

## ORIGINAL ARTICLE

## Risankizumab in Severe Asthma — A Phase 2a, Placebo-Controlled Trial

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## ABSTRACT

## BACKGROUND

Interleukin-23 has been implicated in airway inflammation that is mediated by type 2 and type 17 cytokines. Whether targeting interleukin-23 in the treatment of asthma improves disease control and reduces airway inflammation is unclear.

## METHODS

We conducted a phase 2a, multicenter, randomized, double-blind, placebo-controlled, 24-week, parallel-group trial to assess the efficacy and safety of risankizumab, an anti-interleukin-23p19 monoclonal antibody, in adults with severe asthma. Patients were assigned to receive 90 mg of risankizumab or placebo, administered subcutaneously once every 4 weeks. The primary end point was the time to the first asthma worsening. Asthma worsening was defined as deterioration from baseline on 2 or more consecutive days; deterioration was considered to be a decrease of at least 30% in the morning peak expiratory flow or an increase from baseline of at least 50% in the number of puffs of rescue medication in a 24-hour period (equating to at least four additional puffs), a severe asthma exacerbation, or an increase of 0.75 or more points on the 5-item Asthma Control Questionnaire (ACQ-5; scores range from 0 to 6, with higher scores indicating less control). Secondary end points were the annualized rate of asthma worsening, the annualized rate of severe exacerbations, the ACQ-5 score, and the forced expiratory volume in 1 second. Exploratory end points were assessed with the use of sputum cytologic analysis and gene expression analysis, and safety was assessed.

## RESULTS

A total of 105 patients received risankizumab and 109 received placebo. The clinical characteristics of the patients were similar in the two groups. The time to the first asthma worsening was shorter with risankizumab than with placebo (median, 40 days vs. 86 days; hazard ratio, 1.46; 95% confidence interval [CI], 1.05 to 2.04;  $P=0.03$ ). The rate ratio for annualized asthma worsening with risankizumab as compared with placebo was 1.49 (95% CI, 1.12 to 1.99), and the rate ratio for severe exacerbations was 1.13 (95% CI, 0.75 to 1.70). Sputum transcriptomic pathway analysis showed that genes involved in the activation of natural killer cells and cytotoxic T cells and the activation of the type 1 helper T and type 17 helper T transcription factors were down-regulated by risankizumab. No safety concerns were associated with risankizumab therapy.

## CONCLUSIONS

Risankizumab treatment was not beneficial in severe asthma. The time to the first asthma worsening was shorter and the annualized rate of asthma worsening was higher with risankizumab than with placebo. (Funded by AbbVie and Boehringer Ingelheim; ClinicalTrials.gov number, NCT02443298.)

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ASTHMA AFFECTS MORE THAN 300 MILLION persons worldwide, and its prevalence is increasing.<sup>1,2</sup> Severe asthma affects approximately 5 to 10% of persons with asthma.<sup>3</sup> Patients with severe asthma have poor disease control and frequent exacerbations in spite of treatment with high-dose inhaled glucocorticoids.<sup>1,3</sup> The underlying airway inflammation in severe asthma is typically eosinophilic, with up-regulated type 2 cytokines.<sup>1-4</sup> However, it is recognized that the inflammatory profile in severe asthma is heterogeneous, with increased neutrophilic inflammation and activated type 17 helper T (Th17)–mediated immunity in some patients.<sup>1-7</sup>

Interleukin-23, which has been implicated in the pathogenesis of asthma,<sup>8</sup> is a heterodimeric cytokine composed of an interleukin-12B (interleukin-12p40) subunit, which is shared with interleukin-12, and an interleukin-23A (interleukin-23p19) subunit.<sup>9</sup> A functional receptor for interleukin-23 (interleukin-23R) has been identified and is composed of interleukin-12R $\beta$ 1 and interleukin-23R.<sup>8</sup> Serum interleukin-23 is high in patients with asthma and is associated with a greater impairment in lung function.<sup>10</sup> In animal models, interleukin-23 promotes Th17-cell proliferation to maintain interleukin-17A and interleukin-17F production and neutrophil recruitment,<sup>8</sup> both of which are increased in severe asthma.<sup>5,6,11,12</sup> Independent of the Th17 axis, interleukin-23 mediates type 2 helper T (Th2) cytokine production and eosinophil infiltration.<sup>13</sup> Interleukin-23, either directly or indirectly through the promotion of Th17 cells and the consequent release of interleukin-17, stimulates the proliferation of structural airway cells such as fibroblasts and airway smooth muscle, possibly contributing to airway remodeling.<sup>8</sup> In addition, the lipid mediator resolvin E1 inhibits airway inflammation in part by attenuation of interleukin-23.<sup>14</sup> Inhibition of interleukin-23 is effective in psoriasis<sup>15</sup> and Crohn's disease.<sup>16</sup> Whether targeting interleukin-23 in asthma improves disease control and reduces airway inflammation is unclear.

## METHODS

### TRIAL DESIGN AND OVERSIGHT

We conducted a phase 2a, randomized, double-blind, 24-week, parallel-group, multicenter trial

to assess the efficacy and safety of risankizumab, a humanized, monoclonal antibody directed against subunit p19 of interleukin-23, in adults with severe persistent asthma. Patients were assigned in a 1:1 ratio to receive 90 mg of risankizumab or placebo, administered subcutaneously once every 4 weeks. The 90-mg dose is consistent with a dose used in a previous efficacy trial for plaque psoriasis.<sup>17</sup>

The trial design is summarized in Figure S1 in the Supplementary Appendix (available with the full text of this article at NEJM.org). The trial consisted of a screening period of 3 to 4 weeks, a 24-week treatment period, and a 16-week observation period. Randomization was stratified according to oral glucocorticoid use at baseline, participation in a bronchoscopy sub-study, and history of severe asthma exacerbation in the 12 months before the screening visit. (The bronchoscopy substudy was not completed because of insufficient recruitment.) Patients completed an electronic diary to answer questions related to the use of rescue medication and asthma controller medication and to measure peak expiratory flow. In addition, patients completed the five-item Asthma Control Questionnaire (ACQ-5) with the use of the electronic diary once a week. ACQ-5 scores range from 0 to 6, with higher scores indicating less asthma control. At scheduled clinic visits, spirometry was performed before and after bronchodilator use, the fraction of exhaled nitric oxide (F<sub>ENO</sub>) was measured, and sputum and venous blood samples were obtained.

All the authors had access to the data, which were analyzed by the trial sponsors (AbbVie and Boehringer Ingelheim). The authors conducted the gene pathway analysis. All the authors except for the third author contributed to the trial design. The authors were fully responsible for all content and editorial decisions regarding the manuscript, wrote the first draft, were involved in all stages of manuscript development, approved the final version, and agreed with the decision to submit the manuscript for publication. The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol (available at NEJM.org). The statistical analysis plan is available with the protocol. Medical writing assistance, paid for by the sponsors, was provided by MediTech Media. Independent ethics committees at each trial cen-

ter or central institutional review boards approved the trial protocol, and all the patients provided written informed consent.

## PATIENTS

At the time of the screening visit, eligible patients were adults, 18 to 75 years of age, who had received an asthma diagnosis at least 1 year before screening and were receiving medium- or high-dose inhaled glucocorticoids and at least one additional controller medication. Patients must have had a documented history of at least one of the following: two or more severe asthma exacerbations in the previous 12 months, one severe asthma exacerbation in the previous 12 months that resulted in hospitalization or an emergency department visit, or one severe asthma exacerbation in the previous 6 months that did not result in hospitalization or an emergency department visit. Patients could not have had a severe asthma exacerbation in the 6 weeks before screening. In addition, eligible patients had never smoked or had not smoked for at least 1 year before screening, and had a smoking history of less than 10 pack-years. The forced expiratory volume in 1 second (FEV<sub>1</sub>) before bronchodilator use had to be between 40% and 85% of the predicted normal value. Bronchodilator reversibility (defined as an increase in the FEV<sub>1</sub> of at least 12% and an absolute change of at least 200 ml after administration of 400 µg of albuterol by means of a metered-dose inhaler at screening) or documented history of reversibility or airway hyperresponsiveness (a provocative concentration of inhaled methacholine causing a 20% decrease in the FEV<sub>1</sub> of <8 mg per milliliter) must have been documented in the previous 2 years, or a difference in the FEV<sub>1</sub> (as assessed in the clinic) of at least 20% between the values from two different clinic visits must have been documented in the previous year.

## END POINTS

The primary end point was the time to the first asthma worsening during the planned 24-week treatment period. Asthma worsening was defined as the occurrence of any one of the following: a decrease from baseline of at least 30% in the morning peak expiratory flow on at least 2 consecutive days; an increase from baseline of at least 50% in the number of puffs of rescue medication in a 24-hour period (equating to at

least four additional puffs) on at least 2 consecutive days; an increase from baseline of at least 0.75 points on the ACQ-5 (minimal clinically important difference, 0.5 points); and severe asthma exacerbation, defined as new use of systemic glucocorticoids (prednisone or equivalent) for 3 or more consecutive days, or (in patients who were already receiving systemic glucocorticoids) a doubling of the maintenance dose, which resulted in a total daily dose of 20 mg or more for 3 or more consecutive days.

Subgroup analyses, prespecified in the protocol, were performed to assess the primary end point, with subgroups defined according to oral glucocorticoid use at baseline (yes or no), asthma inflammatory phenotype (classified according to sputum eosinophil and neutrophil cell counts and blood eosinophil counts), and body-mass index (BMI; the weight in kilograms divided by the square of the height in meters, <30 or ≥30). Subgroups according to BMI were included because of the known association of obesity with poorer outcomes in patients with asthma and the association of obesity with both type 1 and type 2 cellular immune responses.<sup>18-20</sup> Details regarding subgroup analyses are provided in the Supplementary Methods section in the Supplementary Appendix.

Secondary efficacy end points during the 24-week treatment period were the time to the first asthma worsening, in days, according to the alternative definition (which included the same criteria as the primary end point with respect to peak expiratory flow, use of rescue medication, and severe asthma exacerbation, but required an increase in ACQ-5 score from baseline of ≥0.5 points rather than ≥0.75 points), the annualized rate of asthma worsening, the time to the first severe asthma exacerbation, the annualized rate of severe asthma exacerbation, the weekly ACQ-5 score, and the change in the FEV<sub>1</sub> from baseline and after bronchodilator use. Exploratory end points included sputum eosinophil and neutrophil counts, sputum cell gene expression (determined by means of RNA sequencing), the results of Ingenuity Pathway Analysis and upstream regulator analysis (Qiagen), the results of post hoc rotation gene set testing (ROAST), selected sputum and serum mediators that are implicated in interleukin-23-mediated inflammation (measured with the use of the Randox multi-analyte assay), host defense and tissue repair (β-defensin 2,

neutrophil gelatinase–associated lipocalin, vascular endothelial growth factor, S100-A8, and interleukin-6),<sup>8,21</sup> and the FeNO. Additional information is provided in the Supplementary Methods section in the Supplementary Appendix.

Safety was assessed on the basis of physical examinations, blood testing, and electrocardiography. Vital signs and administration-site problems were monitored, and adverse events were recorded.

#### STATISTICAL ANALYSIS

Analyses of primary and secondary end points were performed in the intention-to-treat population, which included all the patients who underwent randomization and received at least one dose of risankizumab or placebo. The primary efficacy end point was analyzed with the use of a Cox proportional-hazards model. Assuming that risankizumab would reduce the risk of asthma worsening by 40%, we calculated that a total of 89 events and 100 patients per group would be required to provide the trial with 87% power at a one-sided type I error rate of 0.1 to show a hazard ratio for asthma worsening with risankizumab as compared with placebo of 0.6. Although the trial was designed to use a one-sided test, two-sided 95% confidence intervals are reported.

The annualized rates of asthma worsening and severe asthma exacerbation were analyzed with the use of negative binomial regression, with trial group and oral glucocorticoid use as fixed effects at baseline. A restricted maximum likelihood–based mixed model with repeated measures was used to analyze the mean change from baseline in the trough FEV<sub>1</sub> and the FEV<sub>1</sub> after bronchodilator use at week 24 and at each clinic visit; these analyses included the fixed, categorical effects of trial-group assignment, the stratification factor of oral glucocorticoid use at baseline, the test day and the interaction of trial group with test day, as well as continuous, fixed covariates of baseline and the interaction of baseline with test day. The weekly ACQ-5 score was analyzed with the use of a covariance model. The model included the fixed categorical effect of trial group and the stratification factor of oral glucocorticoid use at baseline and used baseline ACQ-5 score as a continuous covariate. Post hoc analysis of the ACQ-5 score was conducted with the use of the same mixed model with repeated

measures that was used in the analyses of the FEV<sub>1</sub>. No adjustment for multiplicity was prespecified for the secondary end points, so definitive treatment effects cannot be inferred. The analysis of the exploratory end points is described in the Supplementary Methods section in the Supplementary Appendix.

## RESULTS

#### PATIENTS

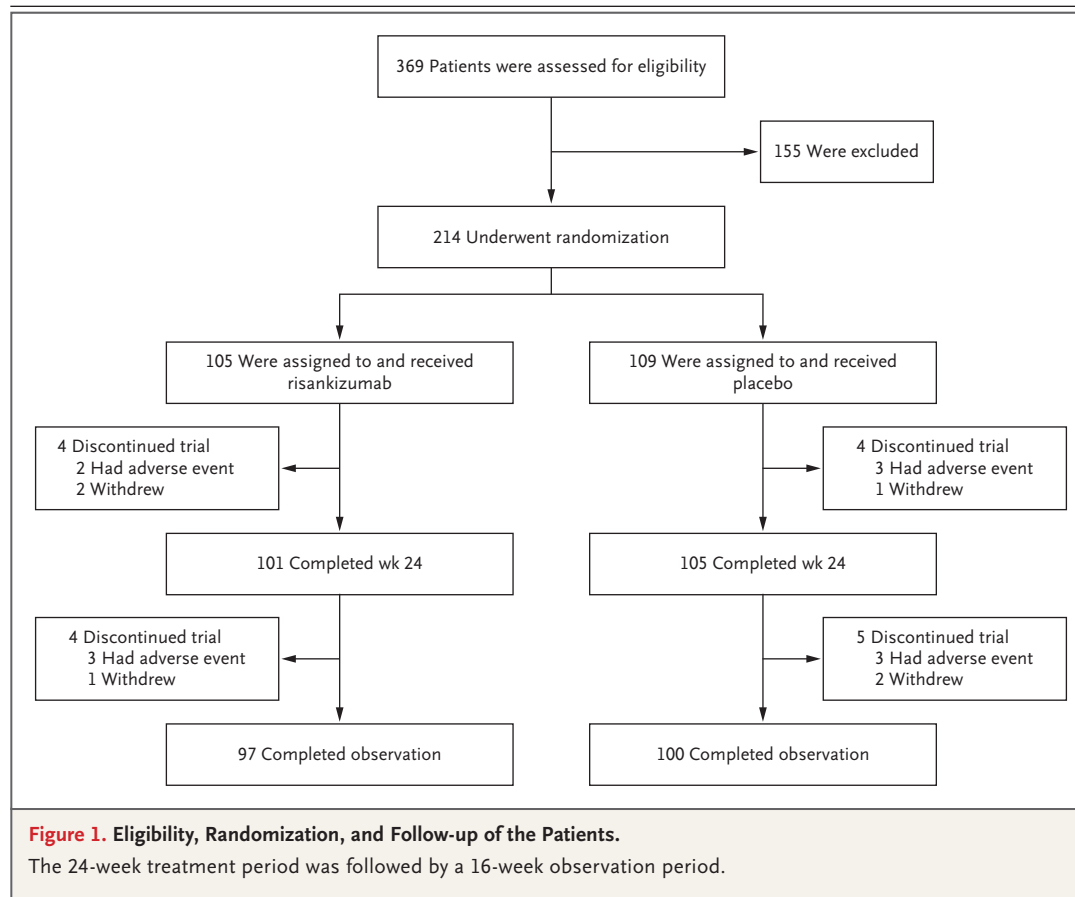
The trial was conducted from August 20, 2015, to February 2, 2018. A total of 369 patients were screened at 48 centers in nine countries. Of those who were screened, 214 patients subsequently underwent randomization; 105 patients received risankizumab and 109 received placebo. No patients were lost to follow-up (Fig. 1). The demographic and clinical characteristics of the patients were well balanced between the two groups (Table 1).

#### PRIMARY AND SECONDARY END POINTS

The median time to the first asthma worsening (the primary end point) was 40 days in the risankizumab group and 86 days in the placebo group (hazard ratio, 1.46; 95% confidence interval [CI], 1.05 to 2.04;  $P=0.03$ ) (Fig. 2). The rate ratio for annualized asthma worsening was 1.49 (95% CI, 1.12 to 1.99) for the comparison of risankizumab with placebo. The time to the first asthma worsening according to the alternative definition was 20 days in the risankizumab group and 37 days in the placebo group (hazard ratio, 1.47; 95% CI, 1.08 to 1.99). The hazard ratio for the time to the first severe exacerbation was 1.18 (95% CI, 0.76 to 1.83), and the rate ratio for annualized severe exacerbations was 1.13 (95% CI, 0.75 to 1.70) (Fig. S2).

Changes in ACQ-5 scores over the trial period are shown in Figure 3A. The adjusted mean ( $\pm$ SE) difference in ACQ-5 scores between the two groups at week 24 was  $0.15 \pm 0.11$  points. A post hoc analysis that used a restricted maximum-likelihood mixed model with repeated measures to assess the mean change from baseline in the ACQ-5 score at week 24 and at each clinic visit yielded similar findings (mean between-group difference in change in ACQ-5 score,  $0.17 \pm 0.11$  points).

The adjusted mean change in the trough FEV<sub>1</sub> from baseline to week 24 was  $-0.05 \pm 0.04$  liters



in the risankizumab group and  $-0.01 \pm 0.03$  liters in the placebo group (mean difference in change,  $-0.04 \pm 0.51$  liters) (Fig. S3). The adjusted mean change in the FEV<sub>1</sub> after bronchodilator use from baseline to week 24 was  $-0.10 \pm 0.03$  liters in the risankizumab group and  $-0.03 \pm 0.03$  liters in the placebo group (adjusted mean difference in change,  $-0.07 \pm 0.04$  liters) (Fig. 3B). The adjusted mean difference in the weekly mean morning peak expiratory flow from baseline to week 24 was  $9.5 \pm 7.5$  ml (Fig. 3C). The adjusted mean change in weekly use of rescue medication in a 24-hour period from baseline to week 24 was  $-0.08 \pm 0.31$  puffs in the risankizumab group and  $-0.55 \pm 0.31$  puffs in the placebo group (mean difference in change,  $0.47 \pm 0.36$  puffs) (Fig. 3D).

#### EXPLORATORY END POINTS

Changes in sputum eosinophil and neutrophil counts are shown in Figure S4. Sputum gene expression pathway and upstream regulator

analyses showed that canonical pathways dominated by molecules involved in the interleukin-12 family, of which interleukin-23 is a member, and the transcription factors regulating Th17 (RORC, the gene encoding retinoic acid–related orphan receptor gamma t) and type 1 helper T (Th1) transcription (T-box transcription factor 21 [TBX21], the gene encoding T-bet) were down-regulated by risankizumab at the end of the treatment period (week 24). (Data are available from the National Center for Biotechnology Information Gene Expression Omnibus [https://www.ncbi.nlm.nih.gov/geo/], accession number GSE167074.) The other genes that were down-regulated after treatment with risankizumab were involved in activation of cytotoxic T cells and natural killer cells (Fig. 4 and Table S1).

We performed a post hoc interleukin-23 pathway ROAST, which showed that interleukin-23–associated genes were apparently down-regulated in response to risankizumab at the end of the treatment period; however, a post hoc ROAST

**Table 1. Baseline Characteristics of the Patients.\***

| Characteristic                                                                                | Risankizumab<br>(N=105) | Placebo<br>(N=109) |
|-----------------------------------------------------------------------------------------------|-------------------------|--------------------|
| Age — yr                                                                                      | 54±11                   | 52±13              |
| Female sex — no. (%)                                                                          | 69 (65.7)               | 64 (58.7)          |
| Body-mass index                                                                               | 30±7                    | 30±8               |
| Race or ethnic group — no. (%)†                                                               |                         |                    |
| American Indian or Alaska Native                                                              | 0                       | 1 (0.9)            |
| Asian                                                                                         | 11 (10.5)               | 11 (10.1)          |
| Black                                                                                         | 9 (8.6)                 | 8 (7.3)            |
| Multiple                                                                                      | 1 (1.0)                 | 1 (0.9)            |
| White                                                                                         | 84 (80.0)               | 88 (80.7)          |
| Dose of inhaled glucocorticoids — no. (%)‡                                                    |                         |                    |
| Medium                                                                                        | 49 (46.7)               | 54 (50.0)          |
| High                                                                                          | 56 (53.3)               | 55 (50.5)          |
| Use of oral glucocorticoids — no. (%)                                                         | 15 (14.3)               | 17 (15.6)          |
| Dose of oral glucocorticoids — mg                                                             | 11.8±9.1                | 10.8±5.4           |
| Allergic asthma — no. (%)§                                                                    | 65 (61.9)               | 75 (68.8)          |
| No. of asthma exacerbations in previous yr that did not result in hospitalization — no. (%)   |                         |                    |
| 0                                                                                             | 3 (2.9)                 | 6 (5.5)            |
| 1                                                                                             | 9 (8.6)                 | 8 (7.3)            |
| 2                                                                                             | 58 (55.2)               | 53 (48.6)          |
| >2                                                                                            | 35 (33.3)               | 42 (38.6)          |
| No. of hospitalizations for asthma in previous yr — no. (%)                                   |                         |                    |
| 1                                                                                             | 10 (9.6)                | 11 (10.1)          |
| 2                                                                                             | 1 (1.0)                 | 1 (0.9)            |
| >2                                                                                            | 2 (1.9)                 | 4 (3.7)            |
| No. of asthma exacerbations in previous 6 mo that did not result in hospitalization — no. (%) |                         |                    |
| 1                                                                                             | 24 (22.9)               | 24 (22.0)          |
| 2                                                                                             | 12 (11.4)               | 7 (6.4)            |
| >2                                                                                            | 3 (2.9)                 | 5 (4.6)            |
| Median total IgE level (IQR) — µg/liter                                                       | 398 (149–1199)          | 485 (190–1504)     |
| Coexisting eczema — no. (%)                                                                   | 15 (14.3)               | 17 (15.6)          |
| Coexisting psoriasis — no. (%)                                                                | 5 (4.8)                 | 1 (0.9)            |
| FEV <sub>1</sub> before bronchodilator use — % of predicted value                             | 65.4±15.0               | 64.0±13.6          |
| Bronchodilator reversibility — %¶                                                             | 14.2±16.3               | 12.1±11.2          |
| ACQ-5 score                                                                                   | 2.15±1.15               | 2.39±1.17          |
| Use of rescue medication in 24-hr period — no. of puffs                                       | 3.2±3.8                 | 3.9±4.8            |
| Morning peak expiratory flow — liters/min                                                     | 299±111                 | 310±115            |
| FeNO** — parts per billion                                                                    | 33.8±3.6                | 34.1±3.2           |
| Median blood eosinophil count (IQR) — cells/mm <sup>3</sup>                                   | 375 (135–630)           | 360 (150–675)      |
| Median sputum eosinophils (IQR) — %                                                           | 4.5 (0.7–16.7)          | 3.0 (0.5–16.2)     |

**Table 1. (Continued.)**

| Characteristic                      | Risankizumab<br>(N=105) | Placebo<br>(N=109) |
|-------------------------------------|-------------------------|--------------------|
| Median sputum neutrophils (IQR) — % | 60 (36–76)              | 54 (27–71)         |

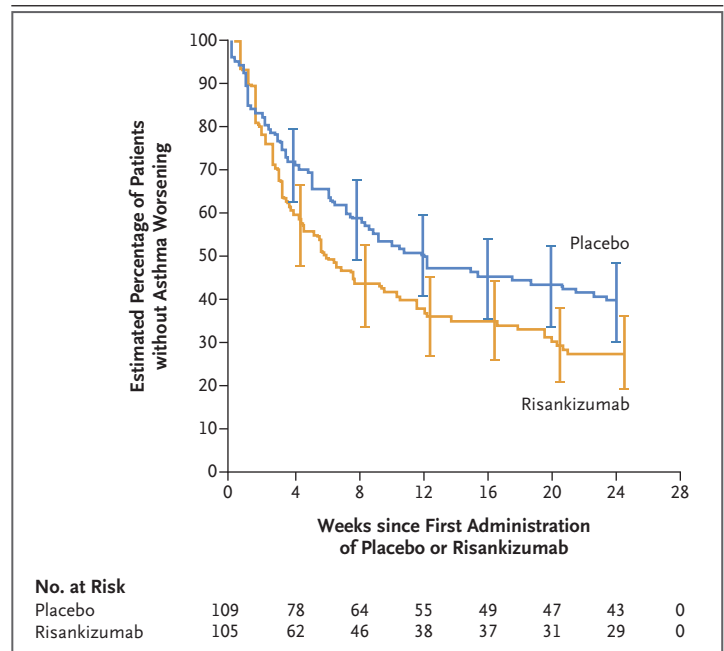
- \* Plus-minus values are means  $\pm$ SD. Percentages may not total 100 because of rounding. FeNO denotes fraction of exhaled nitric oxide, FEV<sub>1</sub> forced expiratory volume in 1 second, and IQR interquartile range.
- † Race or ethnic group was reported by the patients.
- ‡ A medium daily dose was defined as any one of the following: beclomethasone dipropionate (chlorofluorocarbon), more than 500 to 1000  $\mu$ g; beclomethasone dipropionate (hydrofluoroalkane), more than 200 to 400  $\mu$ g; budesonide (dry-powder inhaler), more than 400 to 800  $\mu$ g; ciclesonide (hydrofluoroalkane), more than 160 to 320  $\mu$ g; fluticasone propionate (dry-powder inhaler), more than 250 to 500  $\mu$ g; fluticasone propionate (hydrofluoroalkane), more than 250 to 500  $\mu$ g; mometasone furoate, more than 220 to 400  $\mu$ g; and triamcinolone acetonide, more than 1000 to 2000  $\mu$ g. For patients who were receiving a high dose, there was no restriction on the maximum dose for eligibility to participate in the trial.
- § Allergic asthma status was reported in the clinical history, with the following allergies recorded: animals, house dust, house-dust mites, pollens, fungi and molds, cockroaches, food, and drugs.
- ¶ Bronchodilator response was defined as an increase in the FEV<sub>1</sub> of at least 12% and an absolute change of at least 200 ml after administration of 400  $\mu$ g of albuterol by means of a metered-dose inhaler.
- || Scores on the five-item Asthma Control Questionnaire (ACQ-5) range from 0 to 6, with higher scores indicating less control.
- \*\* Data were available for 104 patients in the risankizumab group and 107 in the placebo group.

comparing gene expression at baseline with that at week 20 did not show similar down-regulation (see the Supplementary Results section in the Supplementary Appendix). Changes in levels of sputum and serum protein mediators are shown in Table S2. The median change in the serum  $\beta$ -defensin level from baseline to week 24 was  $-20.9$  ng per liter (interquartile range,  $-156.8$  to  $22.0$ ) in the risankizumab group and  $-0.4$  ng per liter (interquartile range,  $-86.3$  to  $79.5$ ) in the placebo group. The mean ( $\pm$ SE) change in the FeNO from baseline to week 24 was  $-0.9 \pm 1.9$  parts per billion (ppb) in the risankizumab group and  $-2.4 \pm 1.9$  ppb in the placebo group (mean difference in change in the FeNO,  $1.6 \pm 2.7$  ppb).

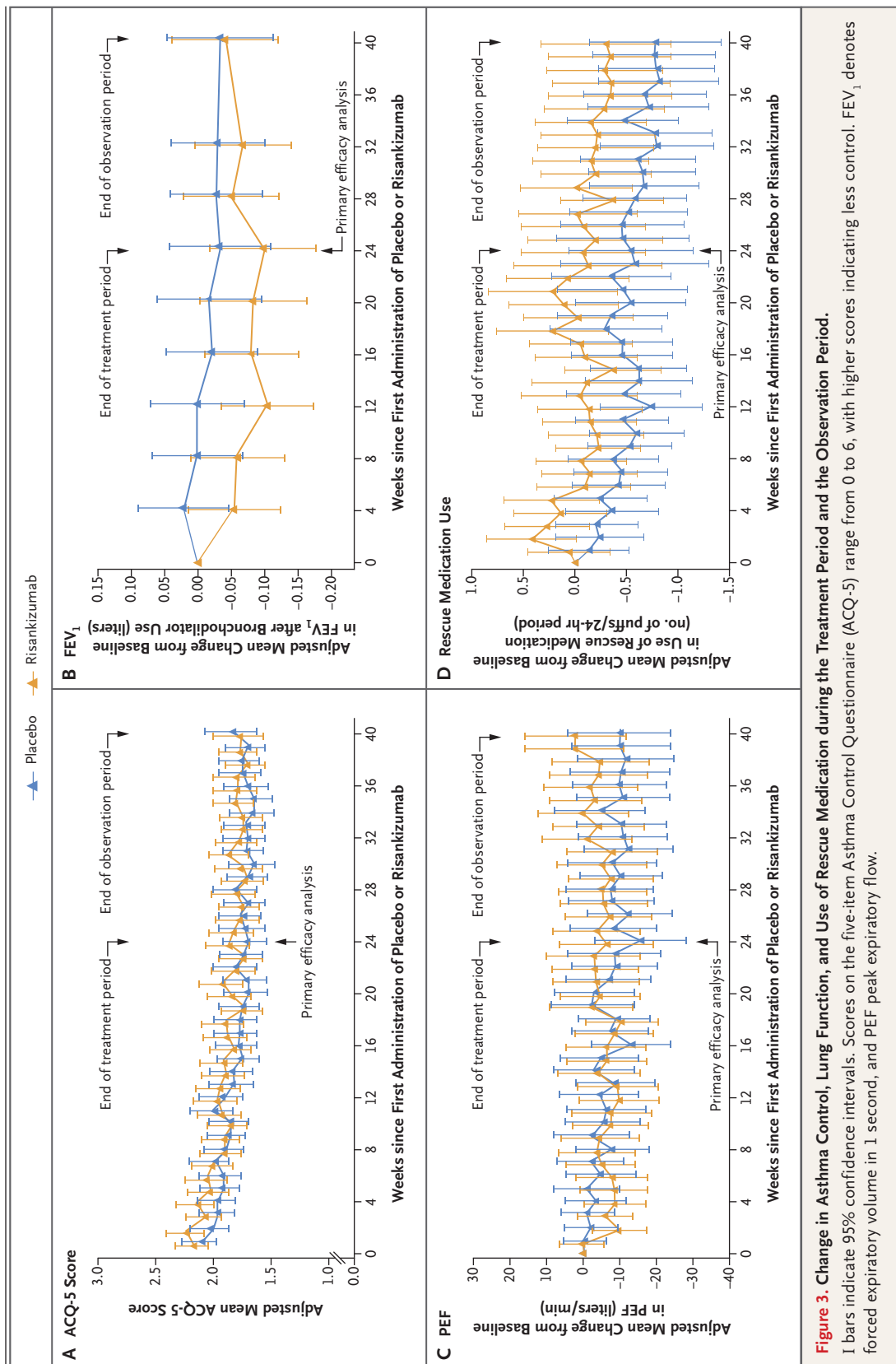
#### PRESPECIFIED SUBGROUP ANALYSES OF THE PRIMARY END POINT

The results of the subgroup analyses showed no apparent differences between the two groups with respect to the primary end point. However, the hazard ratio for the time to the first asthma worsening in the risankizumab group as compared with the placebo group was more marked in patients with higher blood eosinophil counts ( $\geq 200$  cells per cubic millimeter; hazard ratio, 1.76; 95% CI, 1.15 to 2.70) than in those with lower counts ( $< 200$  cells per cubic millimeter; hazard ratio, 1.10; 95% CI, 0.65 to 1.86), and in those with a BMI of less than 30 (hazard ratio,

1.91; 95% CI, 1.20 to 3.01) than in those with a BMI of 30 or more (hazard ratio, 1.04; 95% CI, 0.64 to 1.68) (Figs. S5 and S6).

**Figure 2. Time to the First Asthma Worsening.**

The primary end point was the time to the first asthma worsening during the 24-week treatment period. Patients received either risankizumab at a dose of 90 mg or placebo, administered subcutaneously once every 4 weeks. I bars indicate 95% confidence intervals.



## SAFETY

No safety concerns were identified. The incidence of adverse events and serious adverse events was similar in the two groups (Table S3).

## DISCUSSION

We report here the results of a phase 2a clinical trial of the anti-interleukin-23 monoclonal antibody risankizumab in the treatment of asthma. The primary end point was not met. Indeed, the time to the first asthma worsening was shorter in the risankizumab group than in the placebo group, and the annualized rate of asthma worsenings was higher in the risankizumab group than in the placebo group. Lung function, ACQ-5 scores, and the rate of severe asthma exacerbations were similar in the two groups. Risankizumab therapy did not affect sputum cell counts, but it did attenuate the sputum interleukin-23 gene set and gene pathways associated with activation of cytotoxic T cells and natural killer cells and the Th17 transcription factor *RORC* and the Th1 transcription factor *TBX21*. These findings support the view that risankizumab exerted a biologic effect on airway immunity, which may have contributed to the poor clinical outcome.

The lack of benefit with risankizumab therapy in asthma contrasts with the marked clinical efficacy in psoriasis<sup>15,22</sup> and the good responses in Crohn's disease.<sup>16</sup> The inability of this trial to show clinical improvement in asthma in response to interleukin-23 inhibition also contrasts with the consistent benefit from the type 2-mediated biologic agents targeting IgE, interleukin-5, and interleukin-4 receptor  $\alpha$ .<sup>1,2,4</sup> Our findings therefore challenge the current understanding of the role that Th17- and interleukin-23-mediated pathways play in the pathogenesis of asthma. These findings are consistent with the failure of a phase 2b trial of the anti-interleukin-17 monoclonal antibody brodalumab, which was stopped after an interim futility analysis (ClinicalTrials.gov number, NCT01902290).

It is critical to note that patients who received risankizumab had a poorer outcome with respect to the primary end point than those who received placebo: the time to the first asthma worsening was approximately 50% shorter in the risankizumab group than in the placebo group. Asthma worsening consisted of four components, including a reduction in peak expiratory

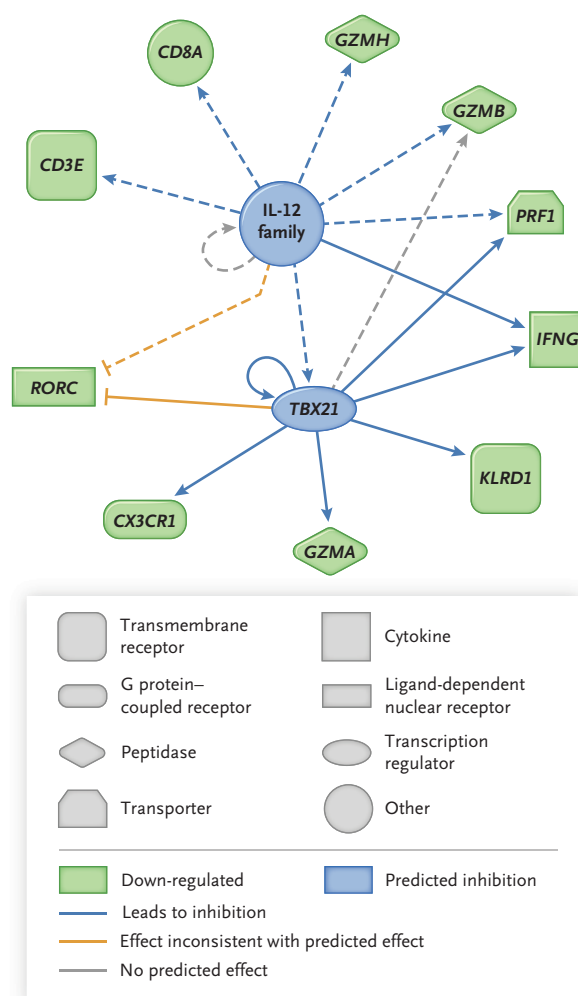


**Figure 4. Upstream Regulator Analysis of Genes Affected by Risankizumab.**

Gene expression analysis was conducted with the use of sputum samples. Upstream regulator analysis was performed, incorporating genes that were differentially expressed after treatment with risankizumab. This analysis predicted that the interleukin-12 (IL-12) family and T-box transcription factor 21 (*TBX21*, the gene encoding T-bet) would be inhibited by risankizumab treatment (shown in blue). Genes that were down-regulated are shown in green. *CD3E* and *CD8A* are T-cell-specific receptors involved in T-cell activation. Interferon- $\gamma$  (*IFNG*) is a critical cytokine for type 1 immune responses involved in antiviral immunity; it is expressed by type 1 helper T cells, CD8+ cytotoxic T cells, and natural killer cells. Granzyme A (*GZMA*), granzyme B (*GZMB*), granzyme H (*GZMH*), and perforin (*PRF1*) are all involved in cytotoxic killing mediated by cytotoxic T cells and natural killer cells. Killer cell lectinlike receptor D1 (*KLRD1*) is expressed by natural killer cells, and it regulates the natural killer cell-mediated killing of target cells. *CX3CR1* is a chemokine receptor that is implicated in CD8+ cytotoxic T-cell memory activity. *RORC* is the gene encoding the transcription factor retinoic acid-related orphan receptor gamma t, the master regulator of the differentiation of type 17 helper T cells. Solid lines indicate a direct effect, and dashed lines indicate an indirect effect. Arrows indicate the known activation relationship in the pathway, and lines ending in bars indicate the known inhibitory relationship in the pathway.

flow, increased use of short-acting  $\beta_2$ -agonist relievers, an increase in the ACQ-5 score (indicating less asthma control), and an increased incidence of severe exacerbations. If each component is explored individually, it can be seen that the effects on asthma worsening were driven mostly by the use of short-acting  $\beta_2$ -agonists and partly by peak expiratory flow rather than by ACQ-5 score and severe asthma exacerbations. One possible explanation is the postulated reciprocal relationship between the Th17 and type 2 pathways,<sup>23</sup> such that targeting interleukin-23 might have led to an increase in some type 2 mediators such as interleukin-13, with consequent increased smooth-muscle tone in the airway.<sup>24</sup> This relationship might also explain the early divergence in the primary end point observed between the risankizumab group and the placebo group. Subgroup analyses showed that the primary end point was not affected by oral glucocorticoid use and was similar across the inflammatory phenotypes defined according to sputum cell counts. The poorer outcome in the risankizumab group was more marked in patients with a higher blood eosinophil count ( $\geq 200$  cells per cubic millimeter vs.  $< 200$  cells per cubic millimeter) and in those with a lower BMI ( $< 30$  vs.  $\geq 30$ ).

It is surprising that in spite of reported roles in both eosinophilic and neutrophilic inflammation,<sup>8</sup> risankizumab therapy had no effect on the sputum cell counts. However, among the selected sputum and serum mediators measured that are implicated in interleukin-23 function, there was a trend toward a reduction in the level of serum  $\beta$ -defensin 2, an important host-defense molecule.<sup>8,21</sup> Risankizumab attenuated the interleukin-23 gene set and gene pathways involved in the interleukin-12 family, which includes the transcription factors regulating Th17 and Th1 signaling and genes involved in the activation of cytotoxic and natural killer cells. These pathways included genes for the granzymes A, B, and H and perforin and interferon- $\gamma$ , which are important for cytotoxic T-cell- and natural killer cell-mediated immune response to airway pathogens.<sup>25</sup> Interferon- $\gamma$  has previously been reported to be down-regulated after treatment with risankizumab in psoriasis.<sup>26</sup> These effects on sputum gene expression were not observed in samples obtained at the week 20 visit (before the end of the treatment period), which suggests

possible technical variability or an inability to consistently attenuate interleukin-23 signaling throughout the whole treatment period. Notwithstanding this limitation, our findings suggest that risankizumab modulated airway immunity. Whether these effects had a consequence on the poorer clinical outcomes after treatment with risankizumab is uncertain and warrants further study.

This trial has a number of limitations. The sample-size calculation for the primary end point was based on a one-sided power calculation with a sample size that was not adequate to provide sufficient power for the secondary and exploratory end points. Even though we extensively investigated the effects on airway inflammation, we were unable to examine the effects on other features of severe asthma, including airway hyperresponsiveness, airway remodeling, airway microbiome, and autoimmunity.<sup>27</sup> Thus, we have not fully explored the possible mechanisms for the greater asthma worsening in patients in the risankizumab group than in those in the placebo group. In addition, we did not specifically enroll patients with severe asthma who had up-regulated interleukin-23 or an interleukin-17A signature<sup>28</sup>; thus, it remains possible that such patients might have had better outcomes than those in our trial population. Furthermore, we cannot rule out a possible role of immunomodulation beyond type 2 inflammation and independent of type 17 immunity, such as type 1-mediated immunity or interleukin-6 trans-signaling.<sup>29</sup>

Risankizumab therapy was not beneficial in severe asthma. The time to the first asthma worsening was shorter with risankizumab than with placebo, and the annualized rate of asthma worsening was higher with risankizumab. Risankizumab did not affect the sputum cell counts, but it did attenuate gene pathways associated with cytotoxic T-cell- and natural killer cell-mediated airway immunity. These findings undermine the role of interleukin-23 and the Th17 axis as targets for the treatment of asthma.

The views expressed in this article are those of the authors and are not necessarily those of the NIHR or the U.K. Department of Health and Social Care.

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## Supplementary Appendix

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

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# 1    **Supplementary Appendix**

## 2    **Contents**

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## Supplementary methods

### Subgroup analyses

Subgroup analyses were performed on the primary endpoint to evaluate oral corticosteroid use at baseline, body mass index (BMI), and asthma phenotype and endotype on these results (**Figure S5**). The subgroups were defined as follows:

1. Oral corticosteroid use at baseline: Yes vs. No
2. BMI:  $<30$  vs.  $\geq 30$  kg/m<sup>2</sup>
3. Asthma phenotype 1:
  - Sputum neutrophils  $<40\%$
  - Sputum neutrophils  $\geq 40\%$
4. Asthma phenotype 2
  - Neutrophilic only (sputum neutrophils  $\geq 40\%$  and eosinophils  $<2\%$ )
  - Eosinophilic only (sputum eosinophils  $\geq 2\%$  and neutrophils  $<40\%$ )
  - Mixed eosinophilic and neutrophilic (sputum eosinophils  $\geq 2\%$  and neutrophils  $\geq 40\%$ )
  - Paucigranulocytic (sputum eosinophils  $<2\%$  and neutrophils  $<40\%$ )
5. Asthma phenotype 3
  - Neutrophilic only (sputum neutrophils  $\geq 60\%$  and eosinophils  $<3\%$ )
  - Eosinophilic only (sputum eosinophils  $\geq 3\%$  and neutrophils  $<60\%$ )
  - Mixed eosinophilic and neutrophilic (sputum eosinophils  $\geq 3\%$  and neutrophils  $\geq 60\%$ )
  - Paucigranulocytic (sputum eosinophils  $<3\%$  and neutrophils  $<60\%$ )

6. Asthma endotype 1

Blood eosinophil cell counts  $>300$  cells/ $\mu$ L

Blood eosinophil cell counts  $<300$  cells/ $\mu$ L

7. Asthma endotype 2

Blood eosinophil cell counts  $>200$  cells/ $\mu$ L

Blood eosinophil cell counts  $<200$  cells/ $\mu$ L

**Gene expression RNA sequencing and pathway analysis**

Due to an insufficient number of patients in the optional bronchoscopy sub-study, transcriptome profiling from epithelial brushings and bronchial biopsies was not performed. Since transcriptome profiling of sputum samples was expected to provide a better assessment of treatment effect on mRNA expression in study patients than blood, only transcriptome profiling of sputum samples was analyzed.

We undertook pre-specified exploratory analysis of genome-wide expression in sputum samples to identify RNA-based gene regulation markers by comparing gene expression in samples collected at Visit 2 (prior to treatment) and Visit 8 (end of treatment) in the placebo versus risankizumab treatment groups. Genome-wide expression profiling of the sputum samples was performed by RNA sequencing. Samples were randomized before RNA extraction, library preparation and sequencing to avoid the introduction of analytical gene expression batches for the respective subgroups such as treatment, dosing or time points.

To assess the differences between risankizumab and placebo, a pre-filtering step was performed to remove transcripts with low expression. The samples were normalized using the 'Trimmed Mean

of M-values' method. The R-package limma in conjunction with voom transformation and observational weights was used to fit a standard linear regression model in a univariate fashion to the observed read counts.

Following differential gene expression analysis to identify altered gene expression between treatments, we performed Ingenuity Canonical Pathway Analysis to examine pathways impacted by treatment, using genes that differed by a log<sub>2</sub>-fold change greater than -1.1 to +1.1 (152 genes in total). Ingenuity Canonical Pathway Analysis uses a right-tailed Fisher's exact test to identify pathways enriched for genes altered by treatment. Only pathways that met a false discovery rate Benjamini-Hochberg ( $FDR_{bh}$ ) corrected p-value <0.05 are shown (**Table S1**). Upstream regulator analysis identified downregulation of pathways involving IL12 family and *TBX21* (T-bet) genes (**Figure 4**).

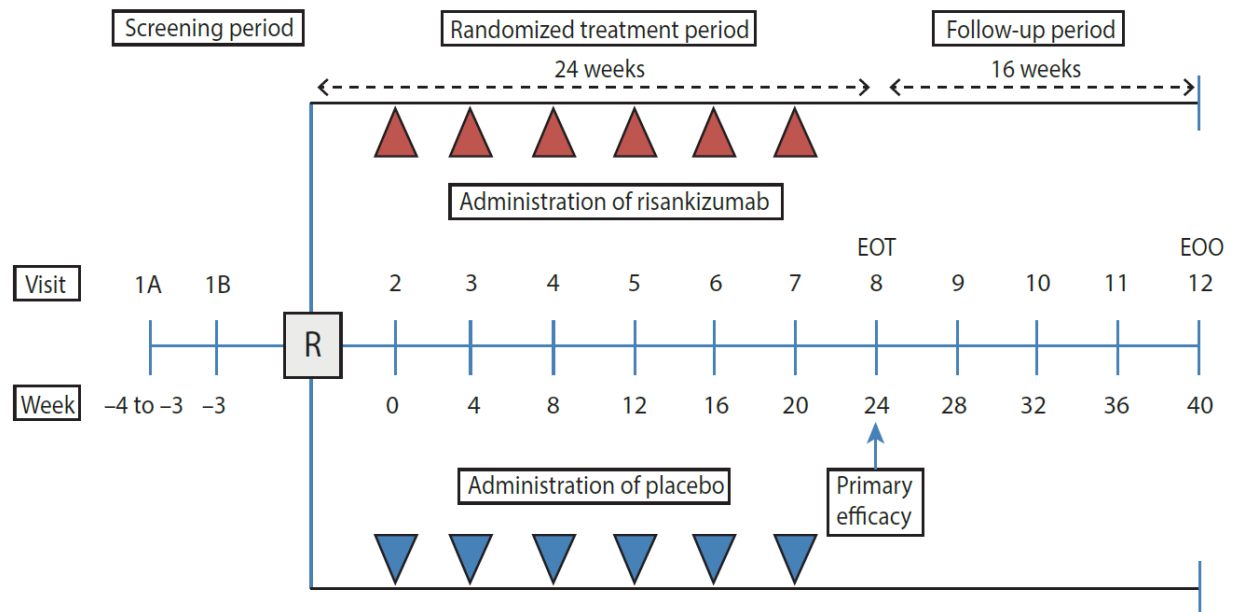
To ensure that our filtering method did not over-represent genes that met fold-change criteria without meeting a p-value criterion or exclude potentially important pathways, we re-analyzed post hoc the data set in the Ingenuity Pathway Analysis using genes that scored p-value (<0.05) with a log<sub>2</sub> >1.1-fold change or with a smaller-fold change (log<sub>2</sub> >1.05). The results of both analyses returned very similar canonical pathways with both Tbx21 and IL12 families in the top 5 upstream pathways inhibited, indicating that we have not eliminated important mechanistic pathways.

We extended our analyses of the pre-specified time points (baseline versus end-of-treatment [Week 24]) to undertake a post hoc ROAST gene-set analysis ([10.1093/bioinformatics/btq401](https://doi.org/10.1093/bioinformatics/btq401))

using an IL23 pathway (MSigDB: PID\_IL23\_PATHWAY). The ROAST gene-set analysis showed an apparent down-regulation of the IL23 pathways ( $p=0.007$ ).

Post hoc, we repeated the ROAST gene-set analysis to compare baseline with samples collected at Week 20. Analysis comparing baseline and Week 20 was not pre-specified in our exploratory analysis plan. In contrast to the findings at end-of-treatment (Week 24), IL23 pathways were apparently not downregulated at Week 20.

102 **Figure S1** Trial design

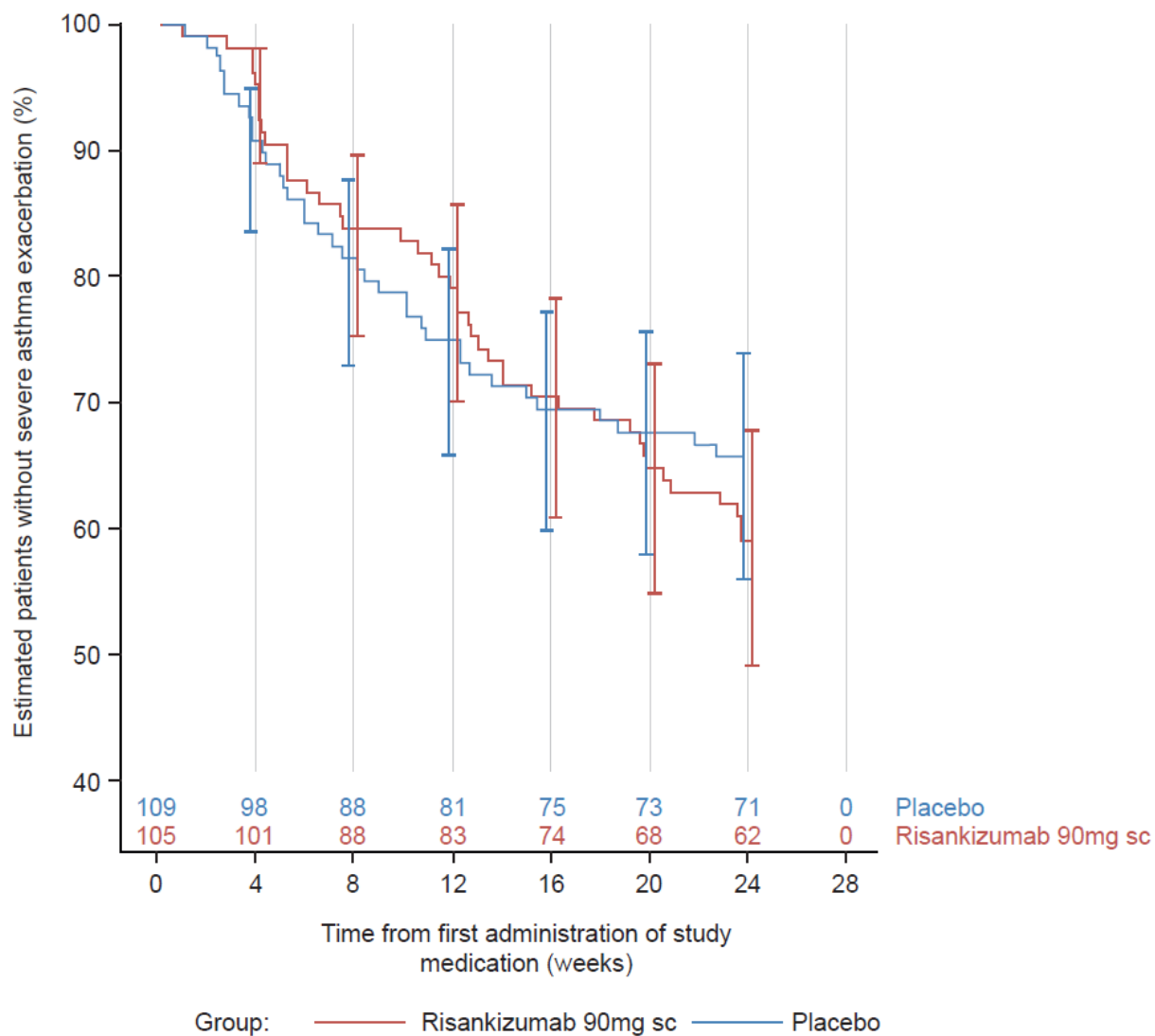


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104 EOO, end of observation; EOT, end of treatment; R, randomization.

105

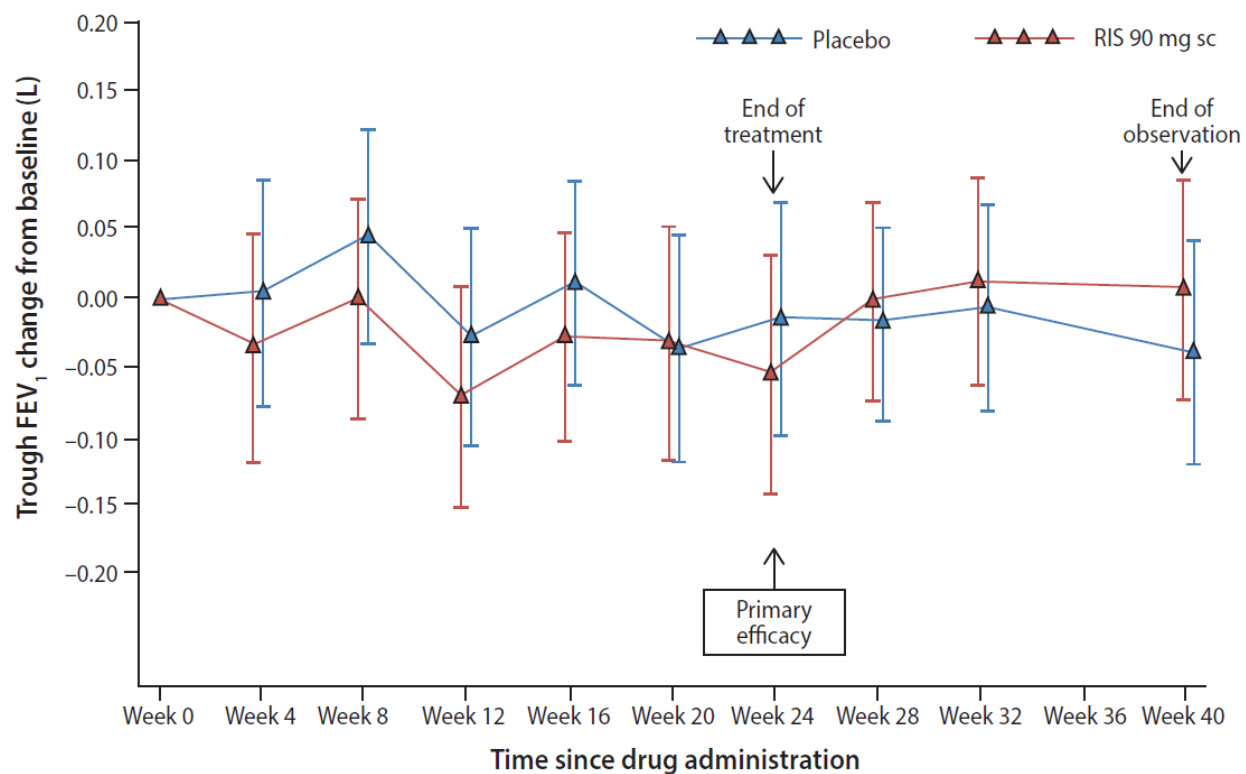
106 **Figure S2** Severe asthma exacerbations during 24-week treatment with risankizumab versus  
 107 placebo



108 Group: — Risankizumab 90mg sc — Placebo

109 SC, subcutaneous.

111 **Figure S3** Change in trough FEV<sub>1</sub> during the 24-week treatment period and washout in those  
 112 subjects that received risankizumab versus placebo

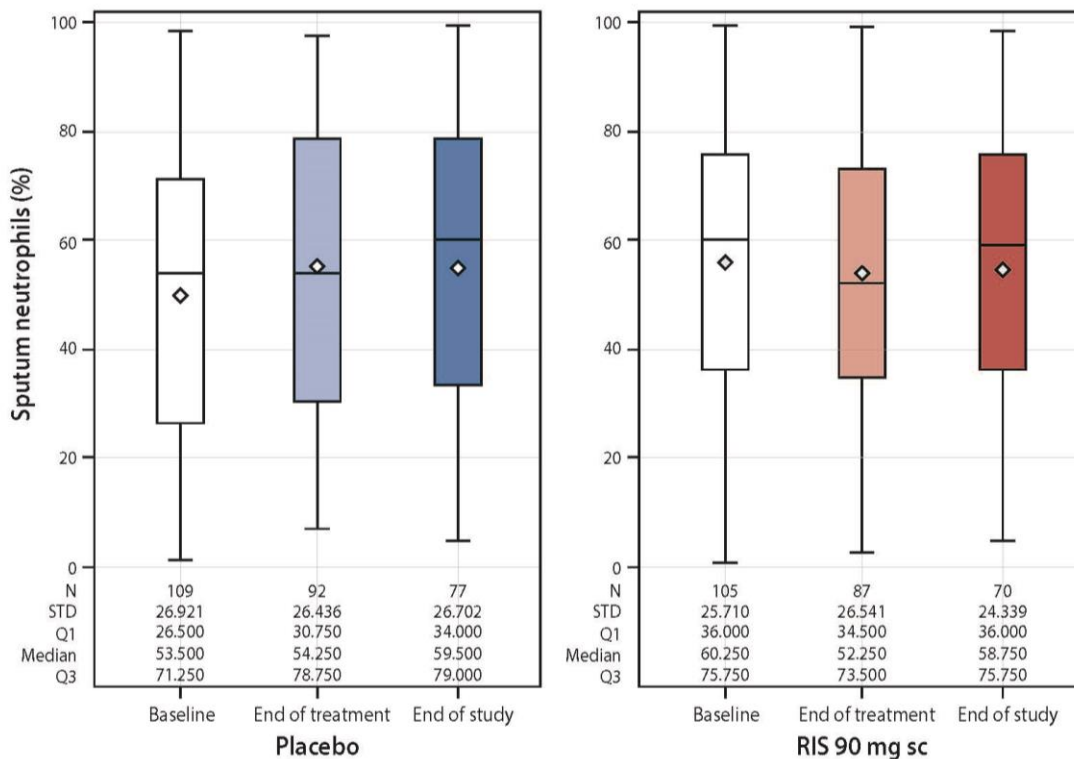


113

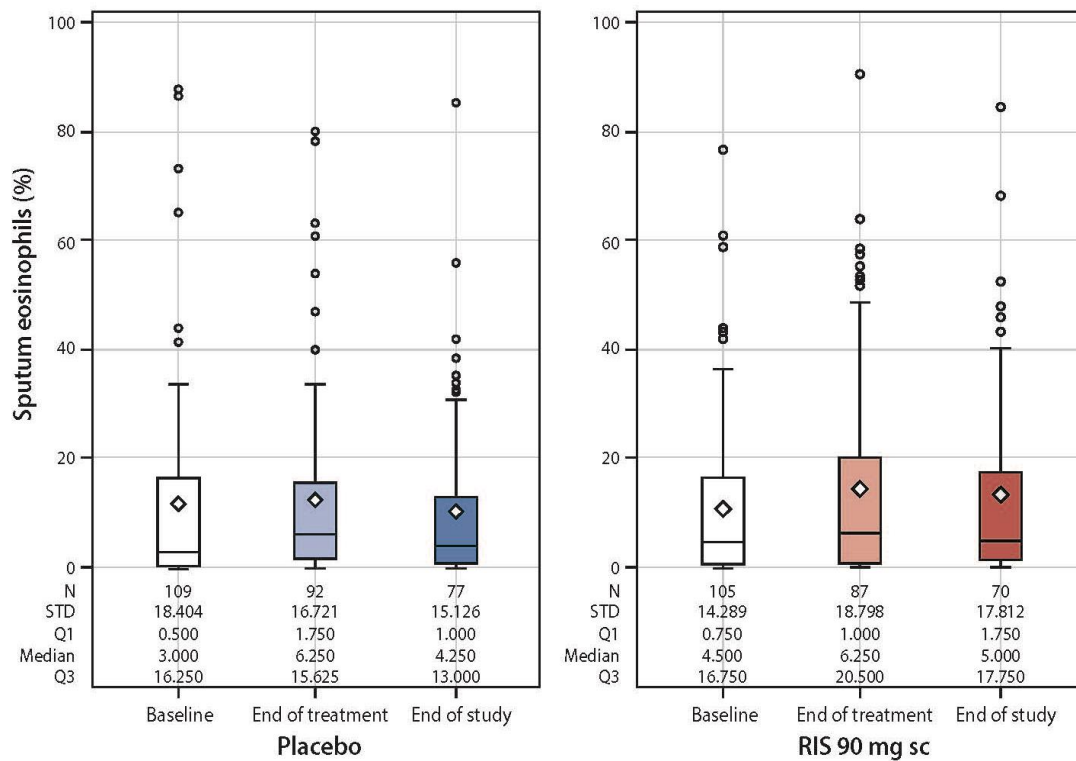
114 FEV<sub>1</sub>, forced expiratory volume in 1 second; RIS, risankizumab; SC, subcutaneous.

**Figure S4** Eosinophil and neutrophil sputum counts (two panels showing change over time for eosinophils and neutrophils)

(A) Distribution of sputum neutrophil percentages at baseline, end of treatment and end of study

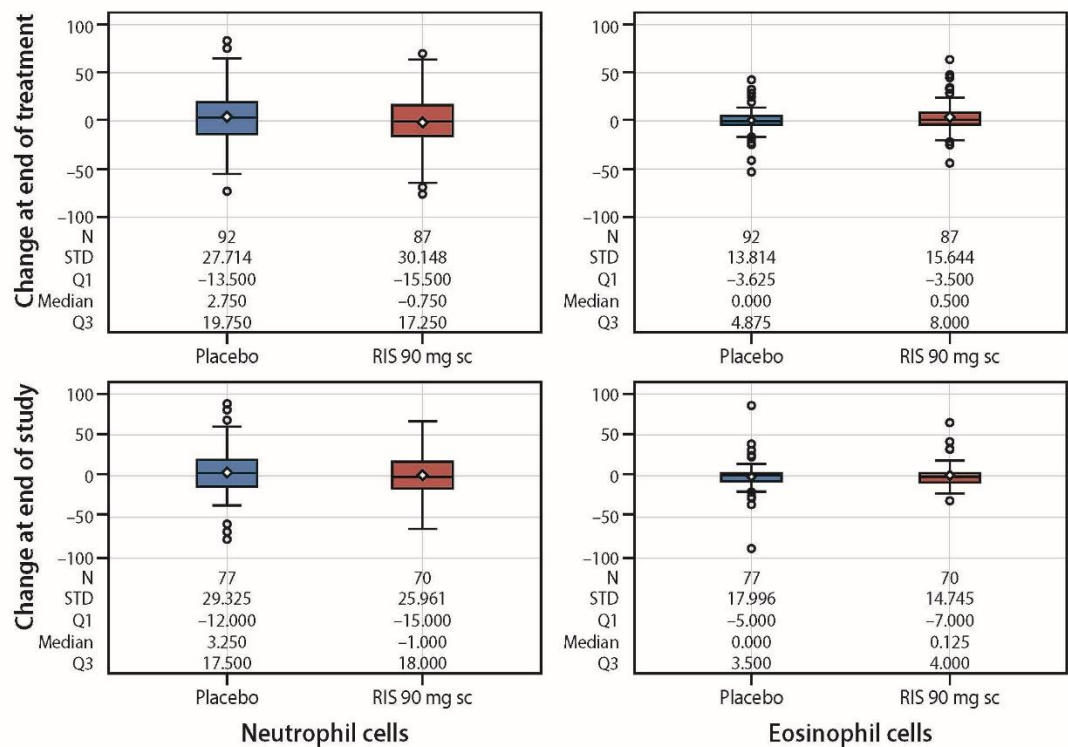


119 (B) Distribution of sputum eosinophil percentages at baseline, end of treatment and end of study



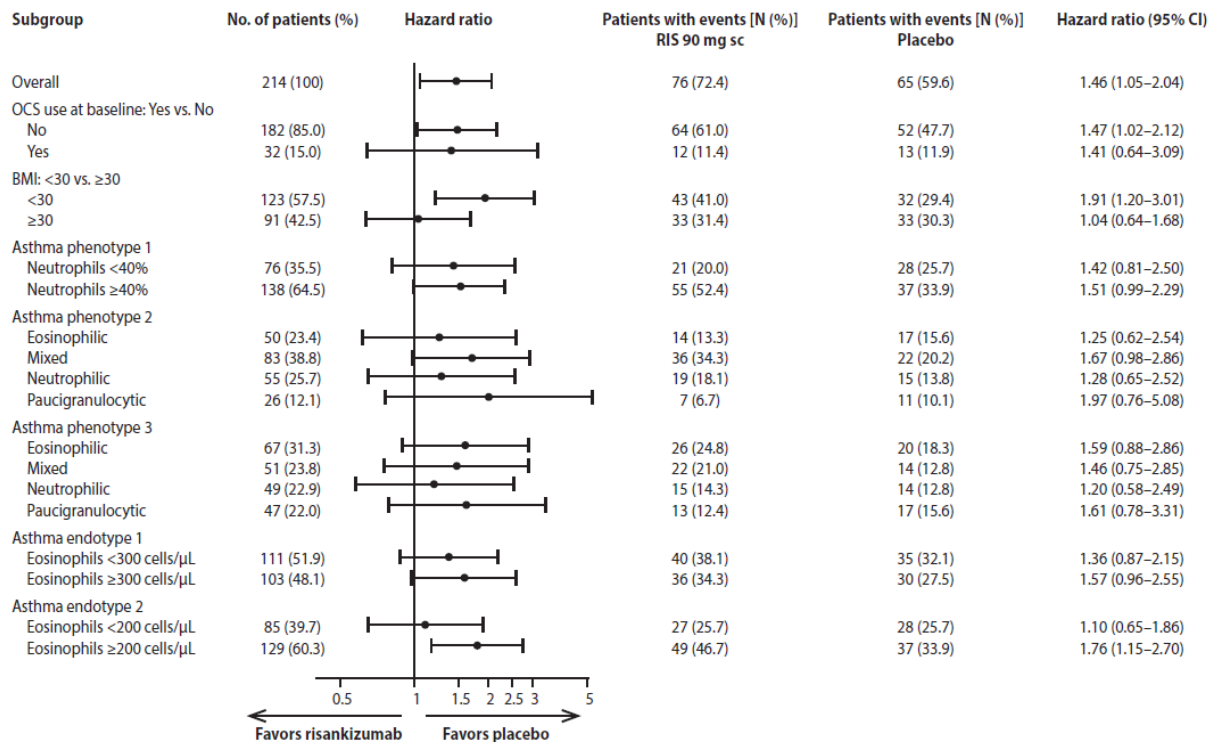
120

(C) Change from baseline in sputum neutrophil and eosinophil percentages at end of treatment  
and end of study by treatment group — ITT



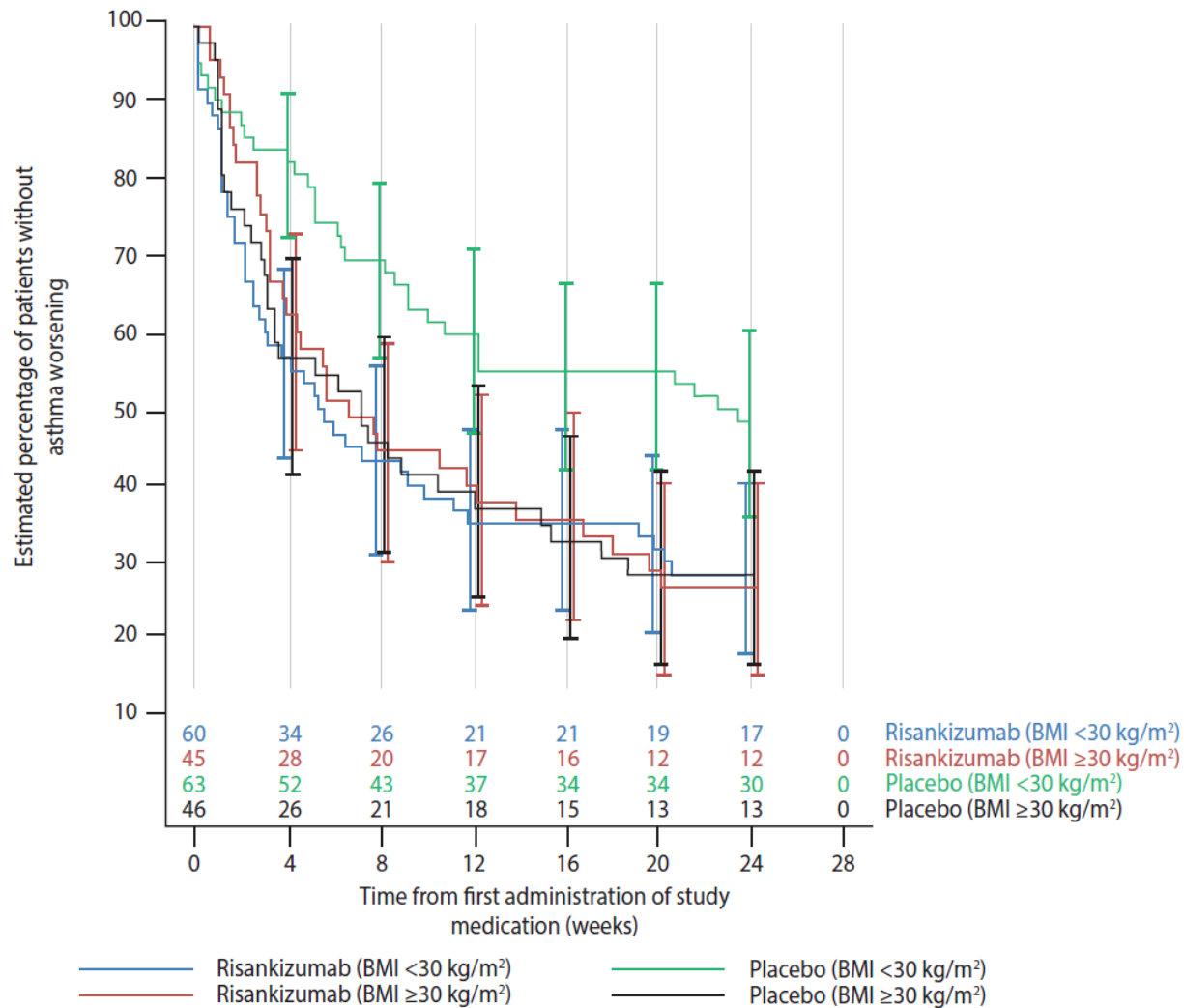
ITT, intention-to-treat; Q1, quartile 1; Q3, quartile 3; RIS, risankizumab; SC, subcutaneous;  
STD, standard deviation.

**Figure S5** Subgroup analysis of time-to-first asthma worsening



BMI, body mass index; CI, confidence interval; OCS, oral corticosteroid; RIS, risankizumab.

**Figure S6** Primary outcome as per BMI – time-to-first asthma worsening during 24-week treatment with risankizumab versus placebo



BMI, body mass index; SC, subcutaneous.

**Table S1.** Ingenuity Canonical Pathway analysis of pathways altered by risankizumab treatment

| Ingenuity Canonical Pathways                               | FDR <sub>bh</sub><br>corrected p-<br>value | Molecules                      |
|------------------------------------------------------------|--------------------------------------------|--------------------------------|
| Type I diabetes mellitus signaling                         | 0.00756                                    | CD3E, GZMB, IFNG, NGFR, PRF1   |
| Neuroprotective role of THOP1 in Alzheimer's disease       | 0.00756                                    | GZMA, GZMB, IFNG, KLK7, PRSS33 |
| Cytotoxic T lymphocyte-mediated apoptosis of target cells  | 0.01750                                    | CD3E, GZMB, PRF1               |
| Crosstalk between dendritic cells and natural killer cells | 0.01750                                    | FSCN2, IFNG, KLRD1, PRF1       |
| Th1 and Th2 activation pathway                             | 0.01840                                    | CD3E, CD8A, CXCR6, IFNG, KLRD1 |
| Primary immunodeficiency signaling                         | 0.02190                                    | CD3E, CD8A, IGHM               |
| Graft-versus-host disease signaling                        | 0.02190                                    | GZMB, IFNG, PRF1               |
| Hematopoiesis from pluripotent stem cells                  | 0.02190                                    | CD3E, CD8A, IGHM               |
| Th1 pathway                                                | 0.02480                                    | CD3E, CD8A, IFNG, KLRD1        |
| Granzyme B signaling                                       | 0.03220                                    | GZMB, PRF1                     |
| Granzyme A signaling                                       | 0.04140                                    | GZMA, PRF1                     |
| T helper cell differentiation                              | 0.04350                                    | IFNG, NGFR, RORC               |

Gene expression analysis was conducted using sputum samples. Pathways are ranked by unadjusted p-value. Only pathways with a false discovery rate Benjamini-Hochberg (FDR<sub>bh</sub>) corrected p-value <0.05 are shown. All pathways identified are dominated by molecules involved in CD8<sup>+</sup> T-cell- and NK-cell-mediated killing. Gene expression analysis was based on sputum samples.

144 **Table S2** Change from baseline of sputum and serum biomarkers at Week 12 and Week 24

|                           | Sputum                   |                          | Serum                   |                         |
|---------------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| Biomarker                 | Risankizumab<br>N=105    | Placebo<br>N=109         | Risankizumab<br>N=105   | Placebo<br>N=109        |
| <b>β-defensin 2, ng/L</b> |                          |                          |                         |                         |
| Baseline                  | N=89<br>270 (37, 929)    | N=92<br>100 (27, 737)    | N=101<br>186 (80, 584)  | N=106<br>226 (100, 502) |
| Change<br>Week 12         | -                        | -                        | N=99<br>-17 (-155, 10)  | N=102<br>1 (-98, 70)    |
| Change<br>Week 24         | N=86<br>0 (-277, 177)    | N=88<br>1 (-50, 245)     | N=99<br>-21 (-157, 22)  | N=106<br>0 (-86, 79)    |
| <b>NGAL, µg/L</b>         |                          |                          |                         |                         |
| Baseline                  | N=89<br>1166 (780, 1652) | N=92<br>1116 (814, 1652) | N=101<br>575 (422, 735) | N=106<br>542 (448, 687) |
| Change<br>Week 12         | -                        | -                        | N=99<br>-6 (-146, 93)   | N=102<br>22 (-118, 107) |
| Change<br>Week 24         | N=86<br>0.00 (-392, 246) | N=88<br>0.00 (-316, 251) | N=99<br>3 (-149, 67)    | N=106<br>-28 (-127, 67) |
| <b>VEGF, ng/L</b>         |                          |                          |                         |                         |
| Baseline                  | N=89<br>566 (349, 859)   | N=92<br>528 (373, 800)   | N=101<br>175 (117, 252) | N=106<br>158 (96, 267)  |
| Change<br>Week 12         | -                        | -                        | N=99<br>2 (-39, 39)     | N=102<br>8 (-24, 44)    |
| Change                    | N=86                     | N=88                     | N=99                    | N=106                   |

|                      |                      |                      |              |              |
|----------------------|----------------------|----------------------|--------------|--------------|
| Week 24              | 29 (–236, 219)       | 15 (–206, 280)       | –4 (–30, 26) | –7 (–35, 30) |
| <b>IL-6, ng/L</b>    |                      |                      |              |              |
| Baseline             | N=84<br>54 (15, 152) | N=86<br>46 (18, 117) | BLQ          | BLQ          |
| Change<br>Week 12    | -                    | -                    | -            | -            |
| Change<br>Week 24    | N=76<br>–8 (–55, 12) | N=79<br>0 (–27, 42)  | -            | -            |
| <b>S-100A8, µg/L</b> |                      |                      |              |              |
| Baseline             | N=89<br>27 (6, 64)   | N=92<br>29 (5, 64)   | BLQ          | BLQ          |
| Change<br>Week 12    | -                    | -                    | -            | -            |
| Change<br>Week 24    | N=86<br>0 (–17, 5)   | N=88<br>0 (–8, 14)   | -            | -            |

145 All data are median (IQR). BLQ, below limit of quantification >50% of samples; IL-6,  
146 interleukin-6; IQR, interquartile range; NGAL, neutrophil gelatinase associated lipocalin; VEGF,  
147 vascular endothelial growth factor.

148

149 **Table S3** Safety of risankizumab (safety analysis set), number of subjects (%)

|                                             | <b>Risankizumab<br/>(n=105)</b> | <b>Placebo<br/>(n=109)</b> |
|---------------------------------------------|---------------------------------|----------------------------|
| Patients with any AE                        | 98 (93)                         | 95 (87)                    |
| AE leading to death                         | 0 (0)                           | 0 (0)                      |
| Any SAE                                     | 14 (13)                         | 28 (26)                    |
| AE leading to discontinuation of trial drug | 3 (3)                           | 3 (3)                      |
| Respiratory                                 |                                 |                            |
| Asthma                                      | 66 (63)                         | 60 (55)                    |
| Dyspnea                                     | 7 (7)                           | 7 (6)                      |
| Cough                                       | 5 (5)                           | 2 (2)                      |
| Infections                                  |                                 |                            |
| Nasopharyngitis                             | 11 (11)                         | 22 (20)                    |
| Bronchitis                                  | 11 (11)                         | 9 (8)                      |
| URTI                                        | 9 (9)                           | 10 (9)                     |
| Sinusitis                                   | 6 (6)                           | 3 (3)                      |
| UTI                                         | 5 (5)                           | 6 (6)                      |
| Respiratory tract infection                 | 4 (4)                           | 2 (2)                      |

150 AE, adverse event; SAE, serious adverse event; URTI, upper respiratory tract infection; UTI,  
151 urinary tract infection.