primary Hypothesis) tio/1xbeclo - 2xbeclo**	tio/1xbeclo - salm/1xbeclo**	salm/1xbeclo - 2xbeclo**
	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value
AM Peak Flow (liters/min)	24.4 (16.0,32.7) < 0.001	-1.4 (-8.4,5.6) 0.69	18.0 (11.5,24.5) < 0.001	25.8 (14.4,37.1) < 0.001	6.4 (-4.8,17.5) 0.26	19.4 (9.4,29.4) < 0.001
PM Peak Flow (liters/min)	29.6 (21.9,37.3) < 0.001	-5.7 (-12.3,0.9) 0.09	19.0 (11.7,26.3) < 0.001	35.3 (24.6,46.0) < 0.001	10.6 (-0.1,21.3) 0.05	24.7 (15.2,34.3) < 0.001
Peak Flow Variability (%)	0.79 (0.23,1.35) 0.006	-1.19 (-1.76,-0.62) < 0.001	-0.94 (-1.46,-0.43) < 0.001	1.98 (1.18,2.78) < 0.001	1.74 (0.98,2.49) < 0.001	0.25 (-0.54,1.03) 0.54
Albuterol Rescue Use (puffs/day)	-0.11 (-0.26,0.03) 0.12	-0.07 (-0.19,0.06) 0.30	-0.16 (-0.28,-0.03) 0.01	-0.05 (-0.24,0.14) 0.63	0.04 (-0.13,0.22) 0.63	-0.09 (-0.27,0.09) 0.33
Albuterol Rescue Use (times dosed/day)	-0.04 (-0.13,0.06) 0.44	-0.08 (-0.17,0.01) 0.08	-0.08 (-0.16,-0.00) 0.04	0.04 (-0.08,0.16) 0.49	0.05 (-0.07,0.16) 0.43	-0.00 (-0.12,0.11) 0.94
Nighttime Symptoms (AM Symptom Diary)	-0.03 (-0.06,0.00) 0.07	0.02 (-0.01,0.05) 0.21	-0.00 (-0.04,0.03) 0.80	-0.05 (-0.09,-0.01) 0.03	-0.02 (-0.07,0.02) 0.29	-0.02 (-0.06,0.02) 0.31
Daytime Symptoms (PM Symptom Diary)	-0.03 (-0.06,0.01) 0.13	0.01 (-0.02,0.04) 0.56	-0.03 (-0.06,0.01) 0.14	-0.04 (-0.09,0.01) 0.13	-0.00 (-0.05,0.05) 0.93	-0.04 (-0.08,0.01) 0.15
Daily Symptoms (0 to 3 scale)	-0.09 (-0.12,-0.05) < 0.001	0.03 (-0.01,0.06) 0.11	-0.04 (-0.08,-0.01) 0.02	-0.11 (-0.16,-0.06) < 0.001	-0.04 (-0.09,0.01) 0.10	-0.07 (-0.12,-0.02) 0.005
Asthma control days (proportion of controlled days)	0.131 (0.090,0.171) < 0.001	0.051 (0.010,0.093) 0.02	0.139 (0.096,0.183) < 0.001	0.079 (0.019,0.140) 0.01	-0.009 (-0.070,0.053) 0.78	0.088 (0.028,0.148) 0.004
Asthma control days (# of controlled days per 14 days)	1.8 (1.3,2.4) < 0.001	0.7 (0.1,1.3) 0.02	2.0 (1.3,2.6) < 0.001	1.1 (0.3,2.0) 0.01	-0.1 (-1.0,0.7) 0.78	1.2 (0.4,2.1) 0.004
Pre-Bronchodilator FEV_1 (liters)	0.12 (0.07,0.17) < 0.001	0.02 (-0.03,0.07) 0.47	0.01 (-0.04,0.06) 0.60	0.10 (0.03,0.17) 0.004	0.11 (0.04,0.18) 0.003	-0.00 (-0.08,0.07) 0.89
Pre-Bronchodilator FEV_1 (% predicted)	3.73 (2.71,4.76) < 0.001	0.63 (-0.28,1.54) 0.17	0.64 (-0.32,1.60) 0.19	3.10 (1.74,4.47) < 0.001	3.10 (1.78,4.41) < 0.001	0.01 (-1.31,1.33) 0.99

	tio/1xbeclo*	2xbeclo*	salm/1xbeclo*	(Primary Hypothesis) tio/1xbeclo - 2xbeclo**	tio/1xbeclo - salm/1xbeclo**	salm/1xbeclo - 2xbeclo**
	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value	Est (95% CI) P-value
ASUI Score	0.03 (0.01,0.05) 0.004	0.00 (-0.02,0.02) 0.77	0.04 (0.03,0.06) < 0.001	0.03 (-0.00,0.06) 0.09	-0.01 (-0.04,0.02) 0.38	0.04 (0.01,0.07) 0.005
Shortened (without FEV_1 % predicted) ACQ Average	-0.18 (-0.30,-0.06) 0.005	-0.02 (-0.13,0.09) 0.67	-0.35 (-0.45,-0.25) < 0.001	-0.16 (-0.33,0.02) 0.08	0.17 (0.01,0.33) 0.04	-0.33 (-0.48,-0.18) < 0.001
ACQ Average	-0.22 (-0.33,-0.11) < 0.001	-0.03 (-0.13,0.06) 0.49	-0.31 (-0.40,-0.22) < 0.001	-0.18 (-0.34,-0.03) 0.02	0.09 (-0.04,0.23) 0.18	-0.28 (-0.41,-0.15) < 0.001
AQLQ Average	0.15 (0.03,0.26) 0.01	0.05 (-0.06,0.15) 0.38	0.28 (0.18,0.38) < 0.001	0.10 (-0.07,0.27) 0.24	-0.13 (-0.28,0.02) 0.09	0.23 (0.09,0.37) 0.002
Ventricular Heart Rate (beats/min)	4.83 (3.38,6.27) < 0.001	2.86 (1.51,4.22) < 0.001	3.46 (1.93,5.00) < 0.001	1.96 (-0.03,3.96) 0.05	1.36 (-0.71,3.43) 0.20	0.60 (-1.36,2.56) 0.55
FEV_1 Post-Albuterol (4 puffs) (liters)	0.02 (-0.01,0.05) 0.16	-0.02 (-0.05,0.01) 0.11	-0.05 (-0.08,-0.03) < 0.001	0.04 (0.01,0.08) 0.01	0.07 (0.05,0.10) < 0.001	-0.03 (-0.06,0.00) 0.06
FEV_1 Albuterol (4 puffs) Reversal (%)	-5.81 (-7.15,-4.47) < 0.001	-2.83 (-4.08,-1.59) < 0.001	-6.40 (-7.82,-4.98) < 0.001	-2.98 (-4.37,-1.59) < 0.001	0.59 (-0.64,1.82) 0.34	-3.57 (-5.01,-2.13) < 0.001
Natural log(FENO) (ppb))	0.07 (-0.01,0.15) 0.07	-0.06 (-0.14,0.02) 0.12	-0.05 (-0.14,0.03) 0.18	0.13 (0.05,0.22) 0.003	0.13 (0.05,0.21) 0.002	0.01 (-0.07,0.09) 0.84
Sputum over-read EOS	0.51 (-0.13,1.14) 0.12	0.20 (-0.40,0.81) 0.51	0.31 (-0.28,0.90) 0.30	0.30 (-0.33,0.94) 0.35	0.20 (-0.36,0.76) 0.49	0.11 (-0.56,0.77) 0.75
Exhaled Breath Condensate pH	-0.30 (-0.48,-0.13) < 0.001	-0.11 (-0.27,0.04) 0.16	-0.14 (-0.30,0.02) 0.08	-0.19 (-0.38,-0.00) 0.05	-0.16 (-0.34,0.02) 0.09	-0.03 (-0.19,0.13) 0.71

* Model-based estimates representing the average of the change between the end and the beginning of each of the three treatment periods.
** Differences between treatments using the model-based estimates.

144

145 Restricted maximum likelihood estimates were determined for the treatment effects (the model-based change between the end and
146 the beginning of each of the three treatment periods). The primary comparison between tio/1xbeclo vs. 2xbeclo for AM PEF showed
147 the treatment with tio/1xbeclo to be 25.8 L/min superior to 2xbeclo (95% confidence interval, [CI] 14.4,37.1;P<0.001). Similar

results favoring tio/1xbeclo were obtained for PM PEF, pre-bronchodilator FEV_1, proportion of Asthma Control Days (ACDs), daily symptoms, ACQ score, and FEV_1 after 4 puffs of albuterol. The secondary comparison between tio/1xbeclo vs. salm/1xbeclo showed no significant difference between groups for AM PEF, PM PEF, proportion of ACDs, daily symptoms, and ACQ score. Pre-bronchodilator FEV_1 favored tio/1xbeclo 0.11 L (CI 0.04,0.18;P=0.003), as did FEV_1 after 4 puffs of albuterol 0.07 L (CI 0.05,0.10;P<0.001). The null hypothesis of inferiority was rejected in favor of the alternative hypothesis of non-inferiority at the 0.025 significance level for AM PEF, pre-bronchodilator FEV_1, and the proportion of ACDs. The comparison between salm/1xbeclo vs. 2xbeclo showed that treatment with sal/1xbeclo is superior to 2xbeclo in terms of AM PEF, PM PEF, proportion of ACDs, daily symptoms, ACQ score, ASUI score, and AQLQ score. Scores on the Asthma Control Questionnaire (ACQ) range between 0 and 6, with lower score indicating better asthma control; minimal clinically important difference (MID) is 0.5. Asthma Symptom Utility Index (ASUI) scores range between 0 and 1, with higher score indicating better asthma control; MID unknown but a difference of 0.3 is suggested to distinguish between mild/moderate and moderate/severe asthma. Asthma-specific quality of life (AQLQ) scores range between 1 and 7, with higher score indicating better asthma quality of life; MID is 0.5.

Table S4. Reasons for Patient Withdrawals from TALC

	tio/1xbeclo	2xbeclo	salm/1xbeclo
TOTAL RANDOMIZED	189	195	195
TOTAL DROP-OUTS	7	14	15
Withdrew Consent	3	10	10
Lost to follow-up	2	2	3
Medication-related adverse event	1	1	0
Ineligible post-randomization	0	1	0
Other reason	1	0	2

This Table lists reasons for patient withdrawals from TALC. As far as could be determined, no participant withdrew from TALC because of the FDA advisory concerning tiotropium and stroke, with the subsequent revision of the informed consent document, and appropriate counseling of study participant.