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CYT003, a TLR9 Agonist, in Persistent Allergic Asthma – A Randomized Placebo-controlled Phase 2b Study

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Short Title: TLR9 Agonism for Moderate-to-Severe Asthma

Abstract

Background: New treatment options are required for patients with asthma not sufficiently controlled with inhaled therapies. In a Phase 2a trial, CYT003, a toll-like receptor-9 agonist immunomodulator, improved asthma control during inhaled glucocorticosteroid reduction in patients with allergic asthma. This double-blind Phase 2b study assessed the efficacy and safety of CYT003 in patients with persistent moderate-to-severe allergic asthma not sufficiently controlled on standard inhaled glucocorticosteroid therapy with/without long-acting beta-agonists.

Methods: Overall, 365 patients received seven doses of subcutaneous CYT003 (0.3, 1, or 2 mg) or placebo as add-on therapy to conventional controller medication. Change from baseline in Asthma Control Questionnaire score was the primary outcome; secondary outcomes included change in forced expiratory volume, Mini Asthma Quality of Life Questionnaire, and safety.

Results: All groups, including placebo, showed a clinically important improvement in Asthma Control Questionnaire score; however, there was no significant difference between the CYT003 and placebo groups at Week 12 (least-squares mean difference 0.3 mg: -0.027 [95% confidence interval -0.259 – 0.204]; 1 mg: 0.097 [-0.131 – 0.325]; 2 mg: 0.081 [-0.148 – 0.315]). No significant differences were seen in secondary outcomes. CYT003 was well tolerated; the most common treatment-emergent adverse events were injection site reactions. Due to lack of efficacy, the study was prematurely terminated at the end of the treatment phase with no further follow-up.

Conclusions: Toll-like receptor-9 agonism with CYT003 showed no additional benefit in patients with insufficiently controlled moderate-to-severe allergic asthma receiving standard inhaled glucocorticosteroid therapy with or without long-acting beta-agonists.

Keywords: ACQ, asthma, CpG-motifs, immunotherapeutic, TLR9-agonist

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways associated with substantial morbidity and characterized by recurrent episodes of wheezing, breathlessness, tightness in the chest, and coughing (1, 2). There are approximately 300 million people with asthma worldwide and its prevalence continues to increase, despite advances in our understanding of disease pathophysiology and the availability of effective therapies (1).

The cornerstone of current asthma treatment is the achievement and maintenance of optimal control via anti-inflammatory therapy with inhaled glucocorticosteroids (ICS) or combination therapy with ICS plus long-acting beta-agonist therapy (LABA) (1, 3). However, many patients remain symptomatic and fail to achieve adequate control, even with increases in the dose of ICS or add-on therapy with leukotriene antagonists and anti-immunoglobulin E (IgE) therapies (1). Furthermore, ICS are associated with poor patient compliance and offer little improved efficacy with increases above medium-to-high doses (1, 4, 5). Asthma control remains a challenge in patients with uncontrolled moderate-to-severe asthma receiving high doses of ICS and LABA in particular (6). Thus, there is an urgent unmet medical need for new treatment options in such patients.

Allergic asthma occurs largely as a result of T-cell helper 2 (Th2) activity in the airways of asthmatic patients (7). Upregulation of the Th2-mediated immune response in allergic asthma provokes an increase in IgE levels and secretion of Th2 cytokines, which inhibit differentiation of counterbalancing Th1 cells. Moreover, secretion of Th2 cytokines such as interleukin-5 and eotaxin is directly linked to the underlying allergic airway inflammation, while interleukin-13 has been shown to be involved in excessive airway mucus production (7–11). Thus, an immune-modifying therapy

that provides a balanced mixed Th1/Th2 response may offer a useful addition to the allergic asthma treatment armamentarium.

CYT003 (formerly QbG10) is a toll-like receptor (TLR)-9 agonist immunomodulator. CYT003 consists of a bacteriophage capsid filled with an A-type CpG (12–14). This synthetically produced oligonucleotide, named G10, has a natural DNA backbone and mimics bacterial DNA. Based on its molecular nature, CYT003 efficiently targets plasmacytoid dendritic cells, where G10 activates TLR-9 leading to the production of interferon alpha (15, 16). This process may directly impact existing Th2 responses as interferon alpha has been shown to suppress the Th2 cells through inhibition of the transcription factor GATA-3 (17). In addition, activation of plasmacytoid dendritic cells through TLR-9 leads to the generation of regulatory T cells, which are known to suppress the activity of effector T lymphocytes (18).

Early clinical trials of CYT003 in allergic diseases, including allergic rhinoconjunctivitis, showed significant clinical benefits when used in combination with specific allergen extracts or alone (19, 20). In a Phase 2a proof-of-concept study, treatment with CYT003 significantly improved patient-reported measures, including asthma control questionnaire (ACQ) score and average daily symptom and medication score, as well as change in forced expiratory volume (FEV₁) in patients with controlled mild-to-moderate persistent allergic asthma undergoing controlled glucocorticosteroid withdrawal (21).

Building upon these promising findings, the present Phase 2b study assessed the therapeutic potential and safety of CYT003 at three dose levels versus placebo in patients with persistent moderate-to-severe allergic asthma not sufficiently controlled on standard ICS therapy.

METHODS

Patient Population and Study Design

This randomized, placebo-controlled, double-blind, parallel-group, multicenter, Phase 2b dose finding study was conducted at 81 centers across the Czech Republic, Germany, Hungary, Israel, Poland, Russia, Ukraine, and the USA. Patients aged 18–65 years were eligible if they had persistent allergic asthma (≥ 6 months) that was not sufficiently controlled (ACQ score ≥ 1.5) despite stable treatment (≥ 4 weeks) with medium-to-high doses of ICS with or without LABA. Other inclusion criteria included: $FEV_1 \geq 40\%$ to $\leq 90\%$ of predicted, reversible airway obstruction (FEV_1 improvement of $\geq 12\%$ and FEV_1 improvement of ≥ 200 mL after inhaled β_2 -agonist), and a positive test for ≥ 1 aero-allergen. Smokers and patients who had received treatment or hospitalization for asthma exacerbation within 2 months of the first visit were ineligible.

The use of short-acting β_2 -agonists (salbutamol or equivalent) for asthma symptom relief was allowed as needed during the study. Controller therapy (ICS, LABA, and regular use of leukotrienes antagonist or sustained-release theophylline) was maintained during the screening (Week -4 to Week 0) and treatment (Week 0 to Week 12) phases. Patients requiring a step-up or step-down of their asthma controller therapy or systemic glucocorticosteroid therapy for ≥ 3 days during the treatment phase were discontinued from receiving further treatment but continued to be followed-up.

Patients were randomized 1:1:1:1, via an interactive voice response system, to one of four treatment arms: placebo, CYT003 0.3 mg, CYT003 1 mg, or CYT003 2 mg administered as seven subcutaneous injections into the lateral side of the upper arm (alternating between right and left from visit to visit) at baseline and Weeks 1, 2, 4, 6, 8, and 10. Further details of the blinding and randomization procedure are included in the supplementary material.

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Total study duration was planned to be 56 weeks, comprising 15 visits per patient from screening to the end of the follow-up phase (Figure 1). The trial was conducted in accordance with the ICH-GCP Guidelines (Directive CPMP/ICH/153/95) and the Declaration of Helsinki (1964) and subsequent revisions. The reporting of the trial follows the CONSORT guidelines. The protocol, revised protocols, informed consent forms, and revised informed consent forms were reviewed by local Independent Ethics Committee/Ethics Review Committees and ratified by local health authorities prior to study initiation. All patients provided informed consent before any study procedures were conducted. The study is registered with www.clinicaltrials.gov (NCT01673672).

Outcome Measures

The primary outcome was the change from baseline in ACQ score at Week 12. Secondary efficacy outcomes included change from baseline in ACQ score at all visits up to Week 12, and change from baseline in pre-bronchodilator FEV₁ and Mini Asthma Quality of Life Questionnaire (MiniAQLQ) score at Week 12 and all visits up to Week 12. At each visit, patients completed the ACQ and MiniAQLQ using the VIAPen device, a portable data capture tool that enables the simultaneous collection of data electronically and on paper. Further details of the ACQ and MiniAQLQ are provided in the supplementary material. Patients also received a patient e-diary with integrated peak flow meter (AM3 device) at the screening visit for daily recording of their nighttime and daytime asthma symptoms and use of reliever medication, and to measure their morning and evening peak expiratory flow during the screening/run-in and treatment phases.

Lung function was measured by centralized spirometry according to the American Thoracic Society/European Respiratory Society 2005 guidelines (22). Predicted FEV₁ values were calculated according to the formula of the European Community for Coal and Steel (23). The fraction of nitric oxide (FeNO) in exhaled air was measured with a NIOX Minoanalyzer (Aerocrine, Sweden) before

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spirometry assessments at baseline and Weeks 4, 8, and 12 (24). Blood samples for assessment of eosinophils were collected during screening/run-in and at Weeks 6 and 12, and analyzed at a central laboratory. Safety and tolerability were assessed by recording the incidence and severity of adverse events (AEs) and monitoring vital signs and standard laboratory parameters at a central laboratory.

Statistical Analysis

Assuming a medically important mean difference in the change in ACQ from baseline between active dose and placebo of 0.5, and a standard deviation (SD) of 1.0, a sample size of 90 patients per treatment arm was estimated allowing for 90% power to detect a statistically significant difference at an α level of 0.05 (25, 26). For evaluation of the primary endpoint, a two-sided test at the 5% significance level for the 'no difference' hypothesis was performed in the intention-to-treat population (defined in the supplementary material). The comparison was carried out with the use of analysis of covariance, with treatment as a fixed effect, baseline values as a continuous covariate, and ICS, long-acting β_2 -agonists, and region as factors. Secondary endpoints were analyzed using the same methods. A standard chi-square test was used to compare the response rate for ACQ (≤ 0.75 and < 1.5) across treatment groups.

RESULTS

Patients

Between October 2012 and April 2014, 423 patients were randomized, of whom 365 received at least one dose of study drug (placebo $n = 89$; CYT003 0.3 mg $n = 91$; CYT003 1 mg $n = 94$; CYT003 2 mg $n = 91$) (Figure 2). Patient baseline characteristics were well balanced between treatment groups (Table 1). At baseline, 244 (67%) patients were receiving high-dose ICS, including 205 (56%) in

combination with long-acting β 2-agonists; 121 (33%) patients were receiving medium-dose ICS, including 117 (32%) in combination with long-acting β 2-agonists.

A total of 29 patients discontinued treatment prematurely for the following reasons (Table E1): voluntarily withdrew ($n = 5$), AEs ($n = 15$), asthma exacerbation ($n = 7$; ≥ 3 days of systemic glucocorticosteroids, discontinuation according to study protocol) and other ($n = 2$; sponsor decision). Of the 29 patients who discontinued treatment, 11 withdrew consent and did not perform any more study visits. Patients who discontinued treatment for any reason were asked to remain in the study and complete all study procedures according to the protocol.

Treatment Phase

There was no significant difference in change from baseline in ACQ score at Week 12 between patients receiving CYT003 and placebo (all $P > 0.05$). Relative to placebo, ACQ score had changed by a least-squares (LS) mean of -0.027 (95% confidence interval [CI] -0.259 to 0.204) in the CYT003 0.3-mg group at Week 12, 0.097 (95% CI -0.131 to 0.325) in the CYT003 1-mg group, and 0.081 (95% CI -0.148 to 0.315) in the CYT003 2-mg group (Table E2). The effect of CYT003 was similar in the per-protocol population and in sensitivity analyses that did not impute missing values according to last observation carried forward.

Although the change in ACQ at all timepoints over the 12-week treatment phase revealed a clinically relevant improvement (decrease in score of 0.5 [25]) across all treatment groups (Figure 3), there was no significant difference between the placebo and CYT003 groups at any timepoint except Week 1, when patients receiving placebo showed a significantly greater improvement in ACQ than patients receiving CYT003 2 mg ($P < 0.05$). In responder analyses assessing the proportion of patients with 'well-controlled' (≤ 0.75) and 'uncontrolled' (< 1.5) asthma, there were no significant differences between the CYT003 groups and placebo (all $P > 0.05$).

After an initial increase in FEV₁ over the first 4 weeks in all treatment groups, values gradually decreased to pretreatment values at the end of the treatment phase (Figure 4). There were no significant differences between the CYT003 groups and placebo in either absolute or % predicted FEV₁ (all $P > 0.05$). At Week 12, the LS mean differences in change from baseline in FEV₁ (absolute) in the CYT003 0.3-mg, 1-mg, and 2-mg groups versus placebo were 0.071 L (–0.029 to 0.171), 0.011 L (–0.088 to 0.110), and 0.066 L (–0.033 to 0.165), respectively (all $P > 0.05$). For FEV₁ (% predicted) these values were 1.1% (–2.0 to 4.1), 0.9% (–2.2 to 3.9), and 2.1% (–1.0 to 5.1), respectively (all $P > 0.05$).

The MiniAQLQ revealed increasing scores over the 12-week study period for all treatment groups (Figure 5A), although there were no significant differences between groups at any timepoint (all $P > 0.05$). At Week 12, the LS mean difference versus placebo in change from baseline in MiniAQLQ was 3.6 (–0.5 to 7.6) in the CYT003 0.3-mg group, 0.6 (–3.5 to 4.6) in the 1-mg group, and 0.1 (–4.0 to 4.1) in the 2-mg group (all $P > 0.05$).

Levels of absolute eosinophils in the peripheral blood did not show clinically relevant changes over the 12-week treatment phase and there were no significant differences between the CYT003 groups and placebo (all $P > 0.05$) (Figure 5B). At Week 12, the LS mean difference versus placebo in change from baseline in eosinophil level was –0.03 cells/nL (–0.10 to 0.03) in the CYT003 0.3-mg group, –0.04 cells/nL (–0.11 to 0.02) in the 1-mg group, and –0.04 cells/nL (–0.10 to 0.03) in the 2-mg group (all $P > 0.05$). There were no significant differences in the change in FeNO between the CYT003 and placebo groups at any timepoint during the 12-week treatment phase (all $P > 0.05$) and the overall change from baseline did not exceed 4 parts per million (ppm) at any timepoint (Figure 5C). At Week 12, the LS mean difference in change from baseline in FeNO (absolute) in the CYT003 0.3-mg, 1-mg, and 2-mg groups versus placebo were –0.82 ppm (–6.21 to 4.56), 4.94 ppm (–0.42 to 10.31), and –0.47 ppm (–5.85 to 4.91), respectively (all $P > 0.05$).

Subgroup Analysis

Subgroup analysis of ACQ with patients stratified according to baseline eosinophil cell count (Figure E1) and FeNO (Figure E2) did not reveal any relevant differences between CYT003 and placebo.

Furthermore, there were no differences in ACQ between patients stratified according to baseline ICS dose, FEV₁ (absolute and % predicted), MiniAQLQ, or body mass index (data not shown).

Safety

Overall, 58% of patients who received at least one injection with study medication experienced at least one treatment-emergent AE, including 40% of patients in the placebo group, 58% of the 0.3-mg group, 66% of the 1-mg group, and 67% of the 2-mg group. The most frequent treatment-emergent AEs were injection site reactions: 3% of patients in the placebo group, 28% of patients in CYT003 0.3-mg group, 40% in CYT003 1-mg group and 44% in CYT003 2-mg group (Table 2). Most treatment-emergent AEs were of mild (64%) or moderate (29%) intensity. Severe gastroenteritis was experienced by 1 patient in the placebo group, severe dyspnea by 1 patient in the CYT003 0.3-mg group, severe asthma by 1 patient in the CYT003 1-mg group, and severe headache by 1 patient in the CYT003 2-mg group. Severe injection site reactions were experienced by 1 patient (one event) in the placebo group; 6 patients experienced 30 events in the 0.3-mg group, 6 patients experienced 12 events in the 1-mg group, and 3 patients experienced three events in the 2-mg group.

Of the 29 patients who discontinued study drug treatment, 15 patients discontinued due to treatment-emergent AEs: elevated double-stranded-DNA (baseline) and herpes zoster (both n = 1) in the placebo group; injection site reaction (n = 1) in the CYT003 0.3-mg group; injection site reaction (n = 3), injection site reaction/enlarged lymph nodes (n = 1), and influenza-like illness (n = 1) in the CYT003 1-mg group; injection site reaction (n = 6) and asthma/injection site reaction (n = 1) in the CYT003 2-mg group. There was one serious AE reported during the treatment phase of

hospitalization due to asthma exacerbation reported by a patient in the CYT003 1-mg group.

Changes in blood laboratory examinations values, vital signs, electrocardiograms, and physical examinations did not reveal any safety concerns and were not different to that reported in previous studies.

Follow-Up Phase

At the end of the treatment phase (Week 12) the study was analyzed. As the primary efficacy endpoint was not met, the study was prematurely terminated on April 14 2014. At that time no patient had completed the follow-up phase according to the protocol. Consequently, follow-up phase and patient e-diary data were not analyzed. Thus, daytime/night-time symptoms, use of reliever medication, and morning and evening peak expiratory flow rate data, as self-reported by patients in e-diaries, were not analyzed.

DISCUSSION

This Phase IIb study investigated the safety and efficacy of CYT003 in patients with insufficiently controlled moderate-to-severe allergic asthma receiving standard ICS therapy with or without LABA. Although, CYT003 was generally well tolerated and demonstrated a good safety profile in this population, no benefit on patient-reported outcomes, FEV₁, eosinophil levels, or FeNO was reported. Due to this lack of efficacy, the clinical trial was prematurely terminated after the analysis of the 12-week results and the follow-up phase was not completed.

In a previous Phase 2a proof-of-concept study in patients with mild-to-moderate persistent allergic asthma undergoing controlled glucocorticosteroid withdrawal, CYT003 significantly improved a range of patient reported outcomes compared with placebo (21). Furthermore, while FEV₁

worsened by a clinically relevant extent in patients treated with placebo, it remained stable in patients receiving CYT003, despite ICS withdrawal (21). There was therefore a strong rationale for investigating CYT003 further in the present Phase 2b study.

There are several potential reasons why the Phase 2a study showed consistently positive data while this Phase 2b study did not. Patients enrolled in the Phase 2a study had mild and well-controlled asthma ($FEV_1 = 92.5\%$ at baseline) and were undergoing controlled steroid withdrawal, whereas those in the current study had insufficiently controlled moderate-to-severe allergic asthma despite standard ICS therapy, with the majority also receiving LABA. Indeed, patients with insufficiently controlled moderate-to-severe asthma receiving medium-to-high doses of ICS pose a potentially huge hurdle in clinical trials as there may only be a narrow window for further improvements. To date, many trials of injected therapies tested in add-on trial designs in patients with insufficiently controlled moderate-to-severe allergic asthma have failed, often due to the strong and persistent placebo effect observed, which has proven insurmountable with active therapies (26–30). Nevertheless, improvements may be possible in this population as studies with the anti-IgE antibody omalizumab have shown significantly reduced exacerbations and improved AQLQ score and FeNO in patients with severe allergic asthma inadequately controlled with standard therapy (31).

Positive findings have also been shown with a range of investigational agents in highly selected and more severe patient subgroups using exacerbations as the key outcome measure (27, 32–36). For example, the anti-interleukin-5 antibody mepolizumab significantly reduced exacerbations and improved FEV_1 and ACQ in a recent 32-week Phase 3 trial in patients with severe asthma who had experienced frequent exacerbations despite treatment with high dose ICS plus at least one other controller medication and had a blood eosinophil count ≥ 150 cells/ μ L at initiation of treatment or ≥ 300 cells/ μ L in the preceding 12 months (37). Although these more focused development approaches have been criticized for the fragmentation of the patient population,

patients in the current study were also stratified according to different parameters at baseline. However, CYT003 had no significant effect versus placebo on any of the evaluated outcomes when patients were stratified by eosinophil count (>0.2 cells/nL vs. ≤ 0.2 cells/nL and >0.1 cells/nL vs. ≤ 0.1 cells/nL) baseline FeNO, ICS dose, FEV₁, MiniAQLQ, or body mass index. Hence, a subgroup that benefits from CYT003 treatment and would warrant further investigation could not be identified in this patient population.

While the design of the current trial aimed to build upon previous trials by including a run-in period of 4 weeks to minimize compliance effects and tighter inclusion criteria to improve patient homogeneity, the magnitude of the placebo effect remained high. An extension to the run-in period or a prolonged follow-up may be worthy of consideration in future trials in this population to minimize the placebo effect. There were no other obvious issues with the design of the trial; the data generated were of good quality and variability was low, indicating that the study was adequately powered. There was also no significant effect of CYT003 on the primary or any of the evaluated secondary endpoints, suggesting that selection of an alternative primary endpoint would have had no impact on the outcome of the study. The e-diary data captured during the treatment phase (nighttime and daytime asthma symptoms, use of reliever medication, and morning and evening peak expiratory flow) were not evaluated due to the premature discontinuation of the study. Exacerbations, planned to be assessed after all patients had finished the 52-week follow-up period, were not analyzed for the same reason.

Thus, the most likely explanation for the results of the current trial is that CYT003 is ineffective as an add-on therapy to ICS plus LABA in patients with moderate-to-severe allergic asthma. Although CYT003 might be effective as initial controller medication in steroid-naïve patients, there is no current unmet medical need in this population and further development of CYT003 for this indication is unlikely. However, the data from the Phase 2a trial appear to be robust and positive findings have also been shown with CYT003 in other allergic diseases (21, 38). Therefore, the results

of the present study should not preclude future studies into targeting TLR-9 in allergic asthma, as this approach is based on convincing scientific rationale.

CYT003 was well tolerated in the present study and had a good safety profile. The majority of AEs were of mild or moderate intensity, the most common treatment-emergent AEs were injection site reactions, and serious AEs were only infrequently observed. This finding is in accordance with the Phase 2a study in patients with allergic asthma (21) and also a previous Phase 2b study in patients with perennial allergic rhinoconjunctivitis (38).

In conclusion, although the TLR-9 agonist CYT003 had a good safety profile in patients with moderate-to-severe allergic asthma receiving standard ICS therapy, there was no evidence of significant efficacy compared with placebo and the study was prematurely terminated after analysis of the 12-week results. Continued clinical development of CYT003 in this indication is unlikely but these results should not preclude further exploration of immune-modifying therapies in allergic asthma.

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FIGURE LEGENDS

Figure 1. Study design.

Figure 2. Disposition of patients enrolled in the study.

The safety set consisted of all patients who received at least one injection of study drug and the full analysis set of those randomized patients who received at least one injection of study drug and had at least one data value post baseline. The safety set and the full analysis set were identical for treatment phase efficacy analysis in this study.

Figure 3. Mean ($\pm$ standard error of the mean) change from baseline in ACQ score at all timepoints.

Only observed values of the full analysis set are shown. There were clinically relevant improvements at all timepoints across all treatment groups; however, there was no significant difference between the placebo and CYT003 groups at any timepoint except Week 1, when patients receiving placebo showed a significantly greater improvement in ACQ score than patients receiving CYT003 2 mg ($P < 0.05$).

Definition of abbreviation: ACQ = asthma control questionnaire.

Figure 4. (A) Mean ($\pm$ standard error of the mean) change in FEV₁ (L) and (B) mean ($\pm$ standard error of the mean) change in FEV₁ (% predicted) at all timepoints. Only observed values of the full analysis set are shown. There were no significant differences between the CYT003 groups and placebo in either absolute or % predicted FEV₁ at any timepoint (all $P > 0.05$).

Definition of abbreviation: FEV₁ = forced expiratory volume.

Figure 5. (A) Mean ($\pm$ standard error of the mean) change in MiniAQLQ, (B) Mean ($\pm$ standard error of the mean) change in eosinophils, and (C) mean ($\pm$ standard error of the mean) change in FeNo in exhaled air at all timepoints. Only observed values of the full analysis set are shown. There were no significant differences between groups in any parameter at any timepoint (all $P > 0.05$).

Definition of abbreviations: Eos, eosinophils; FeNO = fraction of nitric oxide in exhaled air; MiniAQLQ = Mini Asthma Quality of Life Questionnaire.

TABLES

Table 1: Patient Demographics and Baseline Characteristics of Patients Enrolled in the Study

Characteristics	Placebo	CYT003 0.3 mg	CYT003 1 mg	CYT003 2 mg
Sex, n (%)				
Male	37 (42)	35 (38)	36 (38)	34 (37)
Female	52 (58)	56 (62)	58 (62)	57 (63)
Age (years)				
mean (SD)	47.5 (12.37)	47.2 (11.94)	47.3 (12.39)	48.0 (12.05)
Race, n				
White	82	88	83	81
Black or African American	6	3	9	7
Asian	1		2	2
Other				1
Ethnicity, n				
Hispanic or Latino	6	3	4	2
Not Hispanic or Latino	83	88	90	89
Body mass index (kg/m ²)				

Characteristics	Placebo	CYT003 0.3 mg	CYT003 1 mg	CYT003 2 mg
mean (SD)	28.21 (5.58)	28.19 (5.15)	29.10 (5.75)	29.78 (6.82)
Duration of asthma (years)				
mean (SD)	21.8 (16.4)	22.1 (16.4)	22.2 (14.3)	19.2 (13.8)
Smoking Classification n (%)				
Never smoked	74 (83)	72 (79)	81 (86)	73 (80)
Current smoker	0	0	0	0
Ex-smoker	15 (17)	19 (21)	13 (14)	18 (20)
Number of years patient has smoked				
n	15	19	13	18
mean (SD)	5.9 (4.59)	7.4 (4.82)	6.2 (4.78)	3.9 (2.99)
ACQ Score				
mean (SD)	2.63 (0.61)	2.57 (0.61)	2.62 (0.65)	2.56 (0.69)
FEV ₁ (L)				
mean (SD)	2.135 (0.651)	2.140 (0.664)	2.186 (0.606)	2.130 (0.709)
mean % of predicted value (SD)	68.3 (13.8)	69.8 (14.8)	72.4 (14.6)	69.5 (14.14)

Characteristics	Placebo	CYT003 0.3 mg	CYT003 1 mg	CYT003 2 mg
range % of predicted value	33-100	39-108	34-101	34-96
MiniAQLQ Score				
mean (SD)	61.5 (14.3)	63.0 (13.9)	60.5 (14.6)	61.9 (15.4)
FeNO (ppb)				
mean (SD)	23.6 (18.3)	31.8 (30.4)	31.8 (29.2)	32.6 (28.9)
Eosinophil count in blood (10 ⁹ /L)				
mean (SD)	0.28 (0.23)	0.28 (0.26)	0.3 (0.28)	0.32 (0.38)
IgE (IU/mL)				
n	87	84	94	86
geometric mean (SD)	150.9 (1301.6)	160.3 (1973.4)	166.1 (724.5)	172.2 (552.0)

Definition of abbreviations: ACQ = asthma control questionnaire; FeNO = fraction of nitric oxide in exhaled air; FEV₁ = forced expiratory volume; MiniAQLQ = Mini Asthma Quality of Life Questionnaire; SD = standard deviation.

Table 2: Treatment-Emergent AE Occurring in >3% of Patients in Any Group

	Placebo	CYT003	CYT003	CYT003	Total
AE, n (%)		0.3 mg	1 mg	2 mg	
	(N = 89)	(N = 91)	(N = 94)	(N = 91)	(N = 365)
Injection site erythema	0	20 (22)	24 (26)	27 (30)	71 (19)
Injection site swelling	0	20 (22)	27 (29)	23 (25)	70 (19)
Injection site pruritus	2 (2)	11 (12)	19 (20)	11 (12)	43 (12)
Injection site pain	1 (1)	10 (11)	12 (13)	16 (18)	39 (11)
Injection site induration	0	3 (3)	2 (2)	4 (4)	9 (2)
Injection site reaction	0	0	3 (3)	1 (1)	4 (1)
Headache	6 (7)	6 (7)	3 (3)	7 (8)	22 (6)
Nasopharyngitis	4 (4)	5 (5)	5 (5)	6 (7)	20 (5)
Influenza-like illness	2 (2)	0	7 (7)	3 (3)	12 (3)
Asthma (worsening)*	2 (2)	2 (2)	4 (3)	2 (2)	10 (3)
Bronchitis	1 (1)	5 (5)	1 (1)	2 (2)	9 (2)
Pyrexia	2 (2)	0	2 (2)	5 (5)	9 (2)
Upper respiratory tract infection	1 (1)	0	4 (4)	4 (4)	9 (2)
Wheezing	1 (1)	1 (1)	1 (1)	3 (3)	6 (2)

Acute sinusitis	0	2 (2)	3 (3)	0	5 (1)
Oropharyngeal pain	0	2 (2)	3 (3)	0	5 (1)
Blood creatinkinase increased	0	3 (3)	1 (1)	0	4 (1)
Body temperature increased	0	0	0	4 (4)	4 (1)
Rhinitis allergic	0	3 (3)	0	1 (1)	4 (1)
Fatigue	0	3 (3)	0	0	3 (1)
Viral respiratory tract infection	3 (3)	0	0	0	3 (1)

*Asthma exacerbation (≥ 3 days with systemic corticosteroid treatment) was captured independently of AEs and is not included in this table.

Definition of abbreviation: AE = adverse event.

FIGURES

Figure 1

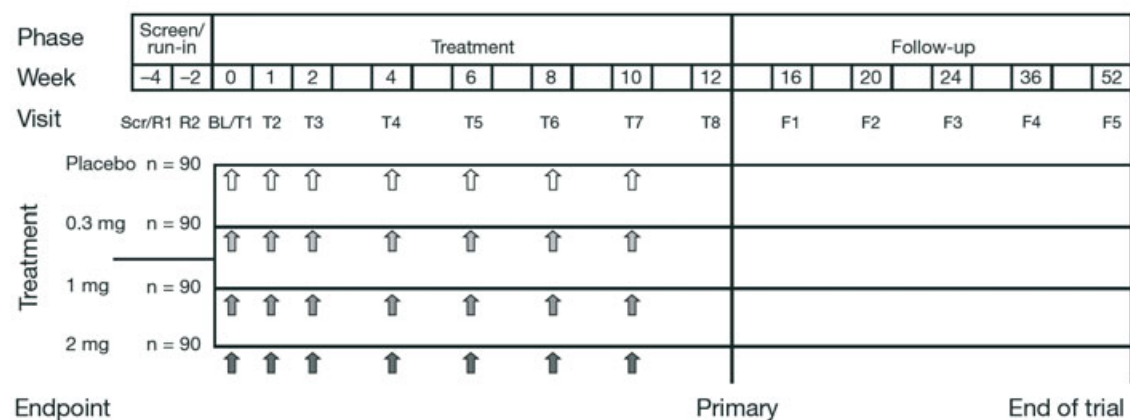


Figure 2

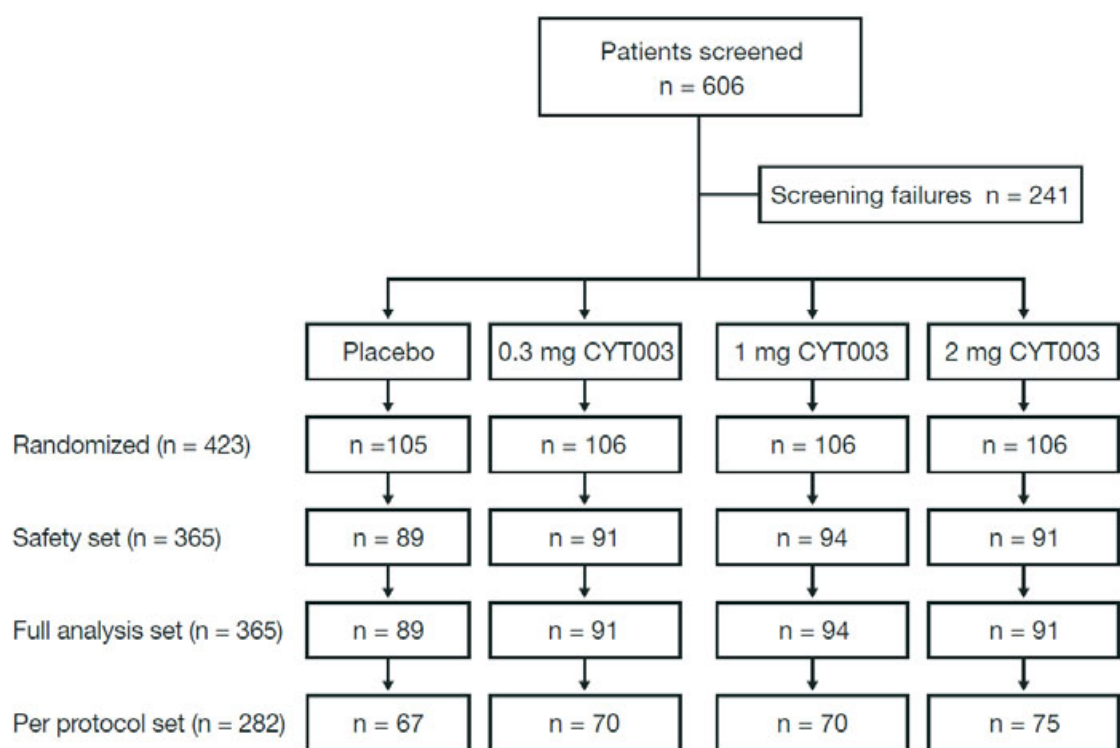


Figure 3

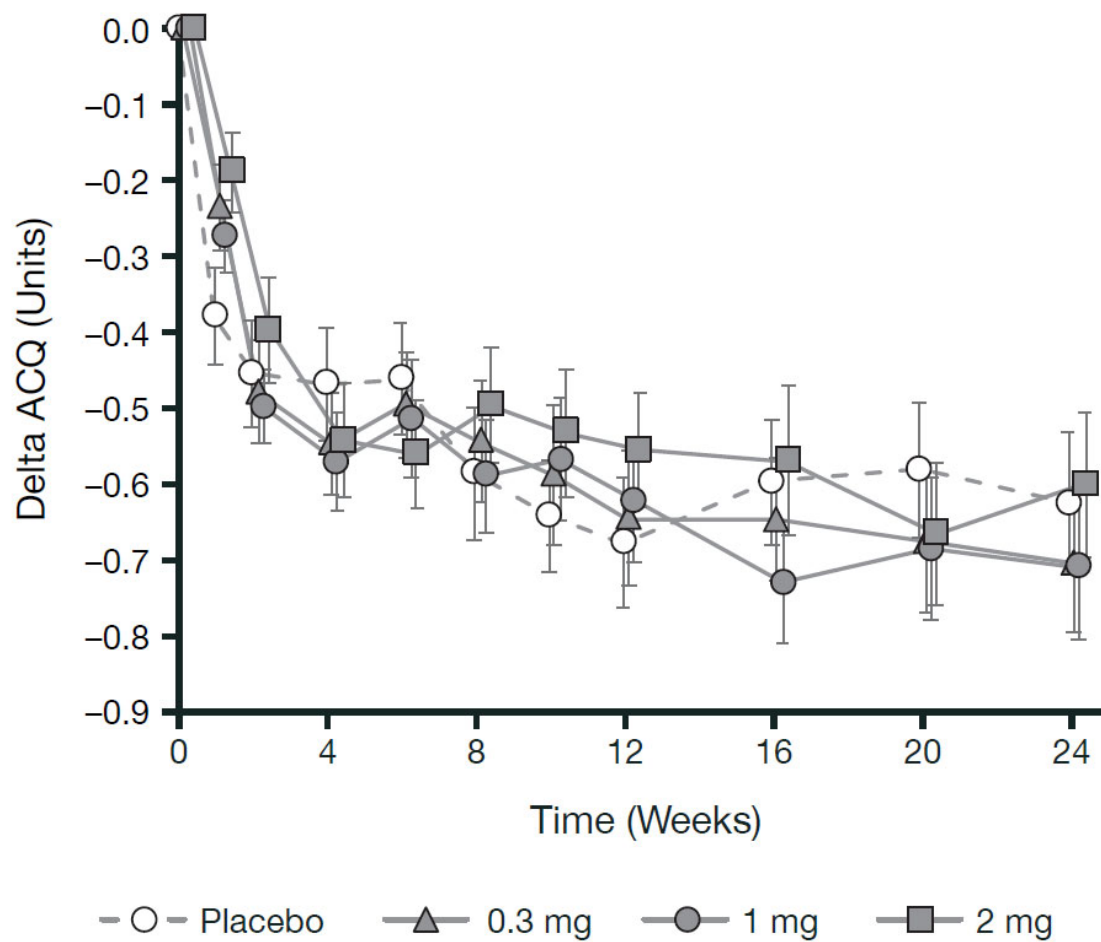


Figure 4

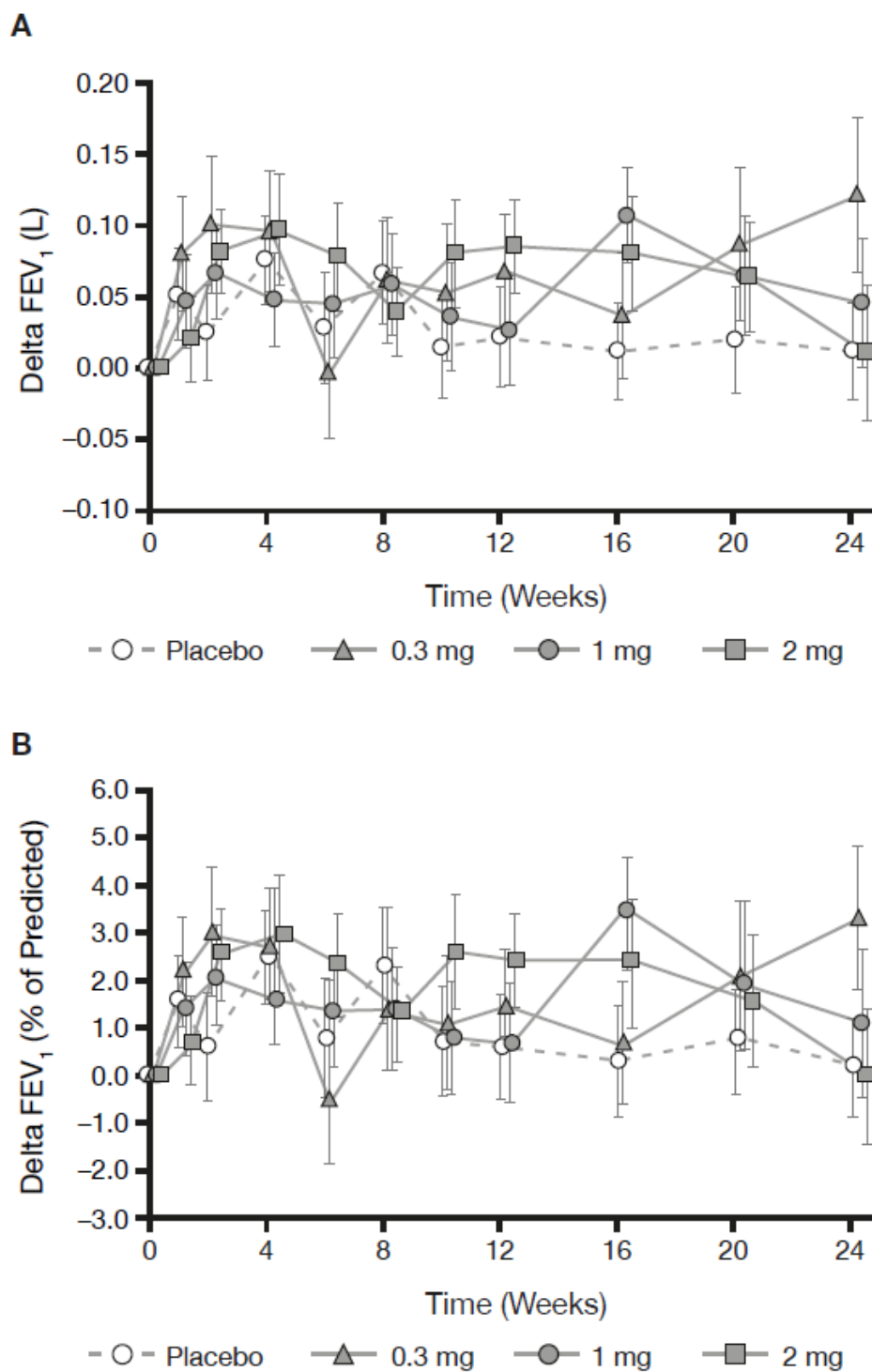
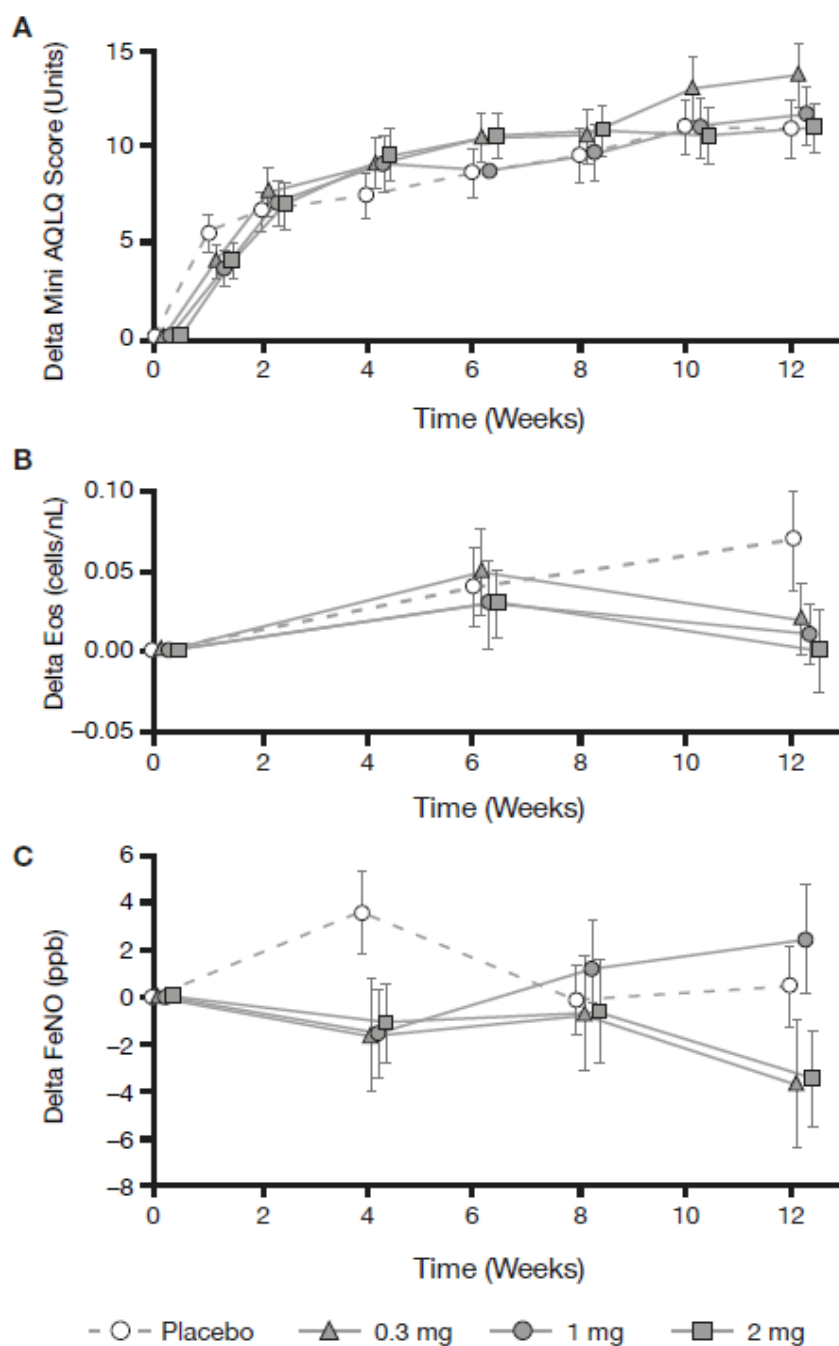


Figure 5



Supplementary tables

Table E1: Number of Patients who Discontinued Treatment and Primary Reasons for Treatment Discontinuation

	Voluntary Withdrawal	Adverse Event	Asthma Exacerbation^a	Others^b	Total
Placebo	2	2	1	1	6
0.3 mg CYT003	2	1	1	0	4
1 mg CYT003	0	5	3	0	8
2 mg CYT003	1	7	2	1	11
Total	15	15	7	2	29

^a≥3 days systemic glucocorticosteroids;

^bSponsor decision after elevation of the storage temperature of the drug (n = 1) and autoimmune disease in medical history (n = 1).

Table E2: Placebo-Corrected Least-Squares Mean Change in ACQ Score at Week 12 in the CYT003 0.3-, 1- and 2-mg Groups

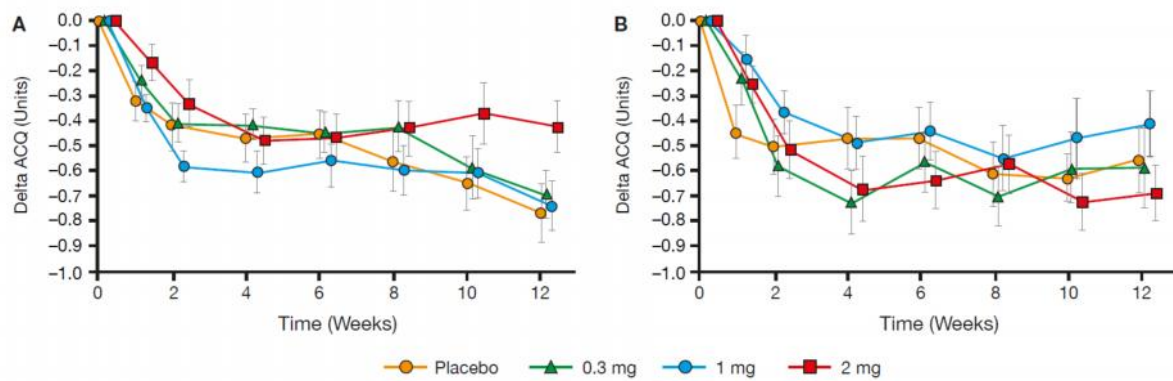
Data from the full analysis set, with and without imputation of missing values, and the per-protocol set are shown. There were no significant differences between the placebo and CYT003 groups in any analysis set.

	CYT003		
	0.3 mg	1 mg	2 mg
Full analysis set (with LOCF)			
Least-squares mean (95% CI)	−0.027 (−0.259 to 0.204)	0.097 (−0.131 to 0.325)	0.081 (−0.148 to 0.315)
P-value	0.8180	0.4025	0.4883
Full analysis set (without LOCF)			
Least-squares mean (95% CI)	−0.007 (−0.234 to 0.221)	0.055 (−0.172 to 0.282)	0.102 (−0.125 to 0.329)
P-value	0.9528	0.6326	0.3777
Per-protocol set			
Least-squares mean (95% CI)	0.016 (−0.232 to 0.264)	−0.054 (−0.301 to 0.192)	0.134 (−0.109 to 0.376)
P-value	0.8979	0.6649	0.2783

Definition of abbreviations: CI = confidence interval; LOCF = last observation carried forward.

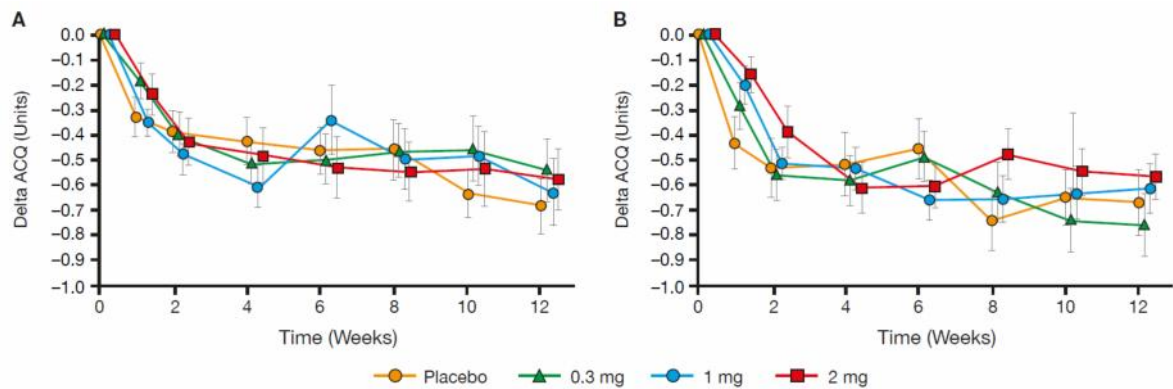
SUPPLEMENTARY FIGURE LEGENDS

Figure E1. Mean ($\pm$ standard error of the mean) change from baseline in ACQ score at all timepoints in (A) patients with low baseline eosinophil counts ($\leq$ median; median = 0.2 cells/nL) and (B) patients with high baseline eosinophil counts ($>$ median). Only observed values of the full analysis set are shown. There were no significant differences between the CYT003 groups and placebo in either subgroup at any timepoint (all $P > 0.05$).



Definition of abbreviation: ACQ = asthma control questionnaire.

Figure E2. Mean ($\pm$ standard error of the mean) change from baseline in ACQ score at all timepoints in (A) patients with low fraction of exhaled nitric oxide ($\leq$ median; median = 20.6 ppb) and (B) patients with high fraction of exhaled nitric oxide ($>$ median). Only observed values of the full analysis set are shown. There were no significant differences between the CYT003 groups and placebo in either subgroup at any timepoint (all $P > 0.05$).



Definition of abbreviation: ACQ = asthma control questionnaire.

SUPPLEMENTARY REFERENCES

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Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. *Eur Respir J* 1999;**14**:32–38.

Supplementary material

Supplementary methods

Asthma Control Questionnaire (ACQ) and Mini Asthma Quality of Life Questionnaire (MiniAQLQ)

ACQ is a validated composite score based on the average of seven items each scored 0 (full asthma control) to 6 (no asthma control), including the patient's scores of five disease-related items (woken at night by asthma, awake in the morning with symptoms, limitation of daily activities, shortness of breath, wheezing) plus a score for on-site measured forced expiratory volume and use of short-acting reliever medication (E1). An ACQ score of ≥ 1.5 indicates 'not sufficiently controlled asthma', and a score ≤ 0.75 indicates 'well-controlled asthma' (E2). The MiniAQLQ is a validated 15-item questionnaire that measures the functional impairment of adult patients with asthma, where a score of 15 indicates the lowest impact and 105 the highest impact of asthma to a patient's life (E3).

Blinding and Randomization

The study was performed with a double-blind design. Neither investigators nor patients could distinguish between placebo and active treatment. An interactive voice response system (IVRS) was used for drug management and permitted rapid unblinding in the event of a medical emergency.

The randomization and stratification was facilitated by IVRS. The randomization schedule for IVRS linked sequential patient randomization numbers to treatment codes allocated at random with a 1:1:1:1 randomization ratio. The randomization numbers were blocked and within each block of 8 patients, the same number of patients were allocated to each treatment group, stratified according to current long-acting β_2 -agonist use and dose of inhaled glucocorticosteroids (medium or high).

Study Populations

241 patients failed the screening process and were not injected with the study drug at baseline.

There were 181 patients who failed the screening process because of the following five main reasons: Not meeting $FEV_1 \geq 40$ to $\leq 90\%$ of predicted value (54 patients), the specified reversibility criteria (48 patients), not able and willing to complete all protocol requirements (44 patients), not meeting at least 80% compliance in the use of the patient e-diary/PEF meter at visit BL/T1 (20 patients), and a ACQ score < 1.5 at screening and at baseline (15 patients).

60 patients who did not enter the trial with treatment were not included mainly because they were not diagnosed with persistent asthma as defined by the protocol, or did not show a positive SPT or RAST for at least 1 aero-allergen, or had a confirmed or suspected current infection with HIV, HBV, or HCV. Some patients had more than one reason for failing the screening process.

The Safety Set included all 365 injected patients and was identical to the FAS which was analyzed according to the ITT principle. In total, 83 patients were excluded from the PP Set due to significant protocol deviations keeping 282 patients in the PP Set.

Statistical analysis

The primary efficacy analysis was performed on the intention-to-treat population (all patients who underwent randomization, received at least one dose of study medication, and had at least one data value post baseline). Missing values, where patients prematurely withdrew or discontinued, were imputed using last observation carried forward (LOCF). Patients who changed (step-up or step-down, or received systemic glucocorticoids for ≥ 3 days) their stable controller treatment were formally

treated as premature discontinuations. The secondary efficacy endpoints were analyzed without imputation of missing values.