Original Article

Tiotropium Respimat Add-on Is Efficacious in Symptomatic Asthma, Independent of T2 Phenotype

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What is already known about this topic? Current research in asthma is directed toward identifying endotypes that predict response to asthma therapies, including biologics. $T2_{high}$ and $T2_{low}$ phenotypes are defined by their differing inflammatory cell composition and biomarkers.

What does this article add to our knowledge? This exploratory subgroup analysis of 4 large randomized trials suggests that the efficacy of the long-acting anticholinergic tiotropium is not predicted by $T2_{high}$ or $T2_{low}$ profile, defined by IgE level or eosinophil count, in patients with asthma.

How does this study impact current management guidelines? Our data suggest that patients being considered for the addition of tiotropium Respimat for symptomatic asthma and/or for the prevention of asthma exacerbations do not require prior phenotyping by T2 status.

BACKGROUND: Adding tiotropium to existing inhaled corticosteroid (ICS) maintenance therapy with or without a long-acting β_2-agonist (LABA) has been shown to be beneficial in patients with symptomatic asthma.
OBJECTIVE: To assess whether responses to tiotropium Respimat add-on therapy were influenced by patients' T2 status.
METHODS: In this exploratory study, data from 4 phase III trials were analyzed: once-daily tiotropium 5 μg or placebo as add-on to ICS + LABA (PrimoTinA-asthma; 2 replicate trials; NCT00772538/NCT00776984; n = 912); once-daily tiotropium 5 μg or 2.5 μg, twice-daily salmeterol 50 μg, or placebo as add-on to ICS (MezzoTinA-asthma; 2 replicate trials; NCT01172808/NCT01172821; n = 2100). The prespecified efficacy outcomes of these studies have been reported previously. Here, further exploratory subgroup analyses were performed to study whether these coprimary end points were influenced by serum IgE levels, blood eosinophil counts, and clinician judgment of allergic asthma. In addition, for the continuous parameters, namely, IgE and blood eosinophils, their influence on the treatment effect was modeled over the whole range of values.
RESULTS: Tiotropium was efficacious in improving peak FEV_1 within 3 hours postdose and trough FEV_1, independent of T2 status. Tiotropium significantly reduced the risk of severe asthma exacerbations and asthma worsening, independent of T2

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Conflicts of interest: T. B. Casale has received research support and consultancy fees from Boehringer Ingelheim and is the American Academy of Allergy, Asthma & Immunology Executive Vice President. E. D. Bateman has received research support and consultancy fees and is on the speaker's bureau for Boehringer Ingelheim; is on the AstraZeneca Asthma & chronic obstructive pulmonary disease (COPD) board; is on the Novartis and Almirall COPD drug development boards; is on the Takeda COPD & asthma studies board; is on the GlaxoSmithKline trial design board; is on the Cipla inhaler design board; has received consultancy fees from AstraZeneca, Almirall, Vectura, Actelion, and Genentech; has received research support from AstraZeneca, Novartis, Almirall, Merck, Takeda, GlaxoSmithKline, Hoffmann la Roche, Actelion, Aeras, Chiesi, Sanofi-Aventis, Cephalon, and Teva; has received lecture fees from AstraZeneca, Novartis, Takeda, GlaxoSmithKline, Chiesi, Cipla, Menarini, and ALK; has received payment for developing educational presentations from PeerVoice; is on the ICON study oversight steering committee; and reports personal fees for lectures, consulting, and advisory board membership and grants to his institution for participation in clinical trials sponsored by Boehringer Ingelheim; personal fees for advisory board membership from Almirall, AstraZeneca, Cipla, Glaxo SmithKline, Novartis, Takeda, and ICON; personal fees for consulting from Actelion, AstraZeneca, Almirall, Genentech, and Vectura; personal fees for lectures from ALK, AstraZeneca, Chiesi, Cipla, Glaxo SmithKline, Menarini, Novartis, and Takeda; personal fees for educational materials from PeerVoice; and grants to his institution for participation in clinical trials sponsored by Actelion, Aeras, Almirall,

Abbreviations used
ACQ-7- 7-question Asthma Control Questionnaire
$FEV_{1(0\text{-}3h)}$- FEV_1 within 3 hours of tiotropium plus maintenance therapy
HFA-MDI- Hydrofluoroalkane metered-dose inhaler
HR- Hazard ratio
ICS- Inhaled corticosteroid
LABA- Long-acting β_2-agonist
MMRM- Restricted maximum likelihood-based repeated measures
OR- Odds ratio
REML- Restricted maximum likelihood
TALC- Tiotropium Bromide as an Alternative to Increased Inhaled Glucocorticoid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid

phenotype; Cox regression modeling supported a beneficial effect of tiotropium on exacerbations, independent of IgE levels or eosinophil counts. Numerical improvements in the 7-question Asthma Control Questionnaire (ACQ-7) responder rate with tiotropium versus placebo were observed in $T2_{high}$ and $T2_{low}$ patients; logistic regression modeling provided further evidence for improvement in ACQ-7 responder rates with tiotropium, independent of IgE levels or eosinophil counts.

CONCLUSIONS: The results of our exploratory analyses suggest that the improvements seen with tiotropium Respimat as add-on to ICS ± LABA in patients with symptomatic asthma on lung function, exacerbation risk, and symptom control are independent of T2 phenotype. **(J Allergy Clin Immunol Pract 2017;■:■-■)**

Key words: *Allergy; Asthma; Tiotropium Respimat; T2 status; Eosinophil; IgE; Subgroup analysis*

Asthma affects around 300 million people worldwide,[1] and at least 40% of individuals diagnosed with asthma remain symptomatic despite treatment with inhaled corticosteroids (ICSs) as monotherapy or in combination with long-acting β_2-agonists (LABAs). Failure to control asthma symptoms increases the risk of serious asthma-related events such as exacerbations,[2-4] which pose a great risk to patients, is associated with significant costs to patients and/or health care systems,[5-8] and may contribute to the accelerated decline in lung function that occurs over time in all patients with asthma.[9] Additional treatment options are therefore needed. The clinical efficacy and safety of tiotropium, a once-daily long-acting anticholinergic bronchodilator (delivered via the Respimat Soft Mist inhaler; Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Germany) as add-on to ICS or ICS plus LABA maintenance therapy, has been demonstrated in a large clinical program involving more than 6000 patients aged 1 to 75 years and with varying asthma severities.[10-20] Consequently, treatment with tiotropium Respimat has been included in the latest (2016) Global Initiative for Asthma treatment strategy as an add-on option at step 4 or 5 for patients aged 12 years or older with a history of exacerbations.[21]

There is a significant allergic component to asthma in a large proportion of patients.[21] Patients with elevated T2 inflammatory cytokines associated with eosinophilic infiltration (such as IL-5 and IL-13) are classified with $T2_{high}$ asthma.[22,23] For these patients, biologic treatment options include anti-IgE (omalizumab) and anti−IL-5 (mepolizumab and reslizumab).[22,24,25] However, eligibility for these treatments is determined on the basis of biomarkers and this phenotyping may involve additional clinic visits. In addition, these medications are applicable to a limited subpopulation of patients with asthma only and are costly. To reduce these additional costs, it is therefore beneficial if safety and efficacy can be demonstrated irrespective of allergic status and additional phenotyping before treatment. Here, we describe the results of exploratory analyses to examine whether responses to tiotropium were influenced by patients' allergic and/or T2 status, in data from these 4 phase III trials in patients with symptomatic asthma. Please see Video 1 (available in this article's Online Repository at www.jaci-inpractice.org) for an overview of trial rationale and findings.

METHODS

Overview of the PrimoTinA-asthma and MezzoTinA-asthma trials

Full details of the study designs, methodologies, and main results from the 4 trials have been published previously.[13,14] The trials were carried out in accordance with the Declaration of Helsinki and Good Clinical Practice, and all participating patients provided written, informed consent.

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Study design and medication. PrimoTinA-asthma and MezzoTinA-asthma were 2 pairs of replicate phase III, randomized, double-blind, placebo-controlled, parallel-group studies assessing the efficacy and safety of tiotropium add-on therapy (5 μg in PrimoTinA-asthma and 5 μg and 2.5 μg in MezzoTinA-asthma) in patients with severe symptomatic asthma or moderate symptomatic asthma, respectively.[13,14]

Briefly, in PrimoTinA-asthma (NCT00772538 and NCT00776984),[13] following a 4-week screening period, patients were randomized 1:1 to receive once-daily tiotropium 5 μg (2 × 2.5 μg) or matching placebo each morning as add-on to ICS (≥800 μg budesonide or equivalent) plus LABA with or without other maintenance therapies for 48 weeks.

In the double-dummy MezzoTinA-asthma studies (NCT01172808 and NCT01172821),[14] patients were randomized 1:1:1:1 following a 4-week screening period to once-daily tiotropium 5 μg (evening, 2 × 2.5 μg), once-daily tiotropium 2.5 μg (evening, 2 × 1.25 μg), twice-daily salmeterol 50 μg (morning, 2 × 25 μg; evening, 2 × 25 μg; via hydrofluoroalkane metered-dose inhaler), or placebo (identical devices in a double-dummy design), each as add-on to ICS (400-800 μg budesonide or equivalent) for 24 weeks.

In all trials, tiotropium or placebo was administered as maintenance therapy with ICS as monotherapy or in combination with other controllers; therefore, patients in the "placebo" arm not only received placebo but also continued to receive background treatment. Open-label salbutamol (albuterol) metered-dose inhalers were provided as rescue medication.

Study population

Patients aged 18 to 75 years with a 5-year or more history of asthma (PrimoTinA-asthma) or a 3-month or more history of asthma (MezzoTinA-asthma) were enrolled. An initial asthma diagnosis before the age of 40 years and symptomatic disease, with a 7-question Asthma Control Questionnaire (ACQ-7) mean score of 1.5 or more, were required.[26] The main exclusion criteria were chronic obstructive pulmonary disease or serious unstable coexisting illnesses.

Study end points

PrimoTinA-asthma: Coprimary lung function end points were peak FEV_1 response within 3 hours ($FEV_{1(0\text{-}3h)}$) of tiotropium (plus maintenance therapy) and trough FEV_1 response, measured at the end of the dosing interval (24 hours postdrug), 10 minutes before the next dose, at week 24. The third coprimary end point, evaluated from the 48-week pooled data from the 2 trials, was time to first severe asthma exacerbation. All coprimary end points were met. Secondary end points included time to first episode of asthma worsening (prespecified as any asthma exacerbation) over 48 weeks and ACQ-7 mean score.

MezzoTinA-asthma: Coprimary end points were peak $FEV_{1(0\text{-}3h)}$ response (change from baseline) and trough FEV_1 response at the end of the 24-week treatment period. The third coprimary end point was the ACQ-7 responder rate at week 24 in the pooled data from the 2 trials. All coprimary end points were met. Secondary end points included time to first severe asthma exacerbation and time to first episode of asthma worsening over 24 weeks, in the pooled data.

In all trials, asthma exacerbation was defined as a progressive increase in 1 or more asthma symptom (ie, shortness of breath, cough, wheezing, chest tightness) beyond the individual's usual daily asthma symptoms and lasting for at least 2 days, and/or a decrease in best morning peak expiratory flow of 30% or more from their mean morning peak expiratory flow on 2 or more consecutive days. For an exacerbation to be classed as severe, the above criteria must have been met and the exacerbation must have required the initiation or at least doubling of systemic (including oral) corticosteroids for at least 3 days.

The analysis of adverse events was based on the concept of treatment-emergent adverse events, that is, all adverse events that were reported after the first dose of study medication in the treatment period and within 30 days after the last dose of study medication.

For completeness, we show data for tiotropium 5 μg, tiotropium 2.5 μg, salmeterol, and placebo; however, to be concise, we do not discuss salmeterol in this article.

Analyses of PrimoTinA-asthma and MezzoTinA-asthma data by T2 status

Exploratory analyses of responses to treatment according to markers of T2 status at baseline (total serum IgE levels and blood eosinophil counts) were performed using 2 data pools: data from the 2 PrimoTinA-asthma trials and data from the 2 MezzoTinA-asthma trials. For these exploratory analyses, all randomized patients who took at least 1 dose of trial drug were included. Analyses from both data pools were not powered for inferential conclusions; hence, *P* values presented in this article must be considered nominal at the 5% alpha level and conclusions are exploratory in nature.

As is standard for subgroup analyses of clinical trial results, categorization of the continuous parameters, total serum IgE levels and blood eosinophil counts, was performed to analyze the treatment effect within each subgroup category as well as the interaction of treatment and subgroup. Such analyses were prespecified for both the PrimoTinA-asthma data pool and the MezzoTinA-asthma data pool for all end points in question, before unblinding of the individual trials (except for analysis of ACQ-7 responders via logistic regression, which was added *post hoc*). Models similar to the primary analyses of the trials were used, appropriate to the end point analyzed, that is, Cox regression for time-to-event end points, logistic regression for binary end points, and a restricted maximum likelihood–based approach of a mixed model for repeated measures (MMRM) for continuous end points. For categorization, a patient's T2 status was assessed in relation to both serum IgE levels, categorized as above or below 430 μg/L (equivalent to 179.2 IU/L), and selected according to defined normal ranges[27] and clinician judgment of allergic asthma, categorized as "Yes" or "No." In addition, blood eosinophil counts categorized by levels above or below 0.6×10^9/L, selected according to defined normal ranges,[27] were used as a putative indicator of eosinophilic asthma phenotype.

In addition to categorical subgroup analysis, modeling using continuous variables, such as serum IgE levels and blood eosinophil counts, was performed over the whole range of the parameter values. For those analyses, no categorization of serum IgE or blood eosinophils was necessary. Treatment effects obtained from the modeling are presented over a range of values: total serum IgE from 2 to 2000 μg/L and blood eosinophils from 0.05 to 2.00×10^9/L.

In the following sections, the analyses are described in more detail for each type of end point.

Peak $FEV_{1(0\text{-}3h)}$ and trough FEV_1. Categorical subgroup analyses of peak $FEV_{1(0\text{-}3h)}$ and trough FEV_1 were performed using a restricted maximum likelihood–based MMRM approach, which included "treatment," "study," "visit," and "treatment-by-visit" as fixed, categorical effects, and "baseline" and "baseline-by-visit" as

fixed, continuous covariates. Patients were included as random effect and a spatial power structure was used to model the within-patient errors. The Kenward-Roger approximation was used to estimate the denominator degrees of freedom. The treatment difference was measured by calculating adjusted means and 95% CIs within subgroup categories. An interaction *P* value was obtained via a separate MMRM model that additionally included "subgroup" and "treatment-by-subgroup" interaction terms, and assessed by a *t* test. In addition, the MMRM model was applied throughout the range of continuous serum IgE levels and blood eosinophil counts (log values), modeled as linear continuous effects including the interaction with treatment, to obtain the treatment difference and respective 95% CIs.

Time to first severe exacerbation and asthma worsening. Categorical subgroup analyses of time to first severe exacerbation and time to first episode of asthma worsening were performed using Cox regression analysis. The regression model included "treatment" and "study" as effects. The treatment effect was measured by calculating hazard ratios (HRs) and 95% CIs within subgroup categories. The validity of the proportional hazards assumption, presumed in Cox regression, was checked graphically through Kaplan-Meier plots and plots of Schoenfeld residuals. All included patients were analyzed from start of treatment until first occurrence of the event (severe asthma exacerbation, worsening of asthma). A patient who did not experience an event during the treatment period was censored at the end of the treatment period. An interaction *P* value was obtained via a separate Cox regression that additionally included "subgroup" and "treatment-by-subgroup" interaction terms, and was assessed by chi-square testing. In addition, the Cox regression model was applied throughout the range of serum IgE levels and blood eosinophil counts (log values), modeled as linear continuous effects including the interaction with treatment, to obtain HRs and respective 95% CIs.

ACQ-7 responder rate. Categorical subgroup analyses of ACQ-7 responder rate were performed using a logistic regression that included "treatment" and "study." Patients were defined as responders if an improvement of 0.5 or more in the ACQ-7 mean score (the minimum clinically important difference[26]) was observed. The treatment effect was measured by calculating odds ratios (ORs) and 95% CIs within subgroup categories. An interaction *P* value was obtained via a separate logistic regression that additionally included "subgroup" and "treatment-by-subgroup" interaction terms, and was assessed by chi-square testing. In addition, the logistic regression model was applied throughout the range of serum IgE levels and blood eosinophil counts (log values), modeled as linear continuous effects including the interaction with treatment, to obtain ORs and respective 95% CIs. All analyses were conducted using SAS v9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Across the PrimoTinA-asthma trials, 912 patients received at least 1 dose of study medication. Total serum IgE levels were more than 430 μg/L in 391 patients (42.9%), blood eosinophil counts were more than 0.6×10^9/L in 189 patients (20.7%), and 559 patients (61.3%) were considered to have allergic asthma according to clinician judgment (Table I).

In the MezzoTinA-asthma trials, 2100 patients received at least 1 dose of study medication. Total serum IgE levels were more than 430 μg/L in 1297 patients (61.8%), blood eosinophil counts were more than 0.6×10^9/L in 429 patients (20.4%), and 1361 patients (64.8%) were considered to have allergic asthma according to clinician judgment (Table I).

Baseline demographic and disease characteristics

Baseline demographic and disease characteristics were balanced between treatment groups within each trial (Table I). Most patients were female (~60%); most patients had never smoked (>75%). Mean age was higher in PrimoTinA-asthma (53.0 years, compared with 43.1 years in MezzoTinA-asthma). In the last 3 months before screening, concomitant leukotriene modifiers had been taken by 22.5% and 10.1% of the patients in PrimoTinA-asthma and MezzoTinA-asthma, respectively. A concomitant diagnosis of allergic rhinitis was reported in 21.3% and 28.9% of the patients in PrimoTinA-asthma and MezzoTinA-asthma, respectively.

Efficacy

To evaluate whether tiotropium provided improvements in lung function, exacerbations, and asthma control versus placebo in both groups (those with $T2_{high}$ or $T2_{low}$ asthma), treatment effects were analyzed (1) by comparing them in categorical subgroups and (2) by representing them graphically across the range of IgE levels and eosinophil counts. For completeness, see Table E1 (available in this article's Online Repository at www.jaci-inpractice.org) for pooled overall analyses of PrimoTinA-asthma and MezzoTinA-asthma with regard to lung function, exacerbations, and asthma control versus placebo.

Peak $FEV_{1(0\text{-}3h)}$ and trough FEV_1. For patients with severe (PrimoTinA-asthma) or moderate (MezzoTinA-asthma) asthma, peak $FEV_{1(0\text{-}3h)}$ and trough FEV_1 responses were significantly improved with tiotropium versus placebo after 24 weeks' treatment, with treatment differences ranging from 93 mL to 223 mL (Figures 1 and 2).

The results of the categorical subgroup analyses reveal that these improvements in lung function with tiotropium versus placebo were independent of serum IgE levels (above or below 430 μg/L), blood eosinophil counts (above or below 0.6×10^9/L), and clinician judgment of allergic asthma ("Yes" or "No") in patients with severe (PrimoTinA-asthma) or moderate (MezzoTinA-asthma) asthma (see Figures E1 and E2 in this article's Online Repository at www.jaci-inpractice.org).

Estimating the treatment differences across the range of IgE levels and eosinophil counts demonstrated mean differences from placebo in peak $FEV_{1(0\text{-}3h)}$ and trough FEV_1 responses that were consistently greater than 0 in patients with severe (PrimoTinA-asthma) and moderate (MezzoTinA-asthma) asthma (Figures 1 and 2); that is, both doses of tiotropium were consistently superior to placebo independent of baseline IgE levels or eosinophil counts across the range of values.

Risk of severe exacerbations. Time to first severe exacerbation for patients with severe (PrimoTinA-asthma) or moderate (MezzoTinA-asthma) asthma was longer with tiotropium versus placebo, with HRs ranging from 0.5 to 0.79 (Figure 3).

Categorical subgroup analyses demonstrated that this was independent of serum IgE levels, blood eosinophil counts, and clinician judgment of allergic asthma in both patients with severe (PrimoTinA-asthma) or moderate (MezzoTinA-asthma) asthma (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org).

TABLE I. Baseline demographic and disease characteristics

Characteristic	PrimoTinA-asthma*		MezzoTinA-asthma†			
	Tiotropium Respimat 5 μg QD (n = 456)	Placebo Respimat QD (n = 456)	Tiotropium Respimat 5 μg QD‡ (n = 517)	Tiotropium Respimat 2.5 μg QD‡ (n = 519)	Salmeterol HFA-MDI 50 μg BID§ (n = 541)	Placebo‖ (n = 523)
Sex, n (%)						
Female	273 (59.9)	278 (61.0)	300 (58.0)	316 (60.9)	312 (57.7)	311 (59.5)
Male	183 (40.1)	178 (39.0)	217 (42.0)	203 (39.1)	229 (42.3)	212 (40.5)
Age (y), mean ± SD	52.2 ± 12.5	53.8 ± 12.2	44.3 ± 12.6	43.4 ± 12.9	42.1 ± 12.9	42.8 ± 13.0
Body mass index (kg/m^2), mean ± SD	28.2 ± 5.9	28.2 ± 6.1	27.1 ± 6.3	26.6 ± 6.1	26.7 ± 6.3	27.0 ± 6.3
Smoking status, n (%)						
Never smoked	340 (74.6)	352 (77.2)	420 (81.2)	437 (84.2)	446 (82.4)	453 (86.6)
Ex-smoker	116 (25.4)	104 (22.8)	97 (18.8)	82 (15.8)	95 (17.6)	70 (13.4)
Smoking history (pack-years), mean ± SD	5.4 ± 2.8	4.8 ± 2.7	4.5 ± 3.0	4.0 ± 2.9	4.1 ± 2.7	4.1 ± 2.5
Median age at asthma onset (y) (range)	26.0 (0-44)	26.0 (0-39)	24.0 (0-40)	24.0 (0-40)	23.0 (0-48)	24.0 (0-39)
Duration of asthma (y), mean ± SD	29.6 ± 13.6	31.0 ± 14.1	23.0 ± 15.0	22.1 ± 14.3	21.0 ± 14.3	21.1 ± 13.7
FEV_1 % predicted, mean ± SD¶	55.9 ± 13.1	56.0 ± 13.2	73.9 ± 11.3	75.4 ± 11.5	75.8 ± 11.7	75.1 ± 11.5
FVC % predicted, mean ± SD¶	79.9 ± 17.3	80.5 ± 16.8	96.1 ± 14.0	96.6 ± 13.4	96.5 ± 13.6	97.5 ± 14.0
FEV_1/FVC %, mean ± SD¶	58.7 ± 10.3	58.1 ± 10.0	65.3 ± 10.2	66.5 ± 10.8	67.0 ± 10.5	65.6 ± 10.4
ICS dose of stable maintenance therapy (μg/d),# mean ± SD¶	1191.7 ± 525.0	1204.6 ± 553.1	663.9 ± 216.0	655.9 ± 213.2	650.8 ± 205.2	668.3 ± 217.3
Potentially allergic asthma, n (%)						
Serum IgE (>430 μg/L)	207 (45.4)	184 (40.4)	322 (62.3)	323 (62.2)	323 (59.7)	329 (62.9)
Blood eosinophils (>0.6 × 10^9/L)	102 (22.4)	87 (19.1)	105 (20.3)	104 (20.0)	111 (20.5)	109 (20.8)
Clinician judgment of allergic asthma (yes)	276 (60.5)	283 (62.1)	329 (63.6)	332 (64.0)	356 (65.8)	344 (65.8)
Concomitant diagnosis of allergic rhinitis, n (%)	103 (22.6)	91 (20.0)	141 (27.3)	154 (29.7)	155 (28.7)	157 (30.0)
Concomitant therapies of interest, n (%)**						
Leukotriene modifiers	96 (21.1)	109 (23.9)	54 (10.4)	53 (10.2)	52 (9.6)	53 (10.1)
Systemic antihistamines	85 (18.6)	59 (12.9)	77 (14.9)	95 (18.3)	85 (15.7)	101 (19.3)
Antiallergic agents (excluding corticosteroids)	18 (3.9)	19 (4.2)	24 (4.6)	31 (6.0)	26 (4.8)	27 (5.2)
Omalizumab	15 (3.3)	28 (6.1)	0	0	0	0
Immune modulatory agents and antibodies	4 (0.9)	4 (0.9)	4 (0.8)	5 (1.0)	3 (0.6)	6 (1.1)

BID, Twice daily; *FVC*, forced vital capacity; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily.
Treated set.
*Pooled data; add-on to ICS (≥800 μg budesonide or equivalent per day) + LABA.
†Pooled data; add-on to ICS (400-800 μg budesonide or equivalent).
‡Plus placebo HFA-MDI BID.
§Plus placebo Respimat QD.
‖Placebo Respimat QD plus placebo HFA-MDI BID.
¶Measured at visit 2 (randomization).
#Budesonide equivalent dose.
**Within the 3 mo before screening.

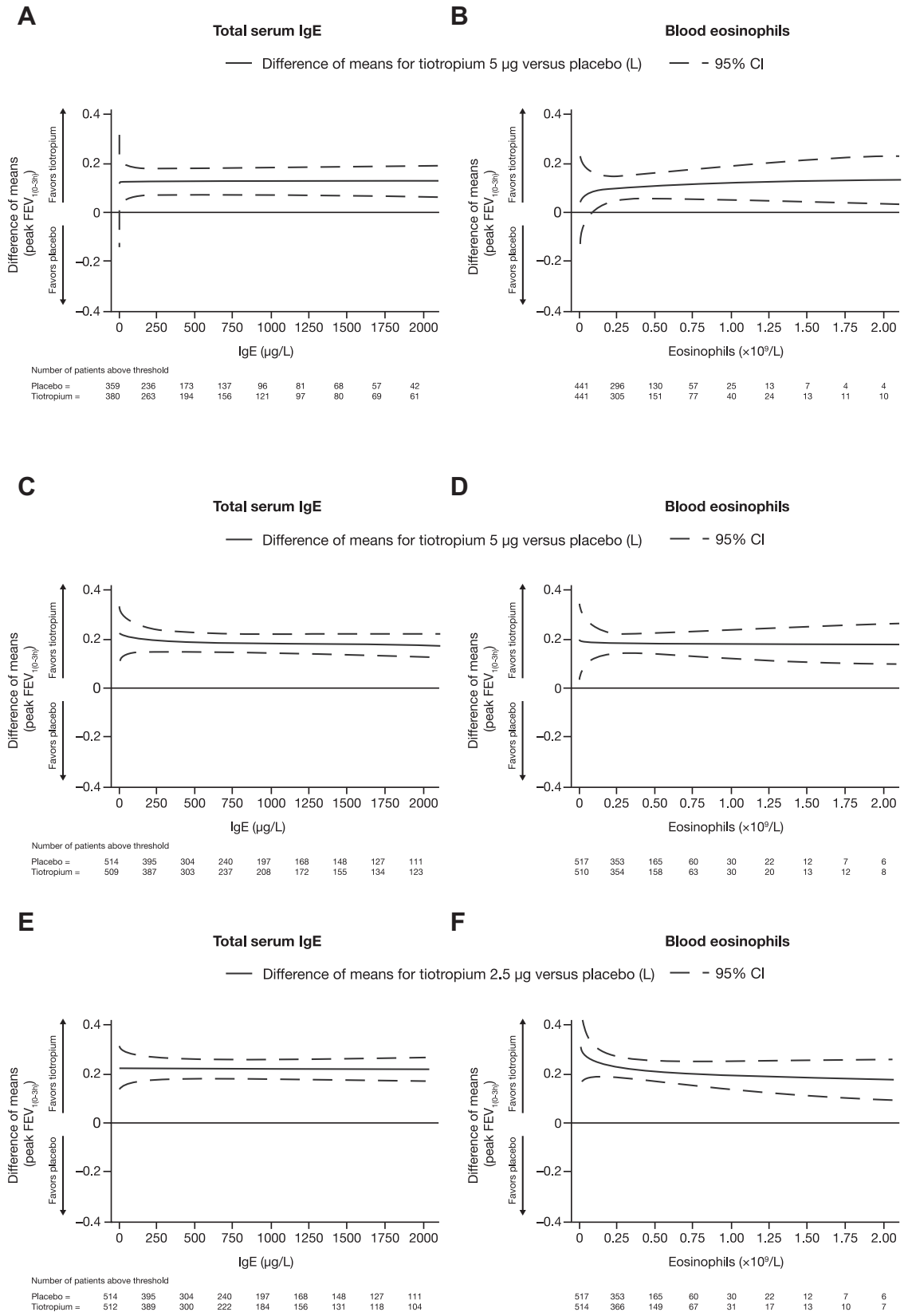

FIGURE 1. Peak $FEV_{1(0\text{-}3h)}$ in PrimoTinA-asthma (**A** and **B**) and MezzoTinA-asthma (**C**-**F**), according to baseline serum IgE levels (Figure 1, *A*, *C*, *E*) and blood eosinophil counts (Figure 1, *B*, *D*, *F*). Full analysis set. Adjusted mean difference (solid line) and 95% CIs (dotted line) are presented.

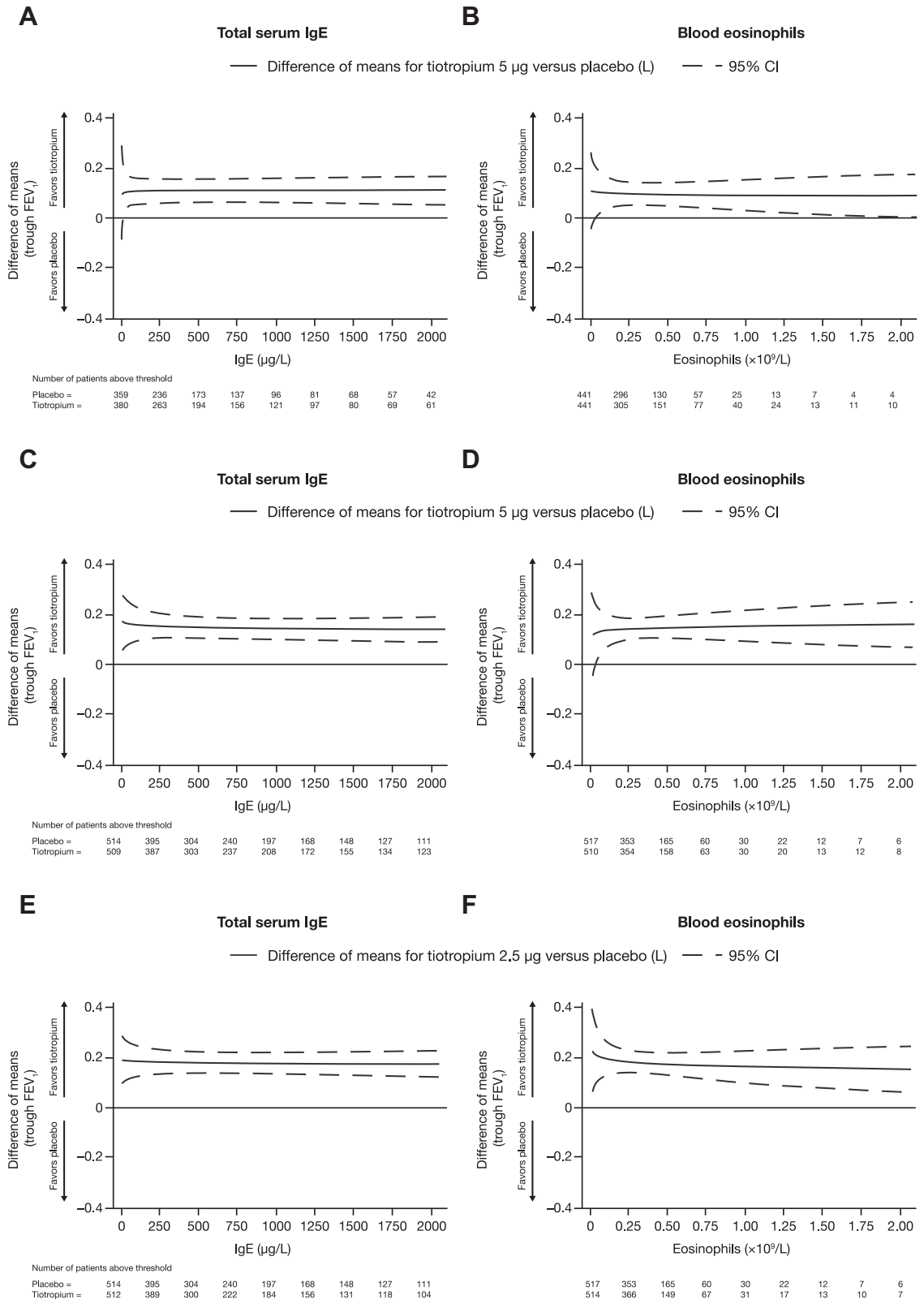

FIGURE 2. Trough FEV_1 in PrimoTinA-asthma (**A** and **B**) and MezzoTinA-asthma (**C**-**F**), according to baseline serum IgE levels (Figure 2, *A*, *C*, *E*) and blood eosinophil counts (Figure 2, *B*, *D*, *F*). Full analysis set. Adjusted mean difference (solid line) and 95% CIs (dotted line) are presented.

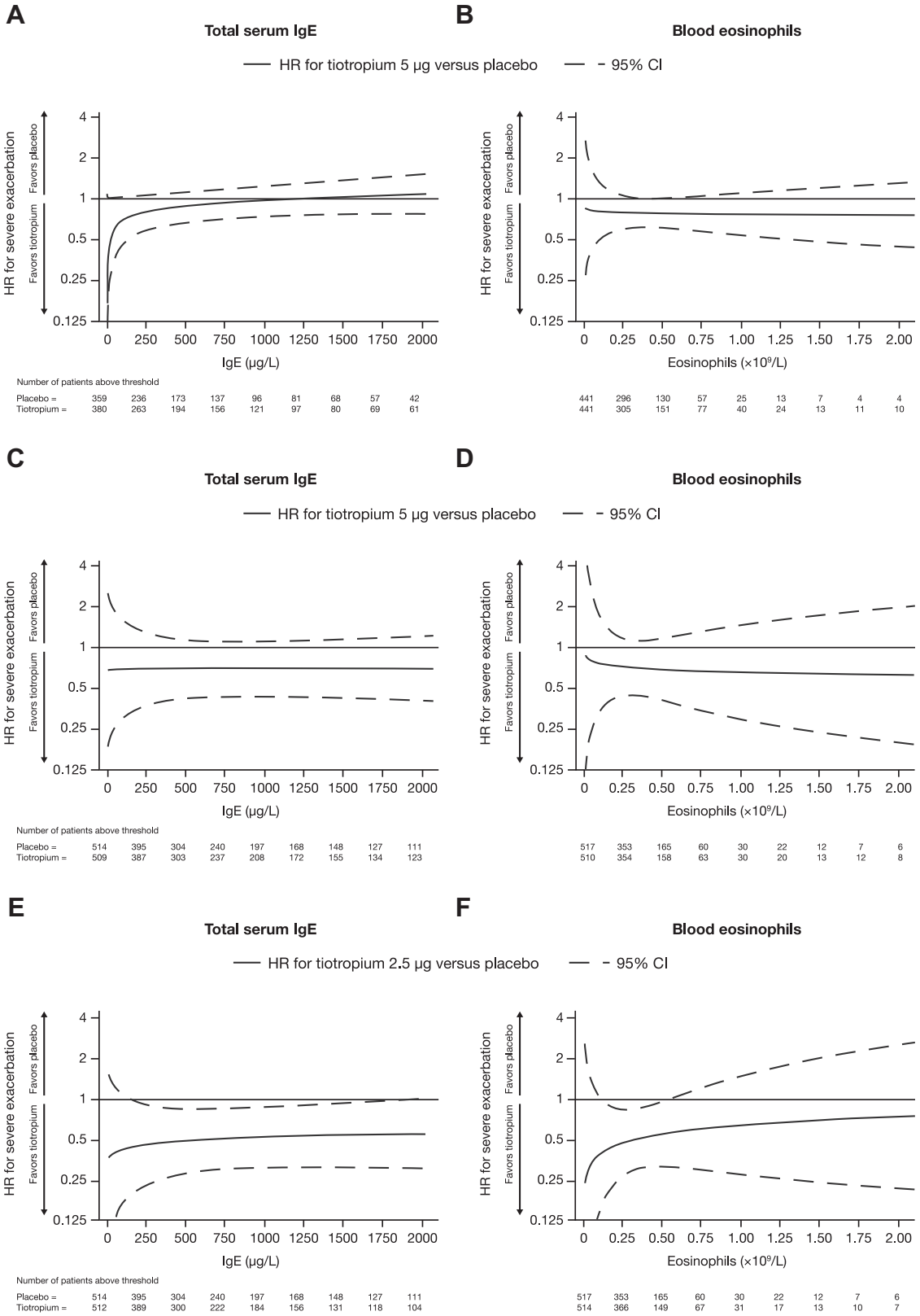

FIGURE 3. Risk of severe asthma exacerbation in PrimoTinA-asthma (**A** and **B**) and MezzoTinA-asthma (**C-F**), according to serum IgE levels (Figure 3, *A*, *C*, *E*) and blood eosinophil counts (Figure 3, *B*, *D*, *F*). Full analysis set. HRs (solid line) and 95% CIs (dotted line) are presented.

Estimating the HRs across the range of IgE levels and eosinophil counts in patients with severe asthma (PrimoTinA-asthma) demonstrated that tiotropium 5 μg reduced the risk of severe exacerbations versus placebo up to an IgE level of approximately 1250 μg/L (Figure 3, *A*) and across the range of eosinophil counts, with an almost constant HR of approximately 0.8 (Figure 3, *B*).

In patients with moderate asthma (MezzoTinA-asthma), similar modeling of HRs indicated that both doses of tiotropium reduced the risk of severe asthma exacerbations versus placebo across the full range of serum IgE levels and blood eosinophil counts measured (Figures 3, *C-F*). HRs generally ranged from approximately 0.5 to 0.75 but were lower at the lower end of eosinophil counts ($\sim 0.25 \times 10^9$/L) for the tiotropium 2.5 μg dose (Figure 3, *F*).

Time to first episode of asthma worsening. Both doses of tiotropium increased the time to first asthma worsening versus placebo for patients with severe (PrimoTinA-asthma) or moderate (MezzoTinA-asthma) asthma, with HRs of between 0.66 and 0.87 (Figure 4).

Categorical subgroup analyses showed that this was independent of IgE levels, blood eosinophil counts, and clinician judgment of allergic asthma for patients with severe asthma (PrimoTinA-asthma; see Figure E4 in this article's Online Repository at www.jaci-inpractice.org). For patients with moderate asthma (MezzoTinA-asthma), this was independent of blood eosinophil counts or clinician judgment of allergic asthma, although in categorical subgroup analysis an interaction *P* value of .04 (χ^2 test) was reported for serum IgE levels in this case (Figure E4).

Estimating the HRs across the range of IgE levels and eosinophil counts in patients with severe asthma (PrimoTinA-asthma) demonstrated that tiotropium 5 μg reduced the risk of asthma worsening versus placebo, independent of IgE levels, with an HR consistently below 1 (Figure 4, *A*), and of eosinophil counts, with an almost constant HR of approximately 0.70 (Figure 4, *B*).

In patients with moderate asthma (MezzoTinA-asthma), the HRs across the range of IgE levels and eosinophil counts showed that tiotropium 5 μg reduced the risk of asthma worsening versus placebo except at the very lowest IgE levels (Figure 4, *C*) and blood eosinophil counts (Figure 4, *D*). Similarly, tiotropium 2.5 μg also reduced the risk of asthma worsening versus placebo in patients with moderate asthma (MezzoTinA-asthma), independent of serum IgE levels, with HRs of approximately 0.65 (Figure 4, *E*), and of blood eosinophil counts. However, these HRs increased toward the higher end of the scale but with wide 95% CIs (Figure 4, *F*).

ACQ-7 responder rate. In the pooled PrimoTinA-asthma data, nominally significant improvements in the ACQ-7 responder rate were observed with tiotropium 5 μg at week 24 (OR, 1.31; Figure 5) and at week 48 (OR, 1.67) versus placebo. In the pooled MezzoTinA-asthma data, nominally significant improvements in the ACQ-7 responder rate (a coprimary end point) were observed with tiotropium 5 μg (OR, 1.32) and 2.5 μg (OR, 1.33) at week 24 versus placebo (Figure 5).[14]

The results of the categorical subgroup analysis in patients with severe asthma (PrimoTinA-asthma) demonstrated that tiotropium 5 μg was associated with numerical improvements in the ACQ-7 responder rate versus placebo at week 24 in $T2_{high}$ and $T2_{low}$ patients (see Figures E5, *A*, and E6, *A*, in this article's Online Repository at www.jaci-inpractice.org), except in patients with blood eosinophil counts of more than 0.6×10^9/L. Further improvements were observed in the ACQ-7 responder rate with tiotropium at week 48 (Figures E5, *B*, and E6, *B*), whereas the responder rate remained stable in the placebo arm with no further improvements at week 48.

In the categorical subgroup analyses of patients with moderate asthma (MezzoTinA-asthma), there was a higher proportion of ACQ-7 responders at week 24 with both doses of tiotropium compared with placebo across subgroups (Figure E6, *C*). ORs for tiotropium 5 μg and 2.5 μg were consistently above 1 in favor of tiotropium versus placebo, and no interaction between treatment and subgroups, IgE level, eosinophil count, or clinician assessment of allergic asthma was observed (see Figure E7 in this article's Online Repository at www.jaci-inpractice.org).

Estimating the ORs across the range of IgE levels and eosinophil counts in patients with severe asthma (PrimoTinA-asthma) showed that improvements in the ACQ-7 responder rate with tiotropium versus placebo were independent of IgE values and eosinophil counts (Figure 5, *A* and *B*). This was indicated by ORs consistently above 1 across the range of IgE levels and eosinophil counts.

In patients with moderate asthma (MezzoTinA-asthma), estimated ORs for tiotropium 5 μg were consistently in the region of 1.2 (Figure 5, *C* and *D*). Tiotropium 2.5 μg was associated with an improvement in the ACQ-7 responder rate versus placebo, except at the very lowest IgE levels (Figure 5, *E*) and eosinophil counts (Figure 5, *F*).

Safety

Full safety data have been presented previously,[13,14] albeit briefly: the incidence of adverse events was comparable between treatment arms in PrimoTinA-asthma and MezzoTinA-asthma, with asthma exacerbations, decreased peak expiratory flow rate, and nasopharyngitis the most frequently reported adverse events (Table E2). The incidence of investigator-defined drug-related adverse events, adverse events leading to discontinuation, and serious adverse events was low in all treatment arms, and no deaths occurred.

DISCUSSION

The Global Initiative for Asthma (GINA) treatment strategy includes tiotropium Respimat as an add-on therapy option in patients with a history of asthma exacerbations at step 4 or 5, with no requirement for prior phenotyping. Because treatment decisions should be optimized on the patient level, we aimed to provide further scientific evidence in line with this guidance, thus enabling physicians to make educated and individualized treatment decisions.

As a bronchodilator, the long-acting muscarinic agonist tiotropium reduces airflow obstruction by its antagonism of M3 receptors, leading to airway smooth muscle relaxation. Airflow obstruction can be observed across different asthma phenotypes, and therefore a consistent benefit of tiotropium can be expected. However, stimulation of muscarinic receptors by acetylcholine has also been shown to have proinflammatory effects. Because asthma is often associated with a significant allergic component, we explored whether responses to tiotropium were influenced by patients' allergic and/or T2 inflammatory status. We present here comprehensive subgroup analyses that investigate the influence of categorized and linear continuous baseline parameters on the treatment effect of tiotropium. These analyses suggest that the improvements versus placebo in lung function and asthma control seen with once-daily tiotropium when added to ICS (with or without other maintenance therapies) and administered via Respimat are independent of T2 phenotype. Furthermore, the risk of severe asthma exacerbations and asthma worsening is also reduced versus placebo, independent of T2 phenotype.

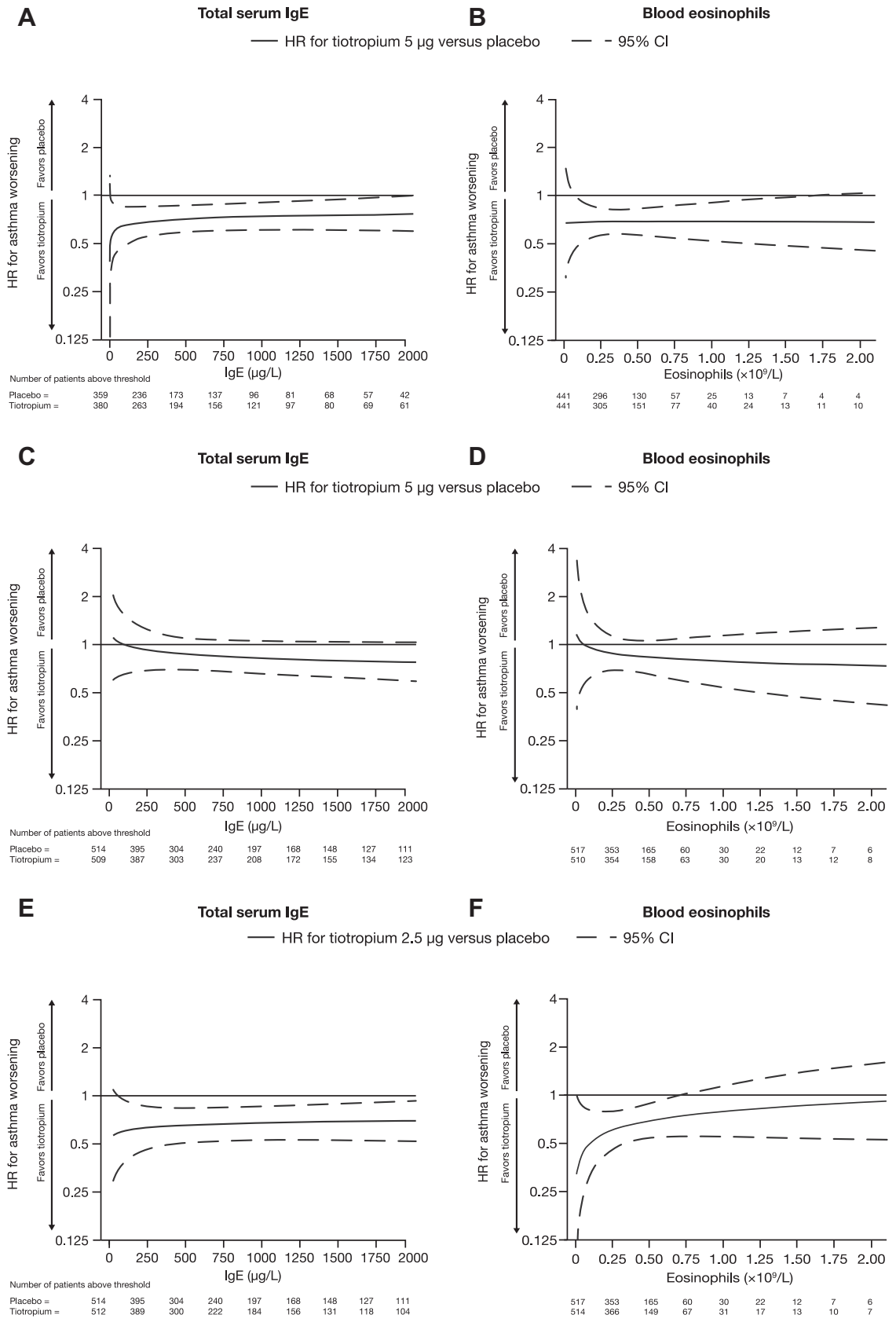

FIGURE 4. Risk of asthma worsening in PrimoTinA-asthma (**A** and **B**) and MezzoTinA-asthma (**C**-**F**), according to serum IgE levels (Figure 4, *A*, *C*, *E*) and blood eosinophil counts (Figure 4, *B*, *D*, *F*). Full analysis set. HRs (solid line) and 95% CIs (dotted line) are presented.

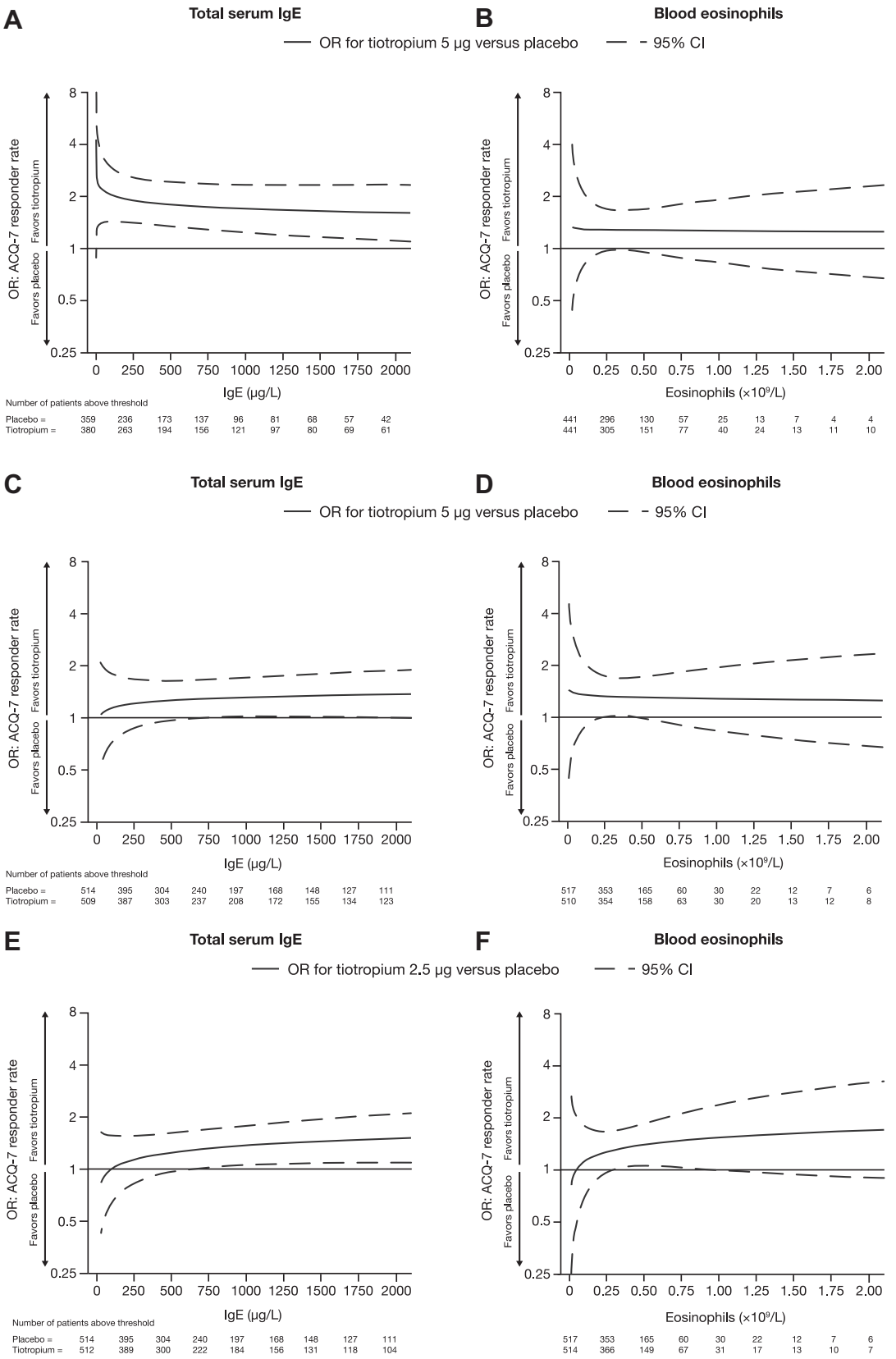

FIGURE 5. Logistic regression analysis of the ACQ-7 responder rate at week 24 in PrimoTinA-asthma (**A** and **B**) and MezzoTinA-asthma (**C-F**), according to serum IgE levels (Figure 5, *A*, *C*, *E*) and blood eosinophil counts (Figure 5, *B*, *D*, *F*). Full analysis set. ORs (solid line) and 95% CIs (dotted line) are presented.

These data support the positioning of tiotropium Respimat as an add-on therapy option in patients with a history of asthma exacerbations at step 4 or 5, with no requirement for prior phenotyping, as per GINA treatment strategy. In contrast, biologic agents that target T2 factors such as eosinophils or IgE require phenotyping as criteria for treatment. Taking the additional costs and resources associated with biologic treatment options into account, it may be appropriate to prescribe tiotropium before biologic agents, and our data provide reassurance regarding the efficacy and safety of tiotropium for patients with $T2_{high}$ and $T2_{low}$ asthma, independent of concomitant controller therapy. However, optimal treatment decisions should be individualized, and it is important to note that our data do not provide head-to-head comparisons of tiotropium and biologic agents.

Stimulation of muscarinic receptors by acetylcholine causes various proinflammatory effects, the blockage of which by tiotropium could account for its efficacy in both $T2_{high}$ and $T2_{low}$ asthma. Therefore, it is interesting to consider the results of our current analysis in the context of nonclinical studies that have investigated the potential anti−T2-inflammatory effects of tiotropium. For example, tiotropium significantly reduced inflammatory cell numbers and T_H2 cytokine levels in an *in vivo* chronic asthma model[28,29] and prevented both antigen-induced eosinophilia and hyperreactivity of airway tissues in antigen-challenged guinea pigs, at least in part via an anti-inflammatory mechanism.[30] Tiotropium prevents allergen-induced goblet cell hyperplasia and mucus gland hypertrophy in guinea pigs[31] and IL-13−induced goblet cell differentiation in human airway epithelial cells *in vitro*,[32] which could contribute to the effect of tiotropium in preventing exacerbations. However, tiotropium could also affect non−$T2_{high}$-driven inflammation by various mechanisms. Tiotropium has been shown to inhibit *ex vivo* neutrophil chemotaxis from patients with chronic obstructive pulmonary disease, as well as acting on alveolar macrophages to inhibit the release of reactive oxygen species.[33] Similar mechanisms may be responsible for the efficacy of tiotropium in patients with $T2_{low}$ asthma; neutrophilic inflammation has been documented in patients with asthma[34] and may contribute to the disease pathogenesis via the release of proteases and reactive oxygen species, both of which damage airways leading to airway hyperresponsiveness and remodeling. In addition, neutrophilic infiltration has been suggested as a marker of uncontrolled asthma and is inversely correlated with FEV_1.[34] Tiotropium has been shown *in vitro* to reduce levels of IL-8 in a human bronchial epithelial cell line, which may reduce not only neutrophil chemotaxis but also bronchial smooth muscle cell chemotaxis and proliferation, known effects of IL-8.[35]

Our studies do have some limitations. The percentage of patients with recorded concomitant allergic rhinitis reported here was lower than expected. This may have been due to underreporting of allergic rhinitis. Although the assessment of allergic asthma status was prompted by a specific "Yes"/"No" question in the case report form, allergic rhinitis was recorded as part of the patients' overall baseline conditions and at the initiative of the individual investigator. However, we consider the patient population to be broadly representative of those encountered in clinical practice because more than 90% of those patients with allergic rhinitis recorded as a baseline condition were also judged to have allergic asthma. In the absence of an agreed definition for the T2 phenotype at the time of the study, the assessment of "potentially allergic asthma" by a composite of serum IgE, blood eosinophil levels, and "investigator judgment for allergic asthma" is a necessary compromise, and mirrors the assessments made by physicians in clinical practice. The categories proposed were those commonly used in clinical practice, but, in retrospect, may be imperfect. However, they are not dissimilar to those used in several studies of biologic and antiallergy treatments.

Another potential limitation of this work is that subgroup analyses performed by categorization of continuous baseline parameters (such as biomarkers) require the selection of cutoff thresholds. We selected cutoff thresholds for serum IgE and blood eosinophils on the basis of reported upper limits of normal ranges.[27] However, there is little consensus on the most appropriate cutoff value for these biomarkers and, in addition, small sample sizes might be obtained in some cutoff groupings. Therefore, we modeled the influence of both parameters on the treatment effect over the whole range of the parameter values for peak and trough FEV_1, time to first severe exacerbation, time to first episode of asthma worsening, and the ACQ-7 responder rate. Our modeling approach is a straightforward *post hoc* investigation to support categorical subgroup analyses. Alternative approaches, including mathematically more complex ones, can be found in the literature.[36-39] The advantage of such modeling is that no explicit cutoff thresholds for subgroup categorization need to be selected and defended *a priori*. These exploratory modeling analyses by serum IgE and blood eosinophils in pooled data from the PrimoTinA-asthma and MezzoTinA-asthma trials support the findings from the categorical subgroup analyses, demonstrating the efficacy of tiotropium independent of patients' T2 status. Overall, our modeling analysis supports the main results and conclusions and adds confidence to the beneficial effect of tiotropium across a wide range of biomarker values.

The 18-month recruitment period, along with the duration of treatment (PrimoTinA-asthma, 48 weeks; MezzoTinA-asthma, 24 weeks), ensured that any seasonal variations in patients' allergic responses were taken into account.

The findings from the analyses presented here are consistent with those from the "Tiotropium Bromide as an Alternative to Increased Inhaled Glucocorticoid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid" (TALC) study, in which the effect of tiotropium was independent of atopy, IgE level, and sputum eosinophil count.[40] The data presented here expand on the evidence from the TALC study by showing a treatment effect of tiotropium on patient-relevant end points including exacerbations and asthma worsening, independent of serum IgE levels or blood eosinophil counts. Furthermore, unlike the TALC study, we assessed the effect of tiotropium on these end points when added to ICS + LABA treatment, as currently recommended by GINA[21] and representative of clinical practice. Finally, patients had more severe asthma, especially in the PrimoTinA-asthma studies, compared with the TALC study.

CONCLUSIONS

Results from the primary clinical trials showed that in patients with moderate or severe symptomatic asthma despite ICS with or without other maintenance therapies, once-daily tiotropium Respimat improved airflow, reduced exacerbation risk, and improved asthma symptom control. The results of our exploratory analyses suggest that these improvements are independent of T2 status as assessed by IgE levels or eosinophil counts—supported by categorical subgroup analysis and modeling of the influence of these parameters on the treatment effects. Modeling analyses like our approach prove valuable as a supportive tool in subgroup analyses where there is no obvious *a priori* choice of cutoff thresholds. Although our findings remain exploratory, they support the potential use of tiotropium Respimat add-on

therapy across a range of asthma severities, independent of patients' T2 status.

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REFERENCES

1. Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469-78.
2. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJH, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med 2004;170:836-44.
3. Partridge MR, Dal Negro RW, Olivieri D. Understanding patients with asthma and COPD: insights from a European study. Prim Care Respir J 2011;20:315-23.
4. Demoly P, Paggiaro P, Plaza V, Bolge SC, Kannan H, Sohier B, et al. Prevalence of asthma control among adults in France, Germany, Italy, Spain and the UK. Eur Respir Rev 2009;18:105-12.
5. Sims EJ, Price D, Haughney J, Ryan D, Thomas M. Current control and future risk in asthma management. Allergy Asthma Immunol Res 2011;3:217-25.
6. Bavbek S, Mungan D, Türktaş H, Mısırlıgil Z, Gemicioğlu B, ADVISE Study Group. A cost-of-illness study estimating the direct cost per asthma exacerbation in Turkey. Respir Med 2011;105:541-8.
7. Ivanova JI, Bergman R, Birnbaum HG, Colice GL, Silverman RA, McLaurin K. Effect of asthma exacerbations on health care costs among asthmatic patients with moderate and severe persistent asthma. J Allergy Clin Immunol 2012;129:1229-35.
8. Lane S, Molina J, Plusa T. An international observational prospective study to determine the Cost Of Asthma eXacerbations (COAX). Respir Med 2006;100:434-50.
9. O'Byrne PM, Pedersen S, Lamm CJ, Tan WC, Busse WW, START Investigators Group. Severe exacerbations and decline in lung function in asthma. Am J Respir Crit Care Med 2009;179:19-24.
10. Ohta K, Ichinose M, Tohda Y, Engel M, Moroni-Zentgraf P, Kunimitsu S, et al. Long-term once-daily tiotropium Respimat® is well tolerated and maintains efficacy over 52 weeks in patients with symptomatic asthma in Japan: a randomised, placebo-controlled study. PLoS One 2015;10:e0124109.
11. Paggiaro P, Halpin DM, Buhl R, Engel M, Zubek VB, Blahova Z, et al. The effect of tiotropium in symptomatic asthma despite low- to medium-dose inhaled corticosteroids: a randomized controlled trial. J Allergy Clin Immunol Pract 2016;4:104-113.e2.
12. Bateman ED, Kornmann O, Schmidt P, Pivovarova A, Engel M, Fabbri LM. Tiotropium is noninferior to salmeterol in maintaining improved lung function in B16-Arg/Arg patients with asthma. J Allergy Clin Immunol 2011;128:315-22.
13. Kerstjens HAM, Engel M, Dahl R, Paggiaro P, Beck E, Vandewalker M, et al. Tiotropium in asthma poorly controlled with standard combination therapy. N Engl J Med 2012;367:1198-207.
14. Kerstjens HAM, Casale TB, Bleecker ER, Meltzer EO, Pizzichini E, Schmidt O, et al. Tiotropium or salmeterol as add-on therapy to inhaled corticosteroids for patients with moderate symptomatic asthma: two replicate, double-blind, placebo-controlled, parallel-group, active-comparator, randomised trials. Lancet Respir Med 2015;3:367-76.
15. Hamelmann E, Bateman ED, Vogelberg C, Szefler SJ, Vandewalker M, Moroni-Zentgraf P, et al. Tiotropium add-on therapy in adolescents with moderate asthma: a 1-year randomized controlled trial. J Allergy Clin Immunol 2016;138:441-450.e8.
16. Bisgaard H, Vandewalker M, Graham LM, Moroni-Zentgraf P, Engel M, El Azzi G, et al. Safety of tiotropium in pre-school children with symptomatic persistent asthma. Poster PA315 presented at the European Respiratory Society International Congress, London, UK; September 3-7, 2016.
17. Vogelberg C, Laki I, Schmidt O, Hamelmann E, El Azzi G, Engel M, et al. Safety and tolerability of once-daily tiotropium Respimat® add-on therapy in children with moderate symptomatic asthma. Poster PA4399 presented at the European Respiratory Society International Congress, London, UK; September 3-7, 2016.
18. Schmidt O, Hamelmann E, Vogelberg C, Laki I, El Azzi G, Engel M, et al. Once-daily tiotropium Respimat® add-on therapy improves lung function in children with moderate symptomatic asthma. Poster PA4398 presented at the European Respiratory Society International Congress, London, UK; September 3-7, 2016.
19. Hamelmann E, Bernstein JA, Vandewalker M, Moroni-Zentgraf P, Verri D, Unseld A, et al. A randomised controlled trial of tiotropium in adolescents with severe symptomatic asthma. Eur Respir J 2017;49:1601100. https://doi.org/10.1183/13993003.01100-2016.
20. Szefler SJ, Murphy K, Harper T III, Boner A, Laki I, Engel M, et al. A phase III randomized controlled trial of tiotropium add-on therapy in children with severe symptomatic asthma [published online ahead of print February 9, 2017]. J Allergy Clin Immunol. http://dx.doi.org/10.1016/j.jaci.2017.01.014.
21. Global Initiative for Asthma. Global strategy for asthma management and prevention. Updated 2016. Available at: http://ginasthma.org/2016-gina-report-global-strategy-for-asthma-management-and-prevention/. Accessed November 7, 2016.
22. Price D. The use of omalizumab in asthma. Prim Care Respir J 2008;17:62-72.
23. Parulekar AD, Diamant Z, Hanania NA. Role of T2 inflammation biomarkers in severe asthma. Curr Opin Pulm Med 2016;22:59-68.
24. Lugogo N, Domingo C, Chanez P, Leigh R, Gilson MJ, Price RG, et al. Long-term efficacy and safety of mepolizumab in patients with severe eosinophilic asthma: a multi-center, open-label, phase IIIb study. Clin Ther 2016;38:2058-2070.e1.
25. Bjermer L, Lemiere C, Maspero J, Weiss S, Zangrilli J, Germinaro M. Reslizumab for inadequately controlled asthma with elevated blood eosinophil levels: a randomized phase 3 study. Chest 2016;150:789-98.
26. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902-7.
27. Fauci AS, Harrison TR. Harrison's manual of medicine. 17th ed. New York: McGraw-Hill Medical; 2009.
28. Ohta S, Oda N, Yokoe T, Tanaka A, Yamamoto Y, Watanabe Y, et al. Effect of tiotropium bromide on airway inflammation and remodelling in a mouse model of asthma. Clin Exp Allergy 2010;40:1266-75.
29. Kang JY, Rhee CK, Kim JS, Park CK, Kim SJ, Lee SH, et al. Effect of tiotropium bromide on airway remodeling in a chronic asthma model. Ann Allergy Asthma Immunol 2012;109:29-35.
30. Buels KS, Jacoby DB, Fryer AD. Non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea-pig model of allergic asthma. Br J Pharmacol 2012;165:1501-14.
31. Bos IST, Gosens R, Zuidhof AB, Schaafsma D, Halayko AJ, Meurs H, et al. Inhibition of allergen-induced airway remodelling by tiotropium and budesonide: a comparison. Eur Respir J 2007;30:653-61.
32. Kistemaker LEM, Hiemstra PS, Bos IST, Bouwman S, van den Berge M, Hylkema MN, et al. Tiotropium attenuates IL-13-induced goblet cell metaplasia of human airway epithelial cells. Thorax 2015;70:668-76.
33. Vacca G, Randerath WJ, Gillissen A. Inhibition of granulocyte migration by tiotropium bromide. Respir Res 2011;12:24.
34. Hosoki K, Ying S, Corrigan C, Qi H, Kurosky A, Jennings K, et al. Analysis of a panel of 48 cytokines in BAL fluids specifically identifies IL-8 levels as the only cytokine that distinguishes controlled asthma from uncontrolled asthma, and correlates inversely with FEV_1. PLoS One 2015;10:e0126035.
35. Montalbano AM, Albano GD, Bonanno A, Riccobono L, Di Sano C, Ferraro M, et al. Autocrine acetylcholine, induced by IL-17A via NFkB and ERK1/2 pathway activation, promotes MUC5AC and IL-8 synthesis in bronchial epithelial cells. Mediators Inflamm 2016;2016:9063842.
36. Royston P, Sauerbrei W. A new approach to modelling interactions between treatment and continuous covariates in clinical trials by using fractional polynomials. Stat Med 2004;23:2509-25.
37. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med 2006;25:127-41.
38. Jiang W, Freidlin B, Simon R. Biomarker-adaptive threshold design: a procedure for evaluating treatment with possible biomarker-defined subset effect. J Natl Cancer Inst 2007;99:1036-43.
39. Thomas M, Bornkamp B. Comparing approaches to treatment effect estimation for subgroups in early phase clinical trials. Stat Biopharm Res 2017;9:160-71.
40. Peters SP, Bleecker ER, Kunselman SJ, Icitovic N, Moore WC, Pascual R, et al. Predictors of response to tiotropium versus salmeterol in asthmatic adults. J Allergy Clin Immunol 2013;132:1068-1074.e1.

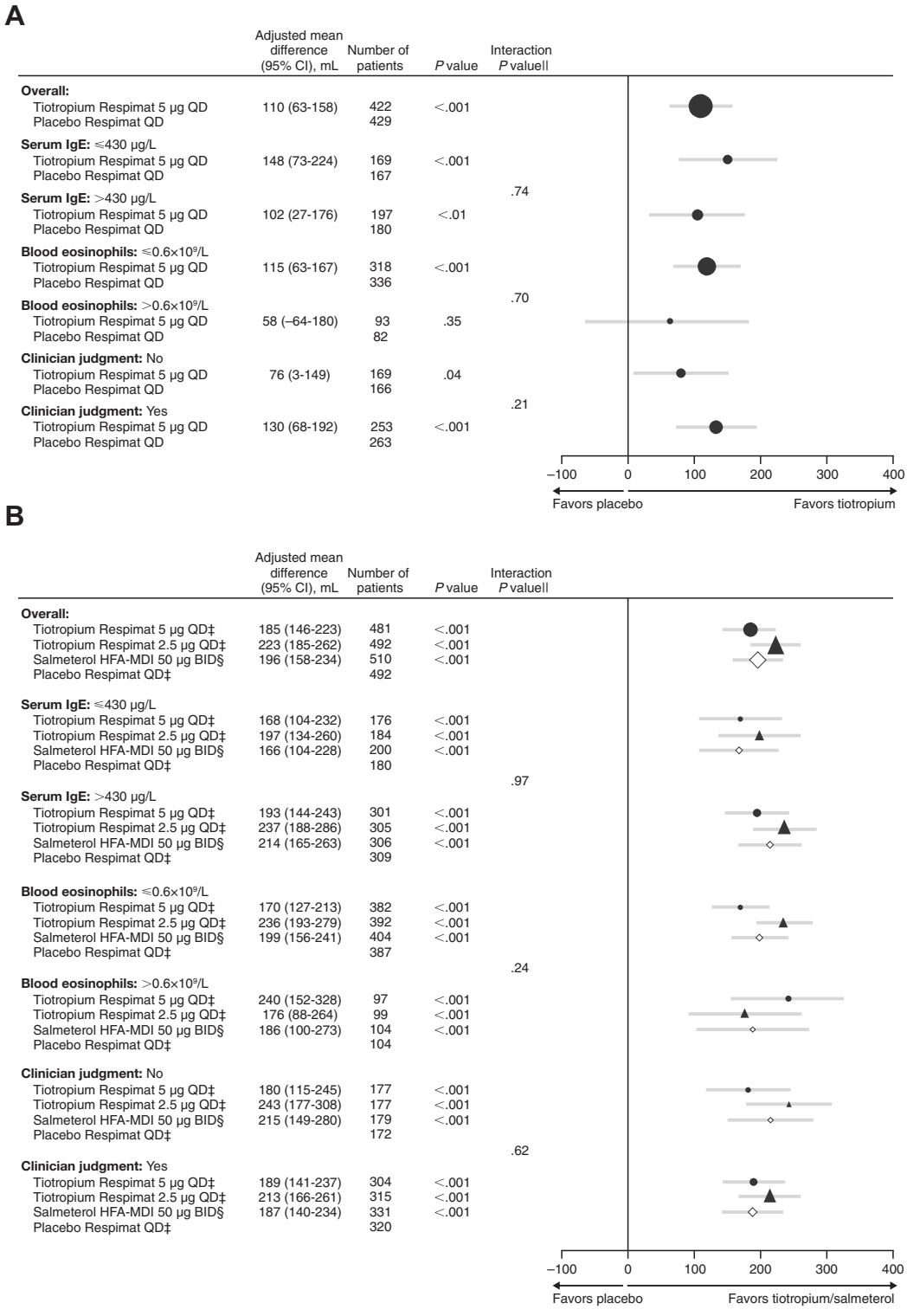
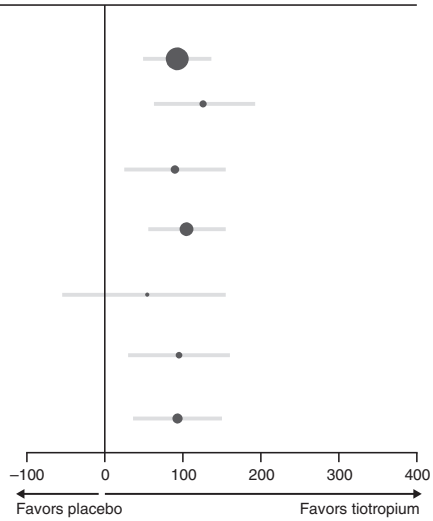


FIGURE E1. Peak $FEV_{1(0\text{-}3h)}$ responses at week 24 in *PrimoTinA-asthma (**A**) and †MezzoTinA-asthma (**B**), by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily. Some of these data have been published previously in Kerstjens et al.[E1] Reproduced according to the license terms of creative commons (https://creativecommons.org/licenses/by-nc-nd/4.0/). Full analysis set. Treatment effect *P* values based on *t* test. *Pooled data; add-on to ICSs (≥800 µg budesonide or equivalent per day) + LABA. †Pooled data; add-on to ICSs (400-800 µg budesonide or equivalent). ‡Plus placebo HFA-MDI BID. §Plus placebo Respimat QD. ||Treatment-by-subgroup interaction *P* value based on *t* test.

A

	Adjusted mean difference (95% CI), mL	Number of patients	*P* value	Interaction *P* value‖
Overall:				
Tiotropium Respimat 5 μg QD	93 (50-137)	421	<.001	
Placebo Respimat QD		429		
Serum IgE: ≤430 μg/L				
Tiotropium Respimat 5 μg QD	127 (61-194)	168	<.001	
Placebo Respimat QD		167		
				.62
Serum IgE: >430 μg/L				
Tiotropium Respimat 5 μg QD	89 (22-156)	197	<.01	
Placebo Respimat QD		180		
Blood eosinophils: ≤0.6×10⁹/L				
Tiotropium Respimat 5 μg QD	103 (53-152)	317	<.001	
Placebo Respimat QD		336		
				.75
Blood eosinophils: >0.6×10⁹/L				
Tiotropium Respimat 5 μg QD	52 (–49-153)	93	.31	
Placebo Respimat QD		82		
Clinician judgment: No				
Tiotropium Respimat 5 μg QD	94 (28-160)	169	<.01	
Placebo Respimat QD		166		
				.41
Clinician judgment: Yes				
Tiotropium Respimat 5 μg QD	91 (33-148)	252	<.01	
Placebo Respimat QD		263		



B

	Adjusted mean difference (95% CI), mL	Number of patients	*P* value	Interaction *P* value‖
Overall:				
Tiotropium Respimat 5 μg QD‡	146 (105-188)	481	<.001	
Tiotropium Respimat 2.5 μg QD‡	180 (138-221)	492	<.001	
Salmeterol HFA-MDI 50 μg BID§	114 (73-155)	510	<.001	
Placebo Respimat QD‡		492		
Serum IgE: ≤430 μg/L				
Tiotropium Respimat 5 μg QD‡	139 (72-206)	176	<.001	
Tiotropium Respimat 2.5 μg QD‡	167 (101-233)	184	<.001	
Salmeterol HFA-MDI 50 μg BID§	115 (50-180)	200	<.001	
Placebo Respimat QD‡		180		
				.84
Serum IgE: >430 μg/L				
Tiotropium Respimat 5 μg QD‡	152 (98-205)	301	<.001	
Tiotropium Respimat 2.5 μg QD‡	188 (135-242)	305	<.001	
Salmeterol HFA-MDI 50 μg BID§	115 (61-168)	306	<.001	
Placebo Respimat QD‡		309		
Blood eosinophils: ≤0.6×10⁹/L				
Tiotropium Respimat 5 μg QD‡	137 (90-183)	382	<.001	
Tiotropium Respimat 2.5 μg QD‡	185 (139-231)	392	<.001	
Salmeterol HFA-MDI 50 μg BID§	115 (69-160)	404	<.001	
Placebo Respimat QD‡		387		
				.51
Blood eosinophils: >0.6×10⁹/L				
Tiotropium Respimat 5 μg QD‡	182 (86-277)	97	<.001	
Tiotropium Respimat 2.5 μg QD‡	158 (62-253)	99	<.01	
Salmeterol HFA-MDI 50 μg BID§	111 (17-205)	104	.02	
Placebo Respimat QD‡		104		
Clinician judgment: No				
Tiotropium Respimat 5 μg QD‡	138 (68-208)	177	<.001	
Tiotropium Respimat 2.5 μg QD‡	209 (139-279)	177	<.001	
Salmeterol HFA-MDI 50 μg BID§	134 (64-204)	179	<.001	
Placebo Respimat QD‡		172		
				.67
Clinician judgment: Yes				
Tiotropium Respimat 5 μg QD‡	153 (101-204)	304	<.001	
Tiotropium Respimat 2.5 μg QD‡	164 (113-215)	315	<.001	
Salmeterol HFA-MDI 50 μg BID§	105 (54-155)	331	<.001	
Placebo Respimat QD‡		320		

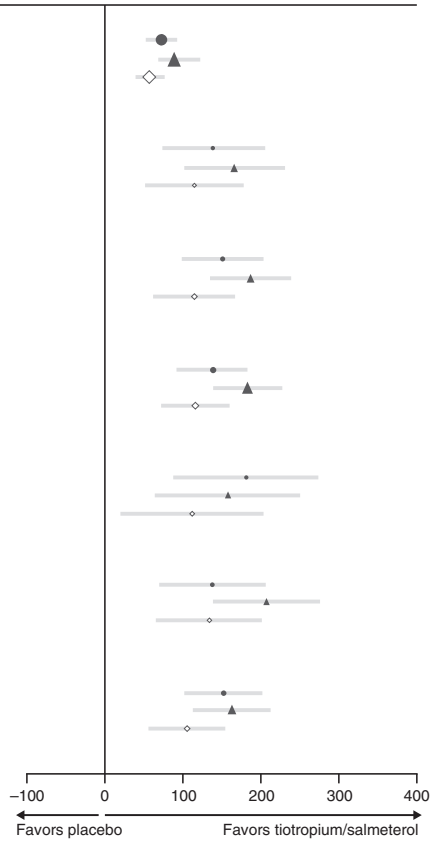


FIGURE E2. Trough FEV_1 responses at week 24 in *PrimoTinA-asthma (**A**) and †MezzoTinA-asthma (**B**), by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily. Some of these data have been published previously in Kerstjens et al.[E1] Reproduced according to the license terms of creative commons (https://creativecommons.org/licenses/by-nc-nd/4.0/). Full analysis set. Treatment effect *P* values based on *t* test. *Pooled data; add-on to ICSs (≥800 μg budesonide or equivalent per day) + LABA. †Pooled data; add-on to ICSs (400-800 μg budesonide or equivalent). ‡Plus placebo HFA-MDI BID. §Plus placebo Respimat QD. ‖Treatment-by-subgroup interaction *P* value based on *t* test.

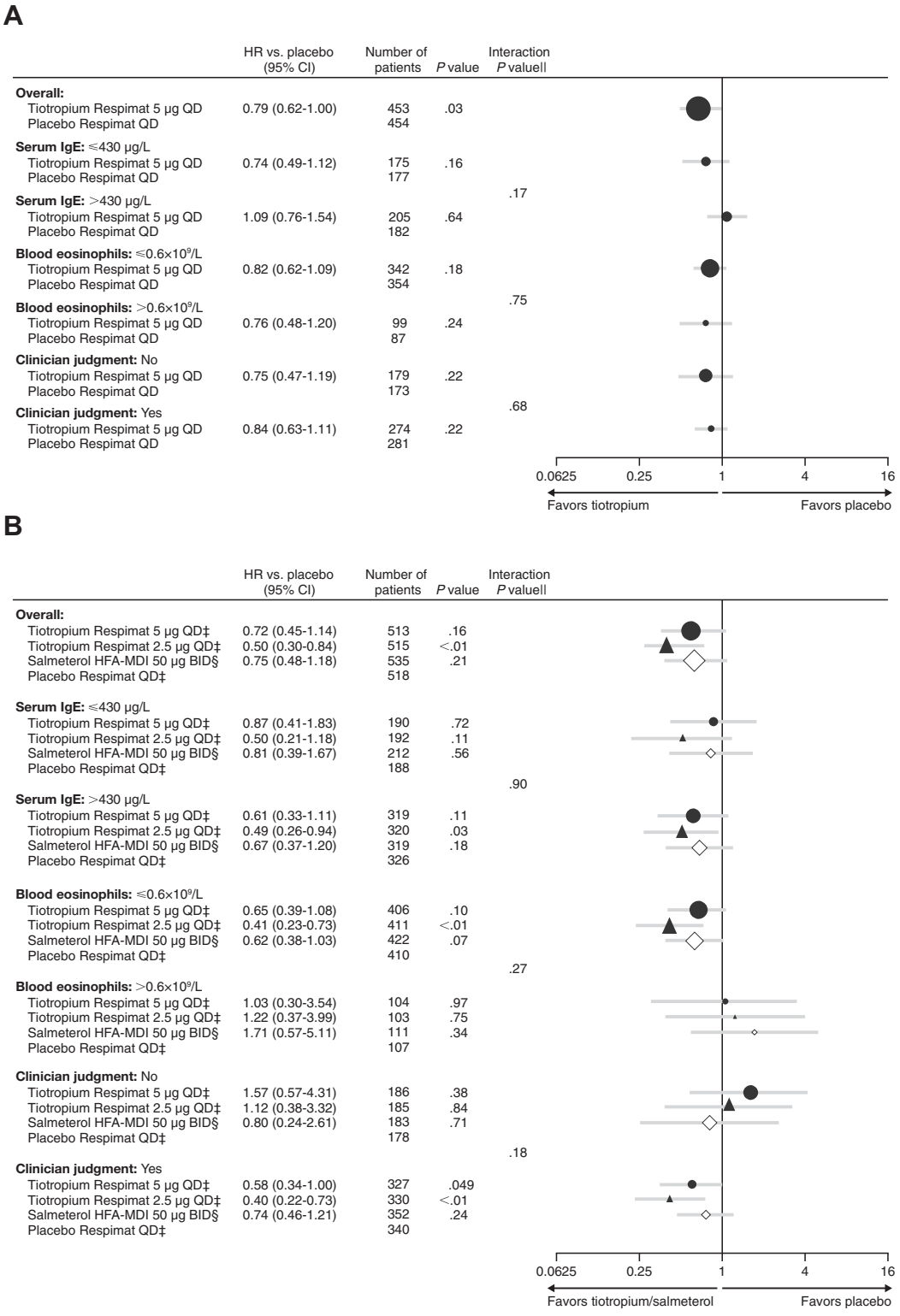
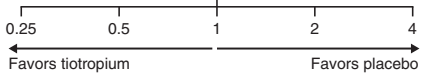


FIGURE E3. Time to severe exacerbation in *PrimoTinA-asthma at week 48 (**A**) and †MezzoTinA-asthma at week 24 (**B**), by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily. Calculation of the HR's point estimate, CI, and *P* value follow the confirmatory result of these studies as described by Kerstjens HA et al,[E1] where some of these data have been published previously. Reproduced according to the license terms of creative commons (https://creativecommons.org/licenses/by-nc-nd/4.0/). Full analysis set. Treatment effect *P* values based on χ^2 test with df = 1. *Pooled data; add-on to ICSs (≥800 µg budesonide or equivalent per day) + LABA. †Pooled data; add-on to ICSs (400-800 µg budesonide or equivalent). ‡Plus placebo HFA-MDI BID. §Plus placebo Respimat QD. ||Treatment-by-subgroup interaction *P* value based on χ^2 with df = 1.

A

	HR vs. placebo (95% CI)	Number of patients	*P* value	Interaction *P* value‖
Overall:				
Tiotropium Respimat 5 μg QD	0.69 (0.58-0.82)	453	<.001	
Placebo Respimat QD		454		
Serum IgE: ≤430 μg/L				
Tiotropium Respimat 5 μg QD	0.72 (0.54-0.97)	175	.03	
Placebo Respimat QD		177		
				1.00
Serum IgE: >430 μg/L				
Tiotropium Respimat 5 μg QD	0.73 (0.57-0.95)	205	.02	
Placebo Respimat QD		182		
Blood eosinophils: ≤0.6×10⁹/L				
Tiotropium Respimat 5 μg QD	0.65 (0.53-0.79)	342	<.001	
Placebo Respimat QD		354		
				.25
Blood eosinophils: >0.6×10⁹/L				
Tiotropium Respimat 5 μg QD	0.85 (0.59-1.21)	99	.36	
Placebo Respimat QD		87		
Clinician judgment: No				
Tiotropium Respimat 5 μg QD	0.69 (0.51-0.93)	179	.01	
Placebo Respimat QD		173		
				.89
Clinician judgment: Yes				
Tiotropium Respimat 5 μg QD	0.70 (0.57-0.87)	274	<.01	
Placebo Respimat QD		281		



B

	HR vs. placebo (95% CI)	Number of patients	*P* value	Interaction *P* value‖
Overall:				
Tiotropium Respimat 5 μg QD‡	0.87 (0.69-1.09)	513	.22	
Tiotropium Respimat 2.5 μg QD‡	0.66 (0.52-0.84)	515	<.001	
Salmeterol HFA-MDI 50 μg BID§	0.75 (0.60-0.94)	535	.01	
Placebo Respimat QD‡		518		
Serum IgE: ≤430 μg/L				
Tiotropium Respimat 5 μg QD‡	0.88 (0.62-1.26)	190	.50	
Tiotropium Respimat 2.5 μg QD‡	0.45 (0.29-0.68)	192	<.001	
Salmeterol HFA-MDI 50 μg BID§	0.60 (0.41-0.88)	212	<.01	
Placebo Respimat QD‡		188		
				.04
Serum IgE: >430 μg/L				
Tiotropium Respimat 5 μg QD‡	0.84 (0.63-1.12)	319	.22	
Tiotropium Respimat 2.5 μg QD‡	0.82 (0.61-1.09)	320	.17	
Salmeterol HFA-MDI 50 μg BID§	0.85 (0.64-1.13)	319	.25	
Placebo Respimat QD‡		326		
Blood eosinophils: ≤0.6×10⁹/L				
Tiotropium Respimat 5 μg QD‡	0.90 (0.70-1.16)	406	.41	
Tiotropium Respimat 2.5 μg QD‡	0.60 (0.46-0.79)	411	<.001	
Salmeterol HFA-MDI 50 μg BID§	0.70 (0.54-0.91)	422	<.01	
Placebo Respimat QD‡		410		
				.12
Blood eosinophils: >0.6×10⁹/L				
Tiotropium Respimat 5 μg QD‡	0.70 (0.42-1.18)	104	.18	
Tiotropium Respimat 2.5 μg QD‡	0.92 (0.56-1.49)	103	.72	
Salmeterol HFA-MDI 50 μg BID§	0.93 (0.58-1.49)	111	.77	
Placebo Respimat QD‡		107		
Clinician judgment: No				
Tiotropium Respimat 5 μg QD‡	0.82 (0.57-1.17)	186	.28	
Tiotropium Respimat 2.5 μg QD‡	0.69 (0.48-1.00)	185	>.05	
Salmeterol HFA-MDI 50 μg BID§	0.74 (0.51-1.07)	183	.11	
Placebo Respimat QD‡		178		
				.94
Clinician judgment: Yes				
Tiotropium Respimat 5 μg QD‡	0.89 (0.67-1.19)	327	.42	
Tiotropium Respimat 2.5 μg QD‡	0.64 (0.47-0.87)	330	<.01	
Salmeterol HFA-MDI 50 μg BID§	0.76 (0.56-1.01)	352	.06	
Placebo Respimat QD‡		340		

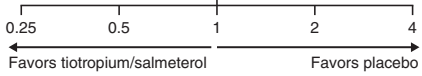


FIGURE E4. Time to asthma worsening in *PrimoTinA-asthma at week 48 (**A**) and †MezzoTinA-asthma at week 24 (**B**), by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily. Some of these data have been published previously in Kerstjens et al.[E1] Reproduced according to the license terms of creative commons (https://creativecommons.org/licenses/by-nc-nd/4.0/). Full analysis set. Treatment effect *P* values based on χ^2 test with df = 1. *Pooled data; add-on to ICSs (≥800 μg budesonide or equivalent per day) + LABA. †Pooled data; add-on to ICSs (400-800 μg budesonide or equivalent). ‡Plus placebo HFA-MDI BID. §Plus placebo Respimat QD. ||Treatment-by-subgroup interaction *P* value based on χ^2 test with df = 1.

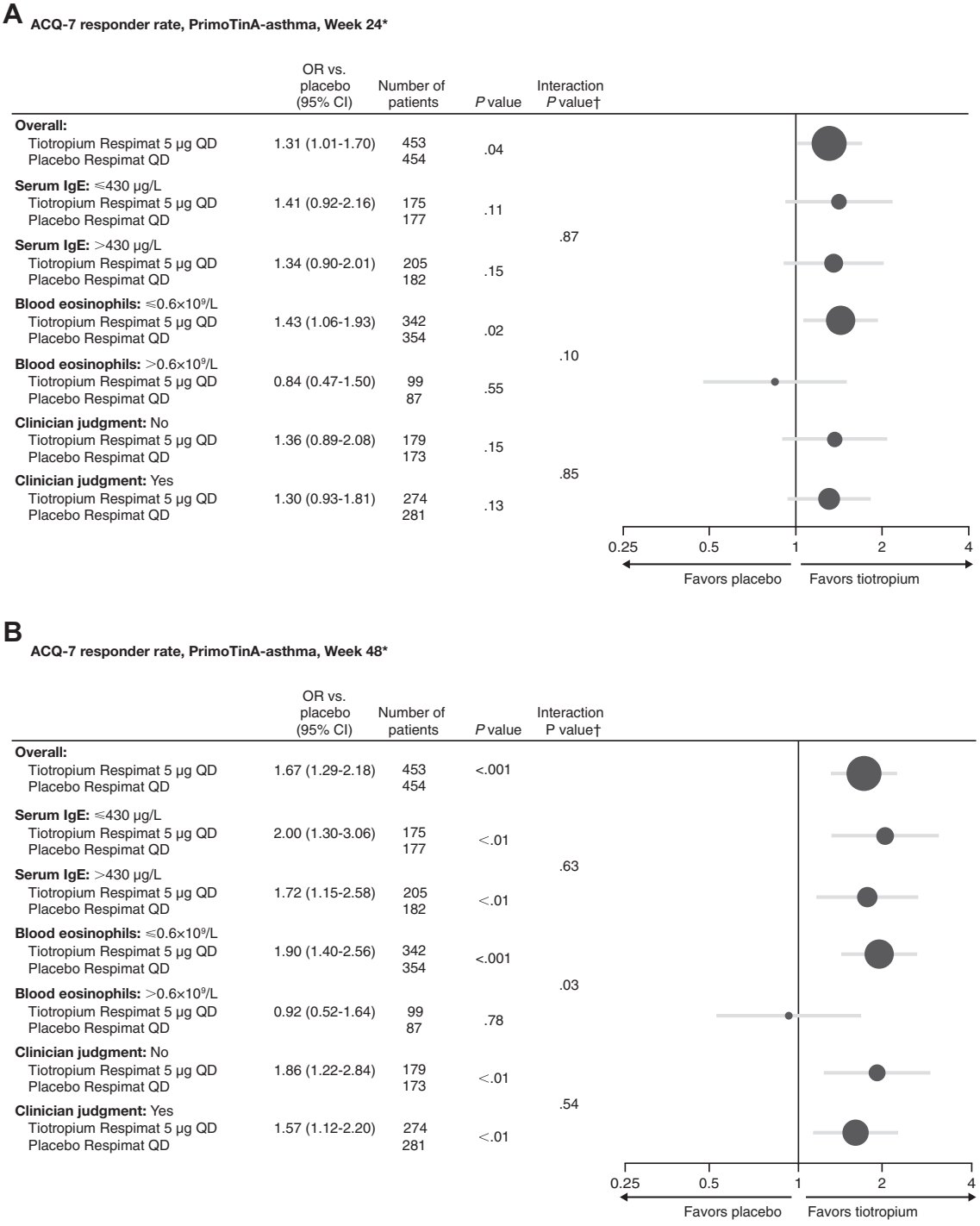


FIGURE E5. ACQ-7 responder rate in PrimoTinA-asthma at weeks 24 and 48, by T2 phenotype. *OR*, Odds ratio; *QD*, once daily. Full analysis set. Treatment effect *P* values based on χ^2 test with df = 1. *Pooled data; add-on to ICSs (≥800 µg budesonide or equivalent per day) + LABA. †Treatment-by-subgroup interaction *P* value based on χ^2 test with df = 1.

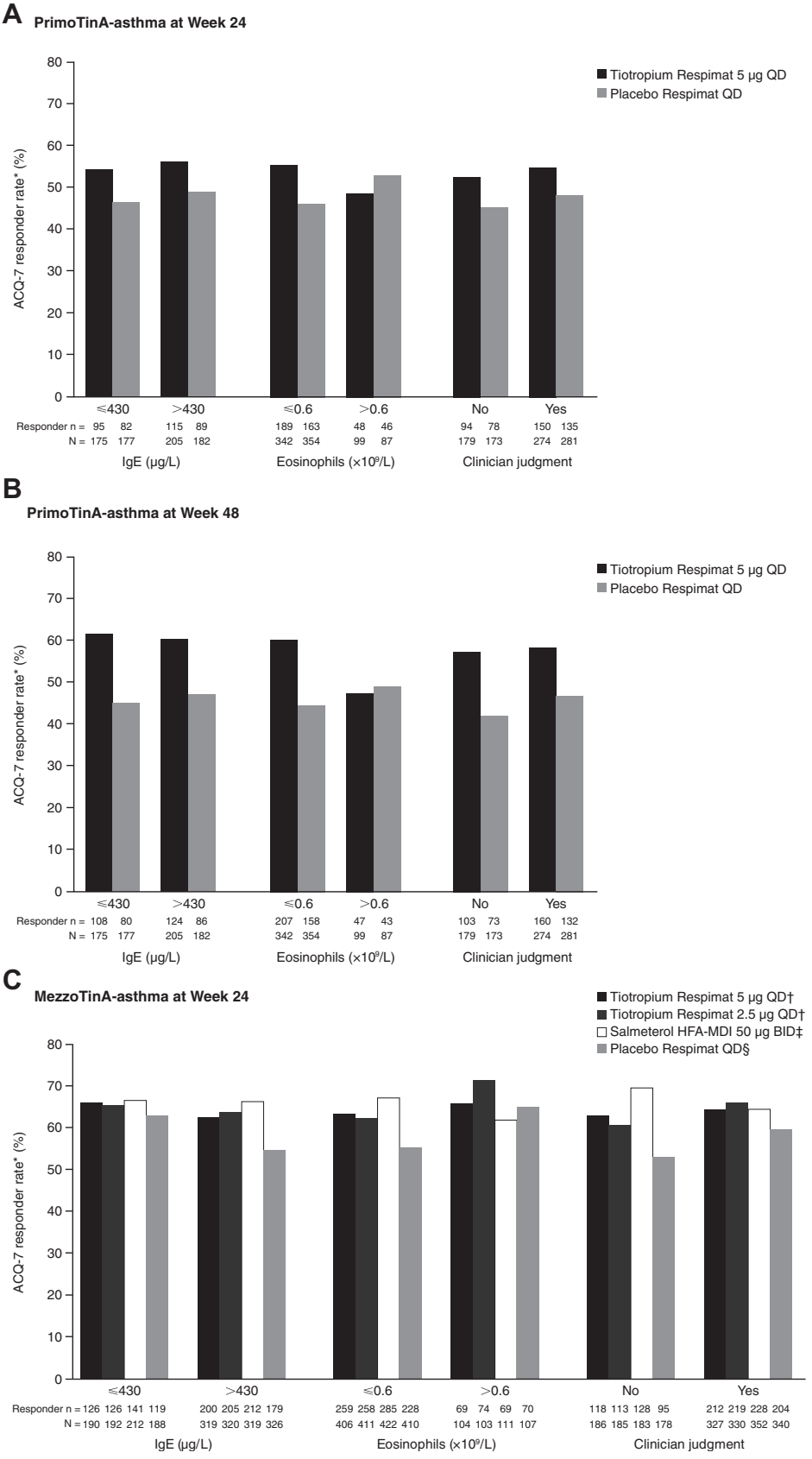


FIGURE E6. ACQ-7 responder rate in PrimoTinA-asthma at week 24 (**A**) and week 48 (**B**), and in MezzoTinA-asthma at week 24 (**C**), by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily. *Patients with a ≥0.5 reduction in the ACQ-7 score. †Plus placebo HFA-MDI BID. ‡Plus placebo Respimat QD. §Placebo Respimat QD plus placebo HFA-MDI BID.

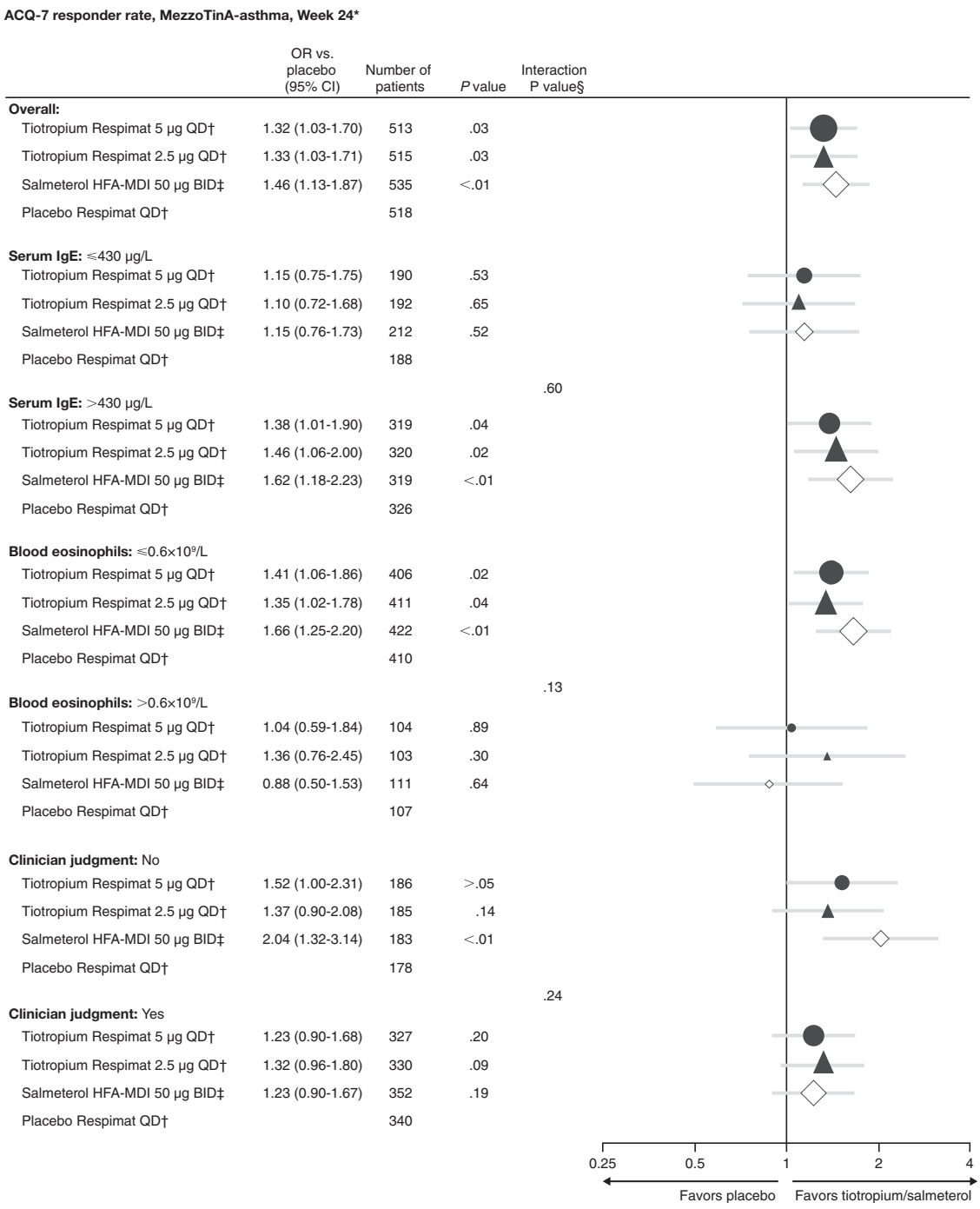


FIGURE E7. ACQ-7 responder rate in MezzoTinA-asthma at week 24, by T2 phenotype. *BID*, Twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *OR*, odds ratio; *QD*, once daily. Full analysis set. Treatment effect *P* values based on χ^2 test with df = 1. *Pooled data; add-on to ICSs (400-800 µg budesonide or equivalent). †Plus placebo HFA-MDI BID. ‡Plus placebo Respimat QD. §Treatment-by-subgroup interaction *P* value based on χ^2 test with df = 1.

TABLE E1. Pooled overall analyses of PrimoTinA-asthma and MezzoTinA-asthma with regard to lung function, exacerbations, and asthma control vs placebo

End point	Trial pool	Treatment effect, 95% CI, *P* value	Model
Peak FEV_1	PrimoTinA-asthma 5 μg	Mean difference 0.110 (0.063-0.158), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Peak FEV_1	MezzoTinA-asthma 5 μg	Mean difference 0.185 (0.146-0.223), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Peak FEV_1	MezzoTinA-asthma 2.5 μg	Mean difference 0.223 (0.185-0.262), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Trough FEV_1	PrimoTinA-asthma 5 μg	Mean difference 0.093 (0.050-0.137), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Trough FEV_1	MezzoTinA-asthma 5 μg	Mean difference 0.146 (0.105-0.188), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Trough FEV_1	MezzoTinA-asthma 2.5 μg	Mean difference 0.180 (0.138-0.221), $P < .0001$	MMRM model adjusted for treatment, study, visit, Visit × Treatment, baseline, Baseline × Visit (week 24 results)
Severe exacerbations	PrimoTinA-asthma 5 μg	HR 0.79 (0.62-1.00), $P = .0343$	Cox proportional hazards model with treatment as effect* (up to week 48)
Severe exacerbations	MezzoTinA-asthma 5 μg	HR 0.72 (0.45-1.14), $P = .1644$	Cox proportional hazards model with treatment as effect (up to week 24)
Severe exacerbations	MezzoTinA-asthma 2.5 μg	HR 0.50 (0.30-0.84), $P = .0084$	Cox proportional hazards model with treatment as effect (up to week 24)
Asthma worsening	PrimoTinA-asthma 5 μg	HR 0.69 (0.58-0.82), $P < .0001$	Cox proportional hazards model with treatment as effect (up to week 48)
Asthma worsening	MezzoTinA-asthma 5 μg	HR 0.87 (0.69-1.08), $P = .2112$	Cox proportional hazards model with treatment as effect (up to week 24)
Asthma worsening	MezzoTinA-asthma 2.5 μg	HR 0.66 (0.52-0.84), $P = .0007$	Cox proportional hazards model with treatment as effect (up to week 24)
ACQ-7 responder	PrimoTinA-asthma 5 μg	OR 1.32 (1.01-1.73), $P = .0427$	Fisher exact test (week 24)
ACQ-7 responder	MezzoTinA-asthma 5 μg	OR 1.32 (1.02-1.71), $P = .0348$	Fisher exact test (week 24)
ACQ-7 responder	MezzoTinA-asthma 2.5 μg	OR 1.33 (1.03-1.72), $P = .0308$	Fisher exact test (week 24)

ACQ-7, 7-question Asthma Control Questionnaire; *HR*, hazard ratio; *MMRM*, restricted maximum likelihood-based repeated measures; *OR*, odds ratio.
*Calculation of the HR's point estimate, CI, and *P* value follow the confirmatory result of these studies as described by Kerstjens et al.[E1]

TABLE E2. Overall summary of patients with adverse events in PrimoTinA-asthma and MezzoTinA-asthma

	PrimoTinA-asthma*		MezzoTinA-asthma†			
n (%)	Tiotropium Respimat 5 μg QD (n = 456)	Placebo Respimat QD (n = 456)	Tiotropium Respimat 5 μg QD‡ (n = 517)	Tiotropium Respimat 2.5 μg QD‡ (n = 519)	Salmeterol HFA-MDI 50 μg BID§ (n = 541)	Placebo‖ (n = 523)
Patients with any AE	335 (73.5)	366 (80.3)	296 (57.3)	302 (58.2)	294 (54.3)	309 (59.1)
Patients with investigator-defined drug-related AEs	26 (5.7)	21 (4.6)	38 (7.4)	36 (6.9)	28 (5.2)	28 (5.4)
Patients with AEs leading to discontinuation	8 (1.8)	14 (3.1)	9 (1.7)	6 (1.2)	10 (1.8)	13 (2.5)
Patients with serious AEs	37 (8.1)	40 (8.8)	11 (2.1)	12 (2.3)	11 (2.0)	14 (2.7)
AEs reported in >5% of patients,¶ by preferred term						
Asthma exacerbation	182 (39.9)	232 (50.9)	111 (21.5)	82 (15.8)	105 (19.4)	115 (22.0)
Decreased peak expiratory flow rate	93 (20.4)	122 (26.8)	59 (11.4)	49 (9.4)	47 (8.7)	79 (15.1)
Nasopharyngitis	51 (11.2)	56 (12.3)	41 (7.9)	49 (9.4)	41 (7.6)	48 (9.2)
Upper respiratory tract infection	21 (4.6)	16 (3.5)	19 (3.7)	27 (5.2)	41 (7.6)	41 (7.8)
Headache	29 (6.4)	33 (7.2)	8 (1.5)	18 (3.5)	6 (1.1)	14 (2.7)
Bronchitis	25 (5.5)	20 (4.4)	11 (2.1)	9 (1.7)	9 (1.7)	5 (1.0)

AE, Adverse event; *BID*, twice daily; *HFA-MDI*, hydrofluoroalkane metered-dose inhaler; *QD*, once daily.
Treated set; treatment period plus 30 days.
*Pooled data; add-on to ICSs (≥800 μg budesonide or equivalent per day) + LABA.
†Pooled data; add-on to ICSs (400-800 μg budesonide or equivalent).
‡Plus placebo HFA-MDI BID.
§Plus placebo Respimat QD.
‖Placebo Respimat QD plus placebo HFA-MDI BID.
¶Reported in >5% of patients in any treatment arm of the PrimoTinA-asthma or MezzoTinA-asthma trials.

REFERENCE

E1. Kerstjens HA, Moroni-Zentgraf P, Tashkin DP, Dahl R, Paggiaro P, Vandewalker M, et al. Tiotropium improves lung function, exacerbation rate, and asthma control, independent of baseline characteristics including age, degree of airway obstruction, and allergic status. Respir Med 2016;117:198-206.